Proceedings

of the 9th International Symposium on Plant-Soil Interactions at Low pH

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Physiological and molecular mechanisms of plant adaptation to acid soils

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Molecular genetics of plant adaptation to acid soils

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Aluminum toxicity, P deficiency, and other acid soil limitations with a focus on their amelioration and remediation

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Sustainable utilization and management of agricultural, forestry and natural ecosystems on acid soils

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Soil Acidity Effects on the Food Chain (Food Quality, Nutrition and Human Health)

Dubrovnik, Croatia
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PREFACE

Acid soils are a significant limitation to crop production worldwide, due to their wide extent especially in the humid tropics and subtropics where food security is often most tenuous. Managing crop production on acid soils requires that we understand how crop plants deal with the multiple acid soil-based limitations which include aluminum, manganese and iron toxicity, phosphorous deficiency and high phosphorous fixation in the soil, and low levels of essential macronutrients that exist as base cations in the soil, including calcium, magnesium and potassium. It is also critical that we better understand the role and impact of agricultural practices in the management of acid soils, both by reducing those practices such as heavy emphasis on nitrogenous fertilizers that can exacerbate soil acidity and by increasing sustainable agricultural practices that can help to ameliorate soil acidity.

The Plant-Soil Interaction at Low pH (PSILPH) symposium series began in 1987 when scientists from Alberta, Canada, realized that soil and plant scientists needed an interdisciplinary forum where they could come together to discuss their research centered around the problems of acid soils. The first PSILPH symposium was held in the city of Grand Prairie in Alberta, Canada, in July, 1987. This inaugural PSILPH symposium was attended by 109 participants from 13 countries. The proceedings were published in a special issue of the journal, *Communications in Soil Science and Plant Analysis*, and the PSILPH symposium participants agreed this should be an ongoing symposium series that would be held every 3-4 years in different international locations. An international PSILPH steering committee was organized to help plan the second PSILPH symposium, and the USDA-ARS Appalachian Soil and Water Conservation Laboratory in Beaver, West Virginia, was chosen to host the second PSILPH. This conference was held at the Pipestem Resort State Park in West Virginia in June of 1990. For the 2cd PSILPH symposium the attendance grew to 220 participants from 28 countries. The subsequent PSILPH symposia have been held in Queensland, Australia in September, 1993, Belo Horizonte, Brazil in 1996, KwaZulu-Natal Province in South Africa in 2001, Sendai, Japan in 2005, Guangzhou, China in 2008, and Bengaluru, India in 2012.

The 9th PSILPH meeting is being held in Dubrovnik, Croatia on October 18-24, 2015. The Local Organizing Committee consists of members of the Faculty of Agriculture, University of Osijek in conjunction with the Croatian Society of Soil Science. The meeting is being held at the Importanne Resort on the Adriatic Sea in Dubrovnik, Croatia. One hundred and forty six participants will be attending the 9th PSILPH symposium, and 48 oral and 53 poster presentations will be made. The 146 delegates come from 25 countries, including Croatia, China, Japan, India, USA, Nigeria, Lithuania, Brazil, Bosnia & Herzegovina, Montenegro, Malaysia, Australia, New Zealand, Serbia, Romania, Kenia, Hungary, France, Ghana, Italy, Indonesia, South Africa, Germany, Poland, and Russia. The conference is organized around 6 symposium topics: 1) Physical, chemical and biological properties of acid soils; 2) Physiological and molecular mechanisms of plant adaptation to acid soils; 3) Molecular genetics of plant adaptation to acid soils; 4) Aluminum toxicity, P deficiency, and other acid soil limitations with a focus on their amelioration and remediation; 5) Sustainable utilization and management of agricultural, forestry and natural ecosystems on acid soils; and 6) Soil Acidity Effects on the Food Chain (Food Quality, Nutrition and Human Health).

"Leon Kochian, Editor in Chief"

Plenary lectures

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Physical, chemical and biological properties of acid soils

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Soil Acidity Effects on the Food Chain (Food Quality, Nutrition and Human Health)

PLENARY LECTURES

Chairpersons: Leon Kochian, Vlado Guberac, Walter Horst

Acid soils, climate change and greenhouse gas emissions

Zed Rengel

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Abstract
Variable and changing climate is likely to alter soil properties depending on the initial soil and ecosystem properties. Improved understanding of the effects of changing and variable climate on soil properties is urgently needed to inform management decisions aimed at preventing loss of environmental functions in soils and terrestrial ecosystems.

INTRODUCTION

As in the past (Grieve, 2001), current climate changes and variability are likely to have significant short- and long-term impacts on soil properties (Rengel, 2011). One of important measures of soil's capacity to fulfil environmental and economic functions is pH (Rengel, 2002; Rengel, 2003) as a dynamic parameter with significant spatial and temporal differences (Gregory and Hinsinger, 1999).
Close to 4 billion hectares (about 30% of the ice-free soils) in the world are acidic (Sumner and Noble, 2003). The worst situation is in the south-east and eastern Asia and South America, with more than 50% of the total land in these areas having low pH. In Australia, soil acidity has been identified as the most serious land degradation issue, with an estimated AU$1 billion in lost production per year (CSIRO, 2004).

THE EFFECTS OF CLIMATE CHANGE AND ALTERED RAINFALL PATTERNS ON SOIL pH

Evans (2005) used the MAGIC (Model of Acidification of Groundwater In Catchments) and current scenarios of rising temperature and falling rainfall and acid deposition in UK, to forecast that increasing dissolved organic carbon (DOC) and elevated organic acidity would lower soil pH and increase leaching of basic cations into the surface water.
Exacerbated soil acidification may arise from greater biomass production (caused by increased temperatures and increased CO_2 partial pressure in the air); given that most plant material contains excess cations, removal of such material in managed ecosystems (that produce food, feed and fibre for economic benefit) removes alkalinity, leaving non-neutralised acidity in soil (unbalanced C cycle). If nitrate produced in decomposition of organic matter is leached (unbalanced N cycle), one equivalent of H^+ is not consumed in soil (ie. absence of nitrate uptake), thus leaving non-neutralised acidity in soil (see Tang and Rengel, 2003).
Soil acidity hampers nitrification (Kemmitt et al., 2006), resulting in an increased proportion of ammonium to nitrate, potentially minimising leaching losses of N (Kemmitt et al., 2005). However, increasing exudation of H^+ from roots in the process of ammonium uptake may acidify soil.
In areas where climate is expected to become warmer and wetter, microbial respiration may increase, resulting in elevated soil air CO_2 concentrations that correlated positively with the soil pH (Kotroczo et al., 2008). Increased soil temperature also increased the flux of N_2O (potent greenhouse gas) from acidic soils (Tokuda and Hayatsu, 2004).
Acidic soils have a high N_2O production activity because soil acidity increases denitrification potential by blocking N_2O reductase and thus transformation of N_2O to N_2 (Li et al., 2013). Indeed, soil acidification increases the ratio $N_2O/(N_2O+N_2)$ (Qu et al., 2014), thus exacerbating greenhouse gas emissions.

ACID SULPHATE SOILS

Climate change and variability may result in decreased rainfall and diminishing runoff, causing a drop in groundwater levels to expose the overlying soil layers to oxygen. Such conditions would cause oxidation of reduced Fe and S minerals (such as pyrite) to generate acidity (=acid sulphate soils). These processes and reactions (eg. on the Swan Coastal Plain, Western Australia) can be quite quick (Salmon et al., 2014), with a decrease in groundwater pH from around 5 to around 3 occurring within weeks (Appleyard, 2005).

AMELIORATING SOIL ACIDITY AND GREENHOUSE GAS EMISSIONS

The primary pathway for dissolution of agricultural lime may result in retention of CO_2 in the form of bicarbonate (HCO_3^-) and/or carbonic acid (H_2CO_3) in soils (West and McBride, 2005). However, depending on soil pH and the form of N fertilizer used, a fraction of agricultural lime may react with strong acids (such as nitric) and release CO_2 (Hamilton et al., 2007). Using the mass-balance approach (all carbon in lime is released into the atmosphere as CO_2), approximately 9 million tonnes of CO_2 were estimated to have been released from 20 million tonnes of lime applied in the US in 2001, but subsequent revised estimates were about ½ that amount (West and McBride, 2005). In contrast, direct CO_2 emissions from grazed pastures in the UK due to liming were measured to be 4-fold greater than from non-limed land (Gibbons et al., 2014), which might have been a consequence of not properly separating biotic (soil respiration) and abiotic (lime dissolution) CO_2 release (Biasi et al., 2008).

CONCLUSIONS

There is no doubt that variable and changing climate will impact on soil pH and thus indirectly on a myriad of biogeochemical and physical processes occurring in the soil-water-microbe-plant continuum in native and managed terrestrial and aquatic ecosystems. However, such impacts would depend on soil properties and environmental factors. Further work in discerning the effects of increasing air and soil temperatures as well as changes in rainfall distribution and intensity on soil pH and other properties is urgently needed to underpin management decisions required to protect soils as one of the most precious natural resources.

REFERENCES

Appleyard, S. 2005. Potential geochemical and environmental changes caused by lowering of water tables. Waters and Rivers Commission, Perth, Western Australia.

Biasi, C., Lind, S. E., Pekkarinen, N. M., Huttunen, J. T., Shurpali, N. J., Hyvonen, N. P., Repo, M. E., Martikainen, P. J. 2008. Direct experimental evidence for the contribution of lime to CO_2 release from managed peat soil. Soil Biol Biochem. 40, 2660-2669.

CSIRO. 2004. Acid soils - a ticking time bomb? CSIRO Plant Industry Communication, Clayton South, Victoria.

Evans, C. D. 2005. Modelling the effects of climate change on an acidic upland stream. Biogeochemistry. 74, 21-46.

Gibbons, J. M., Williamson, J. C., Williams, A. P., et al. 2014. Sustainable nutrient management at field, farm and regional level: soil testing, nutrient budgets and the trade-off between lime application and greenhouse gas emissions. Agriculture, Ecosystems & Environment. 188, 48-56.

Gregory, P. J., Hinsinger, P. 1999. New approaches to studying chemical and physical changes in the rhizosphere: an overview. Plant Soil. 211, 1-9.

Grieve, I. C. 2001. Human impacts on soil properties and their implications for the sensitivity of soil systems in Scotland. Catena. 42, 361-374.

Hamilton, S. K., Kurzman, A. L., Arango, C., Jin, L. X., Robertson, G. P. 2007. Evidence for carbon sequestration by agricultural liming. Global Biogeochemical Cycles. 21, BG20121, DOI: 20110.21029/22006GB002738.

Kemmitt, S. J., Wright, D., Goulding, K. W. T., Jones, D. L. 2006. pH regulation of carbon and nitrogen dynamics in two agricultural soils. Soil Biol Biochem. 38, 898-911.

Kemmitt, S. J., Wright, D., Jones, D. L. 2005. Soil acidification used as a management strategy to reduce nitrate losses from agricultural land. Soil Biol Biochem. 37, 867-875.

Kotroczo, Z., Fekete, I., Toth, J. A., Tothmeresz, B., Balazsy, S. 2008. Effect of leaf- and root-litter manipulation for carbon dioxide efflux in forest soil. Cer Res Commun. 36, 663-666.

Li, X., Hu, F., Bowman, D., Shi, W. 2013. Nitrous oxide production in turfgrass systems: effects of soil properties and grass clipping recycling. Applied Soil Ecology. 67, 61-69.

Qu, Z., Wang, J., Almoy, T., Bakken, L. R. 2014. Excessive use of nitrogen in Chinese agriculture results in high $N_2O/(N_2O+N_2)$ product ratio of denitrification, primarily due to acidification of the soils. Global Change Biol. 20, 1685-1698.

Rengel, Z. 2002. Handbook of Plant Growth. pH as the Master Variable. Marcel Dekker, Inc., New York, USA.

Rengel, Z. 2003. Handbook of Soil Acidity. Marcel Dekker Inc., New York, USA.

Rengel, Z. 2011. Soil pH, soil health and climate change. In: B. P. Singh, A. L. Cowie, K. Y. Chan (ed.) Soil Health and Climate Change. Springer, Berlin Heidelberg, pp: 68-95.

Salmon, S. U., Rate, A. W., Rengel, Z., Appleyard, S., Prommer, H., Hinz, C. 2014. Reactive transport controls on sandy acid sulfate soils and impacts on shallow groundwater quality. Water Resources Research. 50, 4924-4952.

Sumner, M. E., Noble, A. D. 2003. Soil acidification: the world story. In: Z. Rengel (ed.) Handbook of Soil Acidity. Marcel Dekker, New York, pp: 1-28.

Tang, C., Rengel, Z. 2003. Role of plant cation/anion uptake ratio in soil acidification. In: Z. Rengel (ed.) Handbook of Soil Acidity. Marcel Dekker, New York, pp: 57-81.

Tokuda, S.-i., Hayatsu, M. 2004. Nitrous oxide flux from a tea field amended with a large amount of nitrogen fertilizer and soil environmental factors controlling the flux. Soil Sci Plant Nutr. 50, 365-374.

West, T. O., McBride, A. C. 2005. The contribution of agricultural lime to carbon dioxide emissions in the United States: dissolution, transport, and net emissions. Agriculture, Ecosystems & Environment. 108, 145-154.

Why some plants cope with acid soils better than others: What we know and what we don't.

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Abstract

At the turn of last century our understanding of acid soils and how they limited plant growth and production was meagre. Directed breeding programs had not been considered. About one hundred years ago soil scientists in Indiana and Massachusetts in the USA began to publish results linking soluble aluminum in acid soils with reduced plant growth (Abbott et al., 1913; Ruprecht 1915; Miyake 1916). Reports soon emerged from Brazil describing "crestamento" (burning or toasting) symptoms on crops grown on certain soils and in 1942 these were finally attributed to low soil pH (Matzenbacher 1988). Genotypic variation was identified in Brazilian cereals in the 1920s and in 1925 crosses between wheat cultivars Alfredo Chaves and Polyssu to generated new crestamento-tolerant cultivars such as Fronteira, Surpresa and Minuano. Breeding for acid-tolerant crops then developed over the following decades and the cultivar Carazinho was released in 1957. As recently as thirty years ago scientists still had no clear understanding of how acid soils constrained plant growth, apart from some obvious nutrient effects. Similarly there was no model to explain why some species, or even genotypes within species, coped in these conditions better than others. A little over twenty years ago studies convincingly demonstrated for the first time how some plants avoided the toxic effects of Al^{3+} by releasing organic anions from their roots. Exactly how this release occurred or how it was regulated remained unknown. Certainly there was no information on the genetic control although there were ideas that transport proteins were involved. Mechanisms of Al^{3+} toxicity remained an active area of research with many theories being presented but without a clear consensus. It seemed that aluminum interacted with and disrupted most cell processes. About ten years ago the first genes controlling Al^{3+} resistance were identified. These encoded transport proteins from the ALMT and MATE families and were shown to function by releasing organic anions from roots. Around the same time several groups increased the aluminium tolerance of transgenic plants either by manipulating organic acid synthesis and transport or by expressing genes which increased tolerance to oxidative stress. Over the last ten years unprecedented leaps have been made. Novel Al^{3+} resistance mechanisms and genes have been identified in diverse species. Genes were identified through quantitative trait analyses, genome wide mapping as well as through mutational analysis and transcriptomics. Those discoveries exploited the rapid developments in molecular technologies and bioinformatics. Furthermore, robust models are beginning to emerge for how Al^{3+} disrupts root growth either through damaging interactions in the root apoplast or by disrupting cellular mechanisms for DNA repair.

Every three or four years the latest information in this important field is presented to the larger scientific community at the International Symposia of Plant-Soil Interactions at Low pH. This Plenary address will endeavor to provide a summary of the latest set of astonishing developments which explain a little more why acid soils are toxic to plants and how some plants over-come these stresses better than others.

REFERENCES

Abbott JB, Conner SD, Smalley HR (1913) Soil acidity, nitrification, and the toxicity of soluble salts of aluminium. *Indiana Agric. Exp.Sta. Bui.* **170.**

Miyake K (1916) The toxic action of soluble aluminum salts upon the growth of the rice plant. *Journ. Biol. Chem.* 25, 23.

Matzenbacher RG (1988) Advances made in developing wheat with better aluminium toxicity tolerance in Brazil. *In:* AR Klatt (ed.) Wheat production Constraints in Tropical Environments, CIMMYT, D.F.: Mexico. Pp 285-304

Ruprecht W R (1915) Toxic effect of iron and aluminum salts on clover seedlings. *Mass. Agric. Exp. Sta. Bui.* **161.**

Molecular regulatory mechanisms of phosphate starvation response in rice

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Abstract

Phosphorus (P) is an essential macronutrient for plant growth and development. The high chemical fixation rate, slow diffusion and substantial fractions of organically bound P of Pi render it one of the least available nutrients for crop. Developing crop cultivars with increased yield and less dependence on the heavy application of fertilizers is essential for the sustainability of agriculture. Over the past decade, our lab aimed at understanding the molecular regulation of Pi acquisition and homeostasis in the major cereal crop rice. This presentation summarizes the recent progress in our lab in determining the signal networks of Pi starvation response, the roles of SPX proteins in Pi homeostasis, the functions and regulation of rice Pi transporters (PT) and potential genes for improving Pi uptake efficiency.

In rice, OsPHR2 was identified as a central regulator of Pi starvation signaling by binding to P1BS (PHR1 binding sequence) element (GNATATNC). Overexpression of *OsPHR2* in rice mimicked the Pi starvation signal. It induced PSI (Phosphate starvation induced gene) gene expression and resulted in the enhancement of Pi acquisition. The PSI genes that are activated by the overexpression of *OsPHR2* include genes encoding the signaling molecules *OsIPS1/2*, microRNA *osa-miR399* and *osa-miR827*, *SPXs*; *PTs* (*Phosphate transporters*) for Pi uptake and translocation; *PAPs* for releasing Pi from organic P; *SQD2* (*Sulfoquinovosyldiacylglycerol 2*) for recycling Pi from membrane phospholipids etc. We confirmed that existence of P1BS and P1BS-like motifs in promoter are essential for stable binding by OsPHR2. The highest affinity motif was GaATATtC (A–T-type P1BS). Functional redundancy was identified among three PHR1 orthologs in rice (OsPHR1, OsPHR2, and OsPHR3) using phylogenetic and mutation analysis. They function redundantly in transcriptional activation of most Pi starvation-induced genes. However, these three TFs showed differences in DNA binding affinities. The P1BS binding affinity of OsPHR2 was the highest among three proteins, whereas that of OsPHR3 was the lowest. However, unlike OsPHR2-overexpressing lines, which exhibited growth retardation under normal or Pi-deficient conditions, OsPHR3-overexpressing plants exhibited significant tolerance to low-Pi stress but normal growth rates under normal Pi conditions, suggesting that OsPHR3 would be useful for molecular breeding to improve Pi uptake/use efficiency under Pi-deficient conditions.

Recent works in our lab published in Proc Natl Acad Sci USA, Plant Cell and J Exp Bot and the work in Arabidopsis published in Prof. Javier Paz-Ares' lab indicate that SPX proteins play very important role in sensing the external and internal Pi level and negatively regulating of Pi homeostasis in plants. SPX proteins are referenced as proteins exclusively harboring the SPX domain. Six SPX proteins in rice (named OsSPX1-OsSPX6) have been identified. All the SPX genes were Pi starvation induced except *OsSPX4*. Our results demonstrate that SPX proteins function as key components in the Pi-sensing mechanism to control the activity of OsPHR2. Under high cellular Pi conditions, SPX4 interact with OsPHR2 in the cytoplasm which inhibit OsPHR2 from moving into nuclear, while in nucleus, SPX1, SPX2 will further interact with OsPHR2, therefore OsPHR2 can't bind to the promoter of PSI genes, so PSI genes would express in a basal level. However, under low cellular Pi conditions, SPX4 will be degraded, so OsPHR2 can move into nuclear, while in nuclear, interaction of SPX1 and SPX2 with OsPHR2 was weakened and OsPHR2 will preferentially bind to the P1BS motif of PSI gene promoters, allowing OsPHR2 to up-regulate the expression of PSI genes, including OsSPX1 and OsSPX2, OsSPX3, OsSPX5, and OsSPX6. Which make sure the plant cell to adjust itself to the changed Pi environment. The accumulation of SPXs under Pi-deficient conditions also allows plants to shut down the OsPHR2 dependent Pi starvation response rapidly after Pi repletion.

In rice genome, 13 PTs were identified. Among them, OsPT2/6/8/9/10 have been functionally analyzed in detail in our lab. They play a broad role in Pi uptake, translocation and internal transport throughout the plant to enable adaptation to the changing P status of the soil. Our results showed that the trafficking of PT from the endoplasmic reticulum (ER) to plasma membrane requires the PHF1 (PHOSPHATE TRANSPORTER TRAFFIC FACILITATOR1). OsPHF1 can interact with non-phosphorylated PTs but not phosphorylated PTs. Therefore, phosphorylation of PTs affects their trafficking from the endoplasmic reticulum (ER) to the plasma membrane. By yeast two-hybrid screen, we identified a rice kinase subunit, CK2β3, which interacts with PT2 and PT8. The CK2α3/β3 holoenzyme phosphorylates PT8 under phosphate-sufficient conditions. This phosphorylation inhibited the interaction of PT8 with PHF1, which regulating the exit of PTs from the ER to the plasma membrane. On the contrary, phosphorus starvation promoted CK2β3 degradation, relieving the negative regulation of PT. Therefore, more PT protein were trafficked to plasma membrane to transport Pi into plant cell

in phosphorus-deficient conditions. In accordance, transgenic lines expressing a nonphosphorylatable version of OsPT8 resulted in elevated levels of OsPT8 protein at the plasma membrane and enhanced phosphorus accumulation and plant growth under various phosphorus regimes.

Because the PHF1-mediated posttranslational regulation of Pi-transporters does not change the cell- or tissue-specific expression patterns of Pi-transporter genes, modulation of PHF1 on PHT1 may provide a new strategy to improve Pi uptake to enhance crop tolerance to low Pi stress. Overexpression of *OsPHF1* in 9311, an *indica* restorer line of Super Hybrid Rice, led to a significant enhancement of tolerance to low Pi stress in transgenic rice in field test. The field experiment data demonstrated that posttranscriptional regulation of Pi transporters can be used as a novel strategy to improve Pi uptake ability. Furthermore, *OsPHR3*-overexpressing plants exhibited significant tolerance to low-Pi stress but normal growth rates under normal Pi conditions, suggesting that *OsPHR3* would also be useful for molecular breeding to improve Pi uptake/use efficiency under Pi-deficient conditions.

In conclusion, we elucidated the molecular regulation network of phosphate starvation response signaling in rice. It greatly promoted our understanding of how plant sensing the environmental Pi status and reprograming the gene expression profile to adapt to the environment. Our current knowledge of Pi uptake and transport has provided evidence that we can improve Pi uptake ability and tolerance to low Pi stress of rice through molecular manipulation.

Improving crops for agriculture on acid soils: A molecular breeding perspective

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Abstract

Low soil phosphorus (P) availability and aluminum (Al) toxicity are the two major constraints for crop production in tropical regions on acid soils. Considerable progress has been made on fundamental research identifying physiological and molecular determinants of crop Al tolerance and more recently, progress has been made on identifying QTL and associated genes conferring increased P efficiency, which is defined here as increased shoot biomass or grain yield on low P soil compared to the same yield traits on P sufficient soils. Researchers are now beginning to translate this basic understanding of Al tolerance and P efficiency to the molecular breeding of crops with improved performance on acid soils. This talk will present several examples of this type of translational research.

The rice protein kinase, phosphorus-starvation tolerance 1 (OsPSTOL1), was previously shown to be involved in rice P efficiency, enhancing P acquisition and grain yield in rice under P deficiency. We investigated the role of homologs of *OsPSTOL1* in sorghum P efficiency. Association mapping was undertaken in two sorghum association panels phenotyped for P uptake and root system morphology and architecture in hydroponics, and also for grain yield and biomass accumulation on low P soils in Brazil and in Mali, Africa. Root length and root surface area were found to be strongly and positively correlated with grain yield when grown on a low P soil, emphasizing the importance of P acquisition efficiency in sorghum adaptation to low P availability. *SbPSTOL1* alleles linked to sorghum genotypes with longer and finer roots were associated with enhanced P uptake under low P in hydroponics, whereas alleles of two *SbPSTOL1* genes, *Sb03g006765* and *Sb03g0031680*, were associated with increased root surface area and increased grain yield on a low P soil. *SbPSTOL1* genes co-localized with quantitative trait loci for traits underlying root morphology and yield (dry weight accumulation) under low P via linkage mapping. Consistent allelic effects for enhanced sorghum performance under low P for both sorghum association panels, including enhanced grain yield under low P in the field in Brazil, point towards a relatively stable role for *Sb03g006765* across genetic backgrounds and environmental conditions. Our results also indicate that multiple *SbPSTOL1* genes have a more general role in the root system, not only enhancing root morphology traits but also changing root system architecture, which leads to grain yield gain under low P availability in the soil.

Root damage caused by Al toxicity is another major cause of grain yield reduction on acid soils. In sorghum, Al tolerance is conferred by SbMATE, an Al-activated root citrate efflux transporter that underlies the major Al tolerance locus, Alt_{SB}, on sorghum chromosome 3. We used association mapping to gain insights into the origin and evolution of Al tolerance in sorghum and to detect functional variants amenable to allele mining applications. A haplotype network analysis suggested a single geographic and racial origin of causative mutations in primordial guinea domesticates in West Africa. We identified SNP and indel loci within Alt_{SB} that are useful for large-scale germplasm screening aimed at identifying Al tolerant sorghum genotypes by high-throughput genotyping. In this presentation we will emphasize molecular breeding aspects aimed at improving overall sorghum performance on acid soils with low P availability based on Alt_{SB} and *SbPSTOL1*.

Section 01:

Physical, chemical and biological properties of acid soils

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Effect of pH levels on organic carbon status and soil aggregation

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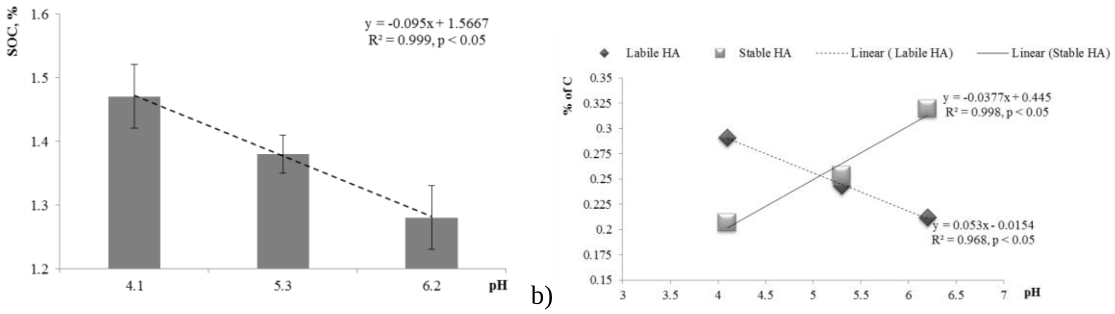
Abstract
Variations of SOC status considering to soil pH levels was investigated in this research. Soil samples were taken in 2011 from the topsoil (0-20 cm). The study showed different quantities of SOC and carbon forms accumulated in the soil. A reduction of SOC content at higher pH was observed. The pH increment changed the SOC stability. The decrease of labile HA and increase of stable HA amount, was established in the soil with increasing soil pH. Water – stable aggregates which are important with respect to long-term SOC sequestration had a tendency to increase parallel to the pH increment.

INTRODUCTION
Considerable effort is dedicated nowadays to determine the effect of agricultural practices on status of soil organic carbon (SOC) and carbon-compound transformations in agricultural soils. The extent of SOC sequestration also varies with land management (Fornara et al. 2011). One of the important indicators seeking to evaluate SOC status is soil pH level. Regarding the potential effect of pH, more favorable pH stimulates soil biological activity and transport into underlying horizons, thus favoring SOC mineralization and very likely accelerating organic matter turnover rates in soil. It was observed that the SOC lost at higher pH was mainly (up to 84% of total loss) in the labile form of SOC occluded within macroaggregates (Paradelo et al., 2015). Fornara et al. (2011) determined that the greater biological activity, despite increasing soil respiration rates, favoured the SOC incorporation into resistant soil organo-mineral pools and improves the efficiency of SOC physical protection. In this way, it favors long-term C sequestration and may provide a mechanism as well as prediction opportunities for soil conservation, sustainability, and protection against degradation (Jokubauskaite et al.,2015). The aim of this paper is to assess the effect of different pH levels on the accumulation and stability of SOC and soil aggregatation.

MATERIALS AND METHODS
The soil *Bathygleyic Dystric Glossic Retisol* (texture-moraine loam with clay-sized particles content of 12-14%) was investigated in this research. Soil samples were taken in 2011 from the topsoil (0-20 cm) with three replicates. Systematic soil liming over 56 years (by 0.5 rates every 7 year (18.1 t ha^{-1} $CaCO_3$) and 2.0 rates every 3-4 year (104.9 t ha^{-1} $CaCO_3$) was done on the background of minimal organic fertilizing and conventional tillage. This influenced the formation of the different soil pH levels: i - pH 4.1 (control); ii - pH 5.3; iii - pH 6.2.
In these essentially different by pH soil the study of soil aggregation and organic carbon compounds was made. Soil structure and aggregate stability in water was estimated according to Savinov. SOC content was determined by photometric procedure at the wavelength of 590 nm using the UV-VIS spectrophotometer Cary 50 (*Varian*) after wet combustion according to Nikitin. SOC fractions were determined by Ponomariova and Plotnikova version of classical Tyurin method.According to this method, the extracted fractions was grouped into the two fractions: labile humic acids (HA1) and stable humic acids (HA2+HA3). The data were processed ($P<0.05$) by the statistical program STAT ENG for EXCEL version 1.55. Each variable (n=3) was displayed as mean ± standard error of the mean.

RESULTS AND DISCUSSION
SOC is the most important indicator of soil quality. The data about the pH effect on the SOC content in soils are often contradictory. Some authors have observed a reduction of SOC content at higher pH, when the rising pH increases microbial activity favoring SOC mineralization (Fornara et al. 2011, Kowalenko et al., 2013). No significant effects of pH levels have also been reported (Costa, 2012). Our study showed that an increasing pH had a negative effect on SOC content in the soil (Fig. 1a). SOC content was 1.47% for the control treatment (pH – 4.1) and at higher pH (5.3 and 6.2) was approximately by 0.09-0.19 percentage points respectively lower compared to the control.



a) b)

Figure 1. The relation between pH and SOC content in soil (a), pH and labile and stable humic acids (b), where SOC – soil organic carbon, HA – humic acids.

The soil with various pH levels differed in organic carbon stability (Fig. 1b). The increasing soil pH, decreased the content of labile HA. Various mechanisms have been suggested to explain this phenomenon, such as increased organic matter solubility, increased microbial activity, an increase in the production of soluble molecules due to the decrease in biologically toxic Al at higher pH. The largest amount of stable HA, which represents the resistance to degradation and the possibility for sequestration, was established in the soil with pH 6.2.

Results showed that macroaggregates which contains active, easy mineralizable carbon had a tendency to decrease at the pH 5.3. In contrast, water – stable aggregates which are more stable in soil and important with respect to long-term SOC sequestration had a tendency to increase parallel to the pH increment. Increased microbial activity at higher pH, may affect aggregate stability, since microorganisms produce extracellular polysaccharides which act as binding agents (Paradelo et al., 2015).

Table 1. The effect of different pH levels on soil aggregatation and carbon dioxide emission (mg g^{-1}) from soil.

Soil pH level	***Soil aggregates, %***				***CO_2 emission, mg g^{-1} from soil through vegetation period***
	Macroaggregates, >5mm	*Mezoaggregates, 0.5-0.25 mm*	*Microaggregates, < 0.25 mm*	*Water-stable aggregates, > 0.25 mm*	
4.1	13.49 ± 4.1	60.35 ± 6.33	26.16 ± 3.75	24.8 ± 1.49	0.03 ± 0.01
5.3	10.80 ± 2.79	62.97 ± 1.19	26.22 ± 3.15	29.8 ± 2.13	0.04 ± 0.01
6.2	14.89 ± 4.13	60.67 ± 5.63	22.55 ± 2.01	28.8 ± 1.97	0.04 ± 0.01

Activity of soil microorganisms was estimated according to carbon dioxide (CO_2) emission from soil through vegetation period. Results showed that increase of pH stimulates the soil biological activity, thus favoring the mineralization of SOC, which results in CO_2 losses.

CONCLUSION

An increase of soil pH had a negative effect on SOC content due to stimulation of microbial activity favoring mineralization processes in soil. The soil with various pH levels differed in SOC stability. Soil with the pH 4.1 contained more labile HA, which shows its higher predisposition to transformation. The pH increment, increased amount of stable HA, which represents the resistance to degradation and the possibility for sequestration. Amount of water – stable aggregates had a tendency to increase parallel to the pH increment which is an important indicator with respect to long-term SOC sequestration.

REFERENCES

Costa M.C.G., 2012. Soil and crop responses to lime and fertilizers in a fire-free land use system for smallholdings in the northern Brazilian Amazon. Soil Tillage Research. 121. 27–37.

Fornara D.A., Steinbeiss S., Mcnamara N.P., Gleixner G., Oakley S., Poulton P.R., MacDonald A.J., Bardgett R.D., 2011. Increases in soil organic carbon sequestration can reduce the global warming potential of long-term liming to permanent grassland. Global Change Biology 17. 1925–1934.

Jokubauskaite I., Slepetiene A., Karcauskiene D., 2015. Influence of different fertilization on the dissolved organic carbon, nitrogen and phosphorus accumulation in acid and limed soils. Eurasian Journal of Soil Science 4(2). 137 – 143.

Kowalenko C.G., Ihnat M., 2013. Residual effects of combinations of limestone, zinc and manganese applications on soil and plant nutrients under mild and wet climatic conditions. Canadian Journal of Soil Science 93. 113–125.

Paradelo R., Virto I., Chenu C., 2015. Net effect of liming on soil organic carbon stocks: A review. Agriculture, Ecosystems and Environment 202. 98–107.

Total and plant available trace elements in acid and calcareous soils of eastern Croatia

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Abstract
Main agricultural region of Croatia, eastern Croatia has soils of wide acidity. The soils vary from very acid to calcareous soils. Liming is often necessary to achieve suitable conditions for agricultural production. Trace element availability also varies due to the wide range of acidity. Two most common extraction methods for available fraction of trace elements (EDTA and DTPA) have been tested on soils of different pH in order to investigate the relationship between these two methods. The results show different extraction values for different elements between the methods, however for all elements there were a strong correlation between extraction methods indicating that one method can be used to easily predict the values of the other method.

INTRODUCTION
Maximum permissible concentrations of trace elements are most commonly defined by total concentrations in soil. However, numerous studies have shown that total concentrations are often not good representative of plant uptake. Soil properties, metal speciation and plant species, especially soil-plant interactions, determine the availability of metals in soils (Ehlken and Kirchner, 2002). Therefore, various one-step extraction methods such as EDTA, DTPA, $CaCl_2$, $NaNO_3$, water extraction etc. have been used to show available fraction. Out of these extraction methods most widely used are EDTA and DTPA extractions. Both of these methods have been used due to their ability to form very stable, water-soluble and well-defined complexes with metal cations (Norvell, 1984; Brun et al., 2001; Hammer and Keller, 2002; Chaignon et al., 2003; Feng et al., 2005). In present study we have observed extraction of trace elements from soil by these two methods on acid and calcareous soils.

MATERIALS AND METHODS
The study was conducted in Danube basin of eastern Croatia where 229 samples from soil depth 0-30 were collected during 2013-2014. Sampling sites included acid and calcareous soils. Samples were analyzed for standard soil properties (pH, organic matter, AL-P, AL-K) as well as for total (*aqua regia*), EDTA and DTPA extractable trace elements (Fe, Mn, Zn, Cu, Ni, Co, Cr, Cd and Pb). Statistical analysis was done in Minitab statistical software and Microsoft Excel.

RESULTS AND DISCUSSION
Analyses of main soil properties show vide variety of soils. Soil pH (in H_2O) was in range 4.4 - 8.7 (avg: 6.8), thus sampling sites included range from very acid to alkaline soils. Organic matter (OM) varied from 0.8-4.5 % (avg: 2.0), most of the soils were poor in OM however soils from farms that use organic manure was high in OM. Similar situation was with available P and K where some extreme values were found on farms using organic fertilizers, otherwise average phosphorous was 31.8 mg/100g and potassium 23.6 mg/100g (Table 1.).

Table 1. Main soil properties of sampling sites

Variable	n	Mean	StDev	Min.	Max.
pH(H_2O)	229	6.8	1.1191	4.4	8.7
pH(KCl)	229	5.9	1.2503	3.7	8.3
OM (%)	229	2.0	0.5329	0.8	4.5
AL-P_2O_5 (mg/100g)	229	31.8	56.62	3.5	460.3
AL-K_2O (mg/100g)	229	23.6	12.241	9.9	132.6

Total concentration of trace elements extracted by *aqua regia* show satisfactory results as not one sample had elevated levels of toxic trace metals (Cd, Cr and Pb) or potentially toxic elements (Zn, Cu and Ni). In that regard all sites satisfy Croatian regulation on pollutants in agricultural fields. All the investigated trace elements (except Pb) extracted by EDTA were correlated with total concentrations, extraction by DTPA shows correlation for all trace elements with their total concentrations.

EDTA and DTPA extraction methods were studied in more details as samples were divided based on soil pH. In order to investigate relationship between two methods we divided samples into three pH groups (4.4-6.5; 6.5-7.5 and 7.5-8.7). For all pH groups EDTA extraction showed significantly higher results for Zn, Cu, Ni, Cr, Cd and Pb, for Co and Mn there was no significant difference between EDTA and DTPA extractions while DTPA extraction showed significantly higher values only for Fe (Table 2). Two extraction methods were correlated for all investigated trace elements, except for Co at pH range between 6.5-7.5 and Cr at pH range at pH range 4.4-6.5.

Table 2. EDTA and DTPA extraction values

	n	pH range	EDTA	DTPA
Fe (mg kg^{-1})	102	pH 4.4-6.5	98.5	122.2***
	42	pH 6.5-7.5	50.2	73.2***
	85	pH 7.5-8.7	22.8	35.3***
Mn (mg kg^{-1})	102	pH 4.4-6.5	42.4	46.9
	42	pH 6.5-7.5	32.2	38.9
	85	pH 7.5-8.7	25.7	25.1
Zn (mg kg^{-1})	102	pH 4.4-6.5	1.67***	0.72
	42	pH 6.5-7.5	1.49***	0.69
	85	pH 7.5-8.7	1.65***	0.72
Cu (mg kg^{-1})	102	pH 4.4-6.5	3.97***	2.30
	42	pH 6.5-7.5	4.41***	2.38
	85	pH 7.5-8.7	5.65***	2.42
Ni (mg kg^{-1})	102	pH 4.4-6.5	1.44***	0.97
	42	pH 6.5-7.5	1.38***	0.94
	85	pH 7.5-8.7	1.39***	0.78
Co (mg kg^{-1})	102	pH 4.4-6.5	0.20	0.19
	42	pH 6.5-7.5	0.14	0.14
	85	pH 7.5-8.7	0.11	0.1
Cr (mg kg^{-1})	102	pH 4.4-6.5	0.16***	0.02
	42	pH 6.5-7.5	0.10***	0.01
	85	pH 7.5-8.7	0.08***	0.01
Cd (mg kg^{-1})	102	pH 4.4-6.5	0.09***	0.05
	42	pH 6.5-7.5	0.09***	0.06
	85	pH 7.5-8.7	0.11***	0.07
Pb (mg kg^{-1})	102	pH 4.4-6.5	2.63***	1.37
	42	pH 6.5-7.5	2.54***	1.34
	85	pH 7.5-8.7	2.92***	1.35

*** indicates significant differences at $p<0.001$

CONCLUSION

Two extraction methods, EDTA and DTPA, are most commonly used for determination of available trace elements. In our study they show good correlation which indicates that if we have the results from one method we can still predict the values of the other one. The pH groups did not effect this relationship, EDTA showed higher values for Zn, Cu, Ni, Cr, Cd and Pb, while DTPA for Fe. Cobalt and Mn had no significant difference between EDTA and DTPA extractions.

REFERENCES

Brun, L.A., Mailler, J., Hinsinger, P., Pe´pin, M., 2001. Evaluation of copper availability to plants in copper-contaminated vineyard soils. Environmental Pollution 111, 293-302

Chaignon, V., Sanchez-Neira, I., Herrmann, P., Jaillard, B., Hinsinger, P., 2003. Copper bioavailability and extractability as related to chemical properties of contaminated soils from a vinegrowing area. Environmental Pollution 123, 229-238.

Ehlken, S., Kirchner, G., 2002. Environmental processes affecting plant root uptake of radioactive trace elements and variability of transfer factor data. A review. Journal of Environmental Radioactivity 58, 97-112.

Feng M-H, Shan, X-Q, Zhang, S., Wen, B. 2005. A comparison of the rhizosphere-based method with DTPA, EDTA, CaCl2, and NaNO3 extraction methods for prediction of bioavailability of metals in soil to barley. Environmental pollution 137; 231-240

Norvell, W.A., 1984. Comparison of chelating agents as extractants for metals in diverse soil materials. Soil Science Society of America Journal 48, 1285-1292.

Role of bacteria in Al-toxicity

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Abstract
The goal of our study was to evaluate the effect of bacteria fertilizer (BF) on the growth of cucumber under aluminium ($Al_2(SO_4)_2$) stress (10^{-3}M, 10^{-4}M). Different responses to Al stress were examined in physiological traits. The applied BF contains *Azotobacter chroococcum* and *Bacillus megaterium*. Due to different Al treatments higher Al concentration was observed in the leaves, while the amounts of other elements (Fe, Mn, Zn, Mg) decreased. This high Al content of the leaves decreased below the control value when BF was applied. According to our results the BF enhances dry matter production and compensates the Al-stress condition.

INTRODUCTION

Some heavy metal-like ions are constituents of the upper soil layer in large amounts, as is the Al. The lowering soil pH makes these compounds more soluble (Matsumoto, 2000). In crop production, aluminium toxicity is one of the major growth limiting factors in acidic soils. The effect of different bacteria on the compensation of Al-stress has already examined (Orhan et al., 2006; Han et al., 2006). The use of plant growth promoting rhizobacteria (PGPR) including phosphate and potassium solubilising bacteria as a bacteria fertilizer (BF) was suggested as a sustainable solution to improve plant nutrition and production (Vessey, 2003) These bacteria vary in their mechanisms of plant growth promotion but generally influence growth via P solubilisation, nutrient uptake enhancement, or plant growth hormone production (Richardson, 2001). Bacteria are common inhabitants of metal-contaminated sites, where they accumulate and immobilize heavy metals. The cell walls of gram-positive bacteria have strong metal-binding properties (Beveridge et al., 1982). Some bacteria also produce extracellular polysaccharide sheaths that bind metals (Matthews et al., 1979). Binding of the siderophore to a heavy metal dramatically changes the free metal concentration. The effect on metal uptake and toxicity are dependent on this siderophore-metal complex being recognized by an uptake receptor (Clarke et al., 1987). Siderophore production is used *in situ* as a protective mechanism against heavy metal toxicity (Fekete and Barton, 1992).
The aim of this study was to examine the compensation effect of living bacteria containing fertilizer which contains two bacteria *Azotobacter chroccoccum* and *Bacillus megaterium* under Al stress conditions.

MATERIALS AND METHODS

The experimental plant was cucumber (*Cucumis sativus* L. *cv. Delicates*). The seeds were soaked in 10 mM $CaSO_4$ for 4 hours after sterilization and then germinated on moistened filter paper at 25 ^oC. The seedlings were transferred to continuously aerate modified Hoagland nutrient solution. The seedlings were grown in growth chamber (light/dark regime 10/14 h at 24/20^{o}C, relative humidity of 65–70%, 300 μmol m^{-2} s^{-1}). The dry matter content was measured by thermogravimetric method. The element (Al, Fe, Mn, Zn) contents were determined using an OPTIMA 3300DV ICP-OA spectrophotometer. The relative chlorophyll contents were measured using a Chlorophyll Meter, SPAD - 502 (Minolta, Japan). The applied BF contains *Azotobacter chroococcum* and *Bacillus megaterium*. The dose of BF was 1ml dm^{-3}. The nutrient solution was completed with $Al_2(SO_4)_3$ (10^{-4}M and 10^{-3} M) when Al-stress was examined. The Al-compounds and BF were added to the nutrient solution at the beginning of the experiments and at every nutrient solution change. The experiment was finished on the 23rd day of experiment. Microsoft Office Excel 2003 and Sigma Plot 8.0 version were used to the statistical analysis.

RESULTS AND DISCUSSION

Usually, plant growth is the most sensitive to heavy metals because of the complexity of the physiological processes involved. Firstly, the toxic effect of Al was observed at the $Al_2(SO_4)_3$ treatments (Table 1).The dry weights of cucumber shoots and roots decreased under Al treatments.

The effect of $Al_2(SO_4)_3$ on the DW depends on the applied concentration. The lower Al concentration caused moderate DW reduction both in the shoot and root. The reduction effect was higher in the case of root (14 %) than in the shoot (6 %). The 10^{-3}M Al treatments resulted in less (40-50 %) dry matter production, than 10^{-4}M Al treatment compared to the control values. The root was more sensitive to the higher Al concentration, as the same affect was experienced in the case of lower Al concentration. The 10^{-3}M Al treatments caused a more than 50 % decline in the dry weight of the root. The additional applied living bacteria containing fertilizer significantly increased the dry matter of cucumber roots compared to the simple Al treatment. The dry matter of shoots and roots increased with 40-50% at the 10^{-4} $Al_2(SO_4)_3$ treatment due to additional BF treatments. In the case of 10^{-3}M Al2$(SO_4)_3$ the BF treatment did not induce any changes.

Table 1. Effects of different Al- concentrations and BF treatment on the dry weight (DW) of cucumber shoots and roots (n=3± S.D.) g plant^{-1}, significant difference compared to the control: *<0.05, and Al treatment to BF application: $^{a}p<0.05$

Treatments	DW of shoot	DW of root
Control	0.17± 0.07	0.15± 0.01
BF	0.31± 0.07*	0.21± 0.07*
10^{-4} M $Al(SO_4)_3$	0.16± 0.06	0.13± 0.06
10^{-4} M $Al(SO_4)_3$+BF	0.23± 0.06*^{a}	0.19± 0.04*^{a}
10^{-3} M $Al(SO_4)_3$	0.10± 0.09	0.07± 0.01*
10^{-3} M $Al(SO_4)_3$+BF	0.11± 0.04	0.05± 0.01*

Examining the favourable impact of BF on the uptake of Al and other elements, the contents were measured during Al-stress (results are not shown). The amounts of Fe, Mn and Zn were analysed, because they have very important role in the redox, detoxification and energy transformation process. Duo to two Al-treatments, higher Al concentration was observed both in the shoot and root, while the amount of Fe, Mn and Zn decreased. The Al-content of shoot and root decreased when BF was added to the Al-treatments and in the case of the shoots, it declined below the control value. The content of Fe, Mn and Zn increased during BF treatment compared to the only Al-treatments. The element composition of leaf may influence chlorophyll content. The toxicity and growth inhibition effect of Al is related to decreased photosynthesis and decreased organic matter production. The relative chlorophyll content (SPAD-value) decreased by 9-14 % under Al-stress in the 17th day of treatments. This decrease of SPAD-value was also experienced compared to the control value when BF was applied on the 17th day of experiment. In older plants (23rd days), the SPAD-value was significantly higher when BF was applied (results are not shown).

CONCLUSION

According to our results the BF is an alternative nutrient supply for replacing chemical fertilizers because it enhances dry matter production. BF usage is also offered under Al polluted environmental conditions. The nutrient solution is a clean system where we can examine the main processes without other effects of natural soils. The soil can modify the results, e.g. the soil born microorganisms have effect on nutrient availability, and also can modify the harmful effects of different heavy metals. The understanding of basic processes will help us to know more about the soil behaviour.

REFERENCES

Beveridge, T. J., Forsberg, C. W., Doyle, R. J., 1982. Major sites of metal binding in Bacillus licheniformis walls. J. Bacterial. 150. 1438-1448.

Fekete, F. A., Barton L. L.. 1992. Effects of iron(III) analogs on growth and pseudobactin synthesis in a chromium-tolerant Pseudomonas isolate. Biol. Metals 4. 211-216.

Han, H. S., Supanjani, Lee, K. D., 2006. Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. Plant Soil Environ. 52 (3). 130-136.

Matsumoto, H., 2000. Cell biology of aluminium toxicity and tolerance in higher plants. Int. Rev. Cytol. 200. 1-47.

Matthews, T. H., Doyle R. J., Streips U. N., 1979. Contribution of peptidoglycan to the binding of metal ions by the cell wall of Bacillus subtilis. Curr. Microbiol. 3. 51-53.

Orhan, E., Esitken, A., Ercisli, S., Turan, M., Sahin, F., 2006. Effects of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry. Scientia Horticulturae 111. 38-43.

Richardson, A. E., 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Australian Journal of Plant Physiology 28. 897-906.

Vessey, K. J., 2003. Plant growth promoting rhizobacteria as biofertilizer. Plant Soil 255. 571-589.

The influence of liming and organic fertilization on microbial activity in the soil

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Abstract
Agricultural production on acid soils is representing a major problem. For this reason, we set up a field experiment on two soil types (Pseudogley, Luvisol). Liming by carbocalk was conducted on the basis of soil analysis with an application of animal manure at the same time, or after 7, 14 or 28 days. All variants of liming with the application of animal manure gave better results compared to the control. The best results of all investigated parameters (total number of bacteria, fungi, actinomycetes, *A. chroococcum*, cellulolytic microorganisms, pH, phosphorus, potassium) were obtained in the variant by application of carbocalk and animal manure at the same time.

INTRODUCTION

Agricultural production on acid soils is representing a major problem for producers. The limited number of plants that tolerate low pH, investment in such production are much higher and finally sown varieties of crops cannot achieve their genetic potential. In acid soils fungi are dominating microorganisms, which as acidophilic microorganisms lead processes of humification of organic residues and produce, so called, acidic humus. Mineralization of acidic humus frees very little nutrients in plants affordable forms. For this reason, acid soils require greater amounts of mineral fertilizers than neutral soils. The only measure to repair acid soils is conducting lime. However, the implementation of this measure results in "stress" among the microbial population, which further adversely affects on all processes in soil that are in connection to microorganisms, primarily on the processes of humification of organic residues and mineralization of humus. For this reason, one extremely important agromeliorative procedure after conducting liming is the incorporation of organic fertilizers, primarily the introduction of mature cattle manure. This is a basic prerequisite for improving the qualitative and quantitative composition of microorganisms in the soil.

MATERIALS AND METHODS

The study was conducted on two types of soil (Pseudogley, Luvisol). Experimental plot was 2000 m^2 (20 x 100 m). The research was conducted in four repetitions, with 10 variants: A. The texture of soil: A1 - medium-heavily textured soil; A2 - heavily textured soil. B. Liming and fertilization with cattle manure: B1 –control; B2 - liming by simultaneous application of cattle manure; B3 - liming + cattle manure application after 7 days; B3 - liming + cattle manure application after 14 days; B3 - liming + cattle manure application after 28 days. Before the implementation of liming we took soil samples and analyse them by standard methods and determinate following parameters: chemical properties of soil (pH_{H2O}, pH_{KCl}, P, K) and microbiological properties of soil (total number of bacteria, fungi and actinomycetes, content of nitro- fixing bacteria in the soil- *Azotobacter chroococcum*, content of cellulolytic bacteria in the soil).

Table1. Chemical properties of soils.

Investigated parameters	Type of soil	
Layer (0 – 0.3 m)	**Pseudogley**	**Luvisol**
pH (H_2O)	5.36	5.70
pH (KCl)	4.55	4.87
Humus (%)	1.83	1.86
P_2O_5 (mg/100 g soil)	17.99	20.93
K_2O (mg/100 g soil)	14.17	22.19

The applied amount of carbocalk was about 10 tonnes for every degree up to pH KCl 7. Amount of applied cattle manure was 30 t / ha. From the beginning up to the end of investigation, every 7 days, soil samples were taken for microbiological analysis. For determining the chemical properties (pH_{H2O}, pH_{KCl} , humus, P, K) of the soil, samples were taken every 14 days.

RESULTS AND DISCUSSION

The total number of bacteria was highest in the variant where carbocalk and cattle manure were applied into the soil at the same time. But, how the application time of cattle manure changed from the application time of carbocalk, so the tendency of increasing the total number of bacteria was prolonged. The same was in the case of total number of actinomycetes, nitrofixing bacteria *A. chroococcum* and cellulolytic microorganisms.

In the variant where carbocalk and cattle manure were applied into the soil at the same time, the fastest and largest reduction of fungi was achieved. But how the appliction time of cattle manure changed from the application time of carbocalk, so the tendency of reducing their number was slower.

Liming with carbocalk has led to an increase in the pH of the soil and increase the amount of humus, phosphorus and potassium. Also, the increase of the investigated parameters was fastest in the variant were carbocalk and cattle manure were applied at the same time.

However, all variants where cattle manure was applied into the soil have given significantly higher values compared to the control variant. At soil type pseudogley in variant B2 (liming by simultaneous application of cattle manure) after 28 days pH in KCl was 6.62 and was for 48.10% higher compared to the control variant; 4.42% higher compared to variant B3; 6.60% higher than the variant B4 and 8.70% higher compared to variant B5. At soil type luvisol the pH in KCl was about 39.80% higher compared to the control, 3.47%; 5.38% and 6.86% higher compared to the variants B3, B4 and B5, respectively.

The same variant (B2) gave the greatest increase in the content of P_2O_5 and K_2O in soil. At soil type pseudogley P_2O_5 content was 22.40 mg / 100 g soil, and 19.50 mg K_2O / 100 g soil. At soil type luvisol the content was 26.00 mg P_2O_5 / 100 g soil and 27.03 mg K_2O / 100 g soil.

The more time passed between liming and fertilization with cattle manure, the slower and fewer was the increase of pH and the content of P_2O_5 and K_2O. The obtained results are in accordance with the research results which were obtained by Haynes and Naidu, 1998; Jarak et all., 2006; Mühlbachová and Tlustoš, 2006; Whalen et all., 2000 and Yagi et all., 2003.

CONCLUSION

Based on the results of numerous investigations on the influence of liming and organic fertilization on soil microbial activity, we can conclude:

- Application of carbocalk, according to the results of chemical analysis, increases the soil reaction (pH) in both types of soil.
- All variants of liming with the application of cattle manure achieved significantly better results of investigated parameters compared to the control.
- The variant in which carbocalk and cattle manure were applied at the same time achieved the best results of all investigated parameters.
- Because of the stress to indigenous strains of microorganisms in the soil, caused by the implementation of carbocalk, it is important to apply cattle manure, slurries or microbial preparation containing beneficial soil microorganisms.

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REFERENCES

Haynes, R. J., Naidu, R. 1998. Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review. Nutrient Cyclingin Agroecosystems, 51(2): 123-137.

Jarak M., Đukic D. and Colo J. 2006. The effect of bacterization and liming in the production of red clover. Sustainable Grassland Productivity, 291-293.

Mühlbachová, G., Tlustoš, P. 2006. Effects of liming on the microbial biomass and its activities in soils long-term contaminated by toxice lements. Plant soil environ., 52(8): 345–352.

Whalen, J. K., Chang, C., Clayton, G. W., Carefoot, P. 2000. Cattle Manure Amendments Can Increase the pH of Acid Soils. Soil Sci. Soc. Am. J., 64:962-966.

Yagi, R., Ferreira, M. E., Pessôa da Cruz, M. C., Barbosa, J. C. 2003. Organic matter fractions and soil fertility under the influence of liming, vermicompost and cattle manure. Sci. agric. (Piracicaba, Braz.), 60(3):28-36.

Plant-Soil interactions at low pH research in South Africa: A review

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Abstract
Soil acidity is a major constraint to plant productivity in South Africa. Consequently, there has been a consistent interest by researchers on the effects of low soil pH on plant production. A systematic review was conducted to investigate various research that has been done on this topic in South Africa. The reviewed literature was found to focus mainly either on soil fertility management or plant sensitivity. Liming is commonly practiced but requires advancement. Currently soil fertility management and plant sensitivity to low soil pH are being addressed through conservation agriculture and breeding programmes respectively.

INTRODUCTION

Soil acidity is a major constraint to plant productivity worldwide. Approximately 30% of the world's total land area and over 50 % of the world's potential arable lands are acidic (von Uexkull and Mutert, 1995a). In South Africa acid soils are widespread in eastern and central parts of the country, with 94% of the soils estimated to be acidic (Sydenham, 2015). There has been concerted efforts by soil and plant scientists alike to understand the effects of acidity on soil fertility and plant production.Whilst soil acidification is caused by natural processes i.e. climate, hydrologic cycle vegetation and parent rocks, it is accelerated by the use of acid-forming fertilizers (Bian et al., 2013). For example, nitrogenous fertilizers release nitrate and hydrogen ions into the soil. The nitrates readily get leached leaving excess hydrogen ions in the root-zone thereby increasing soil acidity (Gazey, 2015). The major effects of low soil pH is both toxicity and deficiency of certain elements e.g. Al, Mn and Fe toxicity and P, Ca, Mg and K deficiency (von Uexkull and Mutert, 1995b). Addition of lime, calcium or magnesium to the soil is the most common approach for alleviating soil acidity (Joubert et al. 2007; Bambara and Ndakidemi, 2010). However, these ameliorants are often too expensive for most smallholder farmers in South Africa (Bian et al., 2013) and alternative approaches, such as breeding for acid and Al tolerance in crops should be investigated (Bian et al., 2013; Sydenham, 2015). This review aims to discuss the current trends, perspectives and future direction of research on plant-soil relations in soils with low pH in South Africa.

MATERIALS AND METHODS

A systematic review has been conducted to select research done on the topic of low pH soils and the effect on plant production in South Africa. We used the search engine Web of Science, with keywords "low pH", "soil" and "South Africa", as well as the local South African journals in which results would most likely be published, such as South African Journal of Plant and Soil (keywords "low pH soil") and South African Journal of Science (key words "low pH soil" and "acid soil"). A total of 31 publications met the criteria of being suitably focussed on low pH plant-soil interactions and were used as a representative sample in this review.

RESULTS AND DISCUSSION

Plant-Soil interactions at low pH research in South Africa

The bulk (32%) of the literature focussed on fertility management issues, especially nutrient dynamics of acid soils. For instance, after studying N and S dynamics, Ngezimana and Agenbag (2014) found canola S application should match S adsorption capacity of the low pH soils. Test plants varied from wheat and various legumes to tree species. About 13% of the surveyed literature focussed on the survey and management of acid soils and some of the studies aimed at using existing data to identify areas prone to acidification (Fey and Dodds 1998) or determining effects of residue management on acidification (Kotze and Du Preez, 2008). The results showed that no tillage combined with chemical weeding was the most beneficial to restrict acidification thus further highlighting an added advantage of implementing conservation agriculture (CA). Similarly, Loke et al. (2013) showed that no tillage and stubble mulch suppressed soil acidification. Therefore, recent studies tend to focus on the management of acidic soils using CA principles (Loke et al., 2013) and nutrient dynamics (Reddy et al., 2014). However, there could be more work done on plant-soil relations in low pH soil in South Africa, which is not available on online databases. This is especially true for most of the work funded by government agencies, where upon completion, the reports are archived. The same also applies for some theses and dissertations.

Several works on this subject are reported in literature from the late 1970`s to 2015 covering mainly soil fertility management and plant sensitivity on a variety of plants such as maize, beans, soya beans, sugarcane, wheat, pastures, trees and mixed systems. Early work focused on the effect of liming (Dee et al., 2003), especially on sugarcane quality and quantity (Moberly and Meyer, 1975). One of the key findings of that research was the importance of Al^{3+} rather than adsorbed H^{+} that constitute the main source of acidity. Several other follow-up studies on Al^{3+} have since been done e.g. Clough (1991), Buhmann et al. (2006) and Haynes and Mokolobate (2001). Studies on Al^{3+} toxicity constituted about 13% of literature. The effects of Al^{3+} toxicity include inhibition of root growth immediately after exposure to micro molar concentrations of Al. Recently, the ARC has embarked on screening programmes to identify Al tolerant wheat cultivars (Sydenham, 2015). Preliminary results suggest that of the 23 cultivars under dryland cultivation, six are moderate resistant whilst eight are resistant types. An earlier study by Bosch et al. (1996) showed that Al tolerant cultivars can be successfully cultivated in low pH soils and high levels of acid saturations, while sensitive cultivars need higher pH and lower acid saturation levels. Consequently, more effort is being put into making Al tolerance a breeding target, which may be a cheaper alternative to liming. Other studies on sensitivity issues include those by Noble and Harding (1989) and Folscher and Barnard (1985). Beukes et al. (2008) recommended that future research on acid soils should focus on quantification of extent and intensity of soil acidity and developing amelioration procedures especially using gypsum. However, this has not been adequately addressed by researchers in the last decade. Instead, interest seems to shift towards acid tolerance in plant breeding programmes. To this effect, work on wheat has identified a common gene called ALMT1, which confers tolerance to Al^{3+} (Sydenham, 2015). However, it may take time to come up with these tolerant genotypes, which leaves liming as the traditional approach. Another area which is gaining attention is the effect of climate smart agriculture approaches such as conservation agriculture (CA) on soil acidity.

CONCLUSION

Soil acidity has significant negative effect on plant production in South Africa. Liming is the main corrective approach for soil acidity but it is expensive and not readily available. Alternative approaches, such as plant breeding for higher acid tolerance or CA farming systems represent the current two-pronged approach to tackle plant sensitivity and soil fertility management in acid soils.

REFERENCES

Bambara, S., Ndakidemi, P.A., 2010. Phaseolus vulgaris response to Rhizobium inoculation, lime and molybdenum in selected low pH soil in Western Cape, South Africa. African Journal of Agricultural Research, 5 (14): 1804-1811

Beukes, D.J., Stronkorst, L.D., Jezile, G.G., 2008. Development of a soil protection strategy and policy for South Africa: An overview of the soil acidity problem in South Africa. Final Report to: Department of Agriculture Report number GW5/A/2008/08.

Bian, M., Zhou, M., Sun, D., Li, C., 2013. Molecular approaches unravel the mechanism of acid soil tolerance in plants. The Crop Journal, 1: 91-104.

Bosch, O.J.H., Otto, W.M., Human, J.J., 1996. Die invloed van grondsuurheid op koringkultivars (Triticum aestivum L.) met verskillende vlakke van alumiumverdraagsaamheid in die Oos-Vrystaat. I. Opbrengs. South Africa Journal of Plant and Soil, 31 (1); 27-30.

Buhmann, C., Beukes, D.J., Turner, D.P., 2006. Acidity/Al aspects in soils of the Lusikisiki area, Eastern Cape Province. SA J Plant Soil, 23 (2): 87-92.

Clough, M.E., 1999. Comparison of aluminium, basic cations and acidity in the soil of indigenous forests and pine stands in the southern Cape. South Africa Journal of Plant and Soil, 8 (4): 177-183.

Dee, B.M., Haynes, R.J., Graham, M.H., 2003. Changes in soil acidity and the size and activity of the microbial biomass in response to the addition of sugar mill wastes. Biology and Fertility of Soils, 37: 47–54.

Fey, M.V., Dodds, H.A. 1998., Classifying the sensitivity of soils of the South African highveld to acidification. South Africa Journal of Plant and Soil, 15 (3): 99-103.

Folscher, W.J., Barnard, R.O. 1985., Groei en chemiese samestelling van verskillende peulgewasse onder suurgrondtoestande. South Africa Journal of Plant and Soil, 2 (2): 93-97.

Gazey, P. 2015. Soil Acidity. Home page address: http://soilquality.org.au/factsheets/soil-acidity. (Accessed August 18, 2015).

Haynes, R.J., Mokolbate, M.S. 2001. Amelioration of Al toxicity and P deficiency in acid soils by additions of organic residues: a critical review of the phenomenon and the mechanisms involved. Nutrient Cycling in Agroecosystems, 59:47-63.

Joubert, M.E., Kotze, W.A.G., Wooldridge, J. 2007. Effect of liming and mineral nutrition on growth of honeybush (Cyclopia spp.) plants. South Africa Journal of Plant and Soil (3): 161-165.

Kotze, E., Du Preez, C.C. 2008. Influence of long-term wheat residue management on acidity and some macronutrients in an Avalon soil. South Africa Journal of Plant and Soil, 25 (1): 14-21.

Loke, P.F., Kotze, E., Du Preez, C.C. 2013. Impact of long-term wheat production management practices on soil acidity, phosphorus and some micronutrients in a semi-arid Plinthosol. Soil Research, 51: 415-426.

Moberly, P.K., Meyer, J.H., 1975. The amelioration of acid soils in the South African sugar industry. Fertiliser Society of South Africa Journal, 2: 57-66.

Ngezimana, W., Agenbag, G.A. 2014. Nitrogen and sulfur effects on macro and micronutrient contents of canola (Brassica napus L.) grown on acidic soils of the Western Cape Province of South Africa. South Africa Journal of Plant and Soil, 45 (13): 1840-1851.

Noble, A.D., Harding, T. 1989. Aluminium and pH effects on dry bean root growth in nutrient solution. South Africa Journal of Plant and Soil, 6(4): 245-249.

Reddy, M., Moodley, R., Jonnalagadda, S.B. 2014. Elemental uptake and distribution of nutrients in avodcado mesocarp and the impact of soil quality. Environ Monit Assess 186: Environtal Monitoring and Assessment, 186 (7): 4519-29.

Sydenham, S. 2015. The importance of aluminium tolerance for production. SA Graan/Grain, Wheat Focus June 2015, 16-19.

Von Uexküll, H.R., Mutert, E. 1995b. Global extent, development and economic impact of acid soils. In: Date R.A., Grundon N.J., Raymet, G.E., Probert, M.E. (eds) Plant–Soil Interactions at Low pH: Principles and Management. Kluwer Academic Publishers, Dordrecht, the Netherlands, p. 5-19.

Von Uexküll, H.R., Mutert, E. 1995a. Global extent, development and economic impact of acid soils. Plant and Soil, 171:1-15.

A Review of Some Physico-Chemical and Biological Properties of Some Acid Soils of Southeastern Nigeria

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Abstract
A review of acid soils within Southeastern Nigeria (4–6°59' N and 7-10°11' E) is made. Some soils are very strongly acid to moderately acid (pH 4.7 to 5.9) while others range from extremely to very strongly acid (pH 3.6 to 5.0) depending on land use and genesis. Cations and NO_3^- are depleted due to excessive leaching. The soils are texturally coarse and structurally weak which indicate degradation reminiscent of climatic condition of the zone. Total porosity is low while bulk density is high giving rise to low water retaining and conducting capacities. Low input tillage systems negatively impact on biodiversity which therefore reduces biological activities. The soils can be rejuvenated with tillage and agronomic practices incorporating application of amendment.

INTRODUCTION
Information on bio-physico-chemical properties of soils is important in evaluating and predicting their capacities to sustain long-term cropping. A key factor in the continuous productivity of tropical soils is organic manuring (Oguike and Mbagwu, 2004). Soil acidity may be attributed to inorganic or organic fertilization, parent material, climatic patterns and acid rain (Brady and Weil, 1999). Across acid soils, properties differ with land use and parent materials. For example, turning forest to cultivated land causes degeneration. Hence, conclusions on management strategies for soils require assessment of differences in properties due to land use and soil origin. This review is focused on low pH and soil properties under differing uses and genesis.

RESULTS AND DISCUSSION
Physical Properties
Coastal plain sand soils are relatively deep with coarse textures, varying from sandy to loamy sand (Lekwa and Whiteside, 1986). Within southeastern Nigeria, texture ranges from sandy loam to loamy sand across land use (Oguike and Mbagwu, 2009) and sandy clay loam to loam across parent materials (Igwe et al., 1999).
The soils are referred to as "structureless", mainly as single grains. The sandy loams range from weak crumb to granular due to amount of clay and organic matter (OM). With low OM content, bulk density (BD) increases, reflecting low total porosity (Pt) ranges (38-51%). Water stable aggregates are low: 1.8 mm to 0.8 mm (MWD) from four-year bush fallow to grassland, respectively (Oguike and Mbagwu, 2009).
Water retained at field capacity (FC) and permanent wilting point (PWP) appears similar with similar textures. However, At PWP, differences are significant. The trend indicates inverse relationship with BD. The low water retention is attributed to low OM and higher percentage of macro pores. Hydraulic conductivity values range from 7.1 to 55.4 cm/h (Obi and Asiegbu, 1980) which are considered slow to rapid respectively.

Chemical Properties
Across parent materials, pH values vary from 4.77 to 5.92 (pH_{H2O}) and from 3.7 to 5.12 (pH_{KCl}). The soils have low base saturation due to nutrient leaching. Exchange Al^{3+} contributes more to low pH values (Oguike and Mbagwu, 2009) in some soils of varying land use.
Comparatively, soils derived from Imo Clay Shale contain more OM than the other parent materials (Igwe et al., 1999). Similarly, forest soils contain more OM than the other land use types. Higher OM of forest soils may be due to plant materials returned to the soil in abundance.
The coarse nature of the soils result to leaching of NO_3^- thereby keeping N content low. The range is from 0.13 to 0.28% across land use (Oguike and Mbagwu, 2009). However, values as low as 0.02% are observed for highly weathered ultisols (Oguike and Mbagwu, 2001).
Complexing with Fe under low pH reduces avail. P. At low pH, there is P-fixation. Where avail. P is observed to be high, Fe^{3+} may have been reduced to Fe^{2+} under redox reaction such that P bound to Fe^{3+} becomes available in the soil as Fe^{2+} is mobile.
In the SE Nigeria, exchange properties are low or moderate indicating degradation. The low levels are due to torrential rainfall and high insolation that quickens OM decomposition and deep leaching (Oguike and Mbagwu, 2009).

Biological properties
Biodiversity is low resulting to reduced bio activity. Earthworm caste counts are affected by conventional tillage practices which are devoid of mulching.

CONCLUSION

The soils are degraded showing poor biodiversity observable in acid soils. The soils are exposed to leaching losses of nutrients due to poor structures. These situations have negative consequences on soil productivity. Liming and organic manuring which improve pH are recommended.

REFERENCES

Oguike, P.C. and Mbagwu, J.S.C. (2004). Changes in some physical properties of two degraded soils treated with water hyacinth residues. Int. Journal of Agric. and Biol. Sci. 3:47-52

Brady, N.C. and Weil, R.R. (1999). *Soil reaction: acidity and alkalinity. The nature and properties of soils (12th Ed.)* Prentice Hall Inc. 343-344

Lekwa, G. and Whiteside, E.P. (1986). Coastal plain Soil of southeastern Nigeria. 1. Morphology, classification and genetic relationships. Soil Sci. Soc. Am. Journ. 50(1): 154-160.

Oguike, P.C. and Mbagwu, J.S.C. (2009). Variations in some physical properties and organic matter content of soils of coastal plain sand under different land use types. World Journal of Agricultural Sciences. 5(1) 63-69.

Igwe, C.A., Akaimigbo, F.O.R. and Mbagwu, J.S.C. (1999). Chemical and mineralogical properties of soils in southeastern Nigeria in relation to aggregarte stability. Geoderma 92:111-123.

Obi, M.E. and Asiegbu,B.O. (1980). The physical properties of some eroded soils of southeastern Nigeria. Soil Sci. 130(1): 39-48.

Oguike, P.C. and Mbagwu, J.S.C. (2001). Effects of water hyacinth residues on chemical properties and productivity of degraded tropical soils. Agro-Science Journ. of Trop. Agric . Food, Environ. and Ext. 2(2) : 44-51

Physical and Chemical Properties of the Sandy Deposits of the Semi-arid Sokoto, Nigeria

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Abstract
Soils resulting from sandy deposits of Quaternary age in Sokoto, Nigeria (Sangiwa, Sokoto and Illela) were identified and characterized for physical and chemical properties. Profile pits were dug in representative areas and samples taken from pedogenic horizons for analyses. The soils are young, generally sandy in nature with poor structural development and because of their geography and granulometry they are undoubtedly aeolic in character. The soils are generally low in inherent fertility, evidenced by low organic matter content and CEC. The soils are acidic with reaction ranging from very strongly acid to slightly acid (4.6 to 5.4). The acidity is caused by intense leaching of the basic cations and the predominance of Al in the exchangeable sites. Incorporation of organic manure, and crop residues and liming were identified as ways of improving the fertility status of the soils and also reduce acidity.

INTRODUCTION
Sokoto located in the Northwest corner of Nigeria has a hot, semi-arid tropical climate with Sudan savanna vegetation The mean annual rainfall varies from 380 mm to 899 mm and falling between June and September, The maximum and minimum temperatures of the area are 40^oC and 15^oC respectively (Arnborg, 1988). Sedimentary rocks of Cretaceous and Tertiary ages are the major geological formation of the present Sokoto State. They were deposited as sediments in a synclinical basin, active from the late Cretaceous to the early Tertiary and are calcareous in nature. In the Quaternary period, deposition of aeolian materials over the sedimentary deposits took place (Sombroek and Zonneveld, 1971). These deposits are Sangiwa, Sokoto, and Illela deposits. The materials are sandy in nature and constitute part of the arable land in Sokoto state (Zonneveld, 1999) and crops grown include cowpea, millet, cassava etc. The soils where these materials have been deposited are described as the poorest soils in the state in terms of fertility rating (Yakubu *et al.*, 2006). Given the calcareous nature of the parent rocks coupled with the Semi-arid climate associated with high temperature and evapotranspiration, the soils are expected to have high pH. The deposition of these sandy materials might influence the soil properties and hence pH. Parent materials influence soil formation by their different rates of weathering, their nutrients and the dominant particle-size they contain. The less developed a soil is, the greater will be the effect of parent material on the properties of the soil (Esu, 1999). It is in realization of the fact that the deposition of the sandy materials on the Sedimentary rocks of Cretaceous and Tertiary ages may influenced the properties of the soils of the area, that is why this research was designed to determined the physical and chemical properties of the soils and to provide management guideline for their better and sustainable utilization.

RESULT AND DISCUSSION
Physical properties of the soils
Sangiwa deposit (Pedon1) have sand weighted average values of 84 % and 95 %, 89 % to 94 % in Pedons 2 (Sokoto deposit) and 95 % to 98 % in Pedon 3 (Illela deposit). The sandy nature of the soils was attributed to the fact that the parent materials are aeolian and colluvial in origin (Sombroek and Zonneveld, 1971). Bulk density weighted values ranged from 1.6 to 1.8 Mg/m^3 in all the pedons. High bulk density values of the soils may be due to compaction caused by the use of heavy machinery during land preparation and grazing animals which is a common phenomenon in Sokoto environment. Porosity values ranged from 62 % to 69 % in all pedons. The porosity values are high and could favour good aeration and free water movement in the soils. The high porosity values of the soils could be attributed to its sandy nature. Average values of the silt/clay ratio are 1.3, 1.3 and 0.5 in pedons 1, 2 and 3. Silt/clay ratio is an important criterion used in the classification of tropical soils. It is also used in the evaluation of weathering stage and age of parent material and soils (Nwaka, 1990). Results of this study show that, all the soils have silt/clay ratio above 0.15 or 0.25 indicating that the soils are young and so the influence of the deposited materials on soil properties.

Chemical characteristics of the soils
The soil pH (H_2O) varies from very strongly acid to strongly acid and ranged from 4.8 to 5.4 in pedon 1 (Sangiwa), 4.6 to 4.9 in pedon 2 (Sokoto) and 4.8 to 5.3 and in pedon 3 (Illela). 1N KCl, it ranged from 3.5 to 4.8 in all the soils. Low pH indicates that the soils have been subjected to a longer period of leaching. Soils derived

from highly siliceous sandstone are very acidic as stated by Esu (1999). Zonneveld, (1999) who classified soils of the general area as “Red Acid Soils” have confirmed the acidic nature of these soils. Organic matter is generally low in the soils and ranged between 0.18 % and 0.60 % in the soils. The generally low organic matter content in the area has been attributed to rapid decomposition and mineralization of organic materials contributed by sparse vegetation in the hot semiarid climate as promoted by the high radiation (Yakubu *et al.*, 2006). Calcium and magnesium are the predominant basic cations in the soils and ranged from 0.14 to 0.88 cmol (+) kg^{-1} and 0.0 to 0.28 cmol (+) kg^{-1} respectively. Exchangeable potassium and sodium are generally low compared to calcium and magnesium. Values as high as 0.11 cmol (+)kg^{-1} for potassium and 0.06 cmol(+)kg^{-1} for sodium were recorded in the soils . The low Exchangeable bases in the soils could be attributed to the sandy nature of the soils in which intense leaching of the available bases might have taken place. The CEC of the soils ranged from 0.9 to 5.1.0 cmol (+) kg^{-1} in all the soils. Cation exchange capacity of the soils is generally low. The low cation exchange capacity in the soil may be attributed to the low organic matter content in the soil and the nature of clay minerals (kaolinite) in the general area (Juo and Moorman, 1981). The effective cation exchange capacity (ECEC) of the soil is also low, an indication that the soils at their natural pH levels remain low in CEC indicating a low capacity of the soils to retain nutrients. Extractable acidity is higher than exchangeable bases in all the soils. Extractable Al ranged from 0.20 to 0.90 cmol (+) kg^{-1} while extractable H ranged from 0.10 to 0.70 cmol (+) kg^{-1}. High extractable Al in soils is an indication of significant amount of acidic cations on the soil exchangeable complex. These values could result to aluminum toxicity and if not ameliorated only acidic tolerant plants can be grown. Base saturation values ranged from 10 % to 43 % in soils derived in pedon 1 (Sangiwa), 37 % to 97 % in soils derived from Sokoto deposit (pedon 2) and 30 % to 75 % in soils derived from Illela deposit (pedons 3). The low base saturation (average of < 50%) in the soils is an indication of low fertility level (FAO, 1998). In general the soils of the deposits have a low base saturation, an indication of leaching of available bases.

Soil limitations and management strategies

The sandy nature, poor structural development and the low inherent fertility of the soils can be improved by the incorporation of organic manure and crop residues. The acidity problem of the soils can be corrected by the application of liming materials. Farmers could use wood ash which is common in the area as liming material to increase the soil pH.

CONCLUSION

The soils are young, extensive in the area, sandy in nature with poor structural development. The soils have a common problem of low inherent fertility, evidenced by low organic matter contents and CEC. In addition they are all acid with reaction ranging from very strongly acid to slightly acid. The acidity is caused by intense leaching of the bases and the predominance of Al in the exchangeable sites.

REFERENCES

Arnborg, T. 1988. Where Savanna Turns Into Desert. International Rural Development Center Swedish University of Agricultural Sciences Rural Development Studies No 24, pp 22-23.

Esu, I.E. 1999. *Fundamentals of Pedology,* stirting Horden pub (Nig) ltd. 136pp.

FAO, 1998. *World Reference Base for Soil Resources (FAO, ISRIC, ISSS)*. World Soil Resource Reports 84, FOA, Rome, Italy. 161 pp.

Sombroek, W.G. and I.S. Zonneveld. 1971. *Ancient dune fields and fluviatile deposits in the Rima-Sokoto River Basin (NW Nigeria).* Soil Survey Paper no.5, Netherlands Soil Survey Institute, Wageningen. 109pp.

Yakubu, M. 2006. Genesis and Classification of Soils over different Geologic Formations and surfaces in the Sokoto plain, Nigeria. A Ph. thesis submitted to the Postgraduate School, Usmanu Danfodiyo University, Sokoto, Nigeria. Pp 222.

Zonneveld, I.S. 1999. A Geomorphological base banded (tiger) vegetation pattern related to former dunes field in Sokoto (Northern Nigeria). *Catena, 37:45-56.*

Distribution of acid-tolerant arbuscular mycorrhizal fungi along a soil-pH gradient suggests a role in plant community resilience in acidic soil

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Abstract
Arbuscular mycorrhizal (AM) fungi play a significant role in plant colonization in acidic soil via supplying mineral nutrients. Soil acidity, however, constrains not only plant but also the fungal growth. We analyzed AM fungal distribution along a pH gradient at the landscape level with reference to LSU rDNA sequences. The fungal communities in lower-pH soils were subsets of those in higher-pH soils, suggesting that AM fungi in strongly acidic soil are not specialists, but generalists. Nestedness in AM fungal community along pH gradients may have important implications for plant community resilience and early primary succession after disturbance in acidic soils.

INTRODUCTION

In acidic soil high-levels of Al^{3+} in the soil solution constrains plant productivity through inhibiting root tip growth and thus water and nutrient uptake (Kochian et al., 2004). Phosphate (Pi) availability in acidic soil is quite low due to formation of sparingly soluble salts with Al^{3+} and Fe^{3+}, leading to serious Pi deficiency in plants (Kochian et al., 2004). Pi acquisition via arbuscular mycorrhizal (AM) associations is an important strategy for plants to adapt to acidic soil. AM fungi are obligate biotrophs that associate with most land plants (Smith and Read, 2008) and play a significant role in the establishment of early-successional species in acidic soil (Cumming and Ning, 2003; Maki et al., 2008).

Phytotoxic levels of Al^{3+} also inhibit spore germination and hyphal growth of AM fungi, but Al^{3+} tolerance is extensively variable among species/isolates (Bartolome-Esteban and Schenck, 1994; Klugh-Stewart and Cumming, 2009). Given that fungi in extreme environments are generally considered to evolve towards specialists (Gostinčar et al., 2010), acidic soil is likely to be an extreme environment in which only a limited range of AM fungal species (i.e. acid-tolerant specialists) are able to survive. On the other hand, in our previous surveys the fungi detected in strongly acidic soils could also be detected in a weakly acidic soil (An et al., 2008), raising a question whether the fungi in acidic soils are specialists or generalists. To answer the question, we analyzed AM fungal distribution along a pH gradient at the landscape level using a synthesized dataset of previous (An et al., 2008; Kawahara & Ezawa, 2013) and recent surveys, in conjunction with a pH-manipulation experiment.

MATERIAL AND METHODS

Field surveys

Six sites that covered a soil pH range of 3.0-7.4 were selected across Japan, and rhizosphere soils of a generalist plant *Miscanthus sinensis* grown in the six sites were collected. *M. sinensis* seedlings were grown on the soils in a glasshouse (soil trap culture), and the community compositions of AM fungi in the roots were investigated with reference to the fungal large subunit ribosomal RNA gene (LSU rDNA) sequences.

pH-manipulation experiment

To examine responses of AM fungal community to soil acidification/neutralization, rhizosphere soils of *M. sinensis* were collected both from a neutral soil and an acidic soil and subjected to trap culture with *M. sinensis* seedlings at three different pH, and the fungal community compositions in the roots were investigated.

Inoculation experiment

To examine the significance of AM fungi in the establishment of plants in acidic soil, *M. sinensis* seedlings were inoculated either with an acid-tolerant AM fungus or an acid-sensitive AM fungus and grown at pH 5.2 and 3.2 in a greenhouse.

RESULTS AND DISCUSSION

Community structure of AM fungi along a soil-pH gradient

In total, over fifty AM fungal phylotypes were defined based on $\geq$ 95% sequence similarity. In the trap culture surveys in the six sites, AM fungal richness was negatively correlated with soil acidity, in agreement with the recent finding (Kohout et al., 2015). The phylotypes detected at more acidic pH were detected in wider ranges of soil pH. The fungal communities showed a significant nestedness pattern along the pH gradient; communities in lower-pH soils were subsets of those in higher-pH soils. These observations suggest that the phylotypes that inhabit strongly acidic soil are not specialists, but generalists.

In the pH-manipulation experiment, acidification of pH had a significant impact on the neutral soil communities, but not on the acidic soil communities, indicating that in acidic soils acid-tolerant fungi predominantly occur, while in neutral soils both acid-tolerant and acid-sensitive fungi coexisted. Nestedness analysis further confirmed that the fungal communities originated from the acidic soil were subsets of those from the neutral soil.

Effect of an acid-tolerant AM fungus on plant survival in acidic soil

M. sinensis seedlings grew sustainably at pH 5.2 regardless of AM fungal acid-tolerance. At pH 3.2, however, only the seedlings inoculated with the acid-tolerant AM fungus that is one of the generalist phylotypes in the community analysis could survive, and those with the acid-sensitive fungus that inhabits at pH 5 and higher could not. These observations indicate that association with acid-tolerant AM fungi is essential for the survival and establishment of plants in strongly acidic soil.

Ecological implications of nestedness in AM fungal community along pH gradients

Community nestedness along environmental gradients, in which species tolerating harsh environments are a subset of those that occur in moderate environments (Chase, 2010), is likely to be relevant to community stability; the tolerant species survive after environmental perturbations and thus can become early-successional species that play a key role in community re-establishment and succession (Connell and Slatyer, 1977). Given the significance of symbiotic associations with acid-tolerant AM fungi in plant survival in strongly acidic soil, the community nestedness along pH gradients in the fungi may have important ecological implications for plant community resilience/establishment in acidic soil; acid-tolerant (early-successional) AM fungi are generally distributed across ecosystems and thus can readily support plant colonization in acidic soil, which would consequently make a significant contribution to rapid recovery of vegetation and succession in acidic soils.

CONCLUSION

The present study demonstrated a distribution pattern of AM fungi along a large pH gradient at the landscape level, with particular emphasis on the fungi in strongly acidic soils. The community nestedness structured across the distant sites indicates that the fungi in acidic soils occur over wide ranges of pH, namely generalists. Furthermore, the pH-manipulation experiment strongly suggests that generalist fungi in neutral soils are as acid-tolerant as those in acidic soils and thus have potential to survive in acidic soils. Nestedness in AM fungal community along pH gradients may have important implications for plant community resilience and early primary succession after disturbance in acidic soils.

REFERENCES

An, G. H., Miyakawa, S., Kawahara, A., Osaki, M., and Ezawa, T. 2008. Community structure of arbuscular mycorrhizal fungi associated with pioneer grass species *Miscanthus sinensis* in acid sulfate soils: Habitat segregation along pH gradients. Soil Science and Plant Nutrition. 54. 517–528.

Bartolome-Esteban, H., and Schenck, N. C. 1994. Spore germination and hyphal growth of arbuscular mycorrhizal fungi in relation to soil aluminum saturation. Mycologia. 86. 217–226.

Chase, J. M. 2010. Stochastic community assembly causes higher biodiversity in more productive environments. Science. 328. 1388–1391.

Connell, J. H., and Slatyer, R. O. 1977. Mechanisms of succession in natural communities and their role in community stability and organization. The American Naturalist. 111. 1119–1144.

Cumming, J. R., and Ning, J. 2003. Arbuscular mycorrhizal fungi enhance aluminium resistance of broomsedge (*Andropogon virginicus* L.). Journal of Experimental Botany. 54. 1447–1459.

Gostinčar, C., Grube, M., de Hoog, S., Zalar, P., and Gunde-Cimerman, N. 2010. Extremotolerance in fungi: evolution on the edge. FEMS Microbiology Ecology. 71. 2–11.

Kawahara, A., and Ezawa, T. 2013. Characterization of arbuscular mycorrhizal fungal communities with respect to zonal vegetation in a coastal dune ecosystem. Oecologia. 173. 533-543.

Klugh-Stewart, K., and Cumming, J. R. 2009. Organic acid exudation by mycorrhizal *Andropogon virginicus* L. (broomsedge) roots in response to aluminum. Soil Biology and Biochemistry. 41. 367–373.

Kochian, L. V, Hoekenga, O. A., and Pineros, M. A. 2004. How do crop plants tolerate acid soils? - Mechanisms of aluminum tolerance and phosphorous efficiency. Annual Review of Plant Biology. 55. 459–493.

Kohout, P., Doubková, P., Bahram, M., Suda, J., Tedersoo, L., Voříšková, J., and Sudová, R. 2015. Niche partitioning in arbuscular mycorrhizal communities in temperate grasslands: a lesson from adjacent serpentine and nonserpentine habitats. Molecular Ecology. 24. 1831–1843

Maki, T., Nomachi, M., Yoshida, S., and Ezawa, T. 2008. Plant symbiotic microorganisms in acid sulfate soil: significance in the growth of pioneer plants. Plant and Soil. 310. 55–65.

Smith, S. E., and Read, D. J. 2008. Mycorrhizal symbiosis. San Diego, California: Academic Press.

Limestone and silicates in soil acidity correction in no-till system

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Abstract
This study assessed acidity correction of a dystrophic Red Latosol with the use of steel slag and limestone in no-till system. The treatments consisted of the surface and incorporated application of correctives for soil acidity correction: steel slag (SS), ladle furnace slag (LFS), blast furnace slag (BFS), stainless steel slag (SSS), wollastonite (W), dolomitic limestone (D) and calcined dolomitic limestone (CDL). Acidity correction was obtained up to the 40-cm layer with the application of limestone, SSS, W and F. SL had a corrective effect limited to the 10-cm layer, while BFS did not correct the soil acidity.

INTRODUCTION
More than 70% of the Brazilian soils are acidic and acidity correction is necessary for adequate productivity of crops. Steel slag (SS) is a good alternative as it brings benefits to chemical properties in the soil. SS increases soil pH due to the base SiO_3^{-2}, base saturation (Brassioli et al., 2009), phosphorus contents, due to competition between SiO_3^{2-} and PO_4^{3-} for the same adsorption places. SS allows P release to soil solution (Prado and Fernandes, 2001) and it is a source of micronutrients (Prado et al., 2001) and silicon (Souza and Korndörfer, 2010), aspects that increase crop yield (Carvalho-Pupato et al., 2004). In Brazil, there is great potential to use SS due to the large production, around 6.25 million ton of SS per year. However, SS use in agriculture in Brazil is still small. This study assessed acidity correction of a dystrophic Red Latosol with the use of steel slag and limestone in no-till system.

MATERIAL AND METHODS
The experiment was carried out in the field at São Paulo State University - UNESP in Botucatu, Brazil, from December 2010 to March 2012. The soil of the experimental area was a dystrophic Red Latosol grown with Brachiaria without maintenance for 20 years. The treatments consisted of two forms of application (surface and incorporated) of seven soil acidity correctives: steel slag (SS), blast furnace slag (BFS), ladle furnace slag (LFS), stainless steel slag (SSS), wollastonite (W), dolomitic limestone (D) and calcined dolomitic limestone (CDL), and one control (C) treatment without corrective application. The corrective materials were characterized according to the Brazilian laws of limes (Table 1).

Table 1. Chemical attributes of sources for soil acidity used in this study.

Corrective Materials [1]	CaO	MgO	RR[2]	NP[3]	ECC[4]
	%	%	%	%E$CaCO_3$	%
stainless steel slag	37.6	9.5	71	84	60
steel slag	28.1	6.1	71	70	50
blast furnace slag	26.6	8.0	19	65	13
ladle furnace slag	36.1	5.8	80	77	62
wollastonite	30.0	3.0	100	60	60
calcined dolomitic limestone	38.4	23.6	98	120	119
dolomitic limestone	27.8	16.4	75	88	67

[2]RR= reactivity rate, express the percentage of corrective material that reacts in three months; [3]NP= neutralization power, [4]ECC = effective calcium carbonate.

Each material dose was calculated to raise the soil base saturation to 70%. Corrective materials were applied in December 2010 with manual distribution. After application, in the treatment with incorporation, materials were incorporated up to 20 cm with rotary tilling. Then, no-till system was established in both treatments. Soil samples were collected 12 months after material application at depths 0-5cm, 5-10 cm, 10-20 cm and 20-40 cm. It was determined pH ($CaCl_2$), calcium (Ca), magnesium (Mg) and base saturation according to Raij et al., 2001. The data were submitted to analysis of variance and means compared by Tukey test ($p < 0.05$).

RESULTS AND DISCUSSION
The forms how the corrective materials were applied, incorporated or surface, did not promote differences to pH values. Surface application showed movement in the soil profile up to depth 10 cm because in this treatment the pH averages were higher than in the control treatment, reaching pH 5.5 and 5.0 at depths 0-5 and 5-10, respectively (Table 2). The corrective materials increased soil pH values mainly at 0-5 cm and effects soil correction decreased in deeper layers. At all depths evaluated, SSS, LFS, W, CDL and CD increased pH and

base saturation compared to control; however, the increase was similar among them. Although SS was similar to SSS and LFS in all layers, SS raised the pH compared to control treatment only up to 10-cm layer and was less efficient than limestone and wollastonite. BFS did not correct soil acidity. This allows inferring that the chemical composition of slag and its solubility interfere in soil acidity correction, since ECC was considered for each material to calculate the dose required to achieve the same correction level to increase base saturation to 70%.

Table 2. Averages of soil pH, base saturation, Ca and Mg contents after 12 months of treatments application*

	Layer (cm)				Layer (cm)			
Treatments	0-5	5-10	10-20	20-40	0-5	5-10	10-20	20-40
Application (A)	pH $CaCl_2$				Base saturation (%)			
Incorporated	5.2 a	5.2 a	4.8 a	4.6 a	61 a	62 a	50 a	48 a
Surface	5.5 a	5.0 a	4.6 a	4.7 a	74 a	58 a	41 a	51 a
F	1.3^{ns}	0.5^{ns}	0.4^{ns}	0.2^{ns}	3.3^{ns}	0.5^{ns}	1.0^{ns}	0.2^{ns}
Correctives(C) [1]								
SSS	5.6 ab	5.3 ab	4.7 bc	4.8 ab	74 a	68 ab	48 ab	52 ab
SS	5.2 b	5.0 bc	4.6 bcd	4.6 abc	73 a	58 b	46 ab	54 ab
BFS	4.5 c	4.4 cd	4.4 cd	4.4 bc	49 b	42 c	40 b	44 b
LFS	5.6 ab	5.2 ab	4.8 abc	4.8 ab	77 a	65 ab	51 ab	55 a
W	6.0 a	5.7 a	5.2 a	5.0 a	78 a	76 a	56 a	56 a
CDL	5.8 a	5.7 a	4.8 abc	4.9 a	75 a	72 a	50 ab	55 a
D	5.7 a	5.5 ab	4.9 ab	4.9 a	78 a	71 a	52 ab	54 ab
C	4.4 c	4.3 d	4.2 d	4.2 c	37 b	29 c	23 c	26 c
C.V. %	6.4	7.0	5.9	5.8	11.5	13.3	16.7	14.1
Application (A)	Ca ($mmol_c\ dm^{-3}$)				Mg ($mmol_c\ dm^{-3}$)			
Incorporated	42 b	50	31	33	17 b	18	15	13
Surface	69 a	41	24	33	27 a	19	12	16
F	19.1*	0.6^{ns}	1.6^{ns}	0.0^{ns}	21.1*	0.4^{ns}	1.1^{ns}	1.8^{ns}
Correctives (C)[1]								
SSS	59 bc	49 b	31 a	37 ab	23 bc	19 bcd	13 bc	12 bc
SS	66 ab	48 b	32 a	43 a	19 bc	14 cde	12 cd	13 bc
BFS	40 d	29 c	25 a	33 bc	17 bc	14 de	12 bc	13 bc
LFS	68 ab	47 b	29 a	39 ab	26 b	21 bc	16 abc	15 b
W	80 a	71 a	29 a	38 ab	13 c	15 cde	12 bcd	12 bc
CDL	47 cd	44 bc	28 a	35 abc	24 b	23 b	17 ab	17 b
D	64 abc	47 b	29 a	27 c	40 a	30 a	21 a	27 a
C	18 e	14 d	10b	13 d	13 c	10 e	6 d	8 c
C.V. %	19.9	21.0	24.1	19.6	28.2	24.5	25.2	22.2

*Means followed by the same letters do not differ significantly in the Tukey test (p de 0.05). ns - non significant. stainless steel slag (SSS), steel slag (SS), blast furnace slag (BFS), ladle furnace slag (LFS), wollastonite (W), calcined dolomitic limestone (CDL), dolomitic limestone (D) and control (C).

SSS and LFS increased pH and base saturation similarly to limestone, therefore, these materials can replace limestone reducing these residues in the environment. BFS did not increase soil pH; however, base saturation increased with its application (Table 2). This is explained by the possible Ca and Mg solubilization in this material during the extraction process in the soil chemical analysis.

CONCLUSIONS

Slags LFS and SSS can be used in no-till system with similar efficiency of limestone to correct soil acidity. The ways how to apply corrective materials do not promote chemical propriety changes in the first year of no-till system. Surface steel slag application is not efficient for soil acidity correction in deeper depths in the first year of no-till system establishment.

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REFERENCES

Brassioli, F.B., Prado, R. M., Fernandes, F. M. 2009. Avaliação agronômica da escória de siderurgia na cana-de-açúcar durante cinco ciclos de produção. Bragantia. 68. 381-387. 2009.

Prado, R. M., Fernandes, F. M. 2001. Efeito da escória de siderurgia e calcário na disponibilidade de fósforo de um Latossolo Vermelho-Amarelo cultivado com cana-de-açúcar. Pesquisa Agropecuária Brasileira. 36.1199-1204.

Prado, R. M., Fernandes, F.M., Natale, W. 2001. Uso agrícola da escória de siderurgia no Brasil – Estudos na cultura da cana-de-açúcar. Jaboticabal, Funep. 68.

Sousa, R. T. X., Korndörfer, G. H. 2010. Slag efficacy as a lime and silicon source for rice crops through the biological method. Journal of Plant Nutrition. 33. 1103-1111.

Soil reaction (pH) and status of mobile phosphorus and potassium in Sava valley area of Bosnia and Herzegovina

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Abstract
Acid soils in Bosnia and Herzegovina occupy 2 256 272 ha or 44.12 % of total soils area of the country and prevail distric cambisols (28.73 %), luvisols (6.90 %) and pseudogleys (4.64 %). Soil pH and plant available phosphorus (P) and potassium (K) status were analysed in 478 soil samples covering 663 ha taken in five municipalities of Sava valley. About 55 % samples were with pH in KCl below 5, while adequate mobile P and K supplies according to AL-method were found in third part of samples only. Liming and the higher P and K fertilization of majority soils of the region could be useful for increases soil fertility.

INTRODUCTION
Excessive soil acidity on agricultural land of Bosnia and Herzegovina (BIH) as an agricultural limiting factor impacts crops production (Komljenovic et al. 2010; Markovic et al., 2006; Komljenovic and Markovic, 2008). Resulovic et al. (2008) estimated that acid soils in BIH occupying 2 256 272 ha or 44.12 % of total soils area of the country and that prevailing distric cambisols (28.73 %), luvisols (6.90 %) and pseudogley (4.64 %). Aim of the study was testing soil pH and plant available phosphorus (P) and potassium (K) status in part of Sava valley area in the entity Republic of Srpska (RS), BIH.

MATERIAL AND METHODS
The area covering five municipalities situated in the Bosnian part of Sava river valley in RS of BIH as follows: Samac (184 km^2), Pelagicevo (178 km^2), Modrica (363 km^2), Srbac (453 km^2) and Gradiska (762 km^2)**.** According the statistical data this area covering 1 940 km^2 and participating with 7.8 % in territory and 13.4 % in arable lands contribution of RS. These municipalities contains 78 427 ha of arable lands and gardens or 13.4 % in level of RS. Main field crops are maize and winter wheat (SYB, 2013).
The analyzed area is part of the Peri-Pannonian region of BIH. Climate of the region is characterised by moderately cold winters and warm summers (Saric et al., 1997). Excessive drought periods as result of recent climatic changes (Kovacevic et al., 2013) have considerable impact on nutrient mobilization to field crops.
In total 478 soil samples covering 663 ha were taken by the auger to 30 cm of depth during 2006 with aim of testing main agrochemical properties. Determination of plant available P was made by AL-method (Egner et al., 1960). Interpretation of the data was made according Vukadinovic and Loncaric (1998) by criterion as follows: for P (mg P_2O_5 100 g^{-1}) = very low (<5.0), low (5.1-12.0), good (12.1-20.0), high (20.1-30.0) and very high (>30.0); for K (mg K_2O 100 g^{-1}) = very low (<12.0), low (12.1-19.0), good (19.1-30.0), high (30.1-40.0) and very high (>40.0).

RESULTS AND DISCUSSION
About 55% samples were with pH in KCl below 5, while adequate mobile P and K supplies were found in third part of samples only (Table 1). Interaction of soil acidity and low supplies with P and K and unfavorable physical properties could be reasons of low yields of maize and wheat. The lowest yields of maize (3.6 t ha^{-1}) and wheat (2.7 t ha^{-1}) in Srbac municipality could be in connection with the something higher share of P and K deficient and acid soils (Table 1).

Growth retardation and chlorosis of maize were found on acid hydromorphic soils of Gradiska municipality. Excessive aluminum and iron, as well as the lower P concentrations are in close connection with this type of disorders (Table 2). Liming and ameliorative fertilization, particularly with P fertilizers, could be recommended for improvement of soil properties. Markovic et al. (2008) applied dolomite up to 20 t ha^{-1} on the acid hydromorphic soil of Gradiska municipality. Maize was grown three years on the experiment and yield was increased in average by 48 %. Komljenovic et al. (2010) applied increasing rates of phosphorus fertilizers up to 1750 kg P_2O_5 ha^{-1} on soil of Gradiska municipality. Phosphorus fertilization resulted mainly by considerable yield increase of maize in the level of 17 %. Yield increases were achieved mainly by application of the P in the level of 750 kg P_2O_5 ha^{-1}.

Table 1. Ranges of soil pH and mobile (AL-method) phosphorus and potassium

Soil properties in the municipality Samac (a), Pelagicevo (b), Modrica (c), Srbac (d) and Gradiska (e): number of samples (N) and area covered by sampling (ha)															
	Municipality								Municipality						
	a	b	c	d	e	Σ	%		a	b	c	d	e	Σ	%
N	25	92	52	173	136	478		ha	40	142	82	221	178	663	
%	5.2	19.2	10.9	36.2	28.5		*100*	%	6.0	21.4	12.4	33.3	26.9		100
Grain yields (t ha^{-1}) of maize (M) and winter wheat (W): 5-year averages 2008 – 2012															
M	5.7	4.8	5.6	3.6	4.9			W	3.5	3.6	4.1	2.7	3.3		
pH H_2O	**Soil pH ranges (pH in H_2O)**							**pH KCl**	**Soil pH ranges (pH in 1n KCl)**						
	a	b	c	d	e	Total			a	b	c	d	e	Total	
	Number of soil samples (N)					Σ N	**%**		Number of soil samples (N)					Σ N	**%**
< 4.0	0	0	0	0	0	0	0	< 4.0	2	16	31	7	21	77	16.1
4 - 5	1	3	0	3	12	19	4.0	4 - 5	14	54	11	42	67	188	39.4
5 - 6	12	60	29	88	68	257	53.8	5 - 6	6	19	10	52	37	124	25.9
6 - 7	9	26	13	37	46	131	27.4	6 - 7	1	3	0	36	11	51	10.7
7 - 8	3	3	10	43	10	69	14.4	7 - 8	2	0	0	35	0	37	7.7
>8.0	0	0	0	2	0	2	0.4	> 8.0	0	0	0	1	0	1	0.2
P_2O_5	**Soil P ranges* (mg P_2O_5 100 g^{-1})**							**K_2O**	**Soil K ranges** (mg K_2O 100 g^{-1})**						
	a	b	c	d	e	Total			a	b	c	d	e	Total	
	Number of soil samples (N)					Σ N	**%**		Number of soil samples (N)					Σ N	%
< 5.0	4	11	35	74	43	167	34.9	< 12	4	26	24	87	30	171	35.8
5.1-12	11	39	13	54	36	153	32.1	12.1-19	10	29	22	48	31	140	29.3
12.1-20	6	23	3	20	28	80	16.7	19.1-30	9	25	5	27	35	101	21.1
20.1-30	2	15	0	12	13	42	8.8	30.1-40	2	8	1	7	24	42	8.8
> 30	2	4	1	13	16	36	7.5	> 40	0	4	0	4	16	24	5.0
***** very low (<5.0), low (5.1-12), good (12.1-20), high (20.1-30) and very high (>30)								****** very low (<12), low (12.1-19), good (19.1-30), high (30.1-40) and very high (>40).							

Table 2. Properties of maize at early growth stage on P-deficient soils (Kovacevic et al., 1988)

Top of maize at 6-9 leaves stage (municipality Gradiska): dry matter yield (DMY), plant height (PH) and P, Fe and Al status (on DM basis) – averages of four samples										
Sample	Chlorotic (majority of plants) maize					Normal (oasis at the same plot) maize				
	DMY	PH	P	Fe	Al	DMY	PH	P	Fe	Al
	g $plant^{-1}$	cm	%	mg kg^{-1}		g $plant^{-1}$	cm	%	mg kg^{-1}	
1-4	2.78	23	0.29	3470	3817	18.84	72	0.46	410	470

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REFERENCES

Egner, H., Riehm, H., Domingo, W.R. 1960. Untersuchungen uber die chemische Bodenanalyse als Grundlage fur die Beurteilung des Nahrstoffzustandes der Boden II. Chemische Extractionsmethoden zu Phosphor- und Kaliumbestimmung. K. Lantbr. Hogsk. Annlr. W.R. 26. 199-215.

Komljenovic, I., Markovic, M., D. 2008. Residual influences of phosphorus fertilization on maize status in Potkozarje area. Cereal Res. Commun. 36 (Supplement). 699-702.

Komljenovic, I., Markovic, M., Kondic, D., Kovacevic, V. 2010. Response of maize to phosphorus fertilization on hydromorphic soil of Bosnian Posavina area. Poljoprivreda/Agriculture.16 (2). 9-13.

Kovacevic, V., Kovacevic, D., Pepo, P., Markovic, M. 2013. Climate change in Croatia, Serbia, Hungary and Bosnia and Herzegovina: comparison the 2010 and 2012 maize growing seasons. Poljoprivreda/Agriculture. 19 (2). 16-22.

Kovacevic, V., Vukadinovic, V., Bertic, B. 1988. Excess in iron and aluminum uptake and nutritional stress in corn (Zea mays L.) plants. Journal of Plant Nutrition. 11 (6-11). 1263-1272.

Markovic, M., Komljenovic, I., Delalic, Z., Kovacevic, V. 2006: Phosphorus as a limiting factor of the field crops yield unde conditions of the northern Bosnia. Lucrari Stiintifice. Seria Agronomie. Universitatea de Stiinte Agricole si Medicina Vetrinara Iasi (1454-7414) 49(1). 218-222.

Markovic, M., Todorovic, J., Biberdzic, M., Delalic, Z. 2008. Response of maize to liming in northern Bosnia. Cereal Res. Commun. 36. 2079-2082.

Resulovic, H., Custovic, H., Cengic, I. 2008. Classification of soils, systematic, development, properties and fertility. University in Sarajevo.

Saric, T., Djikic, M., Gadzo, D. 1997. Two harvest in year. Biblioteka Savremena poljoprivreda, NIP Zadrugar Sarajevo and EDIS Sarajevo.

Vukadinovic, V., Loncaric, Z. 1998. Plant nutrition. Faculty of Agriculture in Osijek.

Seasonal changes in soil acidity and related properties in ginseng artificial bed soils under a plastic shade

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Abstract
In Changbai Mountains, *Panax ginseng* (ginseng) was cultivated in a mixture of the humus and albic horizons of albic luvisol in raised garden with plastic shade. The mixed-bed soils were seasonally collected at three intervals for different-aged ginsengs. Remarkable decrease in pH, concentrations of exchangeable calcium, NH4+, total organic carbon and Alp, as well as a pronounced increase in the bulk density were observed in the different-aged ginseng soils from one spring to the next. The NO_3^- showed remarkable surface accumulation in the summer and even more in the autumn but declined considerably the next spring. Our study revealed a seasonal shift in soil characteristics in ginseng beds with plastic shade.

INTRODUCTION
Panax ginseng C. A. Meyer (ginseng) is a perennial herb cultivated for its highly valued root. Its cultivation is difficult because of its long cultivation period and demand for deep shade and nutrient rich, slightly acidic, deep and well-drained soils. Re-plantation in old fields usually fails, and it takes up to 30 years for previously cultivated fields to recover. Soil conditions might contribute to the problem.
Today, the ginseng supply relies mainly on intensive field-cultivation under artificial-shade structures, which might have the potential to affect the bed soil conditions. Albic luvisol is one of the main soil types used for ginseng cultivation in the Changbai Mountains. A binary mixture of the humus and albic horizons (generally 1:1) was created in an elevated bed (Jilin province soil and fertilizer department, 1990). Ginseng bed soils from albic luvisols have been shown in our research, as well as others, to be acidic (Xie and Xu, 1996; Zhao and Li, 1998). Impacts related to soil acidity, such as Al toxicity, might contribute to ginseng replant disease in albic ginseng garden soils. In this study, the soil conditions were investigated seasonally at a ginseng farm located in the Changbai Mountains in Northeast China.

MATERIALS AND METHODS
The study was carried out in a field (41°32'N, 128°09'E) on the first ginseng farm in Malugou County, Jilin province, China. Soil samples were collected from beds with different-aged ginseng in April (spring) of 2009 before the plastic shades were put into place. The soil was sampled at 0-5 cm (upper roots), 5-10 cm (root zone) and 10-15 cm (down root) using an auger in three replicates. We re-sampled the soils in July (summer) of 2009, September (winter) of 2009 and April of 2010 (the next spring). The bulk density, moisture content, pH in water (w:v, 1:2.5), total organic carbon (TOC), nitrate of the soil was determined using general methods in the lab. Exchangeable cations were extracted with a 1 M NH4Cl (Soil: extractant, 1:50) solution and were determined by atomic absorption (Ca, Mg and Al) and flame photometer (FP640, Shanghai Jingxue Scientific instrument company, Shanghai, China) (Na and K). The effective cation exchange capacity (ECEC) was calculated as a molar ratio of Ex-Al^{3+} to the sum of Ex-$Ca^{2+,}$ Ex-$Mg^{2+,}$ Ex-Na^+, Ex-K^+ and Ex-Al^{3+} (Kamprath, 1970). The Al saturation was calculated as Al/ECEC. The soils were also extracted using 0.1 M Na-pyrophosphate (pH 10.0) (soil ratio: extractant 1:100, with shaking for 16 h) for organic Al (Alp) (Buurman, 1996).

RESULTS AND DISCUSSION
To better understand the potential soil damage caused by the artificial plastic canopy during ginseng cultivation, an annual cycle investigation was conducted to inspect the seasonal dynamics of soil acidity and related parameters in the albic ginseng bed soils. The results showed that ginseng planting resulted in soil acidification, decreased concentrations of Ex-Ca^{2+}, NH_4^+, TOC and Al_p, and an increased bulk densityof soils originating from albic luvisols. There were also marked seasonal changes in the Ex-Al^{3+} and NO_3^- concentrations and spatial variation of water content.
A model was proposed to describe the process of soil acidification and Ex-Al^{3+} dissolution (Fig. 1). Aluminium toxicity might be one important impact on albic ginseng garden soils, especially in the summer and autumn.

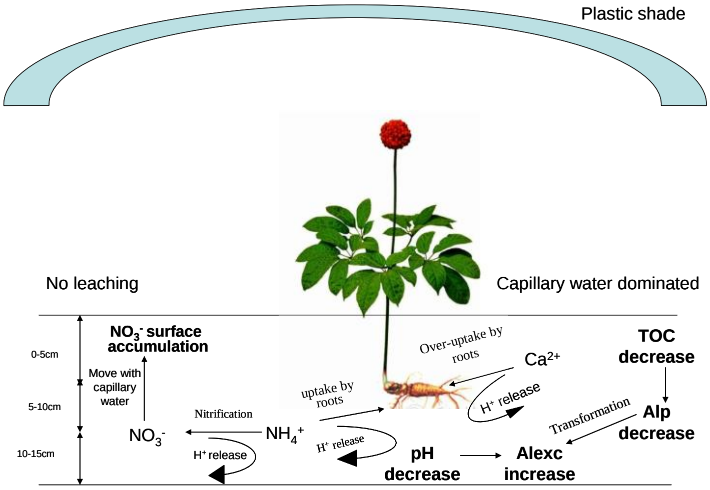

Figure1 The model of soil acidification and Ex-Al^{3+} dissolution in ginseng bed soils under a plastic shade.

A hypothesized picture was plotted according to the experiment results. The over-uptake of Ex-Ca^{2+} and NH_4^+ by ginseng roots and the nitrification process will release a large amount of protons, resulting in a decreased pH. A plastic canopy reduced nutrient leaching and resulted in upward water capillary domination, which promoted NO_3^- surface accumulation. Ginseng planting decreased the TOC concentrations and, subsequently, the Al_p concentrations. The increase in the Ex-Al^{3+} in the summer and autumn might result from a decreased pH, NO_3^- surface accumulation and the transformation of Al_p to Ex-Al^{3+}.

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REFERENCES

Buurman P, Lagen V. B., Velthorst E.J. 1996. Mannual for Soil and water analysis. Backhuys Publishers, Leiden, The Netherlands. 314.

Jilin province soil and fertilizer department, 1990. Jilin soils. Chinese agriculture publisher, Beijing (in Chinese)

Kamprath E. J. 1970. Exchangeable aluminium as a criterion for liming leached mineral soils. Soil Sci Soc Am P. 34. 252–254.

Xie Z. K. and Xu H. L. 1996. The study on ginseng continuous cropping in Changbai mountain area -the report on ginseng soil acidification. Renshen YanJiu 2. 31-34. (in Chinese)

Zhao Y. F, Li X. M. 1998. On the damage of Al^{3+} in soils to ginseng. Special wild Economic Animal and Plant Research. 3. 38-42. (in Chinese).

Properties of eluvial and illuvial soil horizons in Croatian Pseudogleys

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Abstract
Most Croatian Pseudogleys are on agricultural land or in agro-ecosystems. However, many have severe agricultural production constraints. Most notably, water periodically stagnates on/in the subsoil horizon. We determined which differences in soil properties (particle size distribution, bulk density, water holding capacity, air capacity, microaggregates stability, porosity, pH, organic matter content) exist between the topsoil (eluvial Eg horizon) and the subsoil (illuvial Btg horizon) of 33 forest Pseudogleys studied across the Pannonian region of Croatia. The properties of Eg horizon significantly differ from the properties of Btg horizon. Most notably, Btg horizon is distinctly finer-textured and more compacted than Eg horizon.

INTRODUCTION

Pseudogley is the second most widespread soil type in Croatia, developed almost exclusively in its Pannonian region (Bogunović et al., 1998; Husnjak, 2014; Rubinić et al., 2015b). Most Croatian Pseudogleys correlate with Stagnosols (IUSS Working Group WRB, 2014), and their profile may be designated principally as A-Eg-Btg-Cg (FAO, 2006). Given that they developed in relief and climate conditions that are generally favorable for agriculture, 55 % of Croatian Pseudogleys are found on agricultural land or in agro-ecosystems (Husnjak et al., 2011). However, most of these soils have numerous agricultural production constraints (Husnjak, 2014). Primarily, this is due to the unfavorable soil water regime. Namely, precipitation water periodically stagnates on/in the poorly permeable subsoil horizon(s), causing the formation of stagnic properties *sensu* (IUSS Working Group WRB, 2014). Additionally, if not limed, Croatian pseudogleys are acid to very acid (Husnjak, 2014). Average pH_{KCl} values of Pseudogleys studied by Rubinić et al. (2015b) were below 4.5.

In Croatian Pseudogleys, the poorly permeable subsoil is pedogenetically developed (Rubinić et al., 2014; 2015a). Namely, due to pronounced acidification, lessivage, and compaction, the topsoil horizons (A and Eg) are weakly structured and coarser-textured, whereas the subsoil horizons (Btg and Cg) are massive and finer-textured. The aim of this study was to determine which significant differences in soil properties (particle size distribution, bulk density - BD, water holding capacity - WHC, air capacity - AC, microaggregates stability - MS, porosity – P, pH, soil organic matter content – SOM content) exist between the eluvial (Eg) and the illuvial (Btg) horizon of 33 Pseudogleys in the Pannonian region of Croatia.

MATERIALS AND METHODS

Pannonian region of Croatia covers 46 % of the country (Bašić, 2013). Climate in this region is moderate continental and humid (semihumid to semiarid only in the most eastern part of the region). Pseudogleys formed on the Pleistocene terraces that are built largely from loess derivates, and sporadically from brown loess (Rubinić et al., 2015b). Forest community of sessile oak and hornbeam (*Epimedio-carpinetum betuli*) is the prevailing natural vegetation on these terraces (Bašić, 2013) and the climax vegetation on Pseudogley soils (Škorić, 1986).

Total of 11 locations were investigated across the Pannonian region of Croatia. All locations featured sessile oak and hornbeam forests. Six locations were on plateau and five were on slope. The locations on slopes were uniform in terms of inclination (5 %) and soil profile position (middle slope, 50 m from slope summit). At each investigated location, three replicate soil pits were dug within a circle of 50 m radius. Therefore, 33 Pseudogley profiles were investigated across the study region. Each soil pit was dug to the depth of about 1 m. Soils were described according to FAO (2006). Soil samples were collected from each mineral horizon of each soil profile (only the results for Eg and Btg horizons are presented in this paper). Core samples were taken as triplicates.

Disturbed soil samples were air-dried and sieved through a 2 mm sieve. Soil analyzes were conducted according to Škorić (1985). Soil particle size distribution was determined by pipette-method with wet sieving and sedimentation after dispersion with sodium-pyrophosphate ($Na_4P_2O_7$, c=0.4M). Soil MS was determined after the *Vageler's structure factor*. Soil pH was determined in 1:2.5 soil: H_2O suspension. The SOM content was determined as the content of humus by acid-dichromate ($K_2Cr_2O_7$, c=0.4 M) digestion. Core samples were used to determine soil BD, particle density (PD), and WHC. Soil P was calculated as follows: P=(1-BD/PD)*100. Soil AC was calculated as follows: AC=P-WHC. Data were analyzed using the *Mixed Procedure* and the *Corr Procedure* of the SAS® 9.2 statistical package (SAS Institutes, Cary, NC).

RESULTS AND DISCUSSION

The properties of Eg horizon differed from the properties of Btg horizon (Table 1). Namely, the *p* values for the differences between the horizons were <0.05 for WHC, and <0.01 for other analyzed soil properties. Both soil horizons were silt loams (FAO, 2006). However, in the Eg horizon, content of clay was lower (and content of silt was higher), compared with the Btg horizon. In Croatian Pseudogleys, this is primarily the result of clay eluviation/illuviation (Rubinić et al., 2015b). Ratio between the clay content in Btg horizon and the clay content in Eg horizon, which was as high as 1.46, confirms that Btg horizon is an illuvial (argic) horizon (IUSS Working Group WRB, 2014). Furthermore, the difference in sand content between the Eg horizon and the Btg horizon (Table 1) was not high enough to point to lithic discontinuity between the two horizons (IUSS Working Group WRB, 2014).

Table 1. Averaged values of the selected properties of eluvial (Eg) and illuvial (Btg) soil horizons in 33 Pseudogleys studied across the Pannonian region of Croatia

Soil horizon	Contents of particles (%)			BD	WHC	P	AC	MS	SOM	pH (H_2O)
	Sand 2-0.063 mm	Silt 0.063-0.02 mm	Clay <2 mm	g cm^{-3}	% vol			%		
Eg	5,1	77,7	17,3	1,30	39,7	49,9	10,2	61,3	1,81	4,8
Btg	4,1	70,8	25,1	1,47	38,3	43,8	5,5	67	0,82	5,3

BD – bulk density, WHC – water holding capacity, P – porosity, AC – air capacity, MS – microaggregates stability, SOM – soil organic matter (humus)

In Pseudogleys, biological activity typically decreases and soil compaction typically increases with soil depth distinctly (Vučić, 1987; Škorić, 1991). Accordingly, the Btg horizon featured higher BD, lower P, and lower AC, compared with the Eg horizon (Table). This is in line with lower SOM content and higher clay content in the Btg horizon, than in the Eg horizon (Table). Soil BD was strongly correlated with both the clay content ($r=0.61$, $p<0.01$) and the SOM content ($r=-0.67$, $p<0.01$). Soil P also was strongly correlated with both the clay content ($r=0.62$, $p<0.01$) and the SOM content ($r=0.67$, $p<0.01$).

Soil microaggregates were moderately stable (Škorić, 1985) in both soil horizons (Table). However, compared with the Eg horizon, MS in the Btg horizon was higher (Table). This is the result primarily of the higher pH in the Btg horizon, than in the Eg horizon (Table). As the result of decalcification during top-down pedogenesis, soil pH in forest Pseudogleys typically increases with soil depth (Rubinić et al., 2015b).
The obtained results generally agree with those of other researchers (e.g., Čirić, 1984; Vučić, 1987; Škorić, 1991; Husnjak, 2014). Our results confirm that properties of Croatian Pseudogleys vary significantly with soil depth. This is especially true for soil physical properties. Namely, the subsoil is significantly finer-textured and compacted, than the topsoil. This should be borne in mind if Pseudogleys are used for agricultural production.

REFERENCES

Bašić, F. 2013. The Soils of Croatia. World Soils Book Series. Springer. Dordrecht.
Bogunović, M., Vidaček, Ž., Husnjak, S., Sraka, M., 1998. Inventory of soils in Croatia. Agric. Consp. Sci. 63, 3, 105-112.
Ćirić, M. 1984. Pedologija. Svjetlost, Sarajevo.
FAO, 2006. Guidelines for soil description, fourth ed. FAO, Rome.
Husnjak, S. 2014. Sistematika tala Hrvatske. Hrvatska Sveučilišna naklada. Zagreb.
Husnjak, S., Romić, M., Pernar, N., Poljak, M., 2011. Recommendations for soil management in Croatia. Agric. Consp. Sci. 76, 1, 1-8.
IUSS Working Group WRB, 2014. World reference base for soil resources 2014. International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106, FAO, Rome.
Rubinić, V., Durn, G., Husnjak, S., Tadej, N. 2014. Composition, properties and formation of Pseudogley on loess along a precipitation gradient in the Pannonian region of Croatia. Catena. 113. 138-149.
Rubinić, V., Galović, L., Husnjak, S., Durn, G. 2015a. Climate vs. parent material – Which is the key of Stagnosol diversity in Croatia? Geoderma. 241/242, 250-261.
Rubinić, V., Lazarević, B., Husnjak, S., Durn, G. 2015b. Climate and relief influence on particle size distribution and chemical properties of Pseudogley soils in Croatia. Catena. 127C, 340-348.
Škorić, A. 1985. Priručnik za pedološka istraživanja. Fakultet poljoprivrednih znanosti, Zagreb.
Škorić, A. 1986. Postanak, razvoj i sistematika tla. Fakultet poljoprivrednih znanosti Sveučilišta u Zagrebu. Zagreb.
Vučić, N. 1987. Vodni, vazdušni i toplotni režim zemljišta. Matica srpska i Poljoprivredni fakultet u Novom Sadu. Novi Sad.

Long term liming and manuring effect on soil acidity indicators and crop yield

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Abstract
The effect of soil liming and manuring was observed in the long-term treatment, established in 1959. The soil of the experiment was morain loam (*Bathygleyic Dystric Glosic Retisol*). The research showed that the largest effect of systematic manuring (40 and 60 t ha^{-1}) was on the mobile Al content decreasing (by 4.2 and 6.1 times) and increasing of exchangable Ca amount (by 1.5-1.7 times) in the unlimed soil. The limed soil applied with farmyard manure (FYM) had a higher mobile P_2O_5 and K_2O content, respectively by 1.6-1.8 and 1.7-1.5 times higher. Average of accumulated annual plant metabolitic energy (ME) increased by 20 percent in the limed soil with FYM and by 75 percent in the unlimed soil applied with FYM. According to the results, soil liming and manuring combination is the most effective technique to farming in acid soils.

INTRODUCTION
Soil acidification is one of the main problems in point of soil conservation both in Europe and Lithuania. This is one of the form of chemical soil degradation reducing their fertility and ecological stability (Szymanka et all., 2008, Wolsing and Prieme, 2004). Soil reaction directly affects the availability of nutrients for plants and elements leaching, emissions into the atmosphere and finally plant development and productivity. Liming of acid soils is one of the most important factors that can preserve and even to increase their fertility. Liming materials changes the mobility of some nutrients, their accumulation in the soil and the ability to be absorbed by plants (Pagani et Mallarino, 2012). Seeking to maintain the potential soil productivity and to get the stable yield, it is necessary to supplement the organic matter stocks in the optimal level and to regulate the intensity of synthesis and destruction processes in the soil. Farmyard manure (FYM) is one of the most effective organic fertilizers supporting soil fertility. Calcium and magnesium, which is in the FYM, could neutralize soil acidity. These measures – soil liming and manuring – can‘t replace each other, but could complement each other (Tripolskaja, 2001).

MATERIALS AND METHODS
The research of the effect of long-term soil liming and manuring was conducted since 1959. The soil of the this research was morain loam (*Bathygleyic Dystric Glosic Retisol*). Arable soil layer was very acid (pH_{KCl} 4.1), high levels of plant-available aluminium (131 mg kg^{-1}), amount of exchangable calcium was low (724 mg kg^{-1}). Two backgrounds was formed in this research: unlimed (acid) soil and limed soil at 1.0 liming rate according to hydrolitic acidity. The experimental design included the following treatments for both soil backgrounds: 1) not manured; 2) manured by 40 t ha^{-1}; 3) manured by 60 t ha^{-1} FYM during the crop rotation. The crop rotation was: 1) winter wheat (*Triticum aestivum*), 2) lupine (*Lupinus*) - oat (*Avena sativa*) mixture for the green mass, 3) winter rape, 4) spring barley (*Hordeum vulgare*) with perennial grasses undersown, 5) perennial grasses: red clover (*Trifolium pretense*) + timothy (*Phleum pratense*). The mineral fertilization in both acid and limed soil was the same: $N_{60}P_{60}K_{60}$ for cereals, $N_{60}P_{60}K_{60}$ for barley, $N_{30}P_{60}K_{60}$ for lupine + oats mixture, $N_{60}P_{90}K_{90}$ for rape. In the West Lithuania (Coastal lowlands) falls abundant amount of precipitation, 750-800 mm per year, especially in the second semester. All this creates the favorable conditions for soil leaching. Soil agrochemical properties (in 0-20 cm soil layer) were established using the following methods: pH_{KCl} – by electrometric method, mobile aluminium – by Sokolov method, mobile phosphorus and potassium by AL method.

RESULTS AND DISCUSSION
The long-term and systematic fertilization with FYM had a significant effect on soil agrochemical properties (Table 1). Incorporation of FYM by 40 and 60 t ha^{-1} per rotation increased the soil pH_{KCl} in the unlimed (from 4.10 to 4.41-4.49) and in the limed (from 5.66 to 6.05-6.19) soil. Application of FYM in acid soil improves plant growing conditions as Ca and Mg in the manure binds the exchangable aluminium (Repšienė et al., 2005). Systematic and long-term manuring had a particularly significant impact on the reduction of mobile Al in acid soil. The amount of mobile Al was 131.3 mg kg^{-1} in the unfertilized plots. Application of FYM by 40 and 60 t ha^{-1} during the rotation reduced the amount of mobile Al to 31.3 and 21.5 mg kg^{-1} respectively, or 4.2 and 6.1 times less. In the liimed soils the amount of mobile Al were very low – 0.56 mg kg^{-1}, incorporation of FYM in the soil reduced it to the trace 0.01 mg kg^{-1}. The content of the exchangable Ca was low 724.8 mg kg^{-1}, but

application of FYM increased it by 1.5 - 1.7 times. The significant increase of exchangable Ca (from 1816.6 mg kg^{-1} to 2148.2 ir 2378.4 mg kg^{-1} or 1.2-1.3 times higher) was observed in the limed soil fertilized with FYM. Application of FYM had an essential effect on mobile P_2O_5 ir K_2O content in both soil backgrounds. Content of the mobile P_2O_5 and K_2O increased by 1.6-1.8 and 1.4-1.5 times respectively. The increase of these elements in the acid soil was lower. Content of the mobile P_2O_5 increased by 1.2-1.3 times, K_2O by 1.2 times.

Table 1. Effect of liming and of manuring on topsoil agrochemical properties (2006-2010)

Manure rate (t ha^{-1}) per rotation	pH_{KCl}	Mobile Al mg kg^{-1}	Exchangable Ca mg kg^{-1}	Mobile P_2O_5 mg kg^{-1}	Mobile K_2O mg kg^{-1}
Unlimed soil					
Not manured	4.10	131.3	724.8	190.2	217.7
Manured by 40 t ha^{-1}	4.41**	31.8**	1100.4**	234.6**	256.0**
Manured by 60 t ha^{-1}	4.49**	21.5**	1249.0**	242.7**	257.6**
LSD_{05}	0.058	7.361	135.231	12.901	21.383
Limed soil at 1.0 liming rate					
Not manured	5.66	0.56	1816.6	154.8	171.5
Manured by 40 t ha^{-1}	6.05*	0.17*	2148.2**	250.7**	243.6**
Manured by 60 t ha^{-1}	6.19**	0.01**	2378.4**	280.2**	262.9**
LSD_{05}	0.287	0.307	151.413	18.422	21.113

*Note: * and ** – significantly different from control (P<0.005) and (P<0.001).*

Application of FYM (40 or 60 t ha^{-1}) resulted higher accumulated annual plant metabolitic energy (ME) by 1.7 times higher in the unlimed soil and by 1.2 times in the limed soil compared to not manured soil (Table 2). Incorporation of FYM in the limed soil had a less decisive role to accumulated ME. Average of accumulated annual plant metabolitic energy increased to 52.8 GJ ha^{-1} in the unlimed soil applied with FYM and to 69.9 GJ ha^{-1} in the limed soil applied with FYM or increased by 75 and 20 percent respectively.

Table 2. The effect of liming manure on metabolitic energy (ME) accumulated by the crop rotation plants (2006-2010)

Manure rate (t ha^{-1}) per rotation	Metabolitic energy GJ ha^{-1}			
	total accumulated ME per rotation		average of annual ME	
	Unlimed	Limed at 1.0 rate	Unlimed	Limed at 1.0 rate
Not manured	150.7	290.9	30.2	58.2
Manured by 40 t ha^{-1}	254.8**	323.9	51.0**	64.8*
Manured by 60 t ha^{-1}	263.7**	349.7*	52.8**	69.9**
LSD_{05}	31.343	44.84	7.272	5.059

*Note: * and ** – significantly different from control (P<0.005) and (P<0.001).*

CONCLUSIONS

The long-term and systematic fertilization with FYM (40 and 60 t ha^{-1}) had a significant effect on soil agrochemical properties: the amount of mobile Al reduced from 131.38 to 31.8 - 21.5 mg kg^{-1} (4.2 and 6.1 times), pH_{KCl} increased from 4.10 to 4.41-4.49, exchangable Ca increased by 1.5-1.7 times. Application of FYM in the limed soil had a less decisive influence to these indicators: the amount of mobile Al reduced to0,01 mg kg^{-1}, pH_{KCl} increased from 5.66 to 6.05-6.19, exchangable Ca increased by 1.2-1.3 times. Systematic manuring in the limed soil had a significant effect on the accumulation of mobile P_2O_5 and K_2O. The amount of mobile P_2O_5 and K_2O increased by 1.6-1.8 times and 1.7-1.5 times respectively in the limed soil. In the unlimed soil applied with FYM, the amount of mobile P_2O_5 and K_2O increased by 1.2-1.3 times and 1.2 times respectively. Average of accumulated annual plant metabolitic energy increased to 52.8 GJ ha^{-1} or by 75 percent in the unlimed soil. The effect of manuring on accumulated ME in the limed soil was less decisive. The average of accumulated ME was 69.9 GJ ha^{-1} or 20 percent in the limed soil applied with FYM. According to the getted results, soil liming and manuring combination is the most effective technique to farming in acid soils.

REFERENCES

Pagani A., Mallarino A. P. 2012. Soil pH and crop grain yield s as affected by the source and rate of lime. Soil science society of America Journal.76, 5. 1877-1886.

Repšienė R., Plesevičienė A. K., Čiuberkis, S. 2005. The effect of manure rates on soil properties and productivity of agrocenosis. Žemdirbystė-Agriculture. 89. 18-30.

Szymanska M., Korc M., Lebetowicz J. 2008. Effects of single liming of sandy soils not limed for more than 40 years in the light of results of long-term fertilizing experiment. Polish Journal of soil science, Vol XLI/1, 105-114.

Tripolskaja L. 2001. Influence of various manure types on soil fertility and efficiency of crop rotation. Žemdirbystė=Agriculture. 73. 14-26.

Wolsing M., Prieme A. 2004. Observation of high seasonal variation in community structure of denitrifying bacteria in arable soil receiving artificial fertilizer and cattle manure by determining T-RFLP of nir gene fragments. FEMS Microbiology Ecology. 261-271.

Cation exchange capacity of some acid soils in Eastern Croatia

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Abstract

The cation exchange capacity (CEC) or sum of exchangeable cations are an essential measurement in agronomy and soil science to estimate the physicochemical state of a soil, which may be a good indicator of soil quality and productivity. Soil testing laboratories do not usually provide a direct measure of CEC. Instead, often the CEC is calculated based on the quantities of base cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+) extracted in the standard soil test (e.g. ammonium acetate).
The study was conducted on 30 locations and 2 soil types (Luvisol and Stagnic Gleysols) in Eastern Croatia including only topsoil horizon (depth 0-30 cm). The soil samples (30) were analyzed for the following parameters: pH, organic matter, clay content, base cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+), hydrolytic acidity, CEC and base saturation. Descriptive statistics showed that pH varied from 3.71 to 5.16 (average 4.22) on luvisol and 3.81 to 5.41 (average 4.40) on stagnic gleysols. Organic matter varied from 1.31 % to 2.86 % (average 1.97 %) on luvisol and 1.62 % to 3.11 % (average 2.26 %) on stagnic gleysols, while clay content varied from 11.95 % to 25.03 % (average 19.22 %) on luvisol and 10.76 % to 24.02 % (average 16.52 %) on stagnic gleysols.
The highest value of CEC was recorded on stagnic gleysols (11.84 cmol(+)/kg) and values ranged from 7.01 to 17.52 cmol(+)/kg, while average value of luvisol was 11.60 cmol(+)/kg with range from 7.38 to 17.02 cmol(+)/kg. The highest value of exchangeable Ca^{2+} was recorded on stagnic glaysols (2832.0 mg/kg), while highest values of exchangeable Mg^{2+}(460.7 mg/kg), K^+ (452.7 mg/kg), and Na^+ (27.80 mg/kg), was recorded on luvisol. The lowest value of exchangeable Ca^{2+}(291.80 mg/kg) and Mg^{2+} (55.47 mg/kg) was recorded on luvisol, while lowest value of exchangeable K^+ (73.65 mg/kg) and Na^+ (11.69 mg/kg) was recorded on stagnic gleysols. The average determined adsorption complex saturation luvisol amounted to 56.96 %, while the stagnic gleysols is 73.48 %. No significant difference was found between pH, organic matter, base cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+) and CEC. Soil type was found to have significant influence on clay percentage, hydrolytic acidity and base saturation. It was obtained that stagnic gleysols has a significantly higher content of clay particles and hydrolytic acidity compared to luvisol, however it had significantly lower cation exchange capacity base saturation.
According to the obtained results we can state that investigated soil belong to acid soils, with moderate organic matter content and moderate texture, average values of CEC are low (11.60 cmol(+)/kg on luvisol to 11.84 cmol(+)/kg on stagnic gleysols) and it's not dependent on soil type, determined adsorption complex saturation of luvisol is statistically lower compared to stagnic gleysols and composition of cations on the adsorption complex is Ca> Mg> K> Na.

The potential of organic phosphorus in acidic soils of eastern Slavonia

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Abstract
Phosphorus is often sparingly soluble in soils and, consequently, P deficiency in plants represents a major constraint to world agricultural production. Plants possess many potential mechanisms to increase P uptake from soil, including an upregulation of P membrane transport systems, the increased growth of root hairs, enhanced mycorrhizal association, the release of phosphatases, changes in root architecture and the release of organic acids. The concentrations of plant available phosphorus in soil is closely connected with the chemical properties of the soil, basically soil pH. In fact, the relationship of fractions of inorganic phosphorus in the soil depends primarily on the pH and, in acidic soils (especially in pH_{KCl} <5.5), the dominant ions are iron and aluminum, which easily enter into chemical reactions with phosphate ions in the soil and form Al-Fe-phosphates, which are generally plant unavailable forms. In such acidic soils, organic phosphorus has a great potential for plant nutrition. Organic phosphorus in soil is part of more than 50% of total phosphorus, mostly in the form of inositol penta- and hexa- phosphate bound in iron and aluminum. Phosphatase enzyme activity in the soil is crucial for organic phosphorus availability, because most plants can adopt phosphorus exclusively in inorganic form. The organic phosphorus can be released through mineralization processes mediated by soil organisms and plant roots in association with phosphatase secretion. These processes are highly influenced by soil moisture, temperature, surface physical-chemical properties, soil pH and Eh (for redox potential). Also, the amount of organic phosphorus in the soil is closely related to the organic matter content in soil.

The aim of paper was to determine the potential of organic phosphorus in acidic soils of eastern Slavonia due to the organic matter content, as well as total and mineral phosphorus concentrations in the soil.

Soil samples (n=40) were taken from the field of eastern Slavonija (0-30 cm) and laboratory analysis of the common soil chemical properties were determined: the soil pH (ISO 10390, 1994), the content of organic matter in the soil by dichromate method (ISO 14235, 1994), the concentration of AL- available phosphorus and potassium (Egner et al., 1960). The organic phosphorus in the soil was determined by three step extraction and three fractions of phosphorus were determined: mineral phosphorus obtained by extraction (labile, unstable medium, the humic acids), total phosphorus obtained by digestion (labile, medium labile phosphorus in humic acids) and organic phosphorus (labile, medium labile phosphorus in humic acids). The results of soil samples analyzes were statistically analyzed with PC applications SA for Windows (SAS Institute Inc., Cary, NC, USA), StatSoft Statistica and Excel for determination of analysis of variance (ANOVA) and correlation.

Results of actual acidity pH_{H2O} were ranged from 5.10 to 6.69 and substitutional acidity pH_{KCl} from 3.88 to 5.92 which indicating a range of weak acid to very acid soils in the analyzed samples. Furthermore, the results of AL-phosphorus concentration were from 23.33 to 95.42 mg P_2O_5 $(100g)^{-1}$ of soil. The AL- potassium concentrations were in the range of 16.62 to 36.81 mg K_2O $(100\ g)^{-1}$ soil. The organic matter content in acidic soils was from 2.17 to 3.30 %. The extraction of organic phosphorus (stable, bound phosphorus) were within the range of 320.75 mg kg^{-1} to 579.50 mg kg^{-1} with an average of 419.15 mg kg^{-1}. It actually represents the organic fraction of the soil obtained the in humic acids.

In acidic soils of eastern Slavonia were, also, established the concentrations of total phosphorus in soil (ranged from 511.0 mg kg^{-1} to 766.5 mg kg^{-1} average 647.40 mg kg^{-1}), as well as mineral phosphorus fraction concentration which was ranged from 163.75 mg kg^{-1} to 375.0 mg kg^{-1} with average 228.25 mg kg^{-1}. This concentrations represent labile fraction phosphorus in soil (potentially more plant available forms in soils) and were used for calculated the ratio of mineral/total phosphorus and organic/total phosphorus in soil which was 35 % for mineral fraction in total phosphorus and 64.74 % for organic fraction in acid soils. So, compared to mineral labile fraction, in acidic soils were established higher concentration and potential of organic phosphorus, regardless of the content of organic matter in soil. Also, between total and organic phosphorus was found a high correlation, r = 0.98.

Although the correlations between organic phosphorus and organic matter content were not determined, results can be used to create a model for predicting the organic phosphorus concentrations in soil, based on common soil properties, as well as for correction of fertilizer recommendations based on the AL methods. Furthermore, the obtained results can be used for further analysis, to study the influence of organic matter content and available mineral phosphorus to the organic fraction of phosphorus regardless to pH.

The effectiveness of phosphate and liming on acidic soils

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Abstract

In acid soils, availability of certain nutrients like aluminum, iron and manganese increases due to higher dissolution and at times becomes toxic. In strongly acidic conditions, phosphorus reacts with active iron and aluminum forming insoluble phosphates. More than 80 percent of applied phosphate was converted into unavailable forms in acid soils within very short periods. Under such conditions, calcium and magnesium supply is reduced and plant growth suffers. In addition to these, other beneficial nutrients such as nitrogen, phosphorus, and sulfur are also in deficient concentration. To meet the calcium demands, as well as to create favorable conditions for better uptake of other essential nutrients, liming is an important management practice in acid soils. The overall effects of lime on soils include, among others, increased soil pH, Ca and Mg saturation, neutralization of toxic concentrations of aluminum, increase in pH dependent CEC resulting in absorption and hydrolysis of Ca^{2+}, Mg^{2+}, increase in P availability and improved nutrient uptake by plants. Liming also improves microbiological activities of acid soils, which in turn can increase di-nitrogen fixation and liberate nitrogen (N) from incorporated organic materials. However, over-liming may reduce crop yields, due to lime induced P and micronutrient deficiencies. Therefore, crop yield responses to lime and P are often interdependent. Under these situations, an appropriate combination of lime and P is an important strategy for improving crops growth in highly weathered acid soil.

The aim of this paper was to determine the efficiency of phosphorus fertilizer with and without liming to phosphorus concentrations increment in acidic soils of north Bosnia.

An experiment was carried out in the crop field in north Bosnia in spring 2014. According to soil properties (pH_{KCl} average 4.13 organic matter content average 1.54 % AL-P 7.10 mg $100g^{-1}$) the treatments were: I control (no P and no lime), II P - phosphorus was added in concentrations of 156 kg P_2O_5 ha^{-1} by MAP, III P and lime combinations with different amount of lime III (1) 4 t (spring 2014.), III (2) 8 t (2 t spring 2014.+ 6 t will be added in autumn 2015.), III (3)10 t (2 t spring 2014 + 4 t will be added in autumn 2015 + 4 t will be added in spring 2016). Studies were conducted to 100 m^2 on each plot and on all plots organic manure were added in amount of 30 t ha^{-1}. For laboratory analysis soil samples were taken in summer 2015 and air dried and passed through a 2-mm sieve and stored. Soil pH_{KCl} was slightly increment on treatments with lime (from 4.13 to 4.20 III (1), 4.17 III (2), 4.17 III (3) while organic matter content were almost the same 1.57 %. The concentrations of AL-plant available phosphorus after first year of research were low, but difference between phosphorus applications with and without liming were showed. Thus, in plots with P without liming changes in AL-phosphorus concentration were from an initial 7.10 mg $(100g)^{-1}$ to 8.66 mg $(100g)^{-1}$ (average) and at the plots with P and lime combination average AL-phosphorus concentration increased to 10.5 mg $(100g)^{-1}$ (average). The concentration of phosphorus is still very low, but after the first year of research it can be noticed that phosphorus fertilizer efficiency increased on areas that were limed. Thus, per unit area was determined increase of 5.85 kg P_2O_5 ha^{-1} (P) and 12.75 kg P_2O_5 ha^{-1} (P+liming). So, of the total added amount of phosphorus in the first year P-use efficiency was 3.75 % (P) and 8.19 % (P+liming).

To measure P-use efficiency, the difference between initial state and post-fertilization state is inappropriate method because it does not consider the residual effect of added P. The balance method provides a more realistic estimate of P-use efficiency, which can be as high as 90 percent in some situations. Most of the inorganic P added to soils in fertilizers and manures is usually adsorbed initially, but it may become absorbed by diffusive penetration of phosphate ions into soil components. It is considered that this added P is held with a continuum of bonding energies on the surfaces of, or within, soil components, and that this gives rise to the differing extractability of soil P and its differing availability to plants.

The results conclude that application of lime and phosphorus offers a large scope for better soils properties as well as plant nutrition possibilities. Application of lime along with phosphate fertilizer favored the uptake of N, K and Ca under acidic soil condition and this should be taken into consideration for cultivating plants on acid soil. All the plant benefits were observed at the highest level of lime and P tested. If higher levels of lime and P were taken, the results of variation could be different and it would have been convenient to estimate the optimum requirement of lime and P. Further work in this regard is needed because there was not any statistical significant difference between established results, but in next year's difference between treatments would be more obvious. Also, statistically significant difference in the effectiveness of agro-technical measures to increase phosphorus status in the soil will be more expressed.

Section 02:

Physiological and molecular mechanisms of plant adaptation to acid soils

Chairpersons: Zed Rengel, Ren Fang Shen, Hong Liao

Transport and Detoxification of Manganese in Rice

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Abstract
Manganese (Mn) is one of the major factors limiting crop production on acid soil and poorly drained soil. However, rice is able to accumulate high Mn in the shoots without showing toxicity symptoms. We found that rice has developed a unique transport system for uptake, distribution and detoxification of Mn. Two transporters; OsNramp5 and OsMTP9 expressed in the roots are involved in Mn uptake, while OsNramp3 localized in the node is responsible for the distribution. In the leaves, OsYSL6 and OsMTP8.1 are responsible for detoxification of Mn. The detailed function of these transporters will be presented.

INTRODUCTION

Manganese (Mn) is an essential micronutrient for plant growth and development, but shows toxicity when present in excess. Mn toxicity has been considered as one of the major factors limiting crop production on acid soils and poorly drained soils. However, rice (*Oryza sativa*) is highly tolerant of Mn toxicity. Furthermore, it is able to accumulate high concentration of Mn in the shoots without showing toxicity symptoms. Recently, we have identified a number of transporters involved in uptake, distribution and detoxification in rice.

MATERIALS AND METHODS

Gene expression in different tissues was investigated by RT-PCR. The localization was observed using immunostaining. Phenotypic analysis was performed by growing wild-type rice and knockout lines in nutrient solution or soil. Mineral analysis was conducted by ICP-MS.

RESULTS AND DISCUSSION

Transporters involved in Mn uptake

Two transporters (OsNramp5 and OsMTP9) have been found to be involved in Mn uptake in rice roots. OsNramp5, a member of the Nramp (for the Natural Resistance-Associated Macrophage Protein) family, transports Mn, Cd and ferrous Fe in yeast. *Nramp5* was constitutively expressed in the roots and did not respond to external Mn supply levels (Sasaki et al., 2012). It encodes a plasma membrane–localized protein, which was polarly localized at the distal side of both exodermis and endodermis cells of the roots. Knockout of *Nramp5* resulted in a significant reduction in Mn concentration, growth and grain yield. These result indicates that Nramp5 is responsible for the transport of Mn from the external solution to root cells.
On the other hand, we recently found that another transporter, OsMTP9, was also involved in Mn uptake. OsMTP9 is a member of CDF (cation diffusion facilitator). Similar to OsNramp5, it was also mainly expressed in the roots and the coding protein was localized to the plasma membrane. However, different from OsNramp5, OsMTP9 only transported Mn and localized at the proximal side at both exodermis and endodermis cells. Knockout of *OsMTP9* resulted in decreased Mn uptake and translocation to the shoots. These results show that OsMTP9 is required for Mn uptake by releasing Mn out of the cells toward the root stele. A cooperative transport mediated by both OsNramp5 and OsMTP9 is required for efficient Mn uptake in rice.

Transporters involved in Mn distribution

The concentration of Mn in paddy soil solution ranges from submicromolar to hundreds of micromolars levels. We found that a node-localized Mn transporter, OsNramp3, functions as a switch for regulating Mn distribution in rice for dealing with variable changes of Mn in the environment (Yamaji et al., 2013). OsNramp3 is constitutively expressed in the node, a junction of vasculatures connecting leaves, stems and panicles. It is a plasma membrane-localized transporter for Mn. The protein is localized at the xylem and phloem regions of enlarged and diffuse vascular bundles at the nodes. Under Mn-limited conditions, OsNramp3 preferentially transports Mn to the developing young leaf, crown root tips and panicle. However, under Mn-excess condition, OsNramp3 protein is rapidly degraded within a few hours, resulting in altered distribution of Mn to the older leaves following transpiration. These results reveal the OsNramp3-mediated strategy of rice for adapting to a wide change of Mn in the environment.

Transporters involved in Mn detoxification

We found that at last two transporters (OsYSL6 and OsMTP8.1) are required for detoxification of Mn in rice. OsYSL6 (Yellow Stripe-Like (YSL)) belongs to the oligopeptide transporter family. Knockout of OsYSL6 did not affect the growth at low Mn concentration, but resulted in decreased growth of both roots and shoots at high-Mn (Sasaki et al., 2011). There was no difference in the concentration of total Mn and other essential metals between the wild-type rice and the knockout line, but the knockout line showed a higher Mn concentration in the leaf apoplastic solution and a lower Mn concentration in the symplastic solution than wild-type rice. OsYSL6 was constitutively expressed in both the shoots and roots, and the expression level was not affected by either deficiency or toxicity of various metals. Furthermore, the expression level increased with leaf age. Heterogolous expression of OsYSL6 in yeast showed transport activity for the Mn-nicotianamine complex but not for the Mn-mugineic acid complex. These results suggest that OsYSL6 is a Mn-nicotianamine transporter, which is responsible for transporting this complex from apoplast to symplat, thereby contributing to the detoxification of excess Mn in rice.

On the other hand, OsMTP8.1, a member of cation diffusion facilitator (CDF) family, encodes a Mn transporter localized at the tonoplast of all leaf cells. OsMTP8.1 and its transcript were mainly detected in shoots (Chen et al., 2013). High or low supply of Mn moderately induced an increase or decrease in the accumulation of OsMTP8.1, respectively. Disruption of OsMTP8.1 resulted in decreased chlorophyll levels, growth inhibition in the presence of high concentrations of Mn, and decreased accumulation of Mn in shoots and roots. However, there was no difference in the accumulation of other metals, including Zn, Cu, Fe, Mg, Ca, and K. These results suggest that OsMTP8.1 is a Mn-specific transporter that sequesters Mn into vacuoles and is required for Mn tolerance in shoots of rice.

CONCLUSION

Rice as a Mn-tolerant and accumulating species has developed a unique transport system for uptake, distribution and detoxification of Mn.

REFERENCES

Chen, Z., Fujii, Y., Yamaji, N., Masuda, S., Takemoto, Y., Kamiya, T., Yusuyin, Y., Iwasaki, K., Kato, S., Maeshima, M., Ma, J.F. and Ueno, D. 2013. Mn tolerance in rice is mediated by MTP8.1, a member of the cation diffusion facilitator family. Journal of Experimental Botany. 64, 4375–4387

Sasaki, A., Yamaji, N., Xia, J. X. and Ma, J. F. 2011. OsYSL6 is involved in the detoxification of excess manganese in rice. Plant Physiology. 157, 1832-1840.

Sasaki, A., Yamaji, N., Yokosho, K. and Ma, J. F. 2012. Nramp5 is a major transporter responsible for manganese and cadmium uptake in rice. The Plant Cell. 24, 2155–2167.

Yamaji, N., Sasaki, A., Xia, J.X., Yokosho, K and Ma, J.F. 2013. A node-based switch for preferential distribution of manganese in rice. Nature Communications. 4, 2442 doi: 10.1038/ncomms3442.

AtNIP1;2 Is Involved in Aluminum Uptake and Root-to-Shoot Translocation under Aluminum Stress

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Abstract
Aquaporins (AQPs) are ubiquitous super family of membrane intrinsic proteins (MIPs) that facilitate transmembrane transport of water and small uncharged polar compounds (Maurel et al., 2008). Recently, a couple of members of the AQP family in Hydrangea have been identified as aluminum (Al) transporters that facilitate Al transport and accumulation into the vacuole of sepal cells, which leads to flower color change of hydrangea plants grown on acid soils from red to blue. However, the roles of AQPs in Al tolerance are still elusive. In this report, we demonstrate that AtNIP1;2, a member of AQPs in Arabidopsis, functions as an Al transporter that plays an important role in Al absorption and root-to-shoot translocation, which is a critical step for Al tolerance in Arabidopsis.

INTRODUCTION
One of the Al tolerance mechanisms in plant is involved in Al uptake and root-to-shoot translocation under Al stress. These processes move Al from Al sensitive organs/tissues, such as root tips, to the less sensitive parts such as shoots (Kochian et al., 2015; Liu et al., 2014). However, the molecular mechanism underlying Al transport and translocation is still largely elusive. Recently, a couple of members of the *Hydrangea macrophylla* nodulin 26 like intrinsic protein (NIP) subfamily of the AQP family, *HmPALT* and *HmVALT*, have been found to encode a plasma membrane (PM)-localized Al transporter and a vacuole membrane (VM)-localized Al transporter that facilitate Al transport into the cytosol and the vacuole of sepal (flower) cells of hydrangea plants, respectively (Negishi et al., 2012; Negishi et al., 2013). However, it is unclear if HmPALT and HmVALT or any members of the AQP family are involved in Al tolerance in plants. In this report, we used a reverse genetic approach to test if members of the NIP subfamily in Arabidopsis are involved in Al tolerance. Our results indicate that *AtNIP1;2* encodes an Al transporter that plays a critical role in Al uptake, Al root-to-shoot translocation and Al tolerance in Arabidopsis.

MATERIALS AND METHODS
Individual T-DNA insertion lines of the *AtNIP* subfamily were acquired from the Arabidopsis Biological Resource Center (ABRC). The relative root growth (RRG%) was used to evaluate Al tolerance of these lines (Liu et al., 2009). The procedures for real-time reverse transcriptase PCR (RT-PCR), AtNIP1;2 subcellular localization, GUS staining for the AtNIP1;2::GUS transformants, Al content measurement followed the protocols as described in Liu et al. (Liu et al., 2012; Liu et al., 2009)

RESULTS AND DISCUSSION
Screening Al sensitive mutants of the AtNIP subfamily
Blast results indicated that HmPALT belongs to the NIP subfamily. To test the role of the NIP subfamily in Al tolerance in Arabidopsis, individual T-DNA insertion lines from the AtNIP subfamily were tested for their root growth under Al stress. Our results indicated that three independent T-DNA insertion lines of *AtNIP1;2* were hypersensitive to Al stress, but not to the toxic levels of other metals, including Cd, La, Zn, Cu. These results indicated that *AtNIP1;2* is critical for Al tolerance in Arabidopsis.

AtNIP1;2 gene expression in roots is specifically induced by Al stress
To characterize the gene function of *AtNIP1;2*, time course real-time RT-PCT experiments were conducted for the wild-type plants treated with Al stress. Our results indicated that *AtNIP1;2* transcripts were mainly detected in the root tip region of the wild-type plants and the *AtNIP1;2* expression in root tips is quickly induced by Al treatment. The *AtNIP1;2* transcript levels peaked at 4 hr after Al treatment. In addition, *AtNIP1;2* expression is

specifically induced by Al stress, but not by other metals, including Cd, La, Zn, Cu or by low pH stress.

The AtNIP1;2 protein is localized to plasma membrane

To test the subcellular localization of AtNIP1;2 proteins, the 35S promoter:AtNIP1;2::GFP fusion protein construct was transiently expressed in tobacco leaves through agrobacteria-mediated infiltration. The GFP fluorescence was co-localized with the PM marker. Furthermore, DAPI staining of nucleus indicated that GFP fluorescence encompassed the nucleus, indicating that AtNIP1;2 is localized to the PM.

AtNIP1;2 transports Al

To address the cellular function of AtNIP1:2, the *AtNIP1;2* coding sequence was expressed in yeast. The *AtNIP1;2* expressing yeast cells accumulated more Al than the vector control line and the *AtNIP1;2* expressing yeast line was hypersensitive to Al stress. These results suggest that AtNIP1;2 functions as an Al transporter.

AtNIP1;2 is expressed in root tips and root vascular tissues

To evaluate the tissue- and cell type specific expression patterns of AtNIP1;2, The 2.1 kb *AtNIP1;2* promoter was PCR-amplified from Arabidopsis genomic DNA and used for making the *AtNIP1;2p*::*GUS* (β-glucoronidase) construct. The resulting construct was stably transformed into the wild type (Col-0) by Agrobacterium-mediated transformation. GUS staining of the resulting transgenic seedlings indicate that AtNIP1;2 is mainly expressed in the root tip region and the vascular tissue of mature roots. In the root tip region, *AtNIP1;2* is expressed in epidermal cells, cortex cells, endodermal cells, as well as vascular tissues, while, in the mature roots, *AtNIP1;2* is only expressed in the vascular tissues.

AtNIP1;2 is involved in Al uptake and root-to-shoot translocation

The results of Morin staining indicated that more Al was accumulated in the root tip region of the *Atnip1;2* mutant plants than in the same region of the WT plants. Analyses of Al content indicated that Al concentrations in root cell sap of the *Atnip1;2* mutants were significantly lower than those of the wild-type plants. In contrast, the levels of Al content in root cell walls of the *Atnip1;2* mutants were significantly higher than those of the wild-type plants. These results indicate that AtNIP1;2 is involved in Al transport into the root cells in the root tip region. In shoots, Al content in both shoot cell sap and shoot cell wall was significantly lower in the *Atnip1;2* mutants than that in the wild-type plants. In addition, the wild-type plants displayed higher shoot/root Al concentrations in cell sap than the *Atnip1;2* mutants did. These results indicate that AtNIP1;2 is an important factor for Al absorption and root-to-shoot transport.

CONCLUSION

In this study, we demonstrated that AtNIP1;2 functions as a PM-localized Al transporter that facilitates the absorption of Al from soils or the apoplastic flow into root tip cells, including the epidermal, the cortex as well as the endodermal cells. In the pericycle cells, AtNIP1;2 might also facilities Al upload into the xylem cells (xylem uploading). These procedures reduce Al concentrations in the cell walls of root tip cells and facilitate the translocation of Al from root tips (the Al sensitive part of the plant) to shoots (the less Al sensitive part of the plant).

REFERENCES

Kochian, L., Piñeros, M., Liu, J., and Magalhaes, J. (2015). Plant Aluminum Resistance: Molecular Biology, Genetics and Physiology. Annual Review of Plant Biology *66*.

Liu, J., Luo, X., Shaff, J., Liang, C., Jia, X., Li, Z., Magalhaes, J., and Kochian, L.V. (2012). A promoter‐ swap strategy between the AtALMT and AtMATE genes increased Arabidopsis aluminum resistance and improved carbon‐ use efficiency for aluminum resistance. The Plant Journal *71*, 327-337.

Liu, J., Magalhaes, J.V., Shaff, J., and Kochian, L.V. (2009). Aluminum‐ activated citrate and malate transporters from the MATE and ALMT families function independently to confer Arabidopsis aluminum tolerance. The Plant Journal *57*, 389-399.

Liu, J., Piñeros, M.A., and Kochian, L.V. (2014). The role of aluminum sensing and signaling in plant aluminum resistance. Journal of integrative plant biology *56*, 221-230.

Maurel, C., Verdoucq, L., Luu, D.-T., and Santoni, V. (2008). Plant aquaporins: membrane channels with multiple integrated functions. Annu Rev Plant Biol *59*, 595-624.

Negishi, T., Oshima, K., Hattori, M., Kanai, M., Mano, S., Nishimura, M., and Yoshida, K. (2012). Tonoplast-and plasma membrane-localized aquaporin-family transporters in blue hydrangea sepals of aluminum hyperaccumulating plant. PloS one *7*, e43189.

Negishi, T., Oshima, K., Hattori, M., and Yoshida, K. (2013). Plasma membrane‐ localized Al‐ transporter from blue hydrangea sepals is a member of the anion permease family. Genes to Cells *18*, 341-352.

Physiological and molecular analysis of manganese toxicity and manganese leaf-tissue tolerance in rice (*Oryza sativa*)

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Abstract
The physiological and molecular basis of the high manganese (Mn) tolerance of rice is widely unknown. The screening of a recombinant inbred-line (RIL) population for Mn tolerance showed great differences in Mn sensitivity between RILs expressed as typical brown spots (Mn accumulations) in leaves. At elevated Mn supply selected Mn-sensitive and Mn-tolerant RILs did not differ in bulk-leaf and leaf apoplastic washing fluid (AWF) Mn concentrations but greatly in H_2O_2-producing and consuming peroxidase activities in the AWF. 94% of the bulk-leaf Mn was bound in the cell walls independent of the Mn sensitivity of the RILs. Proteomic (native IEF-FFE) and transcriptomic (microarrary) analysis confirmed that high Mn supply induced major quantitative and qualitative changes in the expression of apoplastic peroxidases and genes particularly of the phenylpropanoid pathway in a Mn-sensitive but not Mn-tolerant RIL. The results suggest that leaf apoplastic peroxidases and their activities are involved in the control of the reactions leading to Mn oxidation and related Mn toxicity also in rice.

INTRODUCTION

Manganese (Mn) excess represents an important factor limiting growth and crop yields particularly on acid and insufficiently drained soils with low redox potential. Great differences in Mn tolerance exist between plant species and cultivars within species. Barley is highly Mn-sensitive, whereas rice is one of the most Mn-tolerant crops (Führs et al., 2010). In some plant species such as barley, common bean and cowpea typical Mn toxicity symptoms are brown spots consisting of Mn oxides and oxidized phenolics in leaves (Horst et al., 1999). The close positive relationship between the formation of such brown spots and the activities of H_2O_2-producing and consuming peroxidases in the AWF suggest that the leaf apoplast is the decisive compartment for the development or avoidance of Mn toxicity in cowpea (Fecht-Christoffers et al., 2006). On the other hand the high leaf-tissue Mn tolerance of sunflower has been attributed to the deposition of oxidized Mn in leaf trichomes (Blamey et al., 1986). In other plant species particularly in Mn accumulators, Mn tolerance has been related to the accumulation of Mn in leaf vacuoles (Fernando et al., 2013). Consistent with the assumption that cytosolic Mn^{2+} activity has to be kept low to avoid Mn^{2+} interfering with essential metabolic functions, pumping of Mn^{2+} from the cytosol into other cell compartment has been shown to confer Mn tolerance in yeast and *Arabidopsis* (Delhaize et al., 2003; Peiter et al., 2007). The high Mn tolerance of rice is not well understood neither on the physiological nor the molecular level. Lidon (2001) postulated that the high leaf Mn tolerance of rice is due to the enhanced binding of Mn in a chloroplast-localized protein. Sasaki et al. (2011) concluded that the OsYSL6 is a Mn-nicotianamine transporter required for the detoxification of high Mn by preventing Mn accumulation in the apoplast resulting in Mn toxicity. The objective of the present study was to further clarify the mechanisms of Mn tolerance of rice taking advantage of differences in Mn tolerance within a recombinant inbred-line population reported by Wang et al. (2002).

RESULTS AND DISCUSSION

Physiological studies

To clarify the nature of the high Mn tolerance of rice (*Oryza sativa*) a RIL population characterized for differences in Mn tolerance using potted soil by Wang et al. (2002) has been studied. In a first step the whole population (90 RILs and their parents were grown in a greenhouse in nutrient solution. After pre-culture until the fifth leaf was fully developed the Mn concentration in the nutrient solution was increased from 1 µM to 500 µM until the individual RILs developed toxicity symptoms in form of brown spots in leaves. Great differences existed between the lines in the appearance of Mn toxicity symptoms. Whereas some RILs showed severe Mn toxicity symptoms already after 6 days of treatment with excess Mn, a number of RILS did not show any Mn toxicity symptoms even after 14 days of treatment. The bulk-leaf Mn concentrations also varied greatly between the genotypes, but they did not show any relationship with the severity of the toxicity symptoms. In a second step, four RILs with great differences in the expression of toxicity symptoms were selected for in-depth characterization of Mn tolerance. When the RILs were exposed to optimal or excess Mn for the same duration the bulk-leaf Mn concentrations and the Mn concentration in the AWF did not differ between the genotypes at either Mn supply. The genotypic differences in Mn leaf-tissue tolerance could be due to differences in the compartmentation and binding state of Mn. The symplastic Mn fraction represented only 5-6% of the total bulk-leaf Mn content. The remaining of the Mn was cell wall-bound. In the cell walls the $BaCl_2$-exchangeable fraction represented 48% of the total Mn content followed by the DTPA-extractable fraction (36%). The HCl and the insoluble residue fractions played only minor roles particularly at high Mn supply. There were no differences between the RILs in response to the Mn supply in either fraction. Since the Mn compartmentation could not explain the genotypic differences in Mn tolerance, the activities of the apoplastic H_2O_2-producing and consuming peroxidases which have been previously implicated in the formation of the Mn toxicity symptoms in cowpea were determined. Under control conditions the specific activities of both NADH and guaiacol apoplastic peroxidases of all four RILs were in the same order of magnitude. High Mn supply, however, led to increased peroxidase activities in the Mn-sensitive but not in the tolerant RILs.

Proteomics

Proteins in the AWF were separated in a gel-free matrix using native Iso Electric Focussing – Free Flow Electrophoresis (nIEF-FFE) covering a pH gradient from 1.0 – 9.8. H_2O_2-consuming guiaicol peroxidase activity was determined in the FFE fractions. In both RILs (Mn-sensitive R20 and Mn-tolerant R120) and Mn treatments (optimum, excess) peroxidase activity mainly clustered around fraction 50 (pH 5.2). For R20, another activity peak around fraction 85 (pH 7.4) was observed which was only marginally present in R120. The proteins responsible for the observed peroxidase activities were identified using shotgun tandem-MS. Among all identified peroxidases in the main peroxidase peak, OS04T0677200-01 was standing out in both RILS, but particularly in R20 by its high number of spectral counts, which were additionally strongly enhanced at high Mn supply. Additionally, a range of enzymes with peroxidase function only found in low levels in R20 at high Mn supply may as well contribute towards the higher peroxidase activity in this line in the presence of excess Mn. For the second peroxidase peak mostly 3 peroxidases were responsible in R20. Additionally, in this pool five peroxidases could only be identified in R20 under Mn stress.

Transcriptomics

For the study of transcriptional changes in response to high Mn supply RNA extracted from leaves of the RILs R20 and R120 the Agilent Single Color Microarray was used. The RILs showed major basic differences in the overall expression of genes. Under high Mn supply only the Mn-sensitive R20 reacted with major changes in gene expression particularly in the phenylpropanoid pathway as demonstrated by a MapMan overwiew. A higher number of genes coding for apoplastic peroxidases expressed basically and in response to high Mn supply in the Mn-sensitive R 20 was confirmed.

CONCLUSION

In conclusion, the results suggest that leaf apoplastic peroxidases and their activities are involved in the control of the reactions leading to Mn oxidation and related Mn toxicity also in rice.

REFERENCES

Blamey, F. P. C., Joyce, D. C., Edwards, D. G., Asher, C. J. 1986. Role of trichomes in sunflower tolerance to manganese toxicity. Plant and Soil 91. 171-180.

Delhaize, E., Kataoka, T., Hebb, D.M, White, R.G., Ryan, P.R. 2003. Genes encoding proteins of the cation diffusion facilitator family that confer manganese tolerance. The Plant Cell 15. 1131–1142.

Fecht-Christoffers, M.M., Fuehrs, H., Braun, H.-P., Horst, W.J. 2006. The role of hydrogen peroxide-producing and hydrogen peroxide-consuming peroxidases in the leaf apoplast of cowpea in manganese tolerance. Plant Physiology 140. 1451–1463.

Fernando, D. R., Marshall, A., Baker, A. J. M., Mizuno, T. 2013. Microbeam methodologies as powerfull tools in manganese hyperaccumulation research. Present status and future directions. Frontiers in Plant Science, Article 319.

Fuehrs, H., Behrens, C., Gallien, S., Heintz, D., Van Dorsselaer, A., Braun, H.-P., Horst, W. J. 2010. Physiological and proteomic characterization of manganese sensitivity and tolerance in rice (*Oryza sativa*) in comparison with barley (*Hordeum vulgare*). Annals of Botany 105. 1129–1140.

Horst, W. J., Fecht, M., Naumann, A., Wissemeier, A. H., Maier, P. 1999. Physiology of manganese toxicity and tolerance in *Vigna unguiculata* (L.) Walp. Journal of Plant Nutrition and Soil Science 162. 263–274.

Lidon, F. C. 2001. Tolerance of rice to excess manganese in the early stages of vegetative growth. Characterization of manganese accumulation. Journal of Plant Physiology 158. 1341–1348.

Peiter, E., Montanini, B., Gobert, A., Pedas, P., Husted, S., Maathuis, F. J. M. Blaudez, D., Chalot, M., Sanders, D. 2007. A secretory pathway-localized cation diffusion facilitator confers plant manganese tolerance. Proceedings of the National Academy of Sciences 104. 8532–8537.

Sasaki, A., Yamaji, N., Xia, J., Ma, J.F. 2011. OsYSL6 is involved in the detoxification of excess manganese in rice. Plant Physiology 157. 1832–1840.

Wang, Y. X., Wu, P., Wu, Y. R., Yan, X. L. 2002. Molecular marker analysis of manganese toxicity tolerance in rice under greenhouse conditions. Plant and Soil 238. 227–233.

Enhancement of plants' coadaptation to multiple stresses in acid soils

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Abstract
Acid soils result in a variety of factors limiting plant growth. Plants have to overcome these multiple stresses rather than only one stress for their better growth. Here, we reviewed how the interactions among several stresses in soils and plants confer the enhancement of plants' coadaptation to multiple acid soil stresses. In soils, the alleviation of one stress can benefit from the coexistence of other stresses. In plants, the adaptive ability of plants to several stresses is often inter-related. In terms of plants and soils, two strategies are proposed to enhance plants' coadaptation to multiple acid soil stresses.

INTRODUCTION
Acid soils cover about 30% of the ice-free land in the world (von Uexküll and Mutert, 1995), and 22.7% of the total land area in China (Shen, 2008). Acid soils are distributed mainly in the humid northern temperate and tropical areas (von Uexküll and Mutert, 1995). Acid soils have a huge production potential where a lack of water or heat is rarely limiting for plant growth. Despite the favorable climatic conditions, the productivity of acid soils is limited by soil acidity and a variety of factors induced by low pH (Zhao et al., 2014). Although many researches have been focused on a single acid soil stress, only a single stress rarely occurs in acid soils. The improvement of productivity of acid soils relies on the alleviation of multiple stresses rather than only one stress. Here, we reviewed several aspects of plants' coadaptation to multiple stresses and thereby two strategies are proposed to improve plant growth in acid soil.

Several aspects of plants' coadaptation to multiple stresses in acid soils
Poor plant growth in acid soils results from a combination of factors including deficiencies in nitrogen (N), phosphorus (P), calcium, and magnesium, and toxicities of proton, aluminum (Al), manganese, and iron (Zhao et al., 2014). We noted that the alleviation of one limiting factor often benefit from the existence of another one in acid soils. For instance, Al alleviates manganese toxicity to rice (Wang et al., 2015); the iron plaques of root surface protect rice against Al toxicity (Chen et al., 2006); proton can alleviate Al toxicity (Kinraide et al., 1992; Zhao et al., 2009); ammonium, the dominating inorganic N in acid soils, alleviates Al toxicity, while Al enhances the ammonium utilization of plants (Zhao et al., 2009; Chen et al., 2010). These results collectively indicate that one stress can "help" plant to tolerate another stress in acid soils. P, calcium, and magnesium can also reduce Al toxicity to plants (Zhao et al., 2014).
Moreover, plants are often "versatile" to adapt to multiple acid soil stresses. For instance, Al-tolerant plants are characterized by high P contents or efficient P uptake and translocation to shoots; correspondingly, P-efficient genotypes were more Al-tolerant than P-inefficient genotypes (Zhang et al., 2011; Chen et al., 2011; Zhao et al., 2014). Al-tolerant plants preferentially used ammonium while Al-sensitive ones used nitrate (Zhao et al., 2013, 2014). Thus, three genetic traits (Al tolerance, inorganic N utilization, P efficiency) may be linked in plants under acid conditions.

Strategies for enhancing plants' coadaptation to multiple stresses in acid soils
In order to grow well in acid soils, plants have to deal with various stresses rather than single stress occurring in acid soils. Based on the above knowledge, two strategies are proposed to enhance the capability of plants' coadaptation to multiple stresses coexisting in acid soils (Figure 1).

(1) Grow the plant varieties with the strong capability to adapt to multiple stresses in acid soils. As some genetic traits, at least Al tolerance, inorganic N utilization, P efficiency, are linked in plants, "versatile" plant varieties can be obtained through traditional breeding or molecular genetic modification.

(2) Use the interactions among multiple stresses in acid soils to ameliorate their phytotoxicity. There are two situations: deficient elements and toxic elements. The supplement of deficient elements not only supplies nutrients for plants but also can reduce toxicity of too much elements. The alleviation of toxic elements relies on the interactions between these toxic elements.

The above strategies consider what plants are grown and how chemical supplements are applied in acid soils. Strategy (1) and (2) are not separate and should be applied combined. This combined knowledge will hopefully permit significant increases in plant production in acid soils.

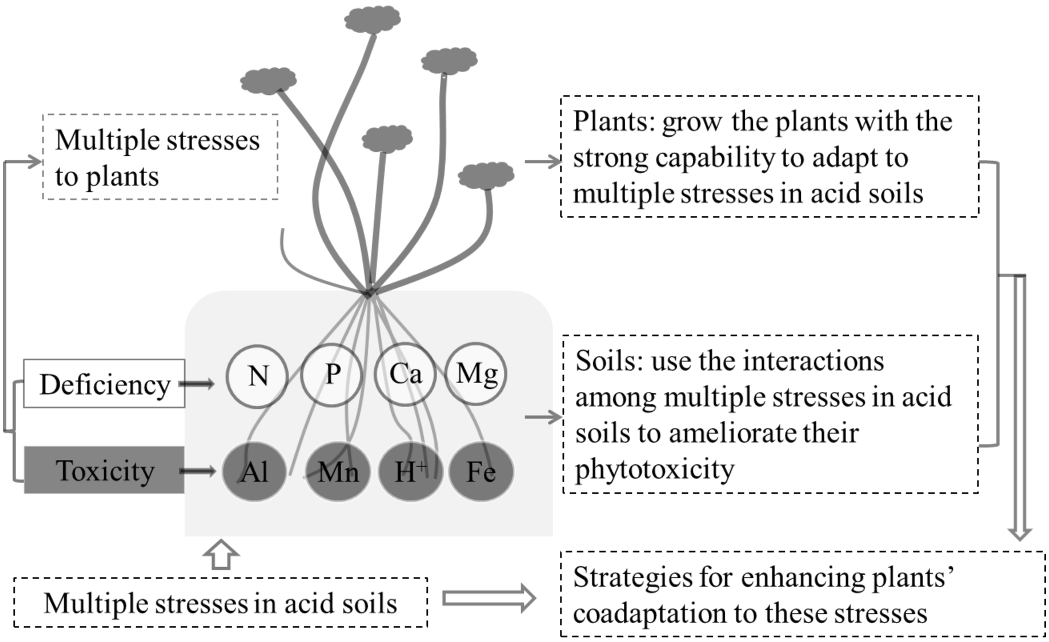

Figure 1. Strategies for enhancing plants' coadaptation to multiple stresses in acid soils

CONCLUDING REMARKS

This mini-review explores the possible strategies for enhancing plants' coadaptation to multiple stresses at the same time in acid soils. They seem feasible, but there are still huge challenges because of the diversity and complexity of soil types, plant species, and stress varieties. As most crop plants are more sensitive to acid soils than native wild plants, much attention should be paid to the enhancement of crop plants' coadaptation capability. Even though we may not completely stop the accelerating soil acidification, it is possible to make a productive acid soil for food security using our knowledge.

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REFERENCES

Chen RF, Shen RF, Gu P, Dong XY, Du CW, Ma JF. 2006. Response of rice (*Oryza sativa*) with root surface iron plaque under aluminium stress. Annals of Botany, 98: 389-395.

Chen RF, Zhang FL, Zhang QM, Sun QB, Dong XY, Shen RF. 2011. Aluminium–phosphorus interactions in plants growing on acid soils – does phosphorus always alleviate aluminium toxicity? Journal of the Science of Food and Agriculture, 92: 995–1000.

Chen ZC, Zhao XQ, Shen RF. 2010. The alleviating effect of ammonium on aluminum toxicity in *Lespedeza bicolor* results in decreased aluminum-induced malate secretion from roots compared with nitrate. Plant and Soil, 337: 389–398.

Kinraide TB, Ryan PR, Kochian LV. 1992. Interactive effects of Al^{3+}, H^+, and other cations on root elongation considered in terms of cell-surface electrical potential. Plant Physiology, 99: 1461–1468.

Shen RF. 2008. The behavior of aluminum in soil-plant systems and its adaptive mechanisms for plants. Beijing: Science Press. (In Chinese)

von Uexküll HR, Mutert E. 1995. Global extent, development and economic impact of acid soils. Plant and Soil, 171: 1–15.

Wang W, Zhao XQ, Hu ZM, Shao JF, Che J, Chen RF, Dong XY, Shen RF. 2015. Aluminum alleviates manganese toxicity to rice by decreasing root symplastic Mn uptake and reducing availability to shoots of Mn stored in roots. Annals of Botany (Accept).

Zhang QM, Chen RF, Zhao XQ, Dong XY, Shen RF. 2011. Effects of phosphorus on growth of rice seedlings under Al stress and relationship between Al tolerance and P efficiency of the rice. Acta Pedologica Sinica, 48: 103–111. (In Chinese)

Zhao XQ, Chen RF, Shen RF. 2014. Co-adaptation of plants to multiple stresses in acidic soils. Soil Science, 179: 503-513.

Zhao XQ, Guo SW, Shinmachi F, Sunairi M, Noguchi A, Hasegawa I, Shen RF. 2013. Aluminum tolerance in rice is antagonistic with nitrate preference and synergistic with ammonium preference. Annals of Botany, 111: 69–77.

Zhao XQ, Shen RF, Sun QB. 2009. Ammonium under solution culture alleviates aluminum toxicity in rice and reduces aluminum accumulation in roots compared with nitrate. Plant and Soil, 315: 107-121.

Significant role of the plasma membrane lipid bilayers in aluminum tolerance of plants

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Abstract
We propose a new aluminum (Al) tolerance mechanism: plasma membrane (PM) lipid bilayers barrier mechanism. Lowering the phospholipid content and the increasing the sterol content of PMs from root tips produces PMs both with less surface negativity and less permeability to Al ions. This mechanism is a common strategy for Al tolerance in several plant species. *PAH* encoding phosphatidate phosphohydrolase and *HMG* encoding 3-hydroxy-3-methylglutaryl CoA reductase are speculated to be promising candidate genes to generate new Al-tolerant plants.

The Direct Evidence of the Significant Role of the Less Permeability for the PM Lipid Bilayers in Al Tolerance
PM Lipid Bilayers Barrier Mechanism as a Significant and Ubiquitous Al Exclusion Mechanism
Al stress responses have been considered to be controlled by two mechanisms: exclusion mechanism and internal tolerance mechanism. Two groups (Kochian and Taylor) have published their PM-lipid related research work, however, further detailed research has not been reported until now. Although the PM lipid bilayers barrier mechanism has not been regarded as a major contribution factor to Al resistance (Yermiyahu et al., 1997), remarkable progress on this mechanism has been accomplished (Khan et al., 2009; Kobayashi et al., 2013; Maejima et al., 2014; Wagatsuma et al., 2015; Additional unpublished related data). Thus, we believe this is an excellent chance to review the studies related to this mechanism.

PM permeability as an indicator for Al sensitivity
In hypotonic medium in the presence of Al ions, only the PMs of the protoplasts from the Al-sensitive pea cultivar were permeabilized considerably in spite of no permeabilization of the PMs from the Al-tolerant cultivar (Ishikawa et al., 2001): permeabilization is speculated to be the result of the enlargement of Al-bound PM.

Development of new simple technique for the isolation of the non-contaminated PM
We developed a technique for PM isolation without any chance of the contamination from other organelle membranes as an alternative to the laborious two-polymer phase partitioning method that was commonly applied, as follows: 1) separation of protoplasts from 1-cm root-tip portions by enzymatic digestion; 2) attachment of the purified protoplasts to glass plates coated with positively charged polylysine; 3) preparation of PM ghosts by successive burst of the attached protoplasts using three separate solutions with slow stirring for 60 s (Wagatsuma et al., 2005b). The PMs were confirmed to be devoid of other organelle membranes. PMs were solubilized, and the isolated lipid fraction was dried and finally solubilized with dodecane.

The direct evidence of the significant role of the less permeability for the PM lipid bilayers in Al tolerance
We established a system to examine lipid permeability using synthesized nylon-2,8 ultrathin and porous capsules trapped previously with methylene blue (MB) solution and coated thereafter with the PM lipid isolated from the root tips (Wagatsuma et al., 2005a). Permeability of the PM lipid was significantly greater in the Al-sensitive triticale line (Wagatsuma et al., 2005a) and the Al-sensitive maize cultivar (unpublished data). This was the first direct evidence showing the primary and early role of the PM lipid in Al tolerance without involvement of organic acid anion (OA) or other protein-related exclusion mechanisms.

PM Surface Negativity, Al Tolerance, and the Related Molecular Background
Relationship between the PM surface negativity and Al tolerance
Higher average zeta potentials of the protoplasts isolated from 0-0.5 cm tip portion of roots were observed in the Al-tolerant plant species compared with the Al-sensitive species (Wagatsuma and Akiba, 1989). The basic MB dye was adsorbed strongly by the PMs of root cells in the tip portions of Al-sensitive plant species, and a simple and rapid technique to discriminate Al-tolerant protoplasts was developed (Wagatsuma et al., 1991). A technique for the collection of Al-tolerant plant cells was also developed: equal volume of freshly prepared, positively charged silica microbeads (PCSMs) and purified protoplasts derived from the root tips were mixed and then

centrifuged on a discontinuous Ficoll gradient. Intact protoplasts from the Al-tolerant plant were recovered mostly in the bottom fraction (Wagatsuma et al., 1995). The largest size of the aggregates of the PCSMs-protoplasts from Al-tolerant plant precipitated based on their relatively low surface negativity.

Higher sterols(S) and lower phospholipids(PL)/sterols ratio in the root-tip cells of the Al-tolerant plants
Al-tolerant rice cultivar had a lower PL/S ratio than the sensitive cultivar, suggesting that the PMs of the tolerant cultivar were less negatively charged and less permeabilized than the PMs of the sensitive cultivar (Khan et al., 2009). Phosphorus (P) deficient rice seedling showed enhanced Al tolerance accompanied by the decrease in Al accumulation in the roots mainly ascribed to the lower PL in the roots (Maejima et al., 2014). The *pah1pah2* (encoding phosphatidate phosphohydrolase) double mutant of Arabidopsis showed enhanced Al sensitivity under low-P conditions where greater levels of negatively charged PL occurred in the PM. The resultant increased PM surface negativity compared with wild-type plants increased $\{Al^{3+}\}_{PM}$ and Al uptake in the roots (Kobayashi et al., 2013). Compared with Al-tolerant pea genotypes, the Al-sensitive genotype accumulated more Al in the root-tips, had less intact PM, and showed a lower expression level of *PsCYP51*, which encodes obtusifoliol-14α-demethylase (OBT 14DM) (a key sterol biosynthetic enzyme, Benveniste 1986) (Wagatsuma et al., 2015). A transgenic Arabidopsis line with knocked-down *AtCYP51* expression showed an Al-sensitive phenotype with greater reduction of root elongation and PM permeability, greater accumulation of Al in the root-tip portion, and lower S and higher PL/S ratios than the wild-type. Uniconazole-P, an inhibitor of OBT 14DM, suppressed the Al tolerance of the Al-tolerant genotypes of maize, sorghum, rice, wheat, and triticale. These results suggest that the higher S, regulated by *CYP51*, with concomitant lower PL in the root tips results in the lower negativity of the PM, lower PM permeability to Al ions, and the final higher Al tolerance.

Significant role of the 3-hydroxy-3-methylglutaryl CoA reductase (HMG) in Al tolerance
Transgenic Arabidopsis plants that overexpressed *CYP51* showed no change in S (Kim et al., 2005). On the other hand, transgenic tobacco lines with the increased expression levels of *HMG* mRNA had 6-fold increased levels of S compared with wild-type lines (Schaller et al., 1995). *HMG* encodes 3-hydroxy-3-methylglutaryl CoA reductase (HMGR), a key enzyme in the upstream biosynthesis of S. *HMG1* expression in Arabidopsis was increased under dark (Enjuto et al., 1994; Learned 1996). The Al tolerance of *japonica*-type rice cultivars with less Al tolerances was increased to the highest level under dark conditions (unpublished data). The S and the expression of *HMG2/HMG3* were increased, and the Al accumulation was decreased in the root-tips. These results may provide promising insights for breeding new Al-tolerant plants in the future.

REFERENCES

Enjuto, M., Balcells, L., Campos, N. et al. 1994. *Arabidopsis thaliana* contains two differentially expressed 3-hydroxy-3-methylglutaryl-CoA reductase genes, which encode microsomal forms of the enzyme. Proc Natl Acad Sci USA. 91. 927-931.

Ishikawa, S., Wagatsuma, T., Takano, T. et al. 2001. The plasma membrane intactness of root-tip cells is a primary factor for Al-tolerance in cultivars of five plant species. Soil Sci Plant Nutri. 47. 489-501.

Khan, MSH., Tawaraya, K., Sekimoto, H. et al. 2009. Relative abundance of Δ^5-sterols in plasma membrane lipids of root-tip cells correlates with aluminum tolerance of rice. Physiol Plant. 135. 73-83.

Kim, HB., Schaller, H., Goh, C-H. et al. 2005. *Arabidopsis cyp51* mutant shows postembryonic seedling lethality associated with lack of membrane integrity. Plant Physiol. 138. 2033-2047.

Kobayashi, Y., Kobayashi, Y., Watanabe, T. et al. 2013. Molecular and physiological analysis of Al^{3+} and H^+ rhizotoxicities at moderately acidic conditions. Plant Physiol. 163. 180-192.

Maejima, E., Watanabe, T., Osaki, M. et al. 2014. Phosphorus deficiency enhances aluminum tolerance of rice (*Oryza sativa*) by changing the physicochemical characteristics of root plasma membranes and cell walls. J Plant Physiol. 171. 9-15.

Schaller, H., Grausem, B., Benveniste, P. et al. 1995. Expression of the *Hevea brasiliensis* (H. B. K.) Müll. Arg. 3-hydroxy-3-methylglutaryl-Coenzyme A reductase 1 in tobacco results in sterol overproduction. Plant Physiol. 109. 761-770.

Wagatsuma, T., Akiba, R. 1989. Low surface negativity of root protoplasts from aluminum-tolerant plant species. Soil Sci Plant Nutr. 35. 443-452.

Wagatsuma, T., Ishikawa, S., Uemura, M. et al. 2005a. Plasma membrane lipids are the powerful components for early stage aluminum tolerance in triticale. Soil Sci Plant Nutr. 51. 701-704.

Wagatsuma, T., Jujo, K., Ishikawa, S. et al. 1995. aluminum-tolerant protoplasts from roots can be collected with positively charged silica microbeads: a method based on differences in surface negativity. Plant Cell Physiol. 36. 1493-1502.

Wagatsuma, T., Khan, MSH. et al. 2015. Higher sterol content regulated by *CYP51* with concomitant lower phospholipid content in membranes is a common strategy for aluminum tolerance in several plant species. J Exp Bot. 66. 907-918

Wagatsuma, T., Nakashima,T., Tawaraya, K. 1991. Identification of aluminum-tolerant protoplasts I the original root ptotoplast population from several plant species differing in aluminum tolerance. In: Plant-Soil Interactions at Low pH. Wright RJ et al. (eds). Kluwer Academic Publishers. Dordrecht. The Netherlands. 789-793.

Wagatsuma, T., Uemura, M., Mitsuhashi, W. et al. 2005b. A new and simple technique for the isolation of plasma membrane lipids from root tips. Soil Sci Plant Nutr. 51. 135-139.

Yermiyahu, U., Brauer, DK., Kinraide, TB. 1997. Sorption of aluminum to plasma membrane vesicles isolated from roots of Scout 66 and Atlas 66 cultivars of wheat. Plant Physiol. 115. 1119-1125.

Arbuscular mycorrhizal fungi provide an alternative pathway of P uptake for Al-damaged roots

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Abstract
Mycorrhizal plants take up P not only through the root epidermis (i.e. direct pathway) but also through the mycorrhizal pathway. The mechanism underlying the enhanced acid-tolerance by arbuscular mycorrhizal (AM) fungi was investigated with respect to plant phosphate transporter genes responsible for these pathways. *Miscanthus sinensis* could not survive in association with an acid-sensitive AM fungus at pH 3.2, but survived with an acid-tolerant fungus. Although the acid-tolerant fungus did not alleviate root damages, expression analysis of the phosphate transporter genes suggests that the fungus supplied P through the mycorrhizal pathway instead of the damaged roots thus secured plant survival.

INTRODUCTION
Plants have developed diverse strategies to overcome Al^{3+} toxicity and P deficiency in acidic soil. A wide-spread strategy for Al^{3+} detoxification in plants is secretion of organic acids, including malate, citrate, and oxalate, from the roots in response to Al^{3+} (Ma et al., 2001a; Kochian et al., 2005). Modification of root architecture and root hair density for enhancing P acquisition from the soil is a typical response to P deficiency (Lynch and Brown, 2001; Ma et al., 2001b). These strategies, however, are likely to be inefficient in strongly acidic soil in which root elongation is seriously inhibited by Al^{3+}. Association with arbuscular mycorrhizal (AM) fungi is an important and effective strategy of plants for enhancing P uptake in acidic soil. The fungi associate with the majority of land plants and in the soil construct extraradical hyphal networks that can provide a large contact area with soil solution (Jakobsen et al., 1992). *Miscanthus sinensis* that is a common pioneer grass in Eastern Eurasia and Pacific Asia is a highly acid-tolerant species, but cannot establish in the soils with pH below 4 without AM fungi (Maki et al., 2008).

Although evidence that AM fungi improve plant growth in acidic soils has been obtained, the mechanism underlying has yet to be elucidated. Mycorrhizal plants have two pathways for P uptake: the direct and mycorrhizal pathways (Smith et al., 2011). In mycorrhizal roots, the high-affinity phosphate transporter gene that is expressed in root epidermis and responsible for the direct pathway is down-regulated. Instead, the mycorrhiza-specific phosphate transporter gene that is responsible for the uptake of P released from the fungi is up-regulated (Bucher, 2007; Javot et al., 2007). Given that root development is largely restricted in acidic soil, the relative importance of the mycorrhizal pathway is likely to increase with increasing in soil acidity. In the present study, we hypothesized that AM fungi would enhance acid-tolerance of the host plant via supplying P through the mycorrhizal pathway, instead of the direct pathway that is dysfunctional in strongly acidic soil, which was tested by employing the associations between *M. sinensis* and AM fungi that differ in acid-tolerance.

RESULTS AND DISCUSSION
Impact of mycorrhizal colonization on plant acid-tolerance and root damages
M. sinensis was inoculate either with the acid-sensitive fungus *Claroideoglomus etunicatum* H1-1 or the acid-tolerant fungus *Rhizophagus clarus* RF1 and grown at pH 5.2 and 3.2 in a greenhouse. At pH 5.2, both fungi, as well as P-fertilizer application, improved plant growth compared with the non-mycorrhizal plants. At pH 3.2, however, only those colonized with the acid-tolerant fungus maintained growth, and the other plants, including those received P-fertilizer, declined 10-13 weeks after sowing. Shoot dry weight was significantly correlated with shoot P concentration, but not with N concentration both at pH 5.2 and 3.2, suggesting that plant growth was limited by P availability under the experimental conditions.

Total root length, Al-deposition on root tips (hematoxylin staining), and membrane integrity of root tips (fluorescein diacetate-propidium iodide staining) were assessed to evaluate the impact of mycorrhizal colonization on root damages at both pH levels. The plants grown at pH 3.2 showed significantly shorter root length, more Al-deposition, and more severe root tip damage than those grown at pH 5.2. Neither of the two fungi reduced these root damages, implying that mycorrhizal formation has no effect on alleviation of the root damages in acidic soil, irrespective of their acid-tolerance.

Expression of phosphate transporter genes reflect the efficiency of mycorrhizal pathway

Phosphate transporter genes were identified in the transcriptome of *M. sinensis* grown with or without *R. clarus* RF1 and characterized based on their expression profiles; *MsPT2* was down-regulated by mycorrhizal colonization and is likely to be responsible for the direct pathway, and *MsPT3;1* and *MsPT3;2* were up-regulated in response to mycorrhizal colonization and are likely to be responsible for the mycorrhizal pathway. At pH 5.2, mycorrhizal colonization generally reduced *MsPT2* expression and increased *MsPT3;1* and *MsPT3;2* expressions, irrespective of fungal acid-tolerance. Whereas, at pH 3.2, *MsPT2* expression was significantly higher in the plants colonized with the acid-sensitive fungus than in those colonized with the acid-tolerant fungus, although *MsPT3;1* and *MsPT3;2* expressions were not different between the plants colonized with the two fungi. Subsequent correlation analysis of these genes revealed that *MsPT2* expression was negatively correlated with shoot P concentration, but *MsPT3;1* and *MsPT3;2* expressions were not. These observations suggest that *MsPT3;1* and *MsPT3;2* that are responsible for the mycorrhizal pathway are expressed simply in response to mycorrhizal colonization, regardless of whether P is supplied through the pathway. On the other hand, the expression of *MsPT2* that is responsible for the direct pathway is regulated by P status at the whole plant level, independent of mycorrhizal status. In this context, the lower expression of *MsPT2* in the plants colonized with the acid-tolerant fungus at pH 3.2 indirectly indicates that the mycorrhizal pathway provided by the fungus made a significant contribution to host P uptake, which consequently secured the survival of the host.

CONCLUSION

The present study demonstrated that acid-tolerant AM fungi significantly enhance acid-tolerance of the host plants beyond their adaptability to acidic soil. The dependency of *M. sinensis* on AM fungi is unlikely to be high under moderate conditions, but the plant could not survive without associating with acid-tolerant fungi in strongly acidic soils, even in the presence of P fertilizer. Acid-tolerance of AM fungi is also a critical factor that affects plant acid-tolerance. In conclusion, our study suggests that AM fungi play a deterministic role in the survival and establishment of plants in strongly acidic soil.

REFERENCES

Bucher, M. 2007. Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. New Phytologist. 173: 11-26.

Jakobsen, I., Abbott L.K., Robson, A.D. 1992. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. New Phytologist. 120: 371-379.

Javot, H., Penmetsa, R.V., Terzaghi, N., Cook, D.R., Harrison, M.J. 2007. A *Medicago truncatula* phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. Proceedings of the National Academy of Sciences of the United States of America. 104: 1720-1725.

Kochian, L.V., Pineros, M.A., Hoekenga, O.A. 2005. The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. Plant and Soil. 274: 175-195.

Lynch, J., Brown, K. 2001. Topsoil foraging–an architectural adaptation of plants to low phosphorus availability. Plant and Soil. 237: 225-237.

Ma, J.F., Ryan, P.R., Delhaize, E. 2001a. Aluminium tolerance in plants and the complexing role of organic acids. Trends in Plant Science. 6: 273-278.

Ma, Z., Bielenberg, D.G., Brown, K.M., Lynch, J.P. 2001b. Regulation of root hair density by phosphorus availability in Arabidopsis thaliana. Plant, Cell and Environment. 24: 459-467.

Maki, T., Nomachi, M., Yoshida, S., Ezawa, T. 2008. Plant symbiotic microorganisms in acid sulfate soil: significance in the growth of pioneer plants. Plant and Soil. 310: 55-65.

Smith, S.E., Jakobsen, I., Grønlund, M., Smith, F.A. 2011. Roles of arbuscular mycorrhizas in plant phosphorus nutrition: Interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiology. 156: 1050-1057.

Localization of aluminium and its toxicity induced alterations in physiological and biochemical traits in lentil

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Abstract
Aluminium tolerant and Al-sensitive genotypes were grown in a nutrient solution (pH-4.5) containing 0, 74 and 148 μM Al. Al stress induced growth inhibition and increased accumulation of Al and callose in all the genotypes. Genotypes 'L-7903' and 'L-4602' (tolerant) showed maximum relative root growth and increased secretion of organic acids, accumulated less Al and callose as compared to sensitive genotypes ('BM-4' and 'L-4147). In tolerant genotypes most of the Al was localized in the epidermal cells, while in sensitive genotypes, Al accumulation was observed in the epidermal, cortical, and even endodermal cells and entered it to outer cell layers. Such traits can be selected as desirable traits for development of Al tolerant genotypes in future plant improvement program in lentil.

INTRODUCTION

Aluminium (Al) is considered as one of the major abiotic stresses and causes 25–80% yield losses in crops grown on soils containing excessive aluminium (Singh et al., 2011). In soils, it is found in the form of insoluble aluminosilicates or oxides. When pH of the soil drops below 5.0, Al is solubilized in the form of phytotoxic Al^{3+} ions which become toxic for many crops. Symptoms of aluminium toxicity such as inhibition of root growth (Singh et al., 2012, Singh et al., 2015), accumulation of Al and callose (Singh et al., 2015), etc. have been used as an indicators of Al sensitivity in crop plants. Plants have developed various mechanisms to minimize the harmful effects of toxic Al. The most documented mechanism of Al resistance is the secretion of organic acid anion from the roots (Kochian et al., 2005). However, no information is available on accumulation of Al and callose and their localization, and secretion of organic in Al stress in lentil. Therefore, the aim of present investigation was to: i) investigates the effects of aluminium toxicity on morpho-physiological traits; ii) better understand the nature of early Al uptake (apoplasmic or symplasmic) using the morin and hematoxylin staining techniques for detection of Al localization in the roots.

MATERIALS AND METHODS

The lentil lines,'L-7903', 'L-4602', 'BM-4' and 'L-4147' were selected at seedling stage on the basis of their tolerance to Al in previous studies (Singh et al., 2012, Singh et al., 2015). Seeds of above 4 genotypes of lentil were disinfected and germinated in the template stand. Eight days old seedlings of almost similar length were placed to a plastic container in low-ionic-strength hydroponic medium as per Simon et al., 1994. The hydroponic solution was aerated and the pH of the nutrient solution was maintained at 4.5 in all the treatments (0, 74 and 148 μM $AlCl_3.6H_2O$) using 1 M HCl or 1 M KOH. Solution was replaced daily to avoid minimize changes in Al concentration and pH. Relative root length (RRL %) was calculated as formula proposed by Anas and Yoshida, 2000. The Al contents were quantified using Perkin-Elmer Atomic Absorption Spectrophotometer and expressed as mg^{-1} g^{-1} on dry weight basis. Evaluation of Al stress induced callose biosynthesis was done using aniline blue staining technique. Localization of Al in roots was visualized by staining with morin and hematoxylin. Secretion of organic acid was determined by standard protocol. All the data presented in this manuscript are the mean values of three replications. The data were statistically verified with analysis of variation (ANOVA) and the significance of difference between the means was tested using Tukey post-hoc test at the significance level $P \leq 0.05$ using SPSS v.10 for Windows (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

Higher Al concentrations in the nutrient solution caused considerable growth inhibition in terms of relative root length. Rapid inhibition of root growth is one of the most important symptoms of Al toxicity which results in stunted root growth. Such growth reduction in roots can restrict absorption of water and nutrients and ultimately causes yield reduction in crop plants in Al toxic soils (Singh et al., 2009, Singh et al., 2015). The effect of Al toxicity was more pronounced on roots as compared with shoot. L-7903' and 'L-4602' (tolerant lines) showed maximum relative root growth as compared to 'BM-4' and 'L-4147' (sensitive genotype). Significantly ($P < 0.05$) higher Al content was detected in roots of sensitive genotypes in comparison to tolerant lines, 'L-7903' and

'L-4602', which showed lower accumulation of Al. Using morin staining method, an aluminium-specific signal was observed mainly in the apical part of the roots exposed to 74 and 148 μM Al concentration for 48 h. No such signals of fluorescence of morin were detected under control conditions. At 74 and 148 μM Al, morin exhibited bright fluorescence. 'L-7903' and 'L-4602' exhibited almost similar fluorescent signal in root tips. At higher concentration (148 μM Al), intensity of fluorescence was more bright in sensitive genotypes ('BM-4' and 'L-4147') compared with tolerant lines ('L-7903 and L-4602') (Fig. 1). In transverse sections of roots of 'L-7903' and 'L-4602', showed Al concentration in the epidermal cells (outer cells) mainly with lesser concentration in inner tissues but small quantity of Al was also detected in cytoplasm. On the other hand, in sensitive genotypes ('BM-4' and 'L-4147'), Al was detected in all the tissues i.e. epidermis, cortex and vascular tissues (Fig. 2). Roots of lentil genotypes showed more callose deposition with increasing A1 concentrations from 0 to 148 μM and it was consistent with the level of Al-induced root growth reduction. Score of fluorescent signals showed increasing trend and was highest at 148 μM Al concentration. Callose staining was much less in tolerant lines ('L-7903' and 'L-4602') in comparison to sensitive genotypes ('BM-4'and 'L-4147'). Exposure to Al caused increased secretion of malate and secrete from roots in all the genotypes, but the amount of secretion were not so large in 'BM-4' and 'L-4147' (sensitive genotypes). Tolerant genotypes ('L-7903' and 'L-4602') were able to secrete more malate and secrete than sensitive ones. Understanding of these physiological and biochemical traits for Al stress tolerance in lentil will allow the design of better technique for reducing the period of developing Al stress tolerant genotypes in future breeding program.

REFERENCES

Anas and Yoshida, T. 2000. Screening of al-tolerant sorghum by hematoxylin staining and growth response. Plant Production Science, 3: 246–253.

Kochian, L.V., M.A. Pin˜ eros and Hoekenga O.A. 2005. The physiology, genetics and molecular biology of plant aluminium resistance and toxicity. Plant Soil 274: 175-195.

Simon, L., Smalley, T.J., Jones JB Jr, Lasseigne, F.T. 1994. Aluminum toxicity in tomato: part I. Growth and mineral nutrition. Journal of Plant Nutrition 17:293–306

Singh, D. Dikshit H.K and Kumar A. 2015. Aluminium tolerance in lentil with monogenic inheritance pattern. Plant Breeding 134: 105–110.

Singh, D., Dikshit, H.K., Singh, R. 2012. Variation of Aluminium tolerance in lentil. Plant Breeding 131: 751-761.

Singh, D., Pal, M, Singh, R., Singh, C.K. and Chaturvedi, A.S. 2015. Physiological and biochemical charactristics of Vigna species for Al tolerance. Acta Physiologia Plantarum, 37:87.

Singh, D., Singh, N.P., Chauhan, S.K., Singh P. 2011. Developing aluminium tolerant crop plants using biotechnological tools. Current Science 100: 1807-1813.

Singh, D., Rai, A.K., Panyang, O. 2009. Hematoxylin staining as a potential screening technique for aluminium tolerance in pea (*Pisum sativum* L.). Current Science 96: 1029-1030.

Advances in technologies for growing, imaging, and analyzing 2-d and 3-d root system architecture

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Abstract
Recent advances in imaging technology and phenotyping software have allowed for greater progress in describing root system architecture. We have designed a hydroponic growth system which retains the 3-dimensional root system architecture (RSA), while allowing for aeration, solution replenishment, and the imposition of nutrient treatments throughout the growth experiment. The simplicity of the system allows for minimal preparation and better throughput. This paper will outline some of the recent improvements and innovations which allow for greater sensitivity, higher efficiency, and growing conditions for plants that more closely mimic those found under field conditions.

INTRODUCTION

How can plants do more with less? How can they be tolerant to insufficient mineral nutrients and water? This is our research focus and we carry out research on root system architecture (RSA) as a way to discover mechanisms for success under these stress conditions. Quantification of 3-dimensional (3-D) plant root architecture is an important approach to investigating plant root growth and function in nutrient acquisition and utilization. The root system is a key organ for plants because it absorbs water and nutrients and provides stability to its aerial parts. Different root system architectures (RSA) can significantly affect many physiological functions, such as water and nutrient acquisition, carbon distribution, and the ability of the plant to adjust to abiotic stresses, such as low phosphorus and high soluble aluminum (Al^{3+}).

Additionally, research shows that certain root qualities in crop plants can improve productivity in resource-limited environments due to improved nutrient and water scavenging abilities. Identifying, evaluating, and selectively introducing both intrinsic and environmentally responsive root architectural characteristics into breeding programs may be a promising area for improving crop production on resource-limited agricultural systems. To that end, the design of a high-throughput screening system to measure RSA, both spatially and temporally, will be an effective tool to help evaluate these important parameters.

In this manuscript we report on the improvements that we have made to some of these existing technologies for describing 3-D root system architecture so that the growth and root reconstruction processes have been enhanced.

RESULTS AND MODEL DESCRIPTIONS

Growing systems

There have been a number of innovations in the way that we grow our plants for 3-D imaging. Currently, all of the plants are grown in "mesh systems" in tubs of nutrient solution in growth chambers with temperature and light controls.

These "mesh systems" consist of ABS plastic mesh discs, made with a 3-D printer, which have been arranged in a series of layers with variable spacing between successive layers, such that the plant roots can grow through the mesh in the discs and the sequential layers of mesh discs help retain root distribution and shape. A 3-D printer has allowed us to customize the shape of the mesh openings (hexagonal) and the size of the discs (diameter and thickness), according to our needs. This mesh growth system has served us well, even for large screening experiments. Our current challenge is to design even larger mesh discs for older or more elaborate root systems, using materials which can make thin, strong, growing systems.

While growing plants in hydroponics is simple, but elegant, we wanted to better simulate root growth under field conditions. In addition, the hydroponic approach limits our research into RSA and drought. Using conventional soils or artificial soil mixes was not possible since both of these could not be completely washed from the roots and they tended to discolor the roots, both of which detract from image analysis and 3-D reconstruction of RSA. We identified a soil amendment called Turface which is inexpensive, well-studied, and commercially available. Turface will not stick to the roots so that RSA imaging is optimized. Turface is a stabilized baked ceramic aggregate, formed by firing non-swelling illite clay at temperatures no less than 650◦C, with 1.0–2.0 mm particle size, (Steinberg et al., 2005). Turface is noncohesive, drains very rapidly, retains large quantity of plant-available water, and can easily be washed off the roots (Van Bavel, 1978). It is compatible with drip irrigation, with no loss of aeration when compared to commercial soil mixtures (Steinberg et al., 2005).

We have established a washing and pre-treatment regime to equilibrate the exchange sites on the Turface particles and to allow a uniform supply of nutrients to the plant. Currently, we are using the Turface with our 3-D mesh growing systems, as well as with drip systems to grow plants on a long term (seed to seed) basis. We are also looking at the hydraulic properties of Turface with the idea of creating an automated system to control "soil" water content in this medium. Feedback from scientists who have grown their plants in this medium is that RSA in Turface is analogous to the very same plants grown in the field.

Engineering and Software Improvements

In an effort to produce high throughput phenotyping via plant root imaging, a new control system is being developed using the Raspberry Pi single-board computer. In addition to being a fully-featured Linux PC, the Raspberry Pi includes a set of general purpose input/output (GPIO) pins allowing for various hardware components to be integrated directly with the computer. The goal is to produce a stand-alone product, packaged as the Raspberry Pi with the necessary software pre-installed, that can be connected to a motor and digital camera for simple image collection and 2D analysis. The 3D root reconstruction is being designed to run on a separate high performance computer. Building on top of the original 3D imaging framework, introduced in Clark et al. (2011), the credit-card sized Raspberry Pi serves as the computer for controlling the camera and turntable. The GPIO pins are connected to a motor controller for adjusting the speed and rotation of the turntable and an open-source digital camera library is used to interface with a USB-connected digital camera. New features are currently being added to the new flexible Raspberry Pi platform. The first development is to automate the camera calibration process, eliminating subjective calibration parameters, reducing the overall process time, and facilitating the repeatability of experiments. Image processing and data extraction methods based, in part, on the RootReader2D program (Clark et al., 2013) are also in development, including: image thresholding, plant root skeletonization, and trait calculation. For data preservation and versatility, raw images, experiment details, and image processing settings are being stored in hierarchical data format (HDF) files. The last update under works is a new graphical user interface (GUI) that will be common amongst the various stages of the phenotyping process (i.e., image capturing, 2D processing, and 3D reconstruction). By creating a common interface, researchers may spend more time on their intended application with less time at the controls (i.e., increasing throughput).

Additional improvements are being developed alongside the Raspberry Pi control system, including image gapfilling, root reconstruction, and data management. As the application of image-based phenotyping moves toward larger and more complex root systems, mesh systems are necessary to protect and preserve root system architecture during the imaging process; however, these mesh systems systematically introduce discontinuities in the root images along root segments during reconstruction. One proposed method for dealing with this is by implementing the valence-driven spatial median method (Wang and Chen, 2008), which, would approximate root distributions across these obstructed regions. For the root reconstruction process, improvements in data throughput include either a re-write of the software in a more efficient programming language (e.g., Python and C++) or the adoption of an existing high-efficiency 3D reconstruction algorithm (e.g., Topp et al., 2013; Zheng et al., 2011). Improvements in data management of root reconstructions are also sought after through the implementation of an octree classification scheme (Szeliski, 1993).

REFERENCES

Clark, R. T., Famoso, A. N., Zhao, K., Shaff, J. E., Craft, E. J., Bustamante, C. D., McCouch, S. R., Aneshansley, D. J., and Kochian, L. V. 2013. High-throughput two-dimensional root system phenotyping platform facilitates genetic analysis of root growth and development. Plant, Cell and Environment, 36:454–466

Clark, R. T., MacCurdy, R. B., Jung, J. K., Shaff, J. E., McCouch, S. R., Aneshansley, D. J., Kochian, L. V. 2011.Three-dimensional root phenotyping with a novel imaging and software platform. Plant Physiol. 156:455–465.

Steinberg, S. L., G. Kluitenberg, S. B. Jones, N. Diadzic, L. Reddi, M. Xiao,M. Tuller, R. Newman, D. Or, and J. I. D. Alexander (2005) Physical and hydraulic properties of baked ceramic aggregates used for plant growth medium, J. Am. Soc. Hortic. Sci., 130:767– 774.

Szeliski, R. (1993). Rapid octree construction from image sequences. *CVIP: Image Understanding*, *58*(1), 23–32.

Topp, C. N., Iyer-Pascuzzi, A. S., Anderson, J. T., Lee, C.-R., Zurek, P. R., Symonova, O., Zheng, Y., Bucksch, A., Mileyko, Y., Galkovsky, T., Moore, B. T., Harer, J., Edelsbrunner, H., Mitchell-Olds, T., Weitz, J. S., & Benfey, P. N. (2013). 3D phenotyping and quantitative trait locus mapping identify core regions of the rice genome controlling root architecture. *PNAS*, *110*(18), E1695–E1704.

Van Bavel, C., Lascano, R., Wilson, D. 1978. Water relations of fritted clays. Soil Sci Soc Amer 42:657-659

Wang, T. & Cheng, I. (2008). Generation of unit-width curve skeletons based on valence driven spatial median (VDSM). In G. Bebis et al. (Ed.), *Advances in Visual Computing* (pp. 1051–1060). Berlin: Springer.

Zheng, Y., Gu, S., Edelsbrunner, H., Tomasi, C., Benfey, P. (2011, Nov. 6–13). Detailed reconstruction of 3D plant root shape. In *Proceedings of the 2011 International Conference on Computer Vision*.

Endophyte bacteria confer habitat-fitness benefits to rice crop in aluminium toxic acid soil

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Abstract

This study assessed whether endophyte inoculation can provide the habitat-fitness benefits to rice crop in Al toxic acid *Inceptisols.* Endophyte inoculated rice plants produced significantly (P<0.001) higher root surface area, volume and length, cell membrane stability, root and shoot biomass and lower root diameter, H_2O_2 activity, Chl-b, carotenoid and malondialdehyde contents in fully expanded leaves than that of uninoculated plants. The interaction effects between endophyte inoculation and Al levels was significant (P<0.001) in terms of malondialdehyde content, carotenoids and cell membrane stability. The toxic effect of an approximate 100ppm Al could be compensated due to endophyte inoculation.

INTRODUCTION

Beneficial bacteria (endophyte and rhizobacteria) colonize surface and tissues of different plant parts in rhizosphere zone, where they live either commensally or execute beneficial functions for the host. Plant seeds usually fall on soil, a complex microbial habitat, and lay dormant waiting for environmental signals to germinate. As seeds begin to germinate, seed endophytes emerge as important founders of the seedling bacterial community. Although, there is no clear picture on overall role of the plant microbiome, there is substantial evidence that these communities are involved in enhanced nutrient acquisition, disease control, growth hormone and benefits to habitat-adaptive fitness of the host plant (Redman et al., 2011; Mitter et al., 2013; Prashar et al., 2014). So, the entophyte-host plant interactions deserve full attention for understanding the mechanisms of soil-plant-microbe continuum for futuristic crop rhizosphere engineering. To what extent such endophytic interactions at host level can influence the key aspects of plant physiology under abiotic stresses are largely unknown. Aluminium toxicity is one of the major hindrances of crop production in acid soils world-wide (Kochain et al., 2005). So, it was hypothesized that the habitat-fitness of rice crop in Al toxic acid soils is independent of endophyte colonization benefits. This study investigated the influence of endophyte inoculation on root morphology and a few important stress indicators of rice plant grown along a toxicity gradient of Al in an acid *Inceptisols*.

MATERIALS AND METHODS

In a microcosm experiment, an acid *Inceptisols* (pH 4.80; soil organic carbon 1.67%; exchangeable acidity, Al and calcium plus magnesium 0.98, 0.3 and 0.7 meq per 100g soil, respectively) was taken in 48 pots (pot diameter 30 cm and bulk density maintained at 1.36 g cc^{-1}) to impose four Al treatments (control, 100, 200 and 300 mg Al kg^{-1} soil as $AlCl_3$) under two conditions i.e. with and without endophyte inoculation. Each treatment contained 6 replicate pots. Each pot contained 4.0 kg soil and soil water was maintained at saturation. *Serratia marcescens* (strain 22WE), a root endophyte bacterium of wild rice species *Zizania latifolia*, was used to inoculate roots of *Kharif* rice (var. CAUR3) seedlings (18 days old) by root-dipping method (Thakuria et al., 2004). Endophyte treated and untreated seedlings were transplanted in pot soils according to treatments. Rice root morphology {diameter (RD), surface area (RSA), volume (RV) and length (RL)}, rhizosphere acidification, chlorophyll-a and -b, carotenoid and diamino-benzidine (DAB) staining assay in fully expanded 3rd leaves at 21 days after transplantation (DAT) and cell membrane stability (CMS) expressed as per cent cell content leakage and lipid peroxidation expressed as malondialdehyde (MDA) content in 3rd leaves, weight of dry root and shoot at 35DAT were determined. For each parameter, influence of factors (with and without endophyte or Al levels) and interactions between endophyte and Al levels were determined by 2-way factorial CRD using SPSS v. 21.0.

RESULTS AND DISCUSSION

Endophyte inoculated rice plants produced significantly (P<0.001) higher RSA, RV and RL and lower RD compared to that of uninoculated plants (Fig.1). Values of RSA, RV and RL decreased and RD increased with the increasing Al levels and these values clearly corroborated with the root architecture depicted in the figure 1. The extent of H_2O_2 production (as indicated by DAB staining) in leaves of endophyte inoculated plants was much lesser than that of uninoculated plants in all Al levels (Fig.1). There were significantly (P<0.001) lesser contents of Chl-b, carotenoids and MDA and higher CMS (in terms of % cell content leakage) in leaves of endophyte inoculated plants compared to that of uninoculated plants (Table 1). The contents of Chl-b, cariotenoids and MDA increased and CMS reduced with the increasing levels of Al (Table 1). The interaction effects between endophyte inoculation and Al levels was significant (P<0.001) in terms of the contents of MDA, carotenoids and CMS (Table 1). Endophyte inoculated rice plants produced significant higher root and shoot biomass compared to uninoculated plants in all Al levels. The root growth reduced to a great extent with the increasing Al levels. Interestingly, the endophyte inoculated plants of 100 ppm Al level and the uninoculated control plants produced comparable root biomass (Fig.1and Table1). Results from rhizosphere acidification also indicated that endophyte inoculation enhanced organic acid secretion in the rhizosphere (data not presented).

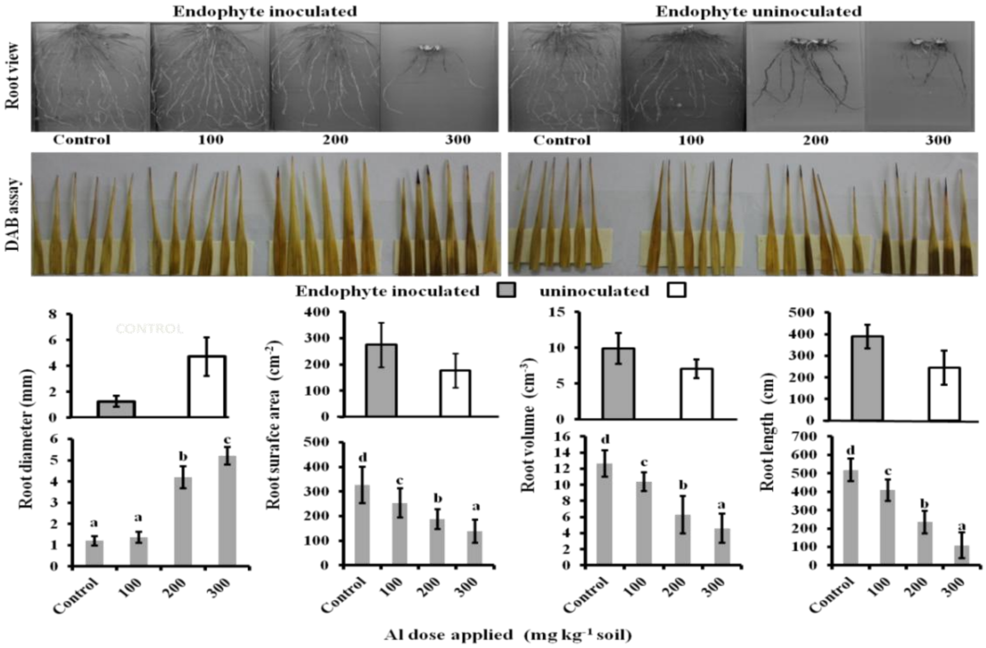

Fig.1. Influence of endophyte colonization and Al levels on root morphological features and H_2O_2 activity in fully expanded 3rd leaf tips determined by DAB assay at 21 DAT of *Kharif* rice grown in an acid *Inceptisols*.

Table 1. Effect of endophyte inoculation and Al levels on leaf pigmentation, plant stress, and root and shoot biomass in rice

Treatment	Al levels	Chl-a	Chl-b	CMS	MDA	Caro	DRW	DSW
Endophyte inoculated	Control	1.51±0.04	0.31±0.04	3.84±0.19	20.7±2.9	0.08±0.003	0.42±0.03	1.47±0.14
	100	1.47±0.01	0.36±0.02	4.37±0.23	27.5±1.7	0.09±0.005	0.32±0.02	1.28±0.03
	200	1.30±0.03	0.42±0.02	5.58±0.20	33.2±2.6	0.12±0.008	0.28±0.01	0.83±0.14
	300	1.26±0.02	0.48±0.03	5.73±0.18	34.6±1.9	0.15±0.014	0.24±0.04	0.78±0.04
Endophyte uninoculated	Control	1.39±0.04	0.39±0.02	5.64±0.23	25.9±2.4	0.10±0.007	0.34±0.01	1.27±0.07
	100	1.31±0.03	0.42±0.02	6.80±0.19	33.4±2.5	0.11±0.005	0.28±0.01	1.03±0.08
	200	1.26±0.06	0.52±0.03	7.04±0.52	37.8±2.6	0.14±0.014	0.21±0.03	0.75±0.06
	300	1.08±0.17	0.62±0.02	8.54±0.46	50.3±4.5	0.14±0.007	0.16±0.01	0.54±0.02
Level of significance								
Endophyte		***	***	***	***	***	***	***
Al level (mg kg^{-1} soil)		**	***	***	***	***	**	**
Endophyte x Al (mg kg^{-1} soil)		ns	ns	**	**	**	ns	ns

Values are means ± SD; Each mean represents average of 5 replicate analyses. Level of significance was determined by performing Univariate 2-Factorial Completely Randomized Design within the General Linear Model using SPSS v.21.
Chl-a, Chl-b: Chlorophyll-a and -b contents in the flag leaf (mg g-1 fresh wt.); CMS: cell membrane stability in the flag leaf (expressed as % cell content leakage); Caro: Carotenoid content in the flag leaf (μg g^{-1} fresh wt.); lipid peroxidation expressed as μM MDA g^{-1} leaf tissue; DRW: dry root weight (g plant^{-1}); DSW: dry shoot weight (g plant^{-1}).

CONCLUSION

This study clearly demonstrates that inoculation of efficient endophytes could exhibit fitness benefits to the host plant against Al toxicity as evident from improved root architecture, lowering stress impact (enhanced CMS and reduced MDA as well as H_2O_2) and incremental increase in shoot and root biomass in endophyte inoculated plants. Previously, Naorem et al. (2015) reported that the endophyte strain 22WE possess the ability to colonize rice roots, dissolve insoluble inorganic phosphate {169.5 P_i μg ml^{-1}h^{-1} in $Ca_3(PO_4)_2$ amended Pikovskaya's broth (pH 6.8)} and organic phosphate {624 P_i μg ml^{-1}h^{-1} in Na-phytate amended Pikovskaya's broth (pH4.6)}, produce indole-acetic-acid like substances (59 μg ml^{-1} h^{-1}) and ACC deaminase activity (5.2 μg ml^{-1} h^{-1}), which are widely recognized plant growth promoting (PGP) mechanisms of beneficial microbes. These possible PGP mechanisms probably helped the endophyte inoculated rice plant to support better root and shoot development, enhanced P_i uptake, and thereby reducing stress factors on the plant under Al toxic acid soil. Further, the root and shoot biomass data indicated that the toxic effect of an approximate 100ppm Al could be compensated due to endophyte inoculation.

REFERENCES

Kochian L.V., Piñeros M.A., Hoekenga O.A. (2005) The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. *Plant Soil*, 274(1-2):175-195.

Mitter B., Brader G., Afzal M., Compant S., Naveed M., Trognitz F., Sessitsch A. (2013) Advances in elucidating beneficial interactions between plants, soil and bacteria. *Adv. Agron.*, 121:381-445.

Naorem A. (2015) Development of multifaceted plant growth promoting microbial consortium for acid soils. A Master Thesis submitted to the College of PG Studies, Central Agricultural University, Umiam, Meghalaya.

Prashar P., Kapoor N., Sachdeva S. (2013) Rhizosphere: its structure, bacterial diversity and significance. Reviews in Environ. Sci. BioTechnol., 1705: 1–15.

Redman R.S., Kim Y.O., Woodwar C.J.D.A., Greer C., Espino L., Doty S.L., Rodriguez R.J. (2011) Increased Fitness of Rice Plants to Abiotic Stress via Habitat Adapted Symbiosis: A Strategy for Mitigating Impacts of Climate Change. PLoS ONE:e14823.

Thakuria D., Talukdar N.C., Goswami C., Hazarika S., Boro R.C. and Khan M.R. (2004) Characterization and screening of bacteria from rhizosphere of rice grown in acidic soils of Assam. *Curr.Sci.*, 86(7): 978-985.

The role of phytohormones in mediating Al-induced root-growth inhibition in the apical root zones

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Abstract
Aluminium (Al) toxicity is the most important soil constraint for plant growth and development in acid soils. It is a matter of debate whether the primary lesions of Al toxicity are apoplastic or symplastic, while the most recent studies have revealed that the local accumulation of auxin in the most Al-sensitive root zone of the root apex is a major factor leading to Al-induced root-growth inhibition, and this process is regulated by ethylene. Evidence suggests that the auxin effect is mediated mainly via modification of cell wall (CW) structural properties. A further in-depth characterization of the phytohormones-Al signaling interaction in the TZ and thus root-growth inhibition under Al stress is urgently required to better understand the phytohormone-mediated signalling network leading to Al-induced inhibition of root growth.

INTRODUCTION
Soil acidity with pH ≤ 5.5 is one of the most important factors limiting crop production worldwide in acid soils. When the soil pH drops below 5, Al^{3+} is solubilized into the soil solution and become a major constraint for plant growth and development in acidic soils (Kinraide et al., 1992). The inhibition of root elongation has been widely used as a bioassay for Al toxicity (Delhaize and Ryan, 1995) and the root apex transition zone (TZ) is the perception site of Al toxicity has been firstly shown in maize (Sivaguru and Horst, 1998). However, until now how external Al interacts with the root apex TZ to inhibit root growth is unclear. The root apex TZ is a critical site for the perception and response to both endogenous phytohormones and environmental cues (Baluška et al., 2010) and phytohormones have been shown to be the key players in root growth, development, and the regulation of root-growth plasticity in response to various stress conditions including Al. Here we summarize the current understanding of the role of phytohormones in Al-induced inhibition of root growth of plants.

Auxin accumulation in the TZ and inhibition of auxin transport into the EZ is involved in the inhibition of root growth
Auxin is a prime regulator of root cell-division, elongation and differentiation as well as, overall root growth. High concentrations of auxin, however, inhibit the elongation of certain cell types. Auxin signaling within the root-apex TZ is sensitive not only to developmental signals but also to environmental cues (Baluška et al., 2010), including Al (Sivaguru and Horst 1998). Several studies have demonstrated that Al may interact with auxin signaling pathways, leading to alterations of auxin accumulation and distribution in roots (Kollmeier et al., 2000; Doncheva et al., 2005; Shen et al., 2008). In maize, the blockage of auxin polar transport and thus auxin signaling from the distal part of TZ (DTZ) to the EZ could be the primary cause of Al-induced root-growth inhibition (Kollmeier et al., 2000), and the inhibition of auxin transport from DTZ to distal part of EZ plays a role in Al-induced alteration of root-cell patterning (Doncheva et al., 2005). In Arabidopsis, Shen et al. (2008) have found that Al inhibited root to shoot auxin transport and thus root growth mainly through the blockage of the transport of PIN2 vesicles from plasma membrane to endosomes. However, the recent study by Yang et al. (2014) suggests that the auxin transporter PIN1 rather than PIN2 is involved in the modulation of Al-induced inhibition of root growth. Especially the *TAA1*-regulated local auxin biosynthesis in the TZ is responsible for the Al-induced root-growth. However, other genes belonging to the YUC family could also contribute to Al-induced local auxin biosynthesis, since the mutant line of *YUC1D* also showed reduced Al toxicity (Yang et al. 2014).

Cell-wall modification is a downstream response to Al stress and contributes to the auxin-mediated root-growth inhibition
The rapid enlargement of cells requires wall loosening, which involves modification of the molecular interactions within the CW network, resulting in the relaxation of wall tension. The involvement of auxin in the CW loosening or expansion via affecting the function of several CW proteins has been emphasized (Perrot-Rechenmann, 2010). The transcriptomic analysis presented by Yang et al. (2014) revealed that many of the differentially transcribed genes associated with CW modification were regulated by the transcription factors ARF10 and ARF16, suggesting that the auxin-regulated Al-induced inhibition of root growth arises from auxin signalling–regulated modification of CW structure and/or structural components. However, the study by Zhu et al. (2013) revealed that auxin enhances Al toxicity via an alteration of *ALUMINUM-SENSITIVE1*–mediated Al distribution in the symplast. In spite of this, the recent study by Wu et al. (2014) in rice indicates that overexpression of *OsPIN2* alleviates the Al-induced cell rigidity in the root apex by modulating PIN2-based

auxin transport, IAA efflux and CW acidification. The reduction of Al accmulation mainly in the CW of the *OsPIN2* overexpression line further supports the hypothesis that the CW modification is probably a downstream response to Al exposure and contributes to the auxin-mediated root-growth inhibition by Al stress.

Interplay of auxin with other phytohormones in the TZ mediates the Al-induced root-growth inhibition

The hormonal cross-talks such as auxin-ethylene, auxin-cytokinin (CK), mediate both developmental cues and diverse environmental signals to control root development or growth responses (Pacifici et al., 2015). Ethylene can regulate auxin biosynthesis via mediating the expression of TAA1/TARs proteins and basipetal auxin transport toward the elongation zone, and thus causes root-growth inhibition (Ruzicka et al., 2007; Stepanova et al., 2008). CK induces transcription factor SHORT HYPOCOTYL2 (SHY2), a member of the auxin-repressor Aux/IAA family, in TZ via a direct activation of transcription by type-B ARRs (Schaller et al., 2015). The role of ethylene and CK in the Al-induced inhibition of root growth has been reported (Massot et al., 2002; Sun et al., 2010). The TAA1-mediated local biosynthesis and thus root-growth inhibition in response to Al stress is dependent on ethylene signaling in Arabidopsis (Yang et al., 2014). And also the critical role of CK especially its interplay with ethylene and auxin signaling in the root apex TZ in the Al-induced root-growth inhibition has been well clarified by our previous results (Yang et al., unpublished data).

CONCLUSION

The phytohormones are key players for the Al-induced inhibition of root growth. Understanding the molecular regulation of phytohormones especially their interplay in Al-induced root-growth inhibition will provide novel insights into how hormone signaling regulates root-growth plasticity in response to environmental cues. In addition, there is increasing evidence that inhibition of root growth is induced by Al directly and indirectly through interaction with CW structure and assembly mediated by phytohormones. An in-depth molecular characterization of hormone signaling regulating root-growth plasticity via modification of CW properties in response to Al stress is urgently required and may represent a prerequisite for an improved understanding of general mechanisms of plant adaptation to a changing environment.

REFERENCES

Baluška, F., Mancuso, S., Volkmann, D., Barlow, P.W., 2010. Root apex transition zone: A signalling-response nexus in the root. Trends in Plant Science. 15:402-408.

Delhaize, E., Ryan, P.R., 1995. Aluminum toxicity and tolerance in plants. Plant Physiology. 107:315-321.

Doncheva, S., Amenós, M., Poschenrieder, C., Barceló, J., 2005. Root cell patterning: a primary target for aluminium toxicity in maize. Journal of Experimental Botany. 56:1213-1220.

Kinraide, T.B., Ryan, P.R., Kochian, L.V., 1992. Interactive effects of Al^{3+}, H^+, and other cations on root elongation considered in terms of cell-surface electrical potential. Plant Physiology. 99:1461-1468.

Kollmeier, M., Felle, H.H., Horst, W.J., 2000. Genotypical differences in aluminum resistance of maize are expressed in the distal part of the transition zone. Is reduced basipetal auxin flow involved in inhibition of root elongation by aluminum? Plant Physiology. 122:945-956.

Massot, N., Poschenrieder, Ch., Barceló, J., 1994. Aluminium-induced increase of zeatin riboside and dihydrozeatin riboside in *Phaseolus vulgaris* L. cultivars. Journal of Plant Nutrition. 17:225-265.

Pacifici, E., Polverari, L., Sabatini, S., 2015. Plant hormone cross-talk: the pivot of root growth. Journal of Experimental Botany. 66(4):1113-1121.

Perrot-Rechenmann, C., 2010. Cellular responses to auxin: division versus expansion. Cold Spring Harbor Perspectives in Biology. 2:a001446

Ruzicka, K., Ljung, K., Vanneste, S., Podhorská, R., Beeckman, T., Friml, J., and Benková, E., 2007. Ethylene regulates root growth through effects on auxin biosynthesis and transport-dependent auxin distribution. The Plant Cell 19:2197-2212.

Schaller, G.E., Bishopp, A., Kieber, J.J., 2015. The yin-yang of hormones: cytokinin and auxin interactions in plant development. The Plant Cell .27:44-63.

Shen H, Hou NY, Schlicht M, Wan Y.L., Mancuso S, Baluska F., 2008. Aluminium toxicity targets PIN2 in Arabidopsis root apices: Effects on PIN2 endocytosis, vesicular recycling, and polar auxin transport. Chinese Science Bulletin. 53:2480-2487.

Sivaguru M., Horst W.J., 1998. The distal part of the transition zone is the most aluminum-sensitive apical root zone of maize. Plant Physiol 116:155-163.

Stepanova, A.N., Robertson-Hoyt, J., Yun, J., Benavente, L.M., Xie, D.Y., Dole_zal, K., Schlereth, A., Jürgens, G., and Alonso, J.M. 2008. TAA1-mediated auxin biosynthesis is essential for hormone crosstalk and plant development. Cell. 133:177-191.

Sun, P., Tian, Q.Y., Chen, J., and Zhang, W.H., 2010. Aluminium induced inhibition of root elongation in Arabidopsis is mediated by ethylene and auxin. Journal of Experimental Botany. 61:347-356.

Wu, D., Shen, H., Yokawa, K., Baluška, F., 2014. Alleviation of aluminium-induced cell rigidity by overexpression of *OsPIN2* in rice roots. Journal of Experimental Botany. 65:5305-5315.

Yang, Z.B., Geng, X., He, C., Zhang, F., Wang, R., Horst, W.J., Ding, Z., 2014. TAA1-regulated local auxin biosynthesis in the root-apex transition zone mediates the aluminum-induced inhibition of root growth in Arabidopsis. The Plant Cell 26:2889-2904.

Zhu, X.F., Lei, G.J., Wang, Z.W., Shi, Y.Z., Braam, J., Li, G.X., Zheng, S.J., 2013. Coordination between apoplastic and symplastic detoxification confers plant aluminum resistance. Plant Physiology. 162:1947-1955.

Physiological variations in aluminum accumulation in different aluminum-accumulator plant species

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Abstract
Aluminum (Al) accumulators are widely distributed in acid soils, particularly in the humid tropics. In addition to their geographical distribution, Al accumulators show a wide phylogenetic distribution. In this study, we discuss the physiological characteristics of Al accumulation among different Al accumulators in various vascular plant taxa. The results of chemical and histochemical analyses suggest that variations in the Al detoxification and accumulation mechanisms in the leaves of Al accumulators seem to be small among vascular plants.

INTRODUCTION
It is well known that high aluminum (Al) ion concentration in soil solutions is the most important factor for restricting plant growth in acid soils. Most plant species have developed the ability to exclude Al from roots (e.g., by organic acid exudation) as a method of adapting to acid soils (Kochian et al., 2015). In contrast, some species accumulate high concentrations of Al in their shoots. These species, called "Al accumulators," are widely distributed throughout the plant kingdom. In angiosperms, Al accumulators are mostly found among woody eudicots, particularly among Ericales, Gentianales, and Myrtales (Jansen et al., 2004). Al accumulators are also found among pteridophytes (Olivares et al., 2009). In this study we investigated the physiological characteristics of Al accumulation among different Al accumulators belonging to various vascular plant taxa.

MATERIALS AND METHODS
Mature leaves of various Al accumulators were sampled at Hokkaido University Botanic Garden or from plants purchased from a garden shop. The samples were lyophilized, ground, and extracted with deionized water and 0.02 M HCl to estimate the water-soluble and total organic acid concentrations, respectively. Organic acid concentrations in the extracts were determined by capillary electrophoresis (Quanta 4000CE, Waters, Milford, MA, USA), as described by Watanabe et al. (1998). In the water extract, Al concentration was also determined by inductively coupled plasma-mass spectroscopy (ICP-MS). To determine the total Al concentration, the samples were digested with HNO_3 and H_2O_2, and Al concentration in the digested solution was determined by ICP-MS. Furthermore, liquid-state ^{27}Al nuclear magnetic resonance (NMR) was used to determine the Al form in the leaves of several Al accumulators. Leaves of several Al accumulators were also sampled to determine Al localization. After washing with deionized water, the leaves were sectioned with a cryomicrotome (CM-3050S, Leica Biosystems, Germany) and then stained with pyrocatechol violet (PCV) on glass slides (Watanabe et al., 1998) or morin (Zheng et al., 2005).

RESULTS AND DISCUSSION
Correlation analysis between Al and organic acid concentrations in leaves and ^{27}Al NMR analysis indicated that oxalate is a common ligand for a part of the Al in the leaves of various Al accumulators, including pteridophytes, whereas a high concentration of non-chelated monomeric Al was also detected in the leaves of woody eudicots. These results imply that internal Al detoxification mechanisms by making Al-oxalate complexes in plant tissue may be very primitive and common in the plant kingdom. Moreover, microscopic analysis revealed that Al localization in pteridophyte leaves was similar to that in angiosperms, although the latter showed higher accumulation of Al in epidermal cells.

REFERENCES
Jansen S., Watanabe T., Dessein S., Robbrecht E., Smets E. 2004. The evolution of aluminium accumulation in angiosperms. In: AR Hemsley, I Poole (eds) The Evolution of Plant Physiology From whole plant to ecosystems. Elsevier Academic Press, London.

Kochian L.V., Piñeros M.A., Liu J., Magalhaes J.V. 2015. Plant adaptation to acid soils: The molecular basis for crop aluminum resistance. Ann. Rev. Plant Biol. 66: 571-598.

Olivares E., Peña E., Marcano E., Mostacero J., Aguiar G., Benítez M., Rengifo E. 2009. Aluminum accumulation and its relationship with mineral plant nutrients in 12 pteridophytes from Venezuela. Environ. Exp. Bot. 65: 132-141.

Watanabe T., Osaki M., Yoshihara T., Tadano T. 1998. Distribution and chemical speciation of aluminum in the Al accumulator plant, Melastoma malabathricum L. Plant Soil 201: 165-173.

Zheng S.J., Yang J.L., He Y.F., Yu X.H., Zhang L., You J.F., Shen R.F., Matsumoto H. 2005. Immobilization of aluminum with phosphorus in roots is associated with high aluminum resistance in buckwheat. Plant Physiol. 138: 297-303.

Isolation and characterization of a rice mutant tolerant to aluminum

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Abstract
Rice is the high Al-tolerance plant species among small-grain cereals, but its molecular mechanisms of Al tolerance remain to be demonstrated. We adopted a forward genetic screen strategy to uncover the Al-tolerance mechanisms in rice. Here we report a rice mutant *tat1* (Tolerance to Al Toxicity 1) that was more tolerant to Al than the wild type at all Al concentrations tested. Physiological experiments revealed that the increased tolerance to Al in the mutant was caused by reduced Al accumulation in the cell wall. We mapped the responsible gene to the long arm of rice chromosome 6.

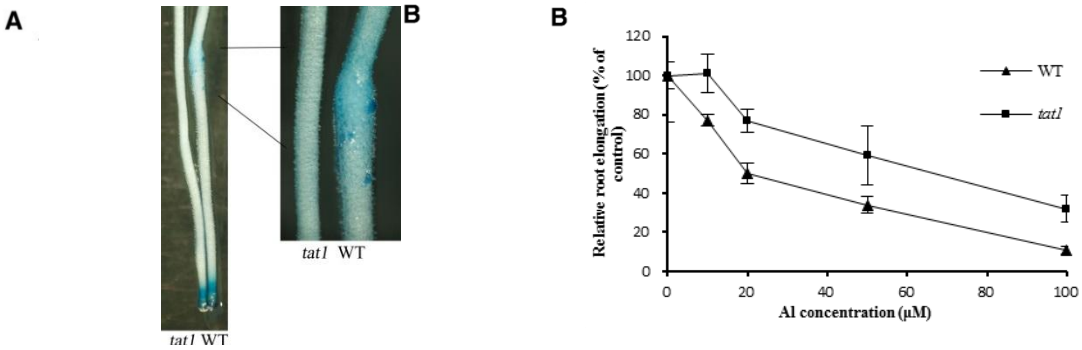
INTRODUCTION
Aluminum (Al) toxicity is a major limiting factor for crop production on acid soils (von Uexkull and Mutert 1995). To survive on acid soils with Al-toxic environments, plants have evolved various mechanisms to detoxify Al. As the most Al-tolerance crop among the small grain cereals, rice employs different Al-tolerance mechanisms from the well-known organic acid-based mechanism to detoxify Al (Ma et al. 2002); nevertheless, the underlying molecular mechanisms of high Al tolerance in rice remain to be demonstrated. Through a forward genetic screen followed by a reverse genetic approach, Ma's group recently identified a series of genes involved in the Al tolerance in rice (Ma et al. 2014), which greatly increases our understanding of Al-tolerance mechanisms in rice. In this study, we adopted a forward genetic screen strategy to further examine the high Al-tolerance mechanisms in rice. Through this screen, we identified a rice mutant with increased tolerance to Al toxicity. We then characterized the mutant at physiological and genetic aspects.

MATERIALS AND METHODS
For mutant screening, an EMS mutagenesis library of an Al-sensitive indica cultivar (cv. Kasalath) was constructed. Root elongation or Al-induced root swelling were used as an index for the initial screening of Al-tolerance mutants in the M2 library. The candidate Al-tolerance mutants were then confirmed in M3 generation based on the increased relative root elongation after exposure to Al stress.
For a dose–response experiment, 5-day-old seedlings of WT and *tat1* mutant were exposed to a 0.5 mM $CaCl_2$ solution containing 0, 10, 20, 50 or 100 μM Al at pH 4.5 for 48 h. Root length was measured before and after treatment. Relative root elongation (RRE) was used to evaluate the sensitivity to Al.To determine Al content in roots, seedlings of the WT and *tat1* mutant were exposed to a 0.5 mM $CaCl_2$ solution containing 20 μM Al for 6 h. Root tips (0-1 cm) and base roots (1-2 cm) were excised and digested with 2N HNO_3 for measurement of total Al content. Al accumulation in cell wall and cell sap was determined according to a previous method described in Xia et al. (2010). The Al concentration in the solution was measured by ICP-MS.
For mapping of *tat1* mutant gene, F2 plants from a cross between *tat1* and another cultivar HJX74 were constructed. Bulked segregant analysis by pooling equal amounts of DNA from 10 Al-tolerance or 10 Al-intolerance plants was used to determine the chromosome location of the mutant gene and then linkage analysis was carried out to map the gene by using 50 F_2 mutants and six polymorphic markers.

RESULTS AND DISCUSSION
After an initial screening of 1600 M2 lines followed by a confirmatory test using M3 generation, a rice mutant named *tat1* (Tolerance to Al Toxicity 1) with increased Al tolerance was obtained. Exposure of wild type (WT) to 20 μM Al caused root swelling, but in the mutant the Al stress did not induce the swelling (Fig. 1A). Evans blue staining showed that Al induced more cell death in WT than in the mutant (Fig. 1A). These results were consistent with the notion that *tat1* mutant was more tolerant to Al than WT. To further compare the Al tolerance phenotype of *tat1* mutant with WT, we exposed roots of WT and the mutant to a series of Al concentrations. Although root elongation of the mutant was slower than that of the wild-type in the absence of Al (WT, 60 ± 4 mm vs *tat1*, 22 ± 5 mm), the mutant showed much higher tolerance to Al than WT at all Al concentrations tested (Fig. 1B).
To examine the mechanism of Al tolerance in *tat1* mutant, we compared Al accumulation pattern between WT and the mutant. Results showed that while total Al content in basal roots of *tat1* was similar to that of WT, the mutant accumulated significantly less Al than WT in root tips (Fig. 2A), suggesting that reduced Al accumulation in the root tips was the cause of increased Al tolerance in the mutant. We further fractionated the root segments into cell wall and cell sap to investigate which component accumulated less Al in the mutant. Results showed that Al content in the cell wall of the root tips was much lower in *tat1* than in the wild type (Fig. 2B), whereas in

the cell sap the Al accumulation was similar between WT and the mutant (Fig. 2C). In the basal roots, the Al accumulation in both the cell wall and cell sap of the mutant did not differ from that of the WT. These results suggest that the cell wall of *tat1* mutant root tips had reduced capability to bind Al, causing the mutant more tolerant to Al.

Fig. 1 Effect of Al on root morphology and elongation of WT and *tat1* mutant. **(A)** Root morphology of WT and the mutant after 20μM Al treatment and Evans blue staining; **(B)** Comparison of Al tolerance of WT and the mutant under various Al concentrations.



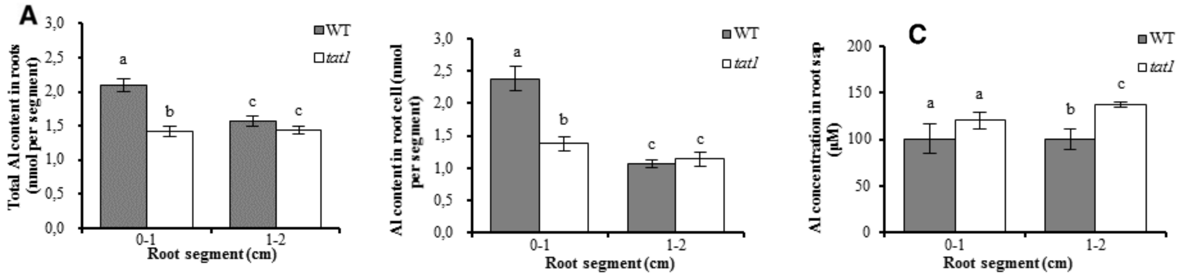


Fig. 2 Al accumulation pattern in WT and *tat1* mutant. (A) Total Al content in root tips (0-1 cm) and base roots (1-2 cm); **(B)** Al accumulation in the cell wall; **(C)** Al concentration in the cell sap

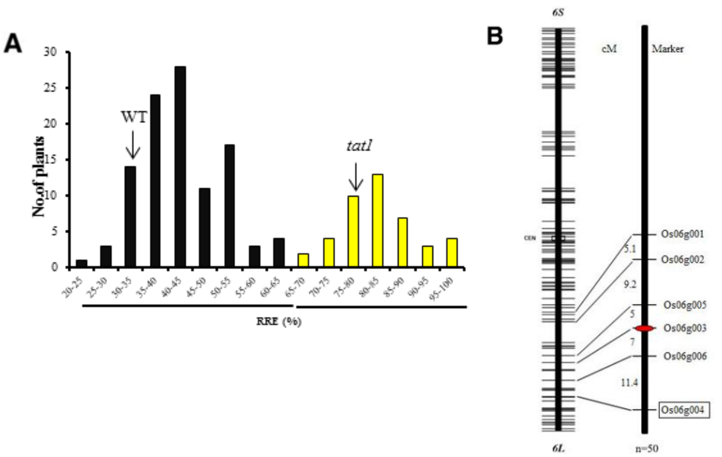


Fig. 3 Genetic analysis and molecular mapping of *tat1*.
(A) Genetic analysis of *tat1* mutant. Relative root elongation (RRE) was used to evaluate Al tolerance of F2 plants. Black and yellow columns indicate plants with normal root elongation and short root, respectively
(B) Rough mapping of *tat1* on the long arm of rice chromosome 6

Genetics analysis of *tat1* mutant was performed by using an F2 population from a cross between *tat1* and its wild type. Of 148 F2 seedlings, 43 seedlings showed Al tolerance and short root phenotype (Fig. 3A), whereas the remained 105 seedlings with normal root elongation were intolerant to Al. The segregation pattern agreed to 1:3 ratio ($\chi 2$=0.62, P$>$0.05), which suggested that Al tolerance and short root phenotype in *tat1* mutant was controlled by a same recessive gene. To map the responsible gene, Al-tolerance F2 mutants from a population of *tat1*/HJX74 was used. Bulked segregant analysis was first used to determine the chromosome location of *TAT1*. The gene was found to be linked to a polymorphic marker Os06g004 on the chromosome 6 (Fig. 3B). By using 50 F2 mutants and developing 5 additional polymorphic markers, *TAT1* gene was further mapped between the two markers Os06g005 and Os06g006 on the long arm of chromosome 6 (Fig. 3B). Further work is currently being undertaken to clone the *TAT1* gene.

REFERENCES

Ma JF, Shen R, Zhao Z, Wissuwa M, Takeuchi Y, Ebitani T and Yano M (2002) *Plant and Cell Physiology, 43*(6), 652-659.
Ma JF, Chen ZC, Shen RF (2014) *Plant Soil* 381: 1-12.
von Uexkull HR and Mutert E (1995) *Plant Soil* 171:1–15.
Xia JX, Yamaji N, Kasai T and Ma JF (2010) *Proceedings of the National Academy of Sciences* 107:18381-18385.

Characteristics of root cell components in aluminum-tolerant woody plants

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Abstract
Melastoma malabathricum and *Melaleuca cajuputi* can grow naturally in highly acidic soils. Although they are highly tolerant to aluminum (Al), the mechanisms are not yet well understood. It has been reported that root cell components such as lipids and phenolics can have some effects on Al tolerance. Therefore, in this study, the characteristics of root cell components in these two species were investigated. Compared with rice, the concentration of phenolics was higher in the root cells of these woody plants. Moreover, phenolics in *M malabathricum* are likely to play some roles in its Al tolerance and/or Al accumulation mechanisms.

INTRODUCTION
Aluminum (Al) stress is one of the most serious stresses in acidic soil. Aluminum binds tightly to the cell wall and plasma membrane of plant root cells, resulting in the inhibition of root elongation. Therefore the Al tolerance mechanisms of plants have been well studied since early days (Kochian et al., 2015). Organic acid anion secretion from root tips has been reported as an important Al tolerance mechanism in many plants. However, this mechanism cannot explain the Al tolerance of some woody plants such as *Melastoma malabathricum* and *Melaleuca cajuputi* (Watanabe and Osaki, 2002, Tahara et al., 2008). *M. malabathricum* and *M. cajuputi* grow naturally in highly acidic soil in tropical and subtropical zones and are both highly Al tolerant. Although internal detoxification mechanisms in the leaves of *M. malabathricum*, an Al accumulator, have been studied extensively (Watanabe et al., 1998), roots, which are directly exposed to soils, have received little attention. It has been suggested that the lipid composition of the plasma membrane in root cells can be responsible for Al tolerance in plants. Lower and higher proportion of phospholipids and sterols, respectively, in the plasma membrane contribute to higher Al tolerance (Khan et al., 2009, Maejima et al., 2014, Wagatsuma et al., 2014). The concentration of phenolics concentration in the root cells of woody plants has a positive correlation with Al tolerance and phenolics can form chelates with Al (Ofei-Manu et al., 2001, Tahara et al., 2013). Therefore we tried to elucidate the mechanisms underlying the high Al tolerance of woody plants by characterizing of root cell components such as lipids and phenolics.

MATERIALS AND METHODS
Melastoma malabathricum (Melastomataceae) and *Melaleuca cajuputi* (Myrtaceae) were grown in hydroponic culture and transferred to Al treatment solutions (phosphorus free nutrient solution containing 0 or 500 μM $AlCl_3$) for 1 week. Aluminum concentration in the roots and leaves and lipid composition, cell wall composition, organic acid concentration, and phenolics concentration in the roots were determined. The functions and profiles of phenolics were also analyzed. To test the chelating ability of the phenolics, the extracted phenolics and $AlCl_3$ were mixed and monomeric Al was determined by the pyrocatechol violet method (Kerven et al., 1989). In addition, the phenolics profile was determined using capillary electrophoresis according to the method described by Wang et al. (2004) with some modifications. Rice (*Oryza sativa*), an Al-tolerant herbaceous plant, was also analyzed for comparison.

RESULTS AND DISCUSSION
Compared with *M. cajuputi*, *M. malabathricum* contained 20 and 3.3 times higher concentrations of Al in the leaves and roots, respectively, and the concentrations of Al in the leaves and roots of *M. cajuputi* was as low as that in rice. The concentration of pectin, which is considered as a primary Al binding site in cell wall, was much higher in the woody plants than in rice. This probably means that cell wall components were not involved in the Al accumulation or exclusion mechanisms of the woody plants. In contrast, *M. malabathricum* and *M. cajuputi* contained lower proportion of phospholipids than rice, suggesting that lipid composition in root cells contributes to their high Al tolerance mechanisms. Moreover, both *M. malabathricum* and *M. cajuputi* contained much higher concentrations of phenolics in their roots than rice, showing their ability to form chelates with Al. The phenolics profile in the roots of *M. cajuputi* did not differ with and without Al treatments, whereas those in the roots of *M. malabathricum* differed completely. It is likely that phenolics in *M. malabathricum* affect its Al tolerance and/or Al accumulation mechanisms.

REFERENCES

Khan MSH, Tawaraya K, Sekimoto H, Koyama H, Kobayashi Y, Murayama T, Chuba M, Kambayashi M, Shiono Y, Uemura M, Ishikawa S, Wagatsuma T. 2009. Relative abundance of delta(5)-sterols in plasma membrane lipids of root-tip cells correlates with aluminum tolerance of rice. Physiologia Plantarum, 135: 73-83.

Kerven G, Edwards D, Asher C, Hallman P, Kokot S. 1989. Aluminum determination in soil solution. 2. Short-term colorimetric procedures for the measurement of inorganic monomeric aluminum in the presence of organic acid ligands. Australian Journal of Soil Research, 27: 91-102.

Kochian LV, Piñeros MA, Liu J, Magalhaes JV. 2015. Plant adaptation to acid soils: The molecular basis for crop aluminum resistance. Annual Review of Plant Biology, 66: 571-598.

Maejima E, Watanabe T, Osaki M, Wagatsuma T. 2014. Phosphorus deficiency enhances aluminum tolerance of rice (*Oryza sativa*) by changing the physicochemical characteristics of root plasma membranes and cell walls. Journal of Plant Physiology 171:9-15.

Ofei-Manu P, Wagatsuma T, Ishikawa S, Tawaraya K. 2001. The plasma membrane strength of the root-tip cells and root phenolic compounds are correlated with Al tolerance in several common woody plants. Soil Science and Plant Nutrition 47:359-75.

Tahara K, Norisada M, Yamanoshita T, Kojima K. 2008. Role of aluminum-binding ligands in aluminum resistance of *Eucalyptus camaldulensis* and *Melaleuca cajuputi*. Plant and Soil 302:175-87.

Tahara K, Hashida K, Otsuka Y, Ohara S, Kojima K, Shinohara K. 2014. Identification of a hydrolyzable tannin, oenothein B, as an aluminum-detoxifying ligand in a highly aluminum-resistant tree, *Eucalyptus camaldulensis*. Plant Physiology, 164: 683-693.

Wagatsuma T, Khan MSH, Watanabe T, Maejima E, Sekimoto H, Yokota T, Nakano T, Toyomasu T, Tawaraya K, Koyama H, Uemura M, Ishikawa S, Ikka T, Ishikawa A, Kawamura T, Murakami S, Ueki N, Umetsu A, Kannari T. 2015. Higher sterol content regulated by *CYP51* with concomitant lower phospholipid content in membranes is a common strategy for aluminium tolerance in several plant species. Journal of Experimental Botany, 66: 907-918.

Wang SF, Zhang JY, Chen XG, Hu ZD. 2004. Study of the electrophoretic behaviour of flavonoids. Chromatographia, 59: 507-511.

Watanabe T, Osaki M. 2002. Role of organic acids in aluminum accumulation and plant growth in *Melastoma malabathricum*. Tree Physiology 22: 785-792.

Watanabe T, Osaki M, Yoshihara T, Tadano T. 1998. Distribution and chemical speciation of aluminum in the Al accumulator plant, *Melastoma malabathricum* L. Plant and Soil 201: 165-173.

Comparative RNA-seq-based transcriptomics of Arabidopsis accessions in response to aluminum rhizotoxicity

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Abstract
Transcriptome of the roots in response to aluminum (Al) was compared between *Arabidopsis thaliana* accessions with contrasting Al tolerance. Transcriptome was determined by RNA-seq for three each of Al-tolerant and -sensitive accessions, and then analyzed by the bioinformatic and the reverse genetic approaches. Fifty-five of Al inducible genes determined by all accessions were highly expressed in the tolerant accessions than that of sensitive ones. Integration of the prediction of *cis*-regulatory elements and the co-expression genes analysis suggested that some transcriptions in the co-expressed gene network were activated by Al in the tolerant accessions. Finally, we identified two Al tolerance genes by the growth test using available T-DNA insertion mutants. Greater expression of these genes could explain a part of natural variation of Al tolerance between Al-tolerant and -sensitive accession groups.

INTRODUCTION
Accessions of *Arabidopsis thaliana* would be useful genetic source to study molecular mechanisms that regulate complex traits because various historical mutations are segregated among the accessions collected from different locations. Aluminum (Al) tolerance is one of the important traits of the world agriculture, and a typical complex trait, which is often regulated by transcriptome changes of Al tolerance genes such as *ALMT1* (aluminum-activated malate transporter 1), *MATE* (multidrug and toxic compound extrusion) and *ALS3* (aluminum sensitive 3) and *STOP1* (sensitive to proton rhizotoxicity 1) regulating these genes. In this study, comparative transcriptome analysis of *A. thaliana* accessions was performed by RNA-seq to identify Al tolerant related genes and their expression regulatory network. The analysis identified a couple of novel genes that control Al tolerance of Al-tolerant accessions.

MATERIALS AND METHODS
Three each of Al tolerant (Br-0, Col-0 and Shigu-2) and sensitive (Dra-2, Valsi-1 and Wei-0) accessions were used in this study. The 10-days-old seedlings grown hydroponically were exposed to 0 or 10 μM Al and then total RNA was extracted from the roots. Thirty six samples, which were obtained from three biological replications, were sequenced by illumina HiSeq2500. Filtered RNA-seq reads was mapped to reference genome sequences by Tuxedo toolkit (Bowtie2 and Tophat). To minimize the read mapping bias among accessions, reference genome sequences for each accession except Col-0 were constructed by replacing TAIR10 genome sequence using public SNP information. Differential expressed genes (DEGs) were detected using DESeq2 R-package. For the purpose of construction of co-regulated gene network, predicted CRE profile of each accession were determined by selecting 336 (1% of whole genome genes) upregulated genes by Al stress and detecting over-representative octamers in the upstream 1,000 bp region from transcription start site (TSS) [4,5] of each gene in the replaced genome.

RESULTS AND DISCUSSION
RNA-seq produced 7,371,609 single-end 100 bp reads per sample on average. The reads were aligned to each reference genome sequence with mapping ratio ranged from 92.2% (Shigu-2) to 96.3% (Col-0). *ALMT1* expression was highly expressed in Col-0, Shigu-2 and Valsi-1 accession compared to other accessions and was not completely correlated with Al tolerance, suggesting other gene expression contributed Al tolerance variation among accessions. In each accession, 2,689 to 3,770 genes were significantly induced or repressed by Al stress (Fig.1A). In addition, splicing variants caused by Al stress were detected in each accession (Fig.1B). Under these conditions, fifty-five genes were selected as candidate Al-tolerance genes by the criteria; (1) upregulated by Al in three tolerant accessions, (2) differentially expressed among six accessions under Al stress and (3) highly upregulated in tolerant accessions compared to sensitive accessions under Al stress. In the molecular function category, "transcription factor activity" and "kinase activity" GO terms were enriched in the fifty-five selected genes. Four co-expression gene clusters containing nine of the fifty-five selected genes were grouped by

ATTED-II co-expression analysis, suggesting that these genes are regulated by the same regulatory mechanisms. The CRE-gene networks constructed in each accession by integration of CRE profile and upregulated genes also suggested that some genes were co-regulated via the same regulatory elements (Fig.2). Finally, root growth of T-DNA insertion mutants of two of the candidate genes were suppressed Al tolerance. Therefore, our analysis successfully identified novel Al-tolerance genes using natural variation of Arabidopsis of Al-tolerance.

A B

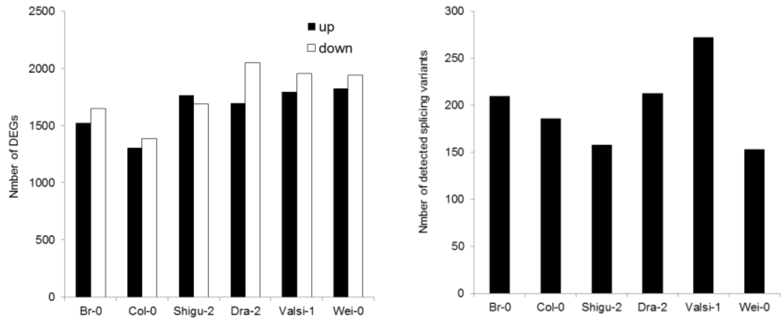

Figure 1. The number of Al-response DEGs (A) and detected splicing variants (B) out of a total of 33,602 TAIR10 genes

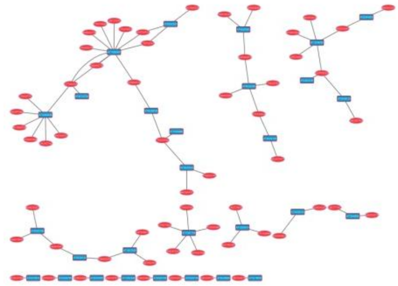

Figure 2. Predicted co-regulated gene network consisting of 55 Al-tolerance candidate genes

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REFERENCES

Hoekenga, O. et al. (2006) AtALMT1, which encodes a malate transporter, is identified as one of several genes critical for aluminum tolerance in Arabidopsis. Proc Natl Acad Sci USA **103**: 9738–9743.

Iuchi, S. et al. (2007) Zinc finger protein STOP1 is critical for proton tolerance in Arabidopsis and coregulates a key gene in aluminum tolerance. Proc Natl Acad Sci USA **104**: 9900–9905.

Tokizawa, M. et al. (2015) SENSITIVE TO PROTON RHIZOTOXICITY1, CALMODULIN BINDING TRANSCRIPTION ACTIVATOR2, and Other Transcription Factors Are Involved in *ALUMINUM-ACTIVATED MALATE TRANSPORTER1* Expression. Plant Physiology **167**: 991-1003.

Yamamoto, Y. Y. et al. (2011) Prediction of transcriptional regulatory elements for plant hormone responses based on microarray data. BMC Plant Biol **11**: 39.

Hieno, A. et al. (2014) Ppdb: Plant Promoter Database Version 3.0. Nucleic Acids Research **42**: D1188–1192.

Transcriptional regulation of Aluminum tolerance genes by STOP1 transcription factor in *Arabidopsis thaliana*

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Abstract
STOP1 (Sensitive TO Proton Rhizotoxicity 1) zinc finger transcription factor plays critical roles of *AtALMT1* (ALuminum-induced Malate Transpoter1)-medicated Al tolerance in Arabidopsis (Sawaki et al., 2009). Recently, we identified that STOP1 protein directly binds to *ALMT1* promoter (Tokizawa et al., 2015). However, mechanism of transcriptional activation of other primary target genes are poorly understood. In the present study, we combined *in silico cis*-element prediction and *in vitro* protein-*cis*-element interaction assay to study the mechanism of transcriptional activation of other STOP1's target genes. Several candidate *cis*-elements are predicted from various target genes, and direct binding of STOP1 were found with the promoters of *STOP2* and *MATE*.

INTRODUCTION
Aluminum ion (Al^{3+}) is most rhizotoxic among the ions solubilized in the acid soils. Various plant species excrete organic acids from the root tips and it protects from Al rhizotoxicity. In *Arabidopsis*, malate excretion from ALMT1 protein has a critical role in Al tolerance. Expression of *ALMT1* is regulated by zinc finger transcription factor STOP1. We recently identified seven cis elements in *ALMT1* promoter, and one of the cis elements is the target binding site of STOP1 protein (Tokizawa et al., 2015). It suggests that binding of STOP1 to promoter is one of the critical steps of transcriptional activation of STOP1's target genes, and which is similar to the transcriptional activation by ART1 (rice STOP1 orthologue) (Tsutsui et al., 2011). In this study, this model was studied with other genes regulated by STOP1 by the combination analyses of *cis*-element prediction and *in vitro* protein-DNA interaction assay.

MATERIALS AND METHODS
Plant materials
Arabidopsis accession Col-0 were obtained from Riken Bio-resource Center.

Cis-element prediction
The relative appearance ratio (RAR) of octamer was calculated by RAR based *cis*-element prediction methods (Yamamoto et al., 2011). To predict STOP1 binding cis elements, the RAR and *p*-value for each octamer was calculated using suppressible genes in the *stop1* mutant from microarray data. The calculated RAR and *p*-value was plotted to *STOP2*, *MATE* and *ALS3* promoters. The significantly overrepresented octamer (RAR>4 and *p*-value<0.05) were defined as candidate cis elements.

In vitro Protein-DNA interaction assay
Interaction of STOP1 protein and predicted cis-elements were evaluated by Alpha screen system. STOP1 protein synthesis were performed by in vitro transcription/translation system.

RESULTS AND DISCUSSION
To identify STOP1 directly regulated genes, we performed RAR based *cis*-element prediction using suppressed genes in *stop1*mutant. The calculated RAR value and *p*-value were plotted to *STOP2, MATE* and *ALS3* promoters which have been identified to be regulated by STOP1 (Sawaki et al., 2009). In the prediction, we identified four candidate *cis*-elements in *STOP2* and each two candidate *cis*-elements in *MATE* and *ALS3* promoter. In vitro binding assays, STOP1 directly binds to one of candidate *cis*-element in *STOP2* and *MATE* promoter. The STOP1 binding sequences, cis-element of *STOP2,* was similar to that of *ALMT1,* but that of *MATE* was different. On the other hands, STOP1 and 2 proteins did not binding to *ALS3* promoter. This results indicate that there is unidentified transcription factor which was regulated by STOP1 for regulation of *ALS3* expression. In our previous study, there were difference of binding capacity of each four zinc finger domains in STOP1 protein for binding to *ALMT1* promoter (Tokizawa et al., 2015). To test binding capacity for *MATE* promoter, we performed binding assay of each zinc fingers mutated STOP1 protein to STOP1 binding *cis*-

element in *MATE*. There is no significant difference of binding capacity of each four zinc fingers for binding to *MATE* promoter. This difference may induce low similarity of STOP1 binding sites in the promoter of these two genes.

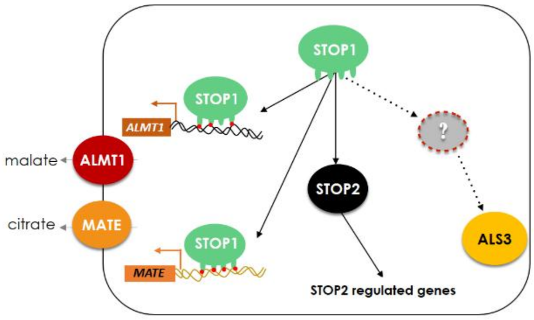

Fig. Model of STOP1 regulatory gene network in Aluminum stress

REFERENCES

Sawaki, Y. et al. (2009) STOP1 regulates multiple genes that protect arabidopsis from proton and aluminum toxicities. Plant Physiology 150: 281-294

Tokizawa, M. et al. (2015) SENSITIVE TO PROTON RHIZOTOXICITY1, CALMODULIN BINDING TRANSCRIPTION ACTIVATOR2, and Other Transcription Factors Are Involved in *ALUMINUM-ACTIVATED MALATE TRANSPORTER1* Expression. Plant Physiology 167: 991-1003.

Tsutsui, T. et al. (2011) Identification of a cis-acting element of ART1, a C2H2-type zinc-finger transcription factor for aluminum tolerance in rice. Plant Physiology 156: 925-931

Yamamoto, Y. Y. et al. (2011) Prediction of transcriptional regulatory elements for plant hormone responses based on microarray data. BMC Plant Biol 11: 39.

Antioxidant induced stimulation of maize plant growth at blockage of calcium channels

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Abstract
Effect of antioxidant ambiol and calcium channel blocker verapamil on growth of shoots and roots of maize plants has been investigated. It was demonstrated that ambiol stimulated growth of these organs. Verapamil suppressed growth of shoots and roots. Suppressing effect of verapamil was more expressed on roots. Verapamil caused a decrease in water pumping activity of roots what resulted in decrease in plant growth rate. Simultaneous addition of ambiol and verapamil to the root zone decreased the growth inhibiting effect of verapamil: in this case the size of plants exceeded the size of plants treated with verapamil. Long-term growing of plants in the presence of verapamil led to appearance of necrosis on leaves, whereas necrosis was not revealed in variants with combined addition of ambiol and verapamil to plants.

INTRODUCTION
Soil acidity limits plant growth. Aluminium toxicity is one of the major factors of inhibition of plant growth and decrease in their productivity (Marschner 1991). Aluminium may inhibit calcium uptake by blocking Ca^{2+} channels in the root cell plasma membrane what may play a role in the cellular mechanism of aluminium toxicity in plants (Huang *et al*., 1992). Calcium channels participate in signal transduction in plant cells. Blockage of calcium channels eliminates the physiological responses (White, 2000). In acid soils calcium deficiency in plants takes place (Marschner 1991). Various stress factors including mineral nutrition deficiency enhanced formation in plants of reactive oxygen species provoking destructive processes (Hendry 1994). Earlier we have demonstrated that blocking of Ca^{2+} channels by calcium channel blocker verapamil caused decrease in the growth rate of buckwheat plants and induced calcium deficiency in their tissues (Budagovskaya, 2010). Besides, blockage of calcium channels induced a decrease in turgor of buckwheat plant shoots, pointing to disturbance of water transport (Budagovskaya, 2010). The present work deals with the study of blocking effect of calcium channels by verapamil and action of antioxidant ambiol on maize plant growth and water transport in the roots.

MATERIALS AND METHODS
Maize plants (*Zea mays*, cv. Dnepropetrovskaya) were grown in specialized chamber at 27°C under 16 h illumination with metal-halogen lamps (20 w/m^2) in water culture using ½ Knopp mixture (pH 6.0). Ambiol and verapamil were introduced to the root zone on the 3rd day of plant growing. Experimental variants contained ambiol (0.001 mM) or verapamil (0.1 mM) or both compounds at the same concentrations. Both ambiol and verapamil were absent in control variants. Sizes of shoots and roots were measured and their state evaluated in the course of plant growth. All measurements were carried out in 3 biological replications. The mean values of obtained results are in the graph.Water transport intensity in plant roots was estimated according to intensity of fluid exudation (xylem exudate) from roots, measured by the method of Anderson and House (1967) with some modification. Maize seeds were germinated at 23°C in the dark on the moistened filter paper in the cuvette with glass cover. Isolated roots of 5-day-old seedlings were used in experiments. The 5 cm cut ends of the roots were placed in the bottles with tap water (control variant) and calcium channel blocker verapamil solution (experimental variant) of different concentrations (0.5, 1.0, 6.0 mM). The basal ends of the roots were inserted into glass capillaries of uniform bore. The rates of fluid exudation from the basal ends of roots were measured every 20 min during few hours at 30°C. Experiments were repeated 6-fold. The graph shows the results obtained in the first 4 h of the experiments.

RESULTS AND DISCUSSION
Fig. 1 and 2 demonstrate results of experiments on investigation of effect of antioxidant ambiol, calcium channel blocker verapamil and their combined action on growth of maize plants. Addition of ambiol to the root zone of plants stimulated growth of shoots and roots. Plants in the variant with ambiol were higher than control plants, and their roots were developed better. Verapamil caused inhibitory effect on the growth of both shoots and roots: plants in the variant with verapamil were significantly smaller than control plants, and their roots were developed worse. Plants treated both with ambiol and verapamil had higher shoots than in variant with verapamil. Ambiol partially neutralized growth inhibiting effect of verapamil. The noted regularity was preserved by increasing residence time of the root system of plants in ambiol and verapamil solutions (Fig. 2). It should be mentioned that growth of the roots of maize plants in variant with verapamil stopped earlier than the shoot growth (Fig. 2).

This could be due to addition of verapamil to the root zone. Since plant growth is connected with water exchange the experiments on investigation of effect of verapamil on water transport have been performed. As verapamil was added to the root zone it was important to evaluate the water pumping activity of roots in the presence of this compound. It was demonstrated that verapamil suppressed this process. Water pumping activity of roots decreased proportionally to increase of verapamil concentration (Fig. 3). High verapamil concentrations (6 mM) caused the change of water transport to the opposite (negative values in Fig. 3). This could point to disturbance in structural integrity of roots. Roots in this state could not keep water and it escaped into outer space.

With increasing concentration of the calcium channel blocker destructive changes in the roots amplified as evidenced by cessation of water pumping activity of roots and increased passive outflow of water from them. Verapamil-induced reduction of water pumping activity of roots is an important factor in slowing or stopping the growth of these organs and the plant as a whole.

At long-term growing of plants in the presence of verapamil necrosis was formed on the leaves, what is associated with the generation of reactive oxygen species. At simultaneous addition of verapamil and ambiol necrosis were not found. Addition of exogenous antioxidant ambiol prevented enhancement of free radical oxidation, reducing the negative effect of the calcium channel blocker on the functional activity of plants.

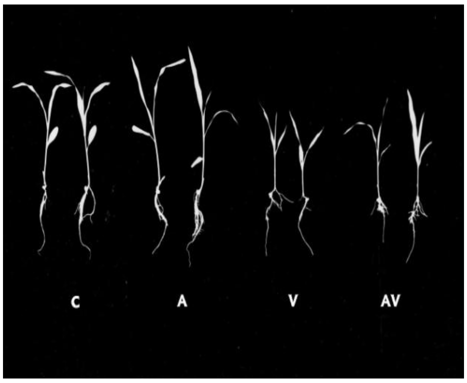

Fig. 1. Effect of ambiol (0.001 mM) and verapamil (0.1 mM) on growth of maize plants. C - control, A - with ambiol, V - with verapamil, AV - with ambiol and verapamil.

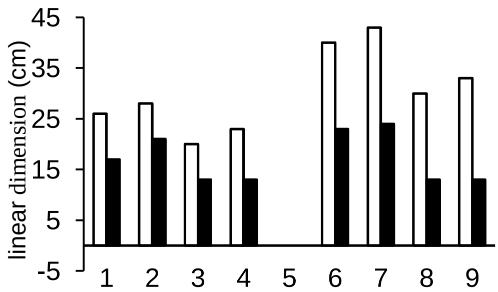

Fig. 2. Effect of ambiol (0.001 mM) and verapamil (0.1 mM) on growth of maize plant shoots and roots. □ – shoots, ■ – roots, 1-4– 10-days plants, 6-9 – 16-days plants, 1, 6 - control, 2, 7 – with ambiol, 3, 8 – with verapamil, 4, 9 – with ambiol and verapamil.

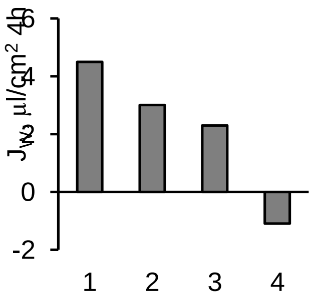

Fig. 3. Effect of verapamil on root exudation for 4 h, 1 - control, 2 – with verapamil (0.5 mM); 3 - with verapamil (1.0 mM); 4 - with verapamil (6.0 mM).

REFERENCES

Anderson W. P., House C.R., 1967. A correlation between structure and function in the root Zea mays L., J. Exp. Bot. 18, 544 - 555.

Budagovskaya N.V., 2010. Rapid response reactions of buckwheat plant shoots on changes in sodium chloride concentration at the root zone and blockage of calcium channels. The European Journal of Plant Science and Biotechnology. 4, 128-130.

Hendry J.A.F., 1994. Oxygen and environmental stress in plants: an evolutionary context. Proc. Royal Soc. Edinburgh, 102B, 155-165.

Huang J.W., Shaff J.E., Grunes D.L., Kochian L.V., 1992. Aluminium effects on calcium fluxes at the root apex of aluminium- tolerant and aluminium-sensitive wheat cultivars. Plant Physiol. 98, 230-237.

Marschner H., 1991. Adaptation of plants to acid soils. Plant Soil, 134, 1-20.

White P.J., 2000. Calcium channels in higher plants. Biochim. Biophys. Acta, 1465, 171-189.

The influence of soil acidity on aluminium and mineral nutrients concentrations in soil solution at different soil water potentials

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Abstract
Soil acidification is a natural process accelerated by agriculture and one of the most important factors limiting crop production worldwide. Concentrations of aluminum and selected mineral nutrients in solution obtained from soil at initial pH 4.5 and after liming at various soil moistures were measured trough out analysis of centrifuged soil solution. Our results showed significant gradual increase of Al and most of mineral nutrients (Ca, Mg and P) concentration with decreasing soil moisture from 14 to 6% (w/w). The results are important in evaluation and interpretation of plant response to aluminum toxicity when accompanied by water deficit.

INTRODUCTION

Approximately 30 % of the world's total land area and as much as 50 % of the world's potentially arable lands (Yang et al., 2013) are affected by soil acidification.

Many of soil properties are affected by changes of soil acidity. In very acid soils (pH<4.5) all the major plant nutrients (nitrogen, phosphorus, potassium, sulphur, calcium, manganese and also the trace element molybdenum) are unavailable, or available in insufficient quantities for most of the plants. Despite of application of fertilizers at high doses plants can show symptoms of deficiency in acid soils. High concentrations of the toxic Al3+ ion in acid soils retards root growth, restricting access to water and nutrients.

One of the most economical of ameliorating soil acidity is liming, however, the use of Al-tolerant crops and combination of both practices is often alternative.

Complex response of crops to Al toxicity is usually altered by co-occurrence of other abiotic stresses. Among these stresses soil water deficit is one of the most important. Soil water content is an important property of soils as it influences soil chemistry and water and nutrient uptake by plants. At the same time soil moisture fluctuations affects the toxicity of aluminum and availability of nutrients for plants (Anderson et al., 2013), however it is dependent on soil type and specified plant characteristic (phenotype).

It has been demonstrated that a decrease in soil water content may increase the toxic Al concentration in the soil solution thus enhancing Al toxicity in plants (Schier and McQuattie, 2000). However there are some reports with opposite conclusions (Slugenowa et al., 2011). These contradiction comes from complex plant response to both simultaneous stresses.

The aim of the research was to analyse the relations between soil water content, soil pH, concentration of Al and mineral nutrients in soil solution.

MATERIALS AND METHODS

Soil preparation

The sandy acid soil was obtained from 0-20 cm depth of arable field in located the south-eastern region of Poland (51º 26' 34.5''N 23 06' 31.4''E). The pH of the soil was 4.2 as determined with 1M KCl. Half of the collected soil was limed by addition of calcium powder to reach pH 6.5.

Extraction of soil solutions

Air dry soil was moistened to selected soil moisture levels: 6, 7, 8, 10, 12 and 14 %, (w/w). These values of soil moisture are in a range of soil water potential from pF 2.2 – 3.4 as a determined by WP4C Water Potential Meter (Decagon Devices, USA) and Tensimeter with tensiometer (Soil Measurement System, Arizona, USA). 70 g of soil at specified moisture was placed into 15 ml specially prepared weighed sample holder with water permeable bottom. Weighed sample holders were transferred into 50 ml collection cups and centrifuged at a speed 9500 rpm for 15 min using Rotanta 460RS centrifuge. After centrifugation, the extracted solution was transferred from the collection cup to small vial using pipette. Obtained soil solution samples were centrifuged again at 14000 rpm for 15 min at MiniSpin Eppendorf centrifuge to remove soil debris. The procedure was repeated for both soil pH and soil water potentials.

Soil mineral properties

The concentration of Al, Ca, Mg and P in acid and limed extracted soil solutions was measured using ICP-OS.

RESULTS AND DISCUSSION

Our results demonstrated that concentration of nutrients varies significantly with changes in soil moisture. Decreasing soil moisture content resulted in gradual increase of almost all analysed minerals concentration. Calcium was the most affected by changes in soil moisture, the increase in the concentration of these mineral with soil drying was up to 3 times in both acid and limed soil.

The decrease in soil Mg concentration was observed with rising soil moisture in both acid and limed soil extracts. However, at all of soil moisture, magnesium concentration was lower than in acid soil as a result of Mg immobilization by e.g. phosphorous ions, becoming much less exchangeable in soil above pH 6.5.

Nevertheless, the soil solution of the limed soil contains much more calcium than acid soil in each variant as a result of calcium powder application.

The concentration of total soluble aluminium in the soil solutions increased significantly under decreasing soil moisture of acid soil. However, in limed soil the concentrations of total soluble Al did not differ significantly between soil moisture variants partly as a consequence of very low concentrations.

CONCLUSION

The results indicates importance of taking into account increase of the concentration of Al with decrease of soil moisture evaluating potential toxicity of Al to crops. It is especially important in acid soils susceptible to drought (e.g. sandy soils). Soil moisture fluctuations are also accompanied by alterations in mineral nutrients availability depending also on soil pH. Result support the hypothesis that soil moisture have important effect on Aluminium toxicity. Observed changes in concentration of analysed chemicals, could become both beneficial (decreases Al availability) and harmful (decreases Mg availability) to the nutrition of plants, depending on the demand of the species.

REFERENCES

Anderson, N.P., Hart, J.M., Sullivan, D.M., Christensen, N.W., Horneck, D.A., and Pirelli, G.J. 2013. Applying Lime to Raise Soil pH for Crop Production (Western Oregon)

Schier, G.A., McQuattie, C.J., (2000.) Effect of water stress on aluminum toxicity in pitch pine seedlings. J Plant Nutr 23:637–647

Slugenova, K., Ditmarowa, L., Kurjak, D., Valka, J., 2011. Drought and aluminium as stress factors in Norway spruce
(Picea abies [L.]Karst) seedlings. Journal of Forest Science, 57(12): 547–554

Yang, Z. B., Rao,rao I. M.,, Horst, W. J., 2013. Interaction of aluminium and drought stress on root growth and crop yield on acid soils. Plant Soil (2013) 372:3–25

OsSultr3;4 expressed at the node is involved in phosphorus distribution in rice

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Abstract
Phosphorus (P) is essential for plant growth and development. Many transporters involved in P uptake have been identified, but the molecular mechanisms underlying P distribution are poorly understood. Our microarray analysis showed that *OsSultr3;4* was highly expressed in rice node. *OsSultr3;4* belongs to sulfate transporter family. Its expression was induced by P deficiency. OsSultr3;4 was localized in the xylem region of enlarged and diffuse vascular bundles of nodes. Knockout of this gene resulted in decreased P concentration in the new leaves, but increased P in the old leaves. These results showed that *OsSultr3;4* is involved in P distribution in rice.

INTRODUCTION
Plants require 14 mineral elements for their growth and development. One of them is phosphorus (P), which is often deficient in soils due to strong fixation with Al, Fe and Ca (Marschner, 2011; Raghothama, 1999). Transport of P from soils to different plant parts is mediated by different P transporters (PT). Many transporters involved in P uptake have been identified in rice, but those involved in P distribution have not been identified. In graminaceous plants including rice, nodes play an important role in the mineral distribution (Yamaji and Ma, 2014). Nodes have a complex and well-organized vascular system. Recently, several mineral transporters involved in the inter-vascular transfer from enlarged vascular bundle to diffuse vascular bundle, have been identified (Yamaji and Ma, 2014). To further understand molecular mechanisms for mineral distribution, we performed a microarray analysis with rice nodes and screened for transporters with high expression in the nodes. In this study, we functionally characterized one of these genes, *OsSultr3;4*, which is a member of sulfate transporter family. However, our detailed analysis showed that *OsSultr3;4* is involved in P distribution, rather than sulfur (S) distribution in rice.

MATERIALS AND METHODS
Wild-type rice (WT, *Oryza sativa* cv. Nipponbare) and two independent Tos17 insertion mutant lines from the Rice Mutant Panel (http://tos.nias.affrc.go.jp/) were used for phenotypic analysis. The concentration of P and S in different organs were determined by ICP-MS. To investigate the response of *OsSultr3;4* expression to P or S, 8-d-old seedlings of WT were exposed to various P concentrations (0, 10, 90, 200, and 2500 μM) or S concentrations (0, 10, 100, 500, and 2500 μM). After one week, total RNA of shoot basal region (0-1 cm from the root-shoot junction) were extracted and cDNA were synthesized for quantitative RT-PCR analysis. The cellular localization of OsSultr3;4 was examined with transgenic lines carrying the promoter (2.02 kb) of *OsSultr3;4* fused with GFP by immunostaining as described previously (Yamaji and Ma, 2007).

RESULTS
Expression level of *OsSultr3;4* in the shoot basal region was induced by P deficiency, but not by P excess (Figure 1). The expression did not respond to both deficiency and excess of S (Figure 1). Immunostaining with an antibody against GFP showed that *OsSultr3;4* promoter was mainly expressed at the xylem region of both enlarged vascular bundles and diffuse vascular bundles in node I (Figure 2). The growth and mineral concentration were compared between WT and two knockout lines. When they were grown in a nutrient solution containing 90 μM P, there was no visible difference in the growth between WT and mutants at the vegetative stage (Figure 3A). There was also no difference in the total uptake of P and S. However, the distribution of P rather than S was altered in the mutants; more P was distributed to the older leaves, but less to the young leaves. By contrast, when grown under P deficiency conditions (1 μM P) for 30 days, the newest leaf of the mutants was much smaller than that of WT and chlorosis was observed. The mutants also accumulated less P in the young leaf, but more P in the old leaves compared with WT (Figure 3B). These results indicate that OsSultr3;4 localized at the nodes is involved in P distribution by unloading P from the xylem of the enlarged vascular bundles.

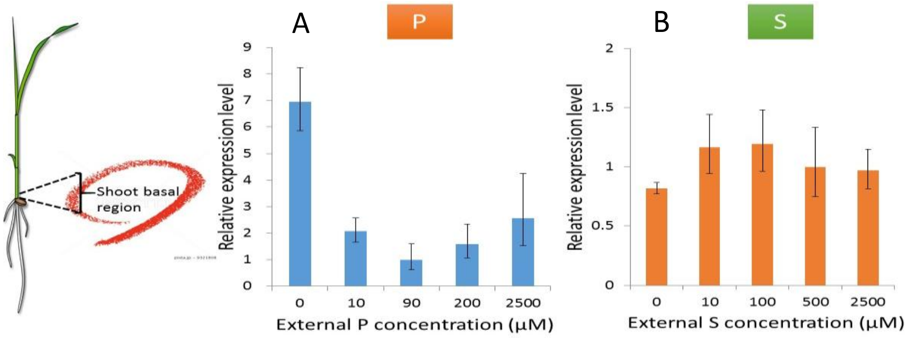

Figure 1. Expression pattern of *OsSultr3;4*
WT seedlings were cultivated in a nutrient solution with various P (A) or S (B) concentrations for 1 week. The expression level of *OsSultr3;4* in the shoot basal region was determined by quantitative RT–PCR.

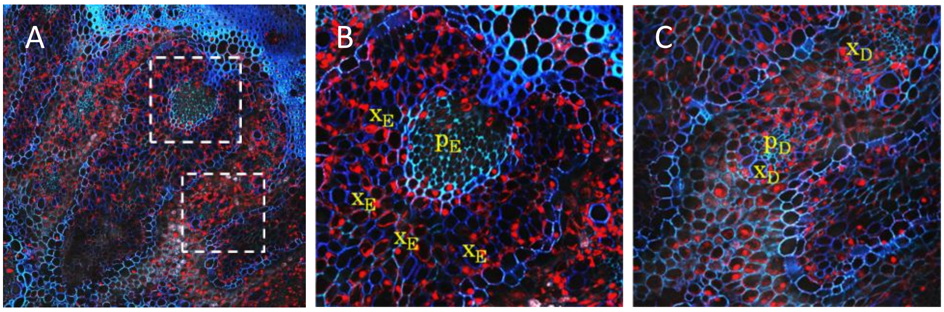

Figure 2. Tissue localization of OsSultr3;4
The node I of transgenic lines carrying *OsSultr3;4* promoter fused with GFP was used for immunostaining using GFP antibody. Red color shows signal from the antibody and cyan color shows auto-fluorescence. B-C represent EVB (B) and DVB (C), respectively, from white dotted boxes in A. The xylem and phloem regions in enlarged vascular bundles (xE and pE), and in diffuse vascular bundles (xD and pD) are shown.

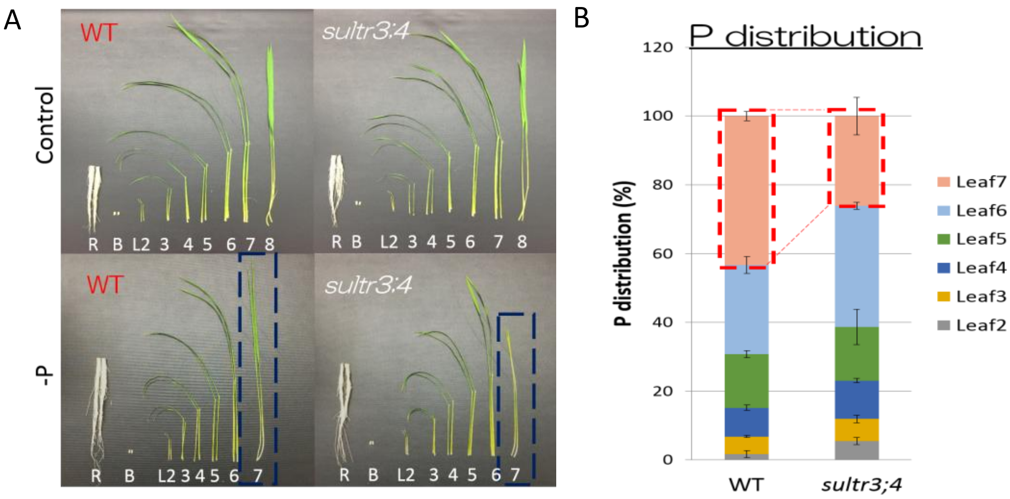

Figure 3. Phenotypical analysis of *OsSultr3;4* mutants at vegetative growth stage
(A)Phenotype of *OsSultr3;4* mutants. WT and *OsSultr3;4* knockout lines (*sultr3;4*) were grown in a nutrient solution containing 90 μM P (control) or 1 μM P (-P) for 30 days. Roots (R), shoot basal regions (B) and individual leaf (L2–7 or 8 from older to younger) are shown.
(B)P distribution in different leaves under -P condition. .

REFERENCES

Marschner, P. 2011. Mineral Nutrition of Higher Plants (3rd edition) Academic Press

Raghothama, K.G. 1999. Phosphate acquisition. Annual Review of Plant Physiology and Plant Molecular Biology 50: 665–693.

Yamaji, N. and Ma, J.F. 2014. The node, a hub for mineral nutrient distribution in graminaceous plants. Trends in Plant Science 19(9): 556–563.

Yamaji, N. and Ma, J.F. 2007. Spatial distribution and temporal variation of the rice silicon transporter Lsi1. Plant Physiology 143: 1306–1313.

Possibility of trade-off between acidic and alkaline soil adaptation in graminaceous plants

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Abstract
Among graminaceous crops, rice is highly tolerant to Al-toxicity in acidic soil but relatively sensitive to Fe-deficiency in alkaline soil. By contrast, barley is the most sensitive species to Al but highly tolerant to Fe-deficiency. We found that a transcription factor ART1-regulated Al tolerance gene network is basically conserved in most graminaceous plants but lost in barley. Moreover, some ART1-reguated genes show different response to Fe-deficiency and Al-toxicity between rice and barley. The possibility of trade-off between acid soil adaptability and alkaline soil adaptability of rice and barley is discussed.

INTRODUCTION

Adaptability of plants to different soil pH largely depends on their native habitats. However, our knowledge on molecular and genetic bases of this adaptation has only been gained from several model species and our understanding on the molecular mechanisms of soil pH adaptability of plants is still poor.

In graminaceous crops, rice (*Oryza sativa*) is the most adaptive species to acid soil, where Al toxicity is the major limiting factor for plant growth. This high adaptability to acid soil is largely attributed to an Al-responsive transcriptional regulator, ART1 (Yamaji et al., 2009). ART1 regulates multiple Al-tolerance genes including *STAR1-STAR2* (Huang et al., 2009), *Nrat1* (Xia et al., 2010), *OsALS1* (Huang et al., 2012) and *OsFRDL4* (Yokosho et al., 2011). These genes are involved in external and internal detoxification of Al at different cell sites. By contrast, barley (*Hordeum vulgare*) is one of the most sensitive species to acidic soil among graminaceous crops although there was also genotypic difference in the Al tolerance. The Al tolerance in barley is only ascribed to one gene, *HvAACT1*, which encodes a citrate transporter (Furukawa et al., 2007). It is responsible for citrate secretion from the roots to rhizosphere for Al detoxification. Interestingly, different from rice Al-tolerance genes, *HvAACT1* expression is not induced by Al. The expression level is consistently higher in the root tips of the tolerance cultivars compared with Al-sensitive cultivars. Further analysis of this gene showed that original function of HvAACT1 is to transport citrate into the root xylem for Fe translocation to shoot as Fe-citrate complex in the root mature zones (Fujii et al., 2012). In some cultivars adapted to acid soil in East Asia, a transposon-like 1-kb was inserted at the 5'-untranslated region of *HvAACT1* gene. This insertion functions a promoter, which alter both the expression location and level (Fujii et al., 2012).

However, barley is known to have high tolerance to Fe deficiency. This is achieved by secretion of phytosiderophore (mugineic acid) and efficient uptake system for Fe-phytosiderophore complex. By contrast, rice is sensitive to Fe deficiency. Although rice is also able to secrete phytosiderophore from the roots, the amount secreted is much less compared with barley. In paddy field where ferrous Fe is dominant form, rice takes up this form rather than Fe-phytosiderophore complex. However, the molecular and genetic bases of these differences in alkaline soil adaptability are poorly understood.

The objective of this study is to find a clue for different soil pH adaptability in graminaceous species. We conducted a phylogenetic analysis of ART1 homologs and compared the response of some major Al tolerance genes to Al-toxicity and Fe-deficiency between barley and rice.

MATERIALS AND METHODS

Predicted amino acid sequences of ART1 homologs were obtained from public database and used for phylogenetic analysis. Barley orthologs of rice Al tolerance genes were also obtained from public database and cloned by PCR. For gene expression analysis, roots were sampled from rice and barley grown hydroponically treated under Al-toxicity or Fe-deficiency conditions.

RESULTS AND DISCUSSION

Phylogenetic analysis of ART1 homologs

ART1 is a C2H2-type Zn finger transcription factor, which is a large gene family conserved in both plants and animals. However, rice ART1 belongs to a different branch from STOP1 (Iuchi et al., 2007), a transcription factor responsible for both Al and proton toxicity in *Arabidopsis* (Fig. 1). The downstream target genes regulated by ART1 and STOP1 are different except two common genes. Close homologs with unknown function belonging to STOP1 branch are also found in various graminaceous plants (Fig. 1). Thus, ART1 seems a transcription factor originally evolved in monocot but not in dicot. Closer homologs within ART1 branch (putative ART1 orthologs) are found in maize (*Zea mays*) and rye (*Secale cereale*), suggesting that *ART1* is at least conserved in 3 major subfamilies containing most important cereal crops (Fig. 1).

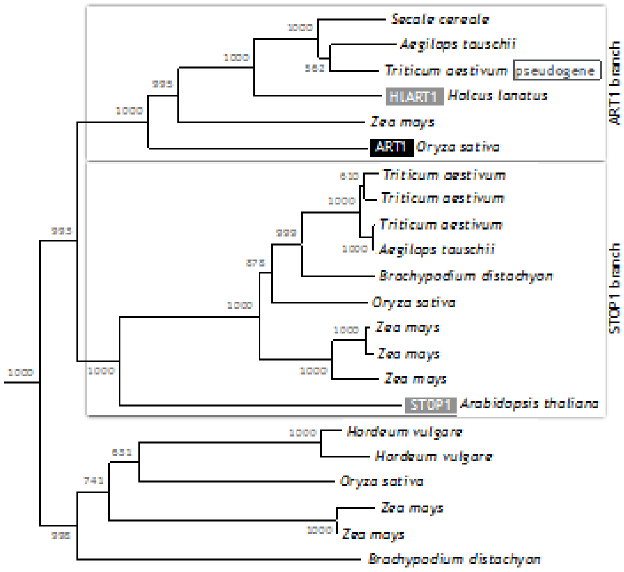

Figure 1. Phylogenetic analysis of ART1

However, *ART1* ortholog gene was not found in barley genome. This may give a good explanation for lower tolerance of barley to acid soil due to lacking of *ART1*-regulated gene networks. Interestingly, *Aegilops tauschii*, which is known as a donor of D genome of common wheat (*Triticum aestivum*), has an *ART1* ortholog, but the corresponding unique copy of *ART1* in common wheat seems to be a pseudogene. Therefore, ART1 function was lost at least two times independently in barley and wheat.

Gene expression response to Al-toxicity and Fe-deficiency in rice and barley

Orthologs of rice Al-tolerance genes; *STAR1*, *STAR2* and *ALS1* were found in barley (*HvSTAR1*, *HvSTAR2* and *HvALS1*), but those of *Nrat1* and *FRDL4* were not present in barley. Different from rice, *HvSTAR1*, *HvSTAR2* and *HvALS1* expression did not respond to Al. However, the expression of these three genes were up-regulated by Fe-deficiency, although the physiological meanings are still unknown. By contrast, in rice, these Al-tolerance genes did not respond to Fe-deficiency. In addition, *HvAACT1*, which was firstly identified as an Al-tolerance gene but originally involved in efficient Fe translocation, was also up-regulated by Fe-deficiency although its expression was not induced by Al. By contrast, *OsFRDL1*, which is a counterpart of *HvAACT1* in rice, did not respond to Fe-deficiency. Taken together, it seems that barley has lost the Al response of tolerance genes, but obtained Fe-deficiency response using the same genes although further studies are required for this speculation.

CONCLUSION

Rice and barley showed contrasting response to Al-toxicity and Fe-deficiency, probably due to loss of ART1 in barley and some differences in unidentified Fe response network. Surprisingly, some of ART1-regulated downstream genes are common among Al-tolerance genes in rice and Fe-deficiency inducible genes in barley. Physiological role of these interchanged genes remains to be investigated in future.

REFERENCES

Furukawa, J., Yamaji, N., Wang, H., Mitani, N., Murata, Y., Sato, K., Katsuhara, M., Takeda, K., Ma, J.F. 2007. An aluminum-activated citrate transporter in barley. Plant Cell Physiol. 48: 1081-1091.

Fujii, M., Yokosho, K., Yamaji, N., Saisho, D., Yamane, M., Takahashi, H., Sato, K., Nakazono, M., Ma, J.F. 2011. Acquisition of aluminium tolerance by modification of a single gene in barley. Nat. Commun. 3: 713.

Huang, C.F., Yamaji, N., Mitani, N., Yano, M., Nagamura, Y., Ma, J.F. 2009. A bacterial-type ABC transporter is involved in aluminum tolerance in rice. Plant Cell 21: 655-667.

Huang, C.F., Yamaji, N., Chen, Z., Ma, J.F. 2012. A tonoplast-localized half-size ABC transporter is required for internal detoxification of aluminum in rice. Plant J. 69: 857-867.

Iuchi, S., Koyama, H., Iuchi, A., Kobayashi, Y., Kitabayashi, S., Kobayashi, Y., Ikka, T., Hirayama, T., Shinozaki, K., Kobayashi, M. 2007. Zinc finger protein STOP1 is critical for proton tolerance in *Arabidopsis* and coregulates a key gene in aluminum tolerance. Proc. Nat. Acad. Sci. USA. 104: 9900-9905.

Xia, J., Yamaji, N., Kasai, T., Ma, J.F. 2010. Plasma membrane-localized transporter for aluminum in rice. Proc. Nat. Acad. Sci. USA. 107: 18381-18385.

Yamaji, N., Hunag, C.F., Nagao, S., Yano, M., Sato, Y., Nagamura, Y., Ma, J.F. 2009. A zinc finger transcription factor ART1 regulates multiple genes implicated in aluminum tolerance in rice. Plant Cell 21: 3339-3349.

Yokosho, K., Yamaji, N., Ueno, D., Mitani, N., Ma, J.F. 2009. OsFRDL1 is a citrate transporter required for efficient translocation of iron in rice. Plant Physiol. 149: 297-305.

Yokosho, K., Yamaji, N., Ma, J.F. 2011. An Al-inducible MATE gene is involved in external detoxification of Al in rice. Plant J. 68: 1061-1069.

Characterization of purple acid phosphatases involved in extracellular organic P utilization in *Stylosanthes*

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Abstract
In this study, two stylo (*Stylosanthes* spp.) genotypes were used to study the molecular mechanisms of exogenous organic phosphorus (P) utilization. The results showed that the P efficient genotype, TPRC2001-1, exhibited higher capability to utilize dNTP as P source, as reflected by higher dry weight and P content than the P inefficient genotype, Fine-stem. Consistently, TPRC2001-1 had higher levels of root-associated acid phosphatase (APase) activity. Subsequently, three *purple acid phosphatases* (*PAPs*) were cloned from TPRC2001-1, including *SgPAP7*, *SgPAP10* and *SgPAP26*. Expression levels of three *SgPAPs* were all up-regulated by phosphate (Pi) starvation in stylo roots. Furthermore, overexpression of three *SgPAPs* could separately result in significantly increased root-associated APase activity, and thus exogenous dNTP utilization in bean hairy roots. Taken together, our results suggested that increased *SgPAP* transcription is a critical mechanism for stylo to exploit exogenous organic P.

INTRODUCTION
Phosphorus is an essential macronutrient for plant growth and development. On soils, applied P is easily fixed into inorganic (e.g., Fe-P) and organic forms, which are not directly unavailable for plants. It has been documented that 30-80% of total soil P exists as organic P, such as phytate and nucleic acids (Richardson, 2009; Bunemann et al., 2011). Purple acid phosphatase (PAP) have been well known to play a critical role in extracellular organic P utilization, such as *PvPAP3* in bean (*Phaseolus vulgaris*), *AtPAP10* and *AtPAP26* in *Arabidopsis thaliana* (Liang et al., 2010; Wang et al., 2011; Liang et al., 2012; Robinson et al., 2012; Tian and Liao, 2015). However, little is known about molecular mechanisms of superior capability of stylo to utilize organic P, which is a major leguminous forage in the tropics and subtropics. The objectives of the study were to clone and characterize *SgPAPs*, which could regulate exogenous dNTP utilization in stylo.

RESULTS AND DISCUSSION
TPRC2001-1 exhibits superior ability to utilize exogenous dNTP
It was observed that dry weight and P content of two stylo genotypes was significantly affected by different P treatments. Phosphorus deficiency significantly inhibited stylo growth, as reflected by the lowest plant dry weight and P content (Figure 1). With application of dNTP and phosphate, plant dry weight and P content was significantly increased. Furthermore, dry weight and P content in TPRC2001-1 was significantly higher than that in Fine-stem with dNTP application, suggesting that TPRC2001-1 has higher capability to utilize dNTP as P source than Fine-stem (Figure 1).

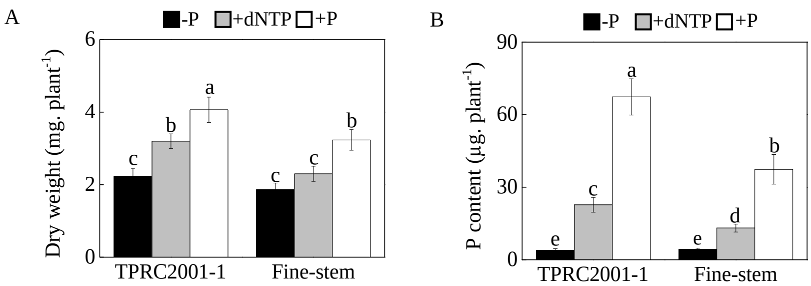

Figure 1 Dry weight (A) and P content (B) of two stylo genotypes applied with different P sources

Root-associated APase activity and SgPAP expression patterns

Root-associated APase activity was significantly increased by P deficiency in both two stylo genotypes (Figure 2A). Furthermore, root-associated APase activity in TPRC2001 was 48% higher than that in Fine-stem under P deficient conditions (Figure 2A). Subsequently, three *SgPAPs* were cloned from TPRC2001-1, including *SgPAP7*, *SgPAP10* and *SgPAP26*. Quantitative RT-PCR results showed that transcripts of *SgPAP7*, *SgPAP10* and *SgPAP26* were significantly increased by P deficiency in roots of both stylo genotypes (Figure 2B). Furthermore, expression levels of *SgPAP7* and *SgPAP10* were significantly higher in TPRC2001-1 roots than those in Fine-stem (Figure 2B).

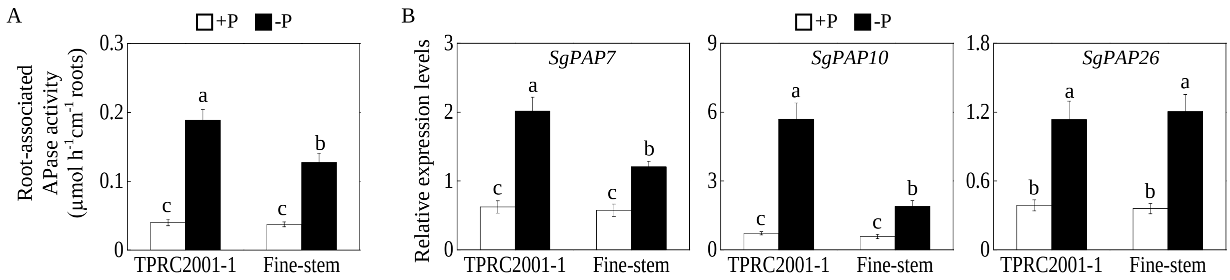

Figure 2 Root-associated APase activity (A) and transcripts of *SgPAPs* (B) in stylo roots at two P levels

SgPAP overexpression enhances extracellular dNTP utilization in bean hairy roots

To investigate the functions of *SgPAPs* in dNTP utilization, *SgPAP7*, *SgPAP10* and *SgPAP26* were separately overexpressed in the bean hairy roots. Results showed that root-associated APase activity was significantly increased in *SgPAP* overexpression lines compared to CK (Figure 3A). Furthermore, all of the transgenic bean hairy roots with *SgPAP* overexpression displayed greater capability in dNTP utilization compared to CK, as reflected by remarkable increases in dry weight and P content (Figure 3B, C). All the results together suggested that superior ability in exogenous organic P utilization in TPRC2001-1was mainly regulated by *SgPAP7* and *SgPAP10*.

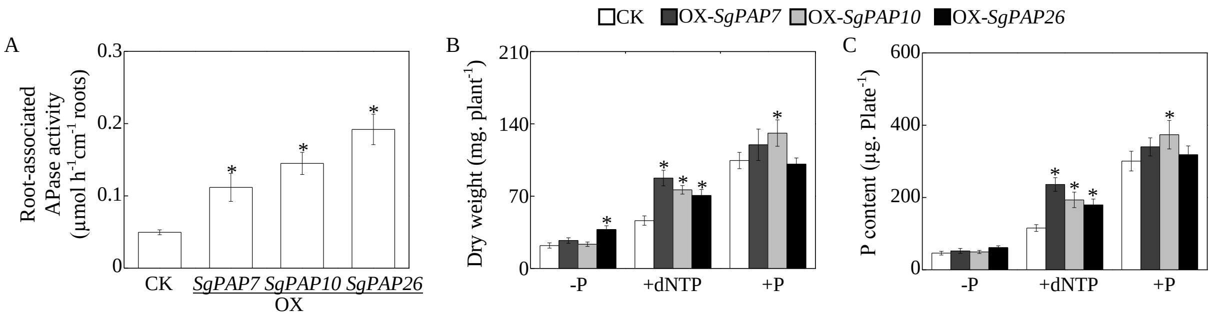

Figure 3 Root-associated APase activity (A), dry weight (B) and P content (C) of bean hairy roots with *SgPAP* overexpression

REFERENCES

Bunemann, E.K., Prusisz, B., Ehlers, K. 2011. Characterization of phosphorus forms in soil microorganisms. In: Bunemann EK, Oberson A, Frossard E. (Eds.). Soil Biology. 26. 37-57.

Liang, C., Sun, L., Yao, Z., Liao, H., Tian, J. 2012. Comparative analysis of *PvPAP* gene family and their functions in response to phosphorus deficiency in common bean. PLoS One. 7. e38106.

Liang, C., Tian, J., Lam, H., Lim, B.L., Yan, X., Liao H. 2010. Biochemical and molecular characterization of PvPAP3, a novel purple acid phosphatase isolated from common bean enhancing extracellular ATP utilization. Plant Physiology. 152. 854-865.

Richardson, A.E. 2009. Regulating the phosphorus nutrition of plants: molecular biology meeting agronomic needs. Plant and Soil. 322. 17-24.

Robinson, W.D., Park J., Tran, H.T., Del Vecchio, H.A., Ying, S., Zins, J.L., Patel, K., McKnight, T.D., Plaxton, W.C. 2012. The secreted purple acid phosphatase isozymes AtPAP12 and AtPAP26 play a pivotal role in extracellular phosphate-scavenging by *Arabidopsis thaliana*. Journal of Experimental Botany. 63. 6531-6542.

Tian, J., Liao, H. 2015. The role of intracellular and secreted purple acid phosphatases in plant phosphorus scavenging and recycling. In: Plaxton WC and Lambers H. (Eds.). Annual Plant Review. 48. 265-288.

Wang, L., Li, Z., Qian, W., Guo, W., Gao, X., Huang, L., Wang, H., Zhu, H., Wu, J., Wang, D., Liu, D. 2011. The Arabidopsis purple acid phosphatase AtPAP10 is predominantly associated with the root surface and plays an important role in plant tolerance to phosphate limitation. Plant Physiology. 157. 1283-1299.

Physiological Response of Buckwheat (*Fagopyrum esculentum L.*) for Nutrient Management Practices in Acid soils of Eastern Himalayas under Climate Change

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Abstract
Under climate change scenario, evaluating the proper NM practices for cultivation of crop plants in acid soil of Eastern Himalayas is urgent need to increase the stress tolerance ability, to intensify the cropping intensity and to increase food security in the region. Buckwheat is one such crop with multi stress tolerance ability suited for the region. In this study, the morpho-physiological response of buckwheat for various NM practices has been evaluated. Among various NM practices, T2(RDF), T6(PLM@5t/ha) and T8(RDF+VC @2.5t/ha) have shown significantly altered chlorophyll pigmentation, (increased chl a and b and carotenoids, reduced anthocyanin content), improved root architecture, increased stomatal distribution and size, increased pollen morphology and pollen viability. Further, these NM treatments reported to increase the cell membrane stability and reduced lipid peroxidation indicating reduced nutrient effect, improved nutrient availability and optimum growth and development of buckwheat to record enhanced yield and yield attributing traits in acid soils of Eastern Himalaya.

Key words: Acid soils, Nutrient Management practices, Stomatal characters,Root architecture, Pollen morphology, and Climate change

Abbreviations: CMS-Cell Membrane Stability, NM –Nutrient Management, VC- Vermi-compost, FYM-Farm Yard Manure, RDF-Recommended Dose of Fertilizer, PLM- Poultry Manure, PGM-Pig Manure, LRWC-Leaf Relative Water Content, SEM-Scanning Electron Microscope, SOC- Soil Organic Carbon, EHR-Eastern Himalayan Region

INTRODUCTION

Farming in EHR is highly complex, diverse and risk prone. Productivity of most of the crops of the region is low due to a number of constraints viz; undulating topography, faulty land use system (Jhum cultivation), soil erosion, soil acidity, poor nutrient status of soil, low moisture retention capacity, lack of appropriate nutrient management is one of the major constraints (Rajkhowa and Manoj-Kumar, 2013). The available N and K status of the soil falls under low to medium range respectively, while available P in the soil is in low range. Further, fertilizer consumption in the region is very poor (average 20 kg ha^{-1}) as against the national average consumption of 133.2 kg ha^{-1} (Indian Fertilizer Scenario, 2010) and moreover farmers are often reluctant in applying recommended dose of chemical fertilizer owing to their poor economic conditions and lack of timely availability of fertilizer. In this context studies on integrated use of fertilizers and manures would not only impart sustenance to the production and improvement of soil health, but also enhance the efficient uptake and use of applied nutrients (Kusro *et al.,* 2014). Integrated nutrient management practices and resource conservation technologies are widely adopted to enhance crop productivity in sustainable agriculture (Liu *et al*, 2005). This has becoming atmost important in the wake of global climate change in HER (Uday *et al*, 2013) which demands judicious use of available resources and to enhance water, soil and energy conservation (Nhemachena *et al*, 2007,)). The effect of physical and chemical degradation of soils are quite obvious, but biological degradation due to the loss of specific soil organic matter fractions and the ‘autochthonous microbial communities’ dependent upon them is insidious (Rao, 2007). Escalating fertilizer cost, growing environmental concerns and need for long term maintenance of soil health necessitates the development of low cost, eco-friendly NM practice(s). VC enhances soil biodiversity by promoting the beneficial microbes, which in turn enhances plant growth directly by production of plant growth regulating substances (hormones and enzymes) and indirectly by controlling plant pathogens, nematodes and other pests, thereby enhancing plant health and minimizing the yield loss (Pathama and Sakthivel, 2012). Further, Sinha *et al*. (2009) reported that VC can significantly influence the growth and productivity of crops due to their macro and micro nutrients, vitamins, enzymes, hormones etc. Anitha (2013) and Bakriamdor (2014) substantially studied the beneficial physiological effect of PLM (5t/ha) and PGM (5t/ha) in terms of increased chlorophyll pigmentation, altered root architecture, differential stomatal characters and yield components variation with better growth and productivity of groundnut in acid soils of EHR. Buckwheat is an annual crop of Polygonaceae family, is a lesser known pseudo-cereal with determinate growth habit but having wider climatic adaptability with shorter duration (2.5-3 months), ability to tolerate multiple stresses with low external inputs requirement and relatively free from pest and diseases. The history of cultivating buckwheat dates back to ancient times in Eastern Tibet and adjacent areas of Sichuan and Yunnan province, China which is probable sites of origin of cultivated buckwheat (Ohnishi, 2002). Even though the buckwheat cultivated extensively in EHR, studies on NM practices, physiological responses for various NM practices in acid soils of the EHR are scanty. In this background this study aims at evaluating various physiological parameters of buckwheat under different NM practices and improved available status of soil nutrients to the buckwheat.

MATERIALS AND METHODS

Experimental Location and Design

An field experiment was conducted during 2013 under terrace land situation of mid altitudes of Eastern Himalaya at our water management farm of our institute. (91^0 55.341 E and 25^0 41.429 N) with an altitude of 3251 feet (991 m) above mean sea level (msl). The soil of the experimental field was acidic (pH: 4.8) containing 2.0% Organic C, 268, 14.6 and 115 kg ha^{-1} of N, P_2O_5 and K_2O, respectively. Treatments comprised of 1. Control (T1), 2. RDF (20:60:40 kg ha^{-1} of N, P_2O_5 and K_2O)- (T2), 3.VC (2.5t/ha)-T3, 4.FYM (2.5t/ha)-T4, 5. VC (2.50 t/ha) + FYM (2.5t/ha)-T5, 6. PLM (5t/ha)-T6, 7. PGM (5t/ha)-T7, 8. RDF+VC (2.5t/ha)-T8. Fertilizers as per the treatment were applied in the form of urea, single super phosphate (SSP) and muriate of potash (MOP). The VC used in the study contained 1.9% N, 1.2% P_2O_5 and 1.6 % K_2O with C: N ratio of 15:1. FYM and PLM contains 0.73% N, 0.18% P_2O_5, 0.71% K_2O, 0.58% S, 16 ppm Zn, 26 % carbon, 13.8% lignin, 38.3% cellulose and 2.51%N, 1.09% P_2O_5 , 1.23% K_2O, 0.72%S, 24 ppm, 31.3% carbon, 14.1% lignin and 30.1% cellulose respectively. PGM has 0.8% N, 0.7% P_2O_5 and 0.5% K_2O (Ghosh *et al*, 2004). The buckwheat crop was sown in the last week of October and harvested in the month of January.

Soil and Plant Observations

Soil samples were collected from each plot (0-20 cm depth), air dried in laboratory, sieved through 2 mm sieve, and analyzed for pH, SOC, available N, P, and K. Soil pH (1:2.5 soil/water) was measured using a glass electrode. The SOC, available N, P, K determined by following standard protocols (Jackson, 1973). Total Chlorophyll and carotenoid levels in fresh leaves was estimated by extracting the leaf pigments with 80% Acetone. The absorbance of filtrate read at 480, 645 and 663 nm. The leaf pigments viz., chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were calculated using appropriate calculations. The CMS of buckwheat leaves assessed by measuring initial absorbance (Ia) and final absorbance (Ta) recorded at 273nm after boiling for 15min. The leaf thickness measured in µm by clamping fresh leaves to vernier calipers. RWC in fresh leaves measured by determining fresh weight (FW), turgid weight (after immersing in water for 4 hrs) and dry weight (DW) after oven drying at 62^oC for 72 hrs. Details of root size and distribution of plants were studied after uprooting the Buckwheat plants from the field by loosening the soil surrounding the root system and washed to remove adhering rhizospheric soil in a smooth flush of water. The fresh and air dried plant roots were scanned using Epson root scanner (Winrhizo[R] software) . Stomatal characters in terms of their distribution, frequency (no. of stomata/unit area), their index (no. of stomata per epidermal cells) and its size, pollen viability were assessed by using compound microscope under 40X (BX-05 Olympus). Pollen structure and morphology studies were performed through SEM (JEOL JSM-6360). The yield and yield components were recorded at harvest.

RESULTS AND DISCUSSION

The physiological data recorded at 30 and 60DAS exhibited differential response for NM practices. The total chlorophyll pigment (Chl a and b) content of buckwheat recorded two stages of growth under different NM practices, showed increased quantities of chlorophyll T2 (RDF), T4 (FYM @5t/ha) and T6(PLM @5t/ha). This increase in total chlorophyll may bedue to increased chl a and b pigments (data not shown) or increased accessory pigments like carotenoid which are estimated in the same leaves (Table.1). Enhanced levels of chlorophyll pigmentation in the above NM practices may be attributed to increased nutrient availability (especially N, Mg and Fe) which are required for chlorophyll synthesis. Anthocyanin pigment being nutrient stress responsive, accumulated higher in T3 (VC @ 2.5t/ha) and T4 (FYM @ 25t/ha) NM practices indicating deficiency of phosphorus availability to buckwheat plant. These accessory pigments enhanced under some NM treatments might act as important part of the plant antioxidant defense system to protect and sustain photochemical processes under stress conditions (Havaux, 1998). The CMS of the fresh leaves of buckwheat varied significantly among various, NM practices. CMS recorded significantly higher under T2, T6 and T8 indicating improved membrane stability and less membrane disturbances with appropriate physical structure and configuration of the membrane. (Gajeswaka *et al*, 2014). Apart from these, leaf thickness also increased in T2, T6 and T8 indicating optimum leaf growth and increased layers of mesophyll cells for photosynthesis.

Table.1 Physiological parameters measured at different stages of Buckwheat under different NM practices

T	Tot Chl		Car		Anth		CMS		LT		LRWC	
	30DAS	60DAS	30DAS	60DAS	30DAS	60DAS	30DAS	60DAS	30DAS	60DAS	30DAS	60DAS
T_1	1.05^{bc}	1.02^{c}	46.1^{bcd}	49.1^{bc}	3.8^{e}	10.9^{a}	9.7^{b}	6.7^{c}	243^{a}	190^{b}	77.8b	77.4bc
T_2	1.50^{a}	1.47^{a}	57.3^{a}	44.2^{cd}	12.3^{b}	10.1^{ab}	12.3^{ab}	9.6^{ab}	277^{b}	217^{b}	84.7ab	78.4bc
T_3	1.15^{bc}	1.25^{abc}	55.3^{a}	64.2^{a}	16.0^{a}	11.4^{a}	11.7^{ab}	8.7^{b}	193^{b}	193^{b}	84.4ab	81.4ab
T_4	1.32^{ab}	1.35^{ab}	56.5^{a}	52.0^{b}	10.0^{c}	9.3^{ab}	11.9^{a}	8.1^{bc}	187^{b}	233^{b}	77.4b	76.8c
T_5	1.22^{bc}	1.19^{bc}	48.1^{abc}	51.6^{b}	8.2^{c}	8.4^{bc}	12.1^{ab}	10.5^{a}	237^{b}	183^{b}	80.3ab	63.5a
T_6	1.26^{abc}	1.27^{abc}	41.4^{cd}	43.6^{d}	6.2^{d}	6.2^{cd}	13.4^{ab}	6.8^{c}	253^{a}	300^{a}	86.5a	80.7bc
T_7	1.11^{bc}	1.15^{bc}	52.8^{ab}	51.8^{b}	5.5^{de}	5.6^{d}	10.5^{ab}	10.7^{a}	173^{b}	203^{b}	78.9ab	78.6bc
T_8	1.03^{c}	1.06^{c}	38.3d	43.8d	6.1d	6.6^{d}	12.8^{ab}	5.0^{d}	260^{b}	227^{b}	81.0ab	79.4bc
Mean	1.21	1.22	49.5	50.0	8.5	8.5	12.2	8.3	228	218	81.4	77.0
Range	1.03-1.50	1.01-1.47	38.3-57.3	43.6-64.2	3.8-16.0	5.60-11.37	9.7-13.4	5.0-10.7	173-277	183-300	77.4-86.5	63.5-81.4

Note: T-treatment, T_1-Control, T_2-RDF, T_3-VC (2.5t/ha), T_4-FYM(5t/ha), T_5-VC (2.5 t/ha) +FYM (2.5t/ha), T_6-PLM (5t/ha), T_7- PGM (5t/ha), T_8- RDF+VC (2.5t/ha), Tot chl-Total chlorophyll(mg/gFW), car-Carotenoids(µg/g FW), Anth-Anthocyanin(µg/g FW), CMS-Cell membrane stability(%), LT-Leaf thickness (µm), LRWC-Leaf relative water content(%).

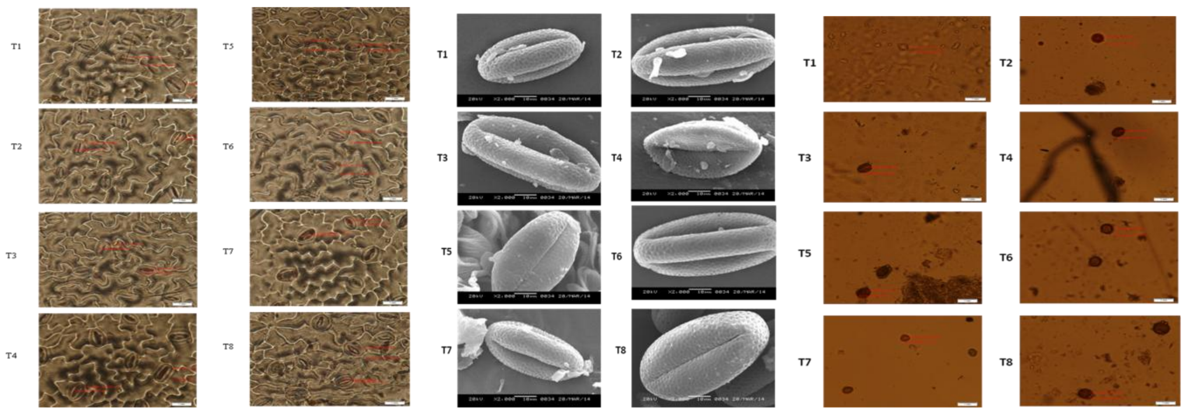

Fig. 1 Stomatal distribution, pollen morphology and viability of buckwheat under different NM practices

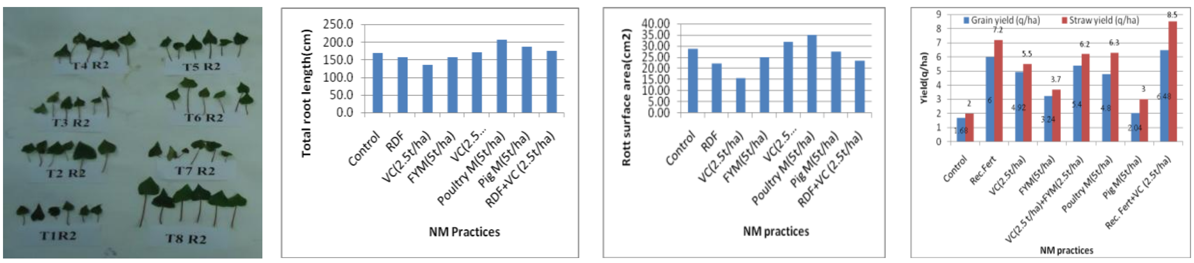

Fig.2 Performance of buckwheat in terms of leaf area, root charcters and yield under NM practices

This result is in corroboration with leaf images presented in Fig.2A. Even it supports the RWC data assessed at two stages (at 30 and 60DAS) of buckwheat (Table.1). The turgid leaves were noticed in T2, T6 and T8, this might due to adequate water uptake and availability for enhanced metabolic processes. Besides this stress responsive leaf traits, increased root architecture parameters of Buckwheat in terms of improved root surface area and total root length assessed at the end of active growth stage (60DAS) in T6 and T8 may acts as stress protective mechanisms, enabling the plant to explore increased quantities of water and essential nutrients from deeper layer of the soil. Similar type of results substantiating the effect of PLM (5t/ha) and PGM (5t/ha) on root growth and productivity of ground nut has been investigated by Anitha (2013) and Bakhriamdor(2014). The stomatal distribution studied in abaxial surface of the buckwheat leaves through compound microscope in different NM practices indicate more number of stomata per unit area of the leaf in T2, T6 and T8(Fig. 1A).

This enhanced stomatal number in the above treatments help in more exchange of gases for enhanced photosynthesis. Similar results with stomatal size and stomatal density variation reported to be considerably influenced by plant species and abiotic environmental perturbations such as changes in atmospheric CO_2 concentration, light intensity, temperature, soil water and nutritional status (Xu and Zhou, 2008). The improved leaf characters important for maximizing light harvesting and utilization of light for higher photosynthesis which is closely associated with higher biomass accumulation and seed yield of the crop (Rajbhari *et al*, 2008).The pollen morphology data recorded through SEM indicate altered pollen size and structure under different NM practices. Bigger and turgid pollens were noticed in T2, T3, T6 and T8. The reduction in pollen size may be due to decreased availability of water and nutrient for better growth and development of buckwheat (Fig.1B). The changes in pollen morphology reflected in altered pollen viability depicted in Fig.1C under different NM practices. The pollen germination data (Data not shown) also show that under above treatments pollen were germinated better with longer pollen tube growth. The yield components recorded indicate higher grain as well as higher straw yield recorded under T2 with RDF, T6 with PLM @ 5t/ha and T8 (RDF+ VC @ 2.5t/ha).

CONCLUSION

This study indicates improved physiological traits recorded in terms of enhanced leaf characters, root attributes better stomatal characters, higher pollen size and viability under T2 (Rec RDF), T6 (PLM @ 5t/ha) and T8 (RDF+VC @2.5t/ha) aided the plant for optimum growth and development and in turn better yield and productivity of buckwheat

REFERENCES

Anitha, 2013. Influence of organic amendments on growth and productivity of Ground nut (Arachis hypogeal L) MSc thesis submitted to Central Agriculture University (CAU), Imphal, Manipur, India.

Bakhriamdor, S, 2014. Influence of organic amendments on phosphorus nutrition and Aluminium Toxicity tolerance in of Ground nut(Arachis hypogeal L). MSc thesis submitted to Central Agriculture University (CAU), Imphal, Manipur, India

Ghosh P.K. , Ajay, Bandyopadhyay, K.K. Manna M.C., Mandal K.G., Misra A.K., Hati K.M. 2004. Comparative effectiveness of cattle manure, poultry manure, phosphocompost and fertilizer-NPK on three cropping systems in Vertisols of semi-arid tropics. II. Dry matter yield, nodulation, chlorophyll content and enzyme activity. Bioresource Technology ,95: 85–93.

Havaux, M., 1998. Carotenoids as membrane stabilizers in chloroplasts. Trends Plant Science, 3: 147–151

Indian Fertilizer Scenario, 2010: Department of fertilizer, Ministry of chemical and fertilizers, Government of India.

Kusro, P.S., Singh, D.P., Paikra, M.S., Deepak K., 2014. Effect of organic and inorganic additions on physicochemical properties in Vertisol. American International Journal of Research in Formal, Applied & Natural Sciences, 5: 51-53.

Liu X., Liu J., Xing B., Herbert S.J., Meng K.H., Xiaozeng H., Zhang X., 2005. Effect of long term continuous cropping, tillage and fertilization on soil organic carbon and nitrogen of black soils in China. Communications in Soil Science and Plant Analysis. 36:1229–1239.

Nhemachena C., Hasran R., 2007. Discussion Paper No.714. Washington, DC: International Food Policy Research Institute;. Micro Level Analysis to Climate Change in Southern Africa, IFPRI.

Ohnishi, O., 2002. Wild buckwheat species in the border area of Sichuan, Yunnan and Tibet and allozyme diversity of wild Tartary buckwheat in this area. Fagopyrum 19: 3-9.

Pathama, J., Sakthivel, N., 2012. Microbial diversity of vermi-compost bacterial that exhibit useful agricultural traits and waste management potential. Springer plus, 1: 26.

Rajbhandari B. P. 2004. Eco-Physiological Aspects of Common Buckwheat. Proceedings of the 9th International Symposium on Buckwheat, Prague, 101-108.

Rajkhowa, D.J.,Manoj-Kumar, 2013. Biowaste utilization for improving health and productivity of acid soils in North-East India. Current Science, 104:11-12.

Rao, D.L.N., 2007. Microbial Diversity, Soil health and sustainability. Journal Indian Society Soil Science, 55: 392-403.

Sinha, R.K., Heart, S., Valani, D. Chauhan, K., 2009. Vermi-culture and sustainable agriculture. Americal Euras. Journal Agricultural and Environmental Science, 5: 1–55.

Uday .S.Saikia, Goswami B., Rajkhowa, D.J., Venkatesh, A. Ramachandran,K Rao V.U.M., Venkateswarlu B. Ngachan S.V. 2013. Shift in monsoon rainfall pattern in the North Eastern region of India post 1991. Journal. of Agrometeorology, 15 (2) : 162-164.

Xu Z and Zhou G. 2008. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. Journal Experiment Botany, 59: 3317–3325.

Physiological performance of Lentil (*Lens culinaris L.*) varieties under residue management practices in Acid soils of Eastern Himalayas

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Abstract

This study aims at investigating the physiological response of lentil varieties for residue management practices in rice fallows of upland conditions of mid altitudes of Eastern Himalaya. Results show that under resource conservation practices, lentil varieties exhibited significantly varied physiological response at whole plant level in terms of varied chlorophyll pigmentation, altered root architecture, differential stress adaptive cellular responses, changes in stomatal characteristics, modification in pollen morphology and germination and in turn impacting the yield and yield attributes. This study indicates early duration lentil variety DPL-62 performed well compare to DPL-15 under different RMP due to its better morpho-physiological traits, increased pollen morphology and better biomass partitioning towards yield in acid soil of Eastern Himalaya.

Abbreviation: EHR-Eastern Himalayan Region, RMP- Residue Management Practices, CMS-Cell Membrane Stability, LRWC- Leaf Relative Water Content, HI-Harvest Index , FW-Fresh Weight, DW-Dry Weight, TW-Total Weight, FP-Farmers practice, SEM-Scanning Electron Microscope.

INTRODUCTION

Although the EHR receives large amount of rainfall (>2000 mm per annum), it majorly distributed over few months of the year (June-October), leaving crops to face moderate to severe drought stress during the remaining winter months i.e. November to April. Low soil moisture content with fast decline in water table over the advancement of winter season after rice harvest results in intermittent to terminal drought at flowering and pod filling stages which adversely affects the productivity of pulses in the region (Turner, 2003). In this situation, growing short duration crop cultivars under conservation tillage and residue retention may help in successful cultivation of second crop (Das *et al.*, 2014a). Lentil, being an edible pulse, self pollinated, determinate type and fixer of atmospheric N, it is most adapted to climatic and soil fertility conditions (Srinivasarao *et al.*, 2012). In EHR, where a large part of the rice cultivated area remains fallow after *kharif* rice, short duration pulse like lentil has a very good potential for increasing farm income as well as cropping intensity. Crop residues in such soil rice fallows increase the soil hydraulic conductivity and infiltration by modifying mainly soil structure, proportion of macropores and aggregate stability. So, if crop residues are retained on the soil surface in combination with suitable planting techniques, it may conserve soil moisture alleviate possible drought condition in pulses. Apart from this, productivity and profitability of rice-lentil cropping system can be improved with efficient utilization of residual soil moisture. But there are less research studies on physiological response of lentil to different RM practices in the acid soils of EHR. Keeping this in view, this study conducted with a major focus on understanding of physiological responses of lentil under RMP in the rice fallows by conserving soil moisture.

MATERIAL AND METHODS

Experimental site and Design

An field experiment was carried out under rainfed conditions during 2013 in upland terraced agronomy farm of our institute (950 m a.s.l., 25◦30'N latitude and 91◦51'E longitude) which is characterized by temperate to subtropical climate with average annual rainfall of 2450 mm. The maximum and minimum temperature during crops (rice-lentil) season was 32^0C and 6^0C respectively. Soil of the experimental site is a *Typic Paleudalf*, low in available N (258.3 kg/ha) and P_2O_5 (9.4 kg/ha) but medium in available K_2O (175.3 kg/ha). The soil is acidic in reaction (pH 5.3) and relatively low in soil organic carbon (SOC) concentration (15.8 g/kg). The bulk density (BD), water holding capacity (WHC) and infiltration rate (IR) of the soil are 1.23 Mg/m^3, 48.9 % and 4.02 mm/hr., respectively (Das *et al.*, 2014b). Treatment and layout includes three RRM practices (residue removal of rice, mulching (residue retention) of rice straw@ 5 t/ha and farmers practice(Non residue management practice(NRMP) with two lentil varieties (early duration DPL- 62 and medium duration DPL- 15) as test crop. Lentil was grown under zero tillage after harvesting of rice with recommended dose of 30 kg N, 60 kg P_2O_5 and

40 kg K_2O /ha, in the form of urea, single super phosphate (SSP), and muriate of potash (MOP) ,respectively and applied in furrows using furrow opener before sowing of lentil and mixed with the soil, The gross plot size was 5.0 x 4.0 m.

Soil and Plant observations

The initial as well as post-harvest soil samples were collected (500 g composite sample, one sample from each plot) at 0–15 cm depth for analyzing the physico-chemical properties of soil. Soil moisture content was measured by gravimetrically at 15 day interval for 0–5, 5–10 and 10-15 cm depths. Soil pH was measured in a 1:2.5 (soil and water suspension) and infiltration rate (IR) was measured by double-ring infiltrometer . Soil BD was measured by core method using cores of 5.8 cm height and 5.4 cm diameter at 0-15cm depth and oven dried at 105^0C(one sample per plot). The observations on morpho-physiological parameters (chlorophyll pigmentation, root architecture, *etc*) at active growth stage (45 DAS) and yield components at harvest were recorded. Chlorophyll and carotenoid pigment levels in fresh leaves were estimated by extracting the leaf pigments with 80% acetone. The CMS, in terms of percent leakage of cell contents in fresh leaves (0.5g) was assessed by recording initial absorbance (Ia) and final absorbance (Ta, after boiling in hot water bath at 100^oC for 15 min.) at 273nm using UV-2450 visible spectrophotometer (Shimadzhu). To study the root size and distribution of lentil, the plants were uprooted from field by loosening the soil surrounding root system and washed to remove adhering soil in a smooth flush of water. The fresh and air dried plant roots were spread on fibre plate (without overlapping of roots) and scanned using root scanner (Winrhizo^R software) to observe finer details of root architectural changes under various -RMP. LRWC is measured by recording weight differences of fresh leaves, turgid leaves (immersed in water for overnight to achieve maximum turgidity) and dried leaves. LRWC calculated using the simple formula (FW-DW)*100/ (TW-DW). The yield parameters of lentil were measured at harvest after sun drying, threshing and cleaning. The moisture content of lentil seed was 9%. The HI was determined by the following standard formula and expressed as percentage (%). The experimental data pertaining to each parameter of study were subjected to statistical analysis by using the technique of analysis of variance and their significance was tested by "F" test. Standard error of means (S.Em±) and critical difference (CD) @ 0.05 were worked out for each parameter.

RESULTS AND DISCUSSION

Lentil varieties (DPL-62 and DPL-15) did not vary significantly in accumulating chl a and total chlorophyll pigments but significant variation is recorded in chl b, carotenoid and anthocyanin (Table 1) among different RMP differentiates the ability of genotype to perform under resource poor condition. Higher quantities of carotenoids and anthocyanin recorded in DPL-62 compare to DPL-15, in FP compare to other RMP. It also significantly reduced chl a/b ratio in DPL-15 and FP and also increase anthocyanin in DPL-15 compare to DPL-62. This increase in carotenoid pigments in DPL-62 compare to DPL-15 under FP underlines the function of above pigments as protective substances during stressful condition occurring in FP(Non Residual Management practice). Increase anthocyanin in DPL-15 compared to DPL-62. Decrease in total chlorophyll contents in DPL-15 under residue removal can be attributed to water stress and sensitivity of pigments (Silva *et al.,* 2007). Improved root architecture parameters in terms of increased root surface area and total root length assessed at the end of active growth stage (60DAS) were noticed in DPL-62 compare to DPL-15 variety and in residue retention compare to other treatments (Table 1 & Fig.1B). Enhanced root traits may help the plant under stress condition (moisture stress)which is predominantly occurring during residue removal and non-residue management (FP) enabling the plant to explore increased quantities of water and essential nutrients from deeper layer of the soil. Under mulching (residue retention) enhanced soil moisture and greater pore-size noticed may increase the root activity of lentil which was in comparison with (Ogban *et al.,* 2008 But root diameter of both varieties was increased significantly under FP and residue removal as compared to mulching because of occurrence of moisture stress conditions (Fig 2C). The extent of increase in root diameter in DPL- 62 variety was 87.5 % and in DPL- 15 it was 43.3%. Between the two varieties of lentil, DPL- 62 has performed well in improving root architecture components indicating the versatile ability of DPL- 62 in enhancing overall root growth in response to sufficient moisture conditions (Table.1 and Fig 1B). Similar studies of assessing the impact of moisture status on plant growth has been studied in many crop plants by previous researchers emphasizing the maintenance of adequate root morphology and distribution as a part of plant resistance responses to possible drought conditions for better water uptake, optimum growth and development of plant (Schroeder *et al.,* 2001). The CMS of the fresh leaves of DPL-62 was higher compare to DPL-15 and it significantly varied among various RRM practices. Significantly higher CMS was recorded in mulching practice with increased membrane stability and less membrane disturbances may be favourable to the optimum plant growth with less changes physical structure and configuration of the membrane (Gajeswaka *et al,* 2012). Apart from these, increased leaf thickness recorded by DPL-62 compare to DPL-15 with optimum shoot growth (Fig. 1A) and drastically reduced leaf thickness observed under FP indicate the extent of abundance of number of layers of mesophyll cells available for photosynthesis (Data not shown). The LRWC assessed at active growth stage (45DAS) of lentil varieties showed that higher LRWC in DPL-62 compare to DPL-15 and in mulching compare to other RMPs. The turgid leaves were noticed in DPL-62 with residue retention, this might be due to adequate uptake of water from conserved soil moisture and also exploration of water by well proliferated root system whereas less LRWC observed in DPL-15 and FP due to weaker root growth and reduced water uptake. Lower LRWC of lentil under residue removal and FP may be due to the moisture stress intensified during the growing season.

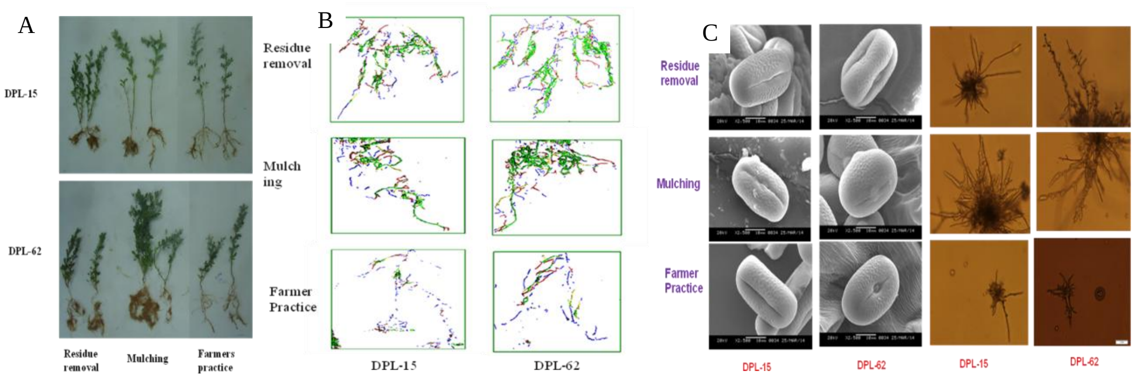

Fig.1 Performance of lentil varieties in terms of whole plant growth, root architecture, pollen size and pollen viability under different RRM practices

Table.1 Physiological parameters of lentil varieties grown under different RRM practices

Treatment	Chl a	Chl b	Tot chl	Caro	Chl a/b	Anth	TRL	TSA	CMS	LRWC
Lentil Varieties										
DPL- 62	0.72	0.51	1.24	72.2	1.51	27.3	91.9	28.5	12.4	75.4
DPL- 15	0.71	0.41	1.16	76.6	1.86	35.2	72.2	21.9	10.7	70.2
S.E(m)±	0.01	0.01	0.04	0.62	0.05	0.8	1.27	0.35	0.42	0.74
C.D. (*p*=0.05)	NS	0.02	NS	1.35	0.11	1.74	2.76	0.77	0.91	1.61
Residue Management Practices										
Residue retention	0.84	0.36	1.29	64.4	2.36	15.9	117.2	34.2	18.2	82.6
Residue removal	0.71	0.45	1.10	73.8	1.61	32.3	79.3	26.4	8.52	73.5
Farmer’s practice	0.60	0.57	1.21	85.0	1.07	45.3	49. 7	15.1	8.03	62.6
S.E(m)±	0.02	0.01	0.04	0.76	0.06	0.98	1.55	0.43	0.51	0.9
C.D. (*p*=0.05)	0.04	0.03	0.10	1.65	0.14	2.13	3.38	0.94	1.12	1.97

Values are means of 3 triplicates. Level of significance was determined by performing Univariate 2-way ANOVA with in FRBD using SPSS v. 21. Chl a and Chl b: Chlorophyll a and b contents (mg/g FW), Tot. Chl: Total chlorophyll content (mg/g FW), Caro and Anth: Carotenoid and Anthocyanin content (µg/gFW), TRL:Total root length(cm/plant), RSA : Root surface area(cm^2/plant), CMS:Cell membrane stability (%), LRWC:Leaf relative water content (%).

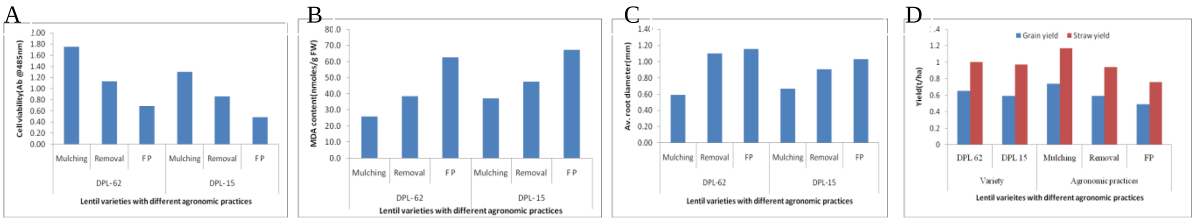

Fig.2 Stress responsive physiological assays and yield parameters of lentil varieties grown with different RMPs.

Improved leaf characters important for maximizing light harvesting and utilisation of light for higher photosynthesis which is closely associated with higher biomass accumulation and seed yield of the crop. The stomatal size and distribution studied in abaxial surface of the lentil varieties through compound microscope under residue management practices indicate more number of stomata per unit area of the leaf of DPL-62 with mulching(Data not shown). This enhanced stomatal number may help for more exchange of gases for enhanced photosynthesis. Similar results with stomatal size and stomatal density variation reported to be considerably influenced by plant species and abiotic environmental perturbations such as changes in atmospheric CO_2 concentration, light intensity, temperature, soil water and nutritional status (Xu and Zhou, 2008). Further, the pollen morphology data recorded through SEM indicate altered pollen size and structure in two lentil varieties and under different residue management practices. Bigger and turgid pollens were noticed in both the lentil varieties under mulching practice. The reduction in pollen size may be due to decreased availability of water and nutrient for better growth and development of lentil at critical stage for better growth and development of lentil (Fig.1C). The changes in pollen morphology reflected in altered pollen viability recorded in the same microscope under different residue management practices. The variation in pollen size and pollen viability affected pollen germination depicted in Fig.1C. The variety and treatments with better pollen viability is extended longer pollen tube growth. Besides, DPL-62 and mulching practice recorded higher cell viability, reduced lipid peroxidation indicating less stress effects at cellular level and healthy growth of the plant (Fig.2A & B). Early duration DPL 62 recorded significantly higher number of pods/plant as well as seed yield, straw yield than medium duration DPL 15 under mulching compare to other residue management practice (Fig.2D). It may possible due to escaping of moisture stress by the early duration lentil variety DPL-62. Higher seeds/pod, pods/plant, test weight and seed yield was recorded in mulching as compared to residue removal and FP. For most of the yield attributes, residue removal was on par with FP. There was not any significant effect of lentil varieties on BD, WHC and IR of soil. However, mulching being at par with 40 cm stubble height recorded significantly lower BD as compared to 20 cm standing stubble and residue removal (Data not shown). It may be due to promotion of aggregation and pore development in the soil system as the residues decomposed (Mulumba and Lal, 2008). The highest WHC was recorded under mulching followed by residue removal and FP (Data not shown).Among the different RRM practices, residue retention recorded significantly higher IR than rest of the treatments (Data not shown). The lowest IR was recorded under residue removal. This result is in accordance with Belder *et al.* (2007) who observed more total infiltration in conservation agriculture systems (reduced tillage and mulching) compared to conventional system. The varieties did not make any significance difference in SOC content of soil in both 0-15 and 15-30 cm soil depth.

CONCLUSION

Early duration lentil variety DPL-62 performed well under different RMP due to its better morpho-physiological traits, increased pollen morphology and better biomass partitioning towards yield in acid soil of Eastern Himalaya.

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REFERENCES

Belder, P., Twomlow, S., Hove, L., 2007. Early evidence of Improved Soil Quality with Conservation Farming Under Smallholder Farming Conditions in Zimbabwe. Paper Presented at the ICID Conference, November 2007, Johannesburg, South Africa

Das A., Ghosh,P. K., Lal, R., Saha,R., Ngachan, S.V. 2014a. Soil quality effect of conservation practices in maize–rapeseed cropping system in Eastern Himalaya. Land Degradation and Development. DOI: 10.1002/ldr.2325

Das, A, Lal, R, Patel, D.P, Ramkrushna, G.I., Layek, J., Ngachan, S.V., Ghosh P.K., Bordoloi J., Kuma,r M.,2014b .Effects of tillage and biomass on soil quality and productivity of lowland rice cultivation by small scale farmers in North Eastern India. *Soil & Tillage Research* 143:50–58

Gajewska, E,, Bernat, P. Długon J., Skłodowska, M., 2012. Effect of Nickel on Membrane Integrity, Lipid Peroxidation and Fatty Acid Composition in Wheat Seedlings. Journal of Agronomy and Crop Science. 198 (4): 286–294.

Mulumba, L.N., Lal, R., 2008. Mulching effects on selected soil physical properties. Soil & Tillage Research 98, 106-111

Ogban, P. I., Ogunewe, W. N., Dike, R. I., Ajaelo, A. C., Ikeata, N. I., Achumba, U. E. and Nyong, E.E. 2008. Effect of tillage and mulching practices on soil properties and growth and yield of cowpea (*Vigna unguiculata*) in South Eastern Nigeria. Agro-*Science Journal of Tropical Agriculture* 7 (2): 118 -128. URL: http://www.agrosciencejournal.com.

Schroeder, J.I., Kwak, J.M., and Allen, G.J. 2001. Guard cell abscisic acid signalling and engineering drought hardiness in plants. Nature 410: 327–330

Silva, M, A, Jifon, J, L, DaSilva J,A.G., Sharma, V., 2007.Use of physiological parameters as fast tools to screen for drought tolerance in sugarcane. Brazilian Journal of Plant Physiology 19:193-201. http://dx.doi.org/10.1590/S1677 04202007000300003.

Srinivasarao C, Venkateswarlu B, Lal R., Singh A.K., Vittal K.P.R.,, Kundu, S, Singh, S. R., Singh, S.P.,2012. Phosphorus Loss Potential and Phosphatase Activity under Phosphorus Fertilization in Long-Term Paddy Wetland Agro-ecosystems. Soil Science Society of American Journal 76:161-167 doi:10.2136/sssaj2011.0078

Turner, N.C.,2003, Adaptation to drought: lessons from studies with chickpea. Indian Journal of Plant Physiology. Special Issue:11-17.

Xu Z, Zhou G. 2008. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *Journal Experiement Botany*, 59:3317–25.

Section 03:

Molecular genetics of plant adaptation to acid soils

Chairpersons: Peter Ryan, Miguel Piñeros, Hiroyuki Koyama

Different mechanisms modulate the transport activity of the MATE and ALMT-type transporters involved in plant aluminum resistance

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Abstract
Structural-functional characterization of membrane transport proteins mediating the release of organic acids underlying aluminum (Al) resistance responses has led to the identification of different mechanisms underlying these processes. While the Al^{3+} triggered regulation of malate efflux via specific anion channels (ALMTs) is an intrinsic characteristic of the transport protein, the modulation of citrate transporters (MATEs) involves the interaction of the transporter with additional elements of a signal transduction cascade. In this study we present evidence for the existence of these two different regulatory mechanisms.

INTRODUCTION
Members of the ALMT (Al-activated malate transporter) and MATE (multidrug and toxin efflux) families confer plant aluminum resistance on acid soils by mediating organic acid (OA) anion efflux, thereby immobilizing toxic aluminum (Al^{3+}) ions at the root surface. Although the similar physiological processes are similar, the structure, functional mechanisms, and posttranslational regulation of these two types of transporters differ significantly. We have integrated electrophysiological analysis with cellular imaging approaches to conduct a structural-functional analysis aimed at determining the transporters' topology, stoichiometry, function and regulation. These studies are allowing us to identify key protein residues/motifs that ensure maximal OA release in the presence of toxic Al in the rhizosphere, thereby limiting unnecessary root carbon loss.

RESULTS AND MODEL DESCRIPTIONS

ALMT and MATE functionally expressed in Xenopus oocytes
As shown in Figure 1, electrophysiological analysis using conventional two electrode voltage clamp approaches in *Xenopus* oocyte cells expressing ALMT or MATE transporters, indicate that both ALMT (Figure 1B) and MATE (Figure 1C) transporters mediate a significant electrogenic transport (i.e. negative currents elicited by test potentials ranging from -160 to 0 mV) relative to the lack of transport recorded in control cells (Figure 1A). This increased electrogenic transport is the product of net organic anion efflux. In the presence of extracellular Al^{3+}, ALMT transporters undergo conformational changes that lead to enhancement of OA transport (Figure 1B). In contrast, exposure to extracellular Al^{3+} significantly reduces the constitutive transport recorded in MATE expressing cells (Figure 1D).

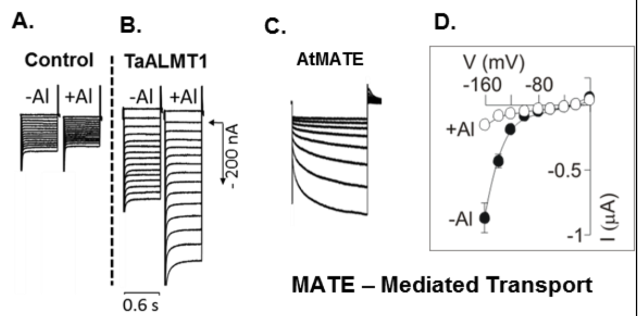

Figure 1. Transport activity of ALMT and MATE transporters expressed in Xenopus oocytes

These observations suggest that while ALMT-mediated transport *in planta* can be modulated by an intrinsic regulatory mechanism, modulation of the MATE transporter activity most likely requires additional cellular mechanisms associated with an upstream Al^{3+} signaling cascade.

Regulation of TaALMT1 activity by extracellular Al^{3+}
Functional analysis of various structurally altered TaALMT1 transporters have suggested specific residues (out of the total 43 negatively charged amino acid residues) are key determinants for the Al^{3+}-dependent intrinsic regulation of TaALMT1 transport activity (Furuichi et al., 2010; Ligaba et al. 2013). However, given that some of the negatively residues identified are highly conserved throughout the entire ALMT family, the significance of these results is confounded by the likeliness of the structural/functional alterations resulting in deleterious changes in protein structure rather than modifications of specific sites involved in Al^{3+} sensing. Therefore, these studies are currently being complemented by evaluating the role/contribution of larger protein domains, rather than single residue substitutions. Preliminary analysis indicates that regions in the N-terminus and a hydrophobic

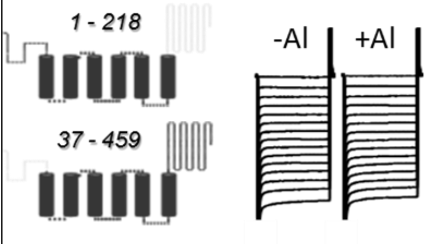

Figure 2. Removal of the N or C–terminal regions of TaALMT1 results in functional transporter lacking Al^{3+} responsiveness.

region at the C-terminus are both involved in Al^{3+}-sensing, as removal of either region leads to modified transporter activity, in that they still mediate anion efflux but the transport is no longer Al^{3+} responsive (Figure 2). We are currently evaluating the role of these and other identified domains (e.g. truncations and swapped domains with functionally distinct ALMT members) to further elucidate the mechanisms/domains underlying the Al^{3+}-responsiveness of TaALMT1, thereby complementing the findings and interpretations from the single residue substitutions.

Regulation of AtMATE activity by phosphorylation

In contrast to TaALMT1, when expressed in X. oocytes the AtMATE transporter mediates a large and constitutive OA transport (Figure 1), which has led us to investigate potential signal transductions mechanisms which could be involved in the post-translational modulation of the MATE transporter.

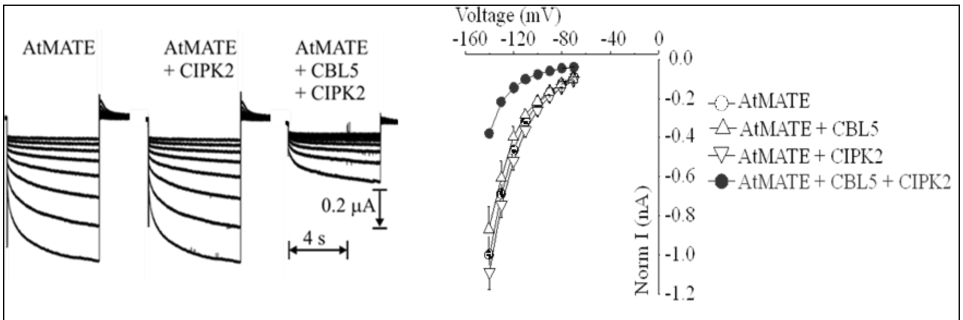

Figure 3. The CBL5/CIPK2 complex is required to modulate the AtMATE transport activity.

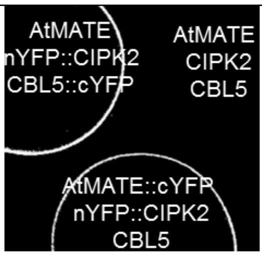

Figure 4. BIFC analysis of AtMATE/CIPK2/CBL5 interactions in X.oocytes.

CBLs (Calcineurin B-like proteins), represent a family of calcium sensor proteins that interact with CIPKS (serine/threonine kinases designated as CBL-interacting protein kinases). As CBL-CIPK complexes have been shown to mediate plant responses to several environmental stress response signals, ultimately regulating ion fluxes, we performed a functional screen to evaluate the role of protein phosphorylation in modulating the transport activity of MATE transporters. Functional screening of the entire *Arabidopsis* CBL and CIPK library in X. oocytes by co-expressing them with AtMATE led to the identification of a unique CBL5/CIPK2 interaction with led to the down regulation of AtMATE transport activity (Figure 3). Bimolecular fluorescence complementation (BiFC) analysis in oocytes (Figure 4) and in tobacco leaves (data not shown) validated the AtMATE/CIPK2 and CIPK2/CBL5 protein-protein interactions. These results indicate CBL5/CIPK2 constitutes a potential Ca^{2+} dependent signaling pathway which can potentially minimize carbon loss (i.e. AtMATE mediated OA exudation), and regulate Al resistance both temporally and spatially as the root grows through the acid soil horizons. Further characterization of the protein phosphorylation mechanism as well as *in planta* studies will be presented.

REFERENCES

Furuichi T, Sasaki T, Tsuchiya Y, Ryan PR, Delhaize E, Yamamoto Y: An extracellular hydrophilic carboxy-terminal domain regulates the activity of taalmt1, the aluminum-activated malate transport protein of wheat. Plant J (2010) 64(1):47-55.

Ligaba A, Dreyer I, Margaryan A, Schneider DJ, Kochian L, Piñeros M: Functional, structural and phylogenetic analysis of domains underlying the al sensitivity of the aluminum-activated malate/anion transporter, Taalmt1. The Plant Journal (2013) 76(5):766-780.

Modification of xyloglucan changes Al sensitivity by affecting Al binding capacity in cell wall

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Abstract
Aluminum toxicity is the major limiting factor for crop production in acidic soils. When plant roots are growing in these soils, cell wall is the first barrier for Al to enter the cell. It is composed of various polysaccachrides such as cellulose, hemicellulose and pectin, and some functional proteins. Under natural conditions, cell wall is usually negatively charged, so it is reasonable to assume that there must have interaction between the cell wall matrix and Al ions. Among the cell wall components, pectin has been implicated as the major Al binding sites. However, when the pectin is removed from the cell wall by pectinase or hot water, the majority of Al is still remained in the residue, indicating that there are still other components in the cell wall which can bind Al effectively. In recent years, our lab tried to elucidate what are the other component that contributes to Al binding capacity in the cell wall.
First of all, we fractionationized the root cell wall into different CW components (pectin, hemicellulose 1 [HC1], and HC2) after exposed to Al treatment (50 mM Al for 24 h) in Arabidopsis, and found that about 75% of CW Al accumulated in the HC1 fraction, suggesting the great contribution of HC in Al retention in the cell wall. xyloglucan endotransglucosylase (XET) is involved in the cell wall loosen during cell wall extension and cell elongation, and Al treatment quickly and greatly inhibited XET activity, which was concomitant with Al-induced callose deposition in roots. Real-time RT-PCR of the genes encoding XET in the roots indicated that XTH14, 15 and 31 may constitute the major contributors to XET activity (Yang et al., 2011). The T-DNA insertion mutants of the above *XTH15* and XTH*31* showed increased Al resistance. The interesting thing is that *xth31* showed significant reduced Al content in the root and root cell wall, while *xth15* showed no change in the root Al content but reduced cell wall Al content, the complementation lines rescued Al sensitivity. however, the underlying mechanisms for these three genes seems completely different. The mutant *xth31* has remarkably lower XET activity and significant less xyloglucan (XyG) content in the cell wall, and exogenous supply of xyloglucan significantly ameliorates Al toxicity by reducing Al accumulation in the roots due to the formation of Al-xyloglucan complex as verified by ^{27}Al-NMR (Zhu et al., 2012). Further investigation demonstrated that in Arabidopsis the predominant XEH function of XTH31 forms heterogeneous dimer with a XET encoded XTH17, as a result, mutation of *XTH17* also resulted in the very similar phenotypes with *XTH31* in Al resistance (Zhu et al., 2014a). However, in the *xth15* mutant, although less Al was accumulated in the cell wall, but more Al was sequestrated into vacuoles via the possible functioning of Al transporter ALS1 in the tonoplast (Zhu et al., 2013).
XyG is the major primary wall hemicellulose in non-poalean monocotyledons and dicotyledons. Structurally, the backbone of XyG consists of (1–4)-linked β-D-glucopyranosyl residues, which can be substituted in a regular pattern with α-D-xylopyranosyl residues at *O*-6. These xylosyl-residues can be further substituted, and the side-chain galactosyl residue can be substituted with an *O*-acetyl-substituent, mainly on the *O*-6 position. we found that the two corresponding *TBL27* mutants, *axy4-1* and *axy4-3*, were more Al sensitive than wild type Col-0 (WT) plants, which was corresponding to the increased Al accumulation in the hemicellulose fraction of the mutants although the total sugar content of hemicellulose fraction showed no difference with WT , suggesting that modulation of the *O*-acetylation level of XyG influences the Al sensitivity in Arabidopsis by affecting the Al binding capacity in the hemicellulose (Zhu et al., 2014b). Besides the effect of acetylation of XyG, recently we further found that reduced fucosylated XyG level of *AXY3* (*XYLOSIDASE1*) resulted in increased Al sensitivity, while two T-DNA insertional mutants with increased fucosylated XyG level of *AXY8* (*FUC95A*) increased Al resistance in Arabidopsis (Data not published). In the future studies, we will tried to investigate the upstream regulators that involve in the modification of the content and structure of XyG, thus elucidate the pathways of how plants modify cell wall properties in order to counteract Al toxicity for their better survive and growth in the acidic soils.

ACKNOWLEDGMENT
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REFERENCES

Zhu XF, Sun Y, Zhang BC, Wan JX, Liu Y, Wang ZW, Shi YZ, Zhou YH, Zheng SJ. 2014. TRICHOME BIREFRINGENCE-LIKE27 Affects Aluminum Sensitivity by Modulating the O-Acetylation of Xyloglucan and Aluminum-Binding Capacity in Arabidopsis. Plant Physiology. 166:181-189

Zhu XF, Wan JX, Sun Y, Shi YZ, Braam J, Li GX, Zheng SJ. 2014. Xyloglucan Endotransglucosylase-Hydrolase17 Interacts with Xyloglucan Endotrans- glucosylase-Hydrolase31 to Confer Xyloglucan Endotransglucosylase Action and Affect Aluminum Sensitivity in Arabidopsis. Plant Physiology. 165:1566-1574.

Zhu XF, Lei GJ, Wang ZW, Shi YZ, Braam J, Li GX, Zheng SJ 2013. Coordination between apoplastic and symplastic detoxification confers plant Al resistance. Plant Physiology. 162:1947-1955.

Zhu XF, Shi YZ, Lei GJ, Fry SC, Zhang BC, Zhou YH, Braam J, Jiang T, Xu XY, Mao CZ, Pan YJ, Yang JL, Wu P, Zheng SJ. 2012. XTH31, encoding an in-vitro XEH/XET-active enzyme, regulates Al sensitivity by modulating in-vivo XET action, cell wall xyloglucan content and Al binding capacity in Arabidopsis. The Plant Cell. 24:4731-4747.

Yang JL, Zhu XF, Peng YX, Zheng C, Li GX, Liu Y, Zheng SJ. 2011. Cell wall hemicellulose contributes significantly to Al adsorption and root growth in Arabidopsis. Plant Physiology. 155: 1885 – 1892.

STOP1, SENSITIVE TO PTOTON RHIZOTOXICITY 1, regulating Al and proton tolerant system in Arabidopsis

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Abstract
A study of Arabidopsis identified a key transcription factor that regulate expression of various genes regulating aluminum (Al) tolerance and proton tolerance. The mutant showed hypersensitivity to proton rhizotoxicity was named as stop1, and showed hypersensitivity to Al rhizotoxicity. The hypersensitiveness was caused by the dysfunctional mutation in a C2H2 zinc finger protein STOP1, which resulted in suppression of various genes control Al and proton tolerance. Various major Al tolerance genes of Arabidopsis such as ALMT1 is regulated the expression by STOP1. Comprehensive studies of the system uncovers complex Al tolerance mechanisms and its evolution in land plant species.

STOP1 regulating system in Arabidopsis
A mutant of Arabidopsis stop1 was first isolated as proton sensitive mutant. Root growth of stop1 was severely suppressed by low pH (proton rhizotoxicity), which was enhanced in the low Ca solutions. The mutant, interestingly, was hypersensitive to Al because of suppression of Al-inducible *AtALMT1* expression (Iuchi *et al*, 2007). STOP1 also regulates expression of *AtMATE* (Liu *et al.,* 2007) and *ALS3* (Larsen *et al*., 2005), which are critical genes for Al tolerance in Arabidopsis. Systems biology identified that various genes regulating ion homeostasis, cell-wall stabilization and N/S metabolism are suppressed in the mutant, and which could explain hypersensitiveness to proton rhizotoxicity (Sawaki *et al*. 2009). A part of STOP1 regulating system is likely regulated by STOP2, a unique homologue in Arabidopsis genome (Kobayashi *et al*., 2014). However, the most critical gene regulating Al tolerance of Arabidopsis, *AtALMT1*, required STOP1 for Al inducible expression (Iuchi *et al*, 2007).

Al sesing and cross-talk of other stress factors in STOP1 regulating system
AtALMT1, encoding Al activated malate transporter in Arabidopsis (Hoekenga OA *et al.*, 2006), expression is sharply induced by Al. Electrostatic modeling study identified that *AtALMT1* expression is regulated by $\{Al^{3+}\}_{PM}$ (Al^{3+} activity at the plasma membrane surface) (Kobayashi *et al.,* 2013). *AtALMT1* expression is activated at lower level of Al that suppresses Al tolerance in the dysfunctional mutant of *AtALMT1*. Characterization of *AtALMT1* promoter identified that binding of the STOP1 is essential step of Al-inducible expression, suggesting that activation of STOP1 by Al signal (Kobayashi *et al.*, 2007). In previous study, we found that *AlALMT1* expression involves protein phosphorylation process (Tokizawa *et al*., 2015), while particular protein kinases have not identified yet. On the other hand, *AtALMT1* and *AtMATE* are inducible by MAMP (microbe associated molecular pattern) peptides and other signal induces such as hydrogen peroxide (Kobayashi *et al*., 2013). It suggests that Al activation and other stress responses may cross talk in STOP1 regulating system.

Conservation of STOP1 regulating system in land plant species
STOP1 orthologue in rice, ART1, regulate expression of various genes control Al tolerance (Yamaji *et al*., 2009). Functional orthologues were identified in wide range of plant species (Fan *et al.,* 2015; Ohyama *et al*., 2013), including bryophyte. It indicates that STOP1 system is an evolutionally old system, and is conserved among land plant species. Proton tolerance would be older than Al tolerance because the trait would be essential for evolutional process when plants' ancestors adapted to land from aquatic environments. Tolerance to submerge stress, which induces cytosolic acidosis, is predicted as essential characteristics of ancestors.

REFERENCES
Fan *et al.* (2015) Characterization of an inducible C2H2-type zinc finger transcription factor VuSTOP1 in rice bean (*Vigna umbellata*) reveals differential regulation between low pH and aluminum tolerance mechanisms. *New Phytol.*

Hoekenga OA *et al.* (2006). AtALMT1, which encodes a malate transporter, is identified as one of several genes critical for aluminum tolerance in Arabidopsis. *Proc Natl Acad Sci U S A*. 103, 9738-9743.

Iuchi, S. *et al* (2007) Zinc finger protein STOP1 is critical for proton tolerance in Arabidopsis and coregulates a key gene in aluminum tolerance. *Proc Natl Acad Sci U S A*. **104**:9900-9905.

Kobayashi et al. (2007) Characterization of AtALMT1 expression in aluminum inducible malate release and its role for rhizotoxic stress tolerance in Arabidopsis thaliana. *Plant Physiol.* 145:843-852

Kobayashi *et al.* (2013) Molecular and physiological analysis of Al3+ and H+ rhizotoxicities at moderately acidic conditions. *Plant Physiol.* 163: 180-92

Kobayashi, Y. *et al*. (2013) Characterization of complex regulation of AtALMT1 expression in response to phytohormones and other inducers. *Plant Physiol*. **162**:732-740.

Kobayashi, Y. *et al*. (2014) STOP2 Activates Transcription of Several Genes for Al- and Low pH-Tolerance that Are Regulated by STOP1 in Arabidopsis. *Mol Plant*. **7**:311-322.

Larsen et al (2005) ALS3 encodes a phloem-localized ABC transporter-like protein that is required for aluminum tolerance in Arabidopsis. *Plant J* 41: 353-363

Liu J *et al.* (2007) Aluminum-activated citrate and malate transporters from the MATE and ALMT families function independently to confer Arabidopsis aluminum tolerance. *Plant Journal* 57: 389-399

Ohyama *et al.* (2013) (2013) Characterization of *AtSTOP1* orthologous genes in tobacco and other plant species. *Plant Physiol.* 162: 1937-1946.

Sawaki, S. *et al*. (2009) STOP1 regulates multiple genes that protect Arabidopsis from proton and aluminum toxicities. *Plant Physiol*. **148**:969-980.

Tokizawa, M. *et al*. (2015) SENSITIVE TO PROTON RHIZOTOXICITY1, CALMODULIN BINDING TRANSCRIPTION ACTIVATOR2, and other transcription factors are involved in *ALUMINUM-ACTIVATED MALATE TRANSPORTER1* expression. *Plant Physiol*. **167**:991-1003

Yamaji *et al*. (2009) A zinc finger transcription factor ART1 regulates multiple genes implicated in aluminum tolerance in Rice. *Plant Cell* 21: 3339-3349

Molecular mechanisms of rice bean (*Vignaumbellata*) in adaptation to acid soils

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Abstract
Aluminum (Al) toxicity is one of the major factors limiting crop production in acid soils. However, there is great variation in Al tolerance among different plant species. Different from model plant species, landraces with long evolutionary history in acid soils must have their unique mechanisms to deal with soil acidity. Understanding of these mechanisms will be helpful for further improvement of crop Al tolerance through genetic modifications. Here we report the recent progress in molecular mechanisms of rice bean to deal with soil acidity.

INTRODUCTION
During the last decades, great progresses have been made in understanding of molecular mechanisms of Al tolerance in model plant species rice and Arabidopsis. However, some native landrace long adaptation to acid soils must have some unique mechanisms that are different from rice and Arabidopsis to deal with Al toxicity. Rice bean (*Vignaumbellata*) is a local landrace growing well in acid soils in South China. In this paper, I will focus on molecular mechanisms of Al-tolerance in this leguminous crop species.

Identification of Al-tolerance genes in rice bean
Rice bean has been cultivated in acid soils for a long history. It has therefore evolved strategies to deal with growth limiting factors frequently occurring in acidic soils such as Al toxicity and proton toxicity. One of the demonstrated mechanisms is Al-induced citrate secretion from root apex (Yang et al., 2006). To understand molecular basis of Al tolerance in rice bean, four SSH libraries (two forward and two reverse) were constructed. A total of 394 unigenes were identified, among which 20 genes were up-regulated at both low and high Al concentrations (Fan et al., 2014). Several of them have been functionally characterized.
VuMATE1 is localized to the plasma membrane and responsible for Al-induced citrate secretion in the root apex (Yang et al., 2011; Liu et al., 2013). Being not expressed in root apex in the absence of Al stress, *VuMATE1* expression was induced after 3 h of exposure to Al stress. Interestingly, characterization of Al-induced citrate secretion revealed a unique biphasic secretion pattern, i.e. an early phase (after 1.5 h of exposure) with lower amount of citrate secretion and a late phase (after 6 h of exposure) with large amount. We found *VuMATE2* is constitutively expressed in root apex and could be further induced by Al stress within 1 h of exposure to Al stress. Furthermore, the regulatory system responsible for Al-induced *VuMATE1* and *VuMATE2* expression differs. Therefore, rice bean has evolved two MATE-type citrate transporters to deal with Al toxicity.
We isolated and characterized a gene encoding STOP1-like protein, VuSTOP1, from rice bean (Fan et al., 2015). The expression of *VuSTOP1* could be induced by both Al and H^+ stress. Although VuSTOP1 could bind to the promoter of *VuMATE1*, the inconsistency in tissue location expression patterns between *VuSTOP1* and *VuMATE1* precludes VuSTOP1 as the major factor regulating *VuMATE1* expression. In planta complementation assay demonstrated that VuSTOP1 could significantly restore H^+ hypersensitivity but not Al^{3+} hypersensitivity of *Atstop1* mutant.
We isolated and characterized a gene encoding NAC protein, *VuNAC1* from rice bean. Overexpression of *VuNAC1* in Arabidopsis resulted in increased tolerance to Al stress. However, the improvement of Al tolerance in transgenic lines is not associated with well-known Al tolerance genes expression, nor does malate secretion. Genome-wide comparative transcriptomic analysis between WT and transgenic lines revealed that there are 128 genes that were specifically up-regulated in transgenic lines under Al stress.
A gene encoding formate dehydrogenase, *VuFDH*, is involved in both Al^{3+} and H^+ tolerance. VuFDH is mitochondrion localized and specifically catalyzeoxidation of formateinto CO_2along withreduction of $NAD^+$and $H^+$to NADH. The expression of *VuFDH* is greatly induced by Al^{3+} and H^+ stress. Overexpression of *VuFDH* in tobacco resulted in a decreased sensitivity to Al^{3+} and H^+ stress in transgenic lines (unpublished data).

ABA-responsive genes are involved in rice bean Al tolerance

We found exogenously application of ABA greatly restored Al-induced root growth inhibition in rice bean. Al stress resulted in the accumulation of ABA in root apex. Interestingly, improvement of Al tolerance by ABA is not associated with citrate secretion and Al exclusion. In addition, increment in Al tolerance appears to be not related to already-known rice bean Al-tolerance genes. Comparative transcriptomic analysis revealed that about one-third of Al-responsive genes are also ABA-responsive, indicating that Al signal overlaps with ABA signal. Furthermore, there are 954 genes that are specifically up-regulated in Al+ABA vs. ABA treatment when compared to Al vs. -Al treatment, indicating that Al stress triggers both ABA-dependent and ABA-independent signal transduction pathways to deal with Al toxicity.

REFERENCES

Fan W., Lou H.Q., Gong Y.L., Liu M.Y., Wang Z.Q., Yang .JL., Zheng S.J., 2014. Identification of early Al-responsive genes in rice bean (Vignaumbellata) roots provides new clues to molecular mechanisms of Al toxicity and tolerance. Plant Cell Environ 37: 1586-1597.

Fan W., Lou H.Q., Gong Y.L., Liu M.Y., Liu Y., MengJie Cao, Yang J.L., Zheng SJ., 2015. Characterization of an inducible C2H2-type zinc finger transcription factor VuSTOP1 in rice bean (Vignaumbellata) reveals differential regulation between low pH and Al tolerance mechanisms. New Phytol in press.

Liu M.Y., Chen W.W., Xu J.M., Fan W., Yang J.L., Zheng .SJ., 2013. The role of VuMATE1 expression in aluminium-inducible citrate secretion in rice bean (Vignaumbellata) roots. J Exp Bot 64: 1795-1804.

Yang J.L., Zhang L, Li Y.Y., You J..F, Wu P., Zheng S.J., 2006. Citrate transporters play a critical role in aluminium-stimulated citrate efflux in rice bean (Vignaumbellata) roots. Anna Bot 97: 579-584.

Yang X.Y., Yang J.L..Zhou Y., Pineros M.A., Kochian L.V., Li G.X., Zheng S.J., 2011. A de novo synthesis citrate transporter, Vignaumbellataand toxic compound extrusion, implicates in Al-activated citrate efflux in rice bean (Vignaumbellata) root apex. Plant Cell Environ 34: 2138-2148.

Investigating the transcriptional regulation of aluminium (Al) tolerance genes in triticale (x *Tritosecale*)

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Abstract
This study investigated the physiology and molecular biology of aluminium (Al) resistance in wheat and rye and in the triticale lines generated by crossing them. We compared relative root growth, organic anion efflux and relative expression of the major Al-resistance genes in these lines. Results indicate that the Al resistance of rye and wheat are not always additive in triticale. We conclude that the resistance genes from rye were expressed poorly in triticale compared to the wheat genes. This could be related to hexaploid wheat being less susceptible than diploid species to modifications that affect gene expression in newly-formed polyploid species.

INTRODUCTION

Rye (*Secale cereale*) grows well in poor soils and is known for its resistance to a range of other abiotic stresses including cold stress and Al toxicity. Triticale (× *Triticosecale* Wittmack) is a hybrid of wheat (*Triticum aestivum*) and rye. Triticale is amphidiploid (and allotetraploid) which means the plants are diploid for the two parental genomes and some tritcales resemble one parent more than the other. "Octoploid" triticale is generated from crossing a hexaploid wheat with rye and "hexaploid" triticale is generated from crossing tetraploid wheat with rye. The known mechanisms of tolerance in wheat and rye rely on the release (efflux) of organic anions from roots (Ma *et al.*, 2001; Ryan *et al.*, 2011). In wheat, the Al-activated malate efflux is controlled by *TaALMT1* (Sasaki *et al.*, 2004) which encodes an anion channel, and citrate efflux is controlled by *TaMATE1B* which encodes transport protein from the multidrug and toxic compound exudation (MATE) family (Ryan *et al.*, 2009; Stass *et al.*, 2008; Tovkach *et al.*, 2013). Homologues of these genes control Al-activated organic anion efflux in rye: *ScALMT1-M39.1* is a major gene controlling malate efflux (Collins *et al.*, 2008) and *ScFRD2* likely controls citrate efflux (Yokosho *et al.*, 2010). Stass *et al* (2008) compared the Al tolerance and anion efflux of triticale lines with the wheat and rye parents used to generate them. They concluded that tolerance of triticale was largely determined by citrate efflux which was mainly controlled by the wheat parent. This study investigates the physiology and molecular biology of this observation further.

MATERIAL AND METHODS

Aluminium resistance, organic anion efflux and expression of the major Al tolerance genes was measured in the Al-tolerant rye *L185*, the two hexaploid wheats *Carazhino* (Al-resistant) and *Egret* (Al-sensitive) and the two primary triticale lines generated by crossing them *L185*x*Carazinho* and *L185*x*Egret*. Al tolerance in *Carazinho* relies on the efflux of malate which is controlled by *TaALMT1* and citrate efflux controlled by *TaMATE1B*. *Egret* shows little or no organic anion efflux. The high Al tolerance of the rye *L185* depends on malate release, controlled by *ScALMT1-M39.1*, and citrate efflux which is controlled by *ScFRDL2*.

RESULTS

Al resistance was compared in the two primary triticale lines (*L185*x*Carazinho* and *L185*x*Egret*) and the rye (*L185*) and wheat (*Carazhino* and *Egret*) parental lines used to generate them. Al resistance was estimated by relative root length after 4 d growth in 100 and 150 μM Al to separate the lines (**Figure 1A**). Rye *L185* was more resistant than *Carazinho* and both triticale lines. Two conclusions emerge from these data: the Al-resistant phenotypes in the rye and wheat parents are not additive in triticale, and neither of the triticale lines was as resistant as the rye parent. Since Al treatment induces organic anion efflux from roots over several hours in some species (Delhaize *et al.*, 2012; Ma *et al.*, 2001) anion efflux was measured in the presence of 30 μM $AlCl_3$ with or without a 24 h pretreatment in the same solution. Malate efflux was greatest from *Carazinho* and the *L185*x*Carazinho* triticale and low from *Egret* wheat, the *L185*x*Egret* triticale and rye (**Figure 1B**). Al pretreatment did not induce malate efflux from any genotype. Citrate efflux without a pretreatment in Al was highest from *Carazinho* and *L185*x*Carazinho* and low in the other genotypes (**Figure 1B**). Al pretreatment did not increase citrate efflux from either wheat genotype or the *L185*x*Carazinho* triticale, but it did induce an almost four-fold increase in efflux from rye. Efflux from *L185*x*Egret* also increased but remained at a low level. These data show: (i) Al-induced malate and constitutive citrate efflux is transferred from the Al-resistant *Carazinho* wheat to triticale (*L185*x*Carazinho*), (ii) Al pretreatment in rye strongly enhanced citrate efflux but not malate efflux, (iii) the rye contributed less to the citrate efflux phenotype in the triticale than *Carazinho* wheat. The Al-activated efflux of malate from roots is controlled by the *TaALMT1* gene in wheat and the *ScALMT1-M39.1* in rye. Relative expression of these genes were compared in the lines (**Figure 1C**). *TaALMT1* expression was high in *Carazinho* and in *L185*x*Carazinho* triticale indicating that this gene functions similarly in the hexaploid and octoploid genomes (**Figure 1C**). *TaALMT1* expression was low in *Egret* and *L185*x*Egret* as expected. *ScALMT1-M39.1* expression was significantly higher in the roots of *L185* rye than in any of the other genotypes including the two triticale lines (**Figure 1C**) indicating that expression of the *ScALMT1-M39.1* gene was suppressed in both octoploid backgrounds.

Citrate efflux is controlled by *TaMATE1B* in wheat and *ScFRDL2* in rye. *TaMATE1B* expression was high in *Carazinho* and *L185*x*Carazinho* triticale but low in *Egret* and absent from all other genotypes (**Figure 1D**). This indicates that *TaMATE1B* functions similarly in the hexaploid and octoploid genomes. *ScFRDL2* was expressed in *L185* rye and *L185*x*Egret* triticale but low in *L185*x*Carazinho* (**Figure 1D**). These data indicate that expression of the rye *ScFRDL2* was suppressed in triticale containing the *Carazinho* genome but not with the *Egret* genome.

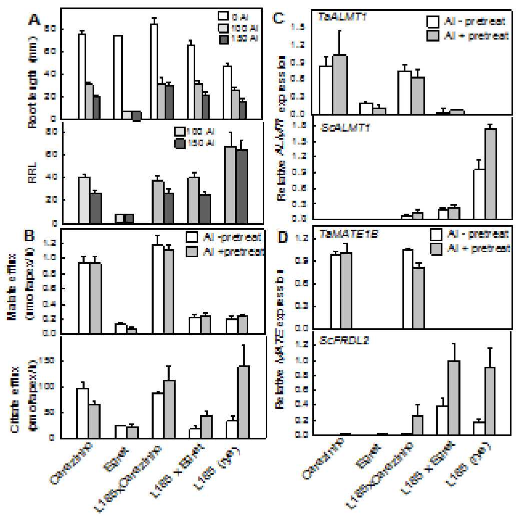

Figure 1 Comparison of relative root length (RRL), organic anion efflux and gene expression in the wheat cvs Carazinho and Egret, the rye line L185 and the triticale lines generated from them. (A) Net root growth (upper) and relative root growth (lower) of lines. (B) Malate efflux and citrate efflux in 30 μM $AlCl_3$ with and without a pretreatment in the same solution. (C) Relative expression of the wheat (*TaALMT1*) and rye (*ScALMT1-M39.1*) genes controlling Al-activated malate efflux. Reference gene was GAPDH in each species. (D) Relative expression of wheat (*TaMATE1B*) and rye (*ScFRDL2*) genes controlling citrate efflux from roots. Reference genes were GAPDH from each species. Data show mean and SE (n=5-8 for A, n=4 for B, n=3 biological reps for C and D).

DISCUSSION

These results show that the Al-resistance of rye and wheat are not always additive in triticale. The full resistance of the rye parent was not conferred to triticale because the resistance genes *ScALMT1-M39.1* and *ScFRDL2* from rye were not expressed as highly in all triticale lines. Rye *ScFRDL2* was expressed in *L185*x*Egret* but not the *L185*x*Carazinho*. The Al-induced efflux of citrate measured in rye was not detected in either triticale, despite the high expression of *ScFRDL2* in *L185*x*Egret*. In wheat, by comparison, the expression of *TaALMT1* and *TaMATE1B* from *Carazinho* were both high in *L185*x*Carazinho* triticale and the Al-activated efflux of malate and constitutive efflux of citrate controlled by these genes were also detected. In conclusion, the Al-resistance genes derived from wheat were more highly expressed and functional in triticale than were the genes from rye.

Hybridization of two species and whole-genome duplication (allopolyploidy) leads to genetic and epigenetic modifications in the merged genome. The genome becomes a target to transcription factors and DNA modifying proteins from the other species which can induce modifications to chromatin and methylation of DNA. Genes can be silenced and even lost in newly-generated polyploid species. Therefore, gene behaviour in polyploid species can vary from their parental lines. The altered expression of Al resistance genes in rye and triticale reported here is likely to be linked with these changes. The finding that the rye genes were expressed poorly in triticale compared to the wheat genes might be because wheat is already a polyploid species and, therefore, its genome is less susceptible to the changes and modifications that affect gene expression in new polyploid species.

REFERENCES

Collins *et al.* 2008. Genetics 179, 669-692; Delhaize *et al.* 2012. Trends Plant Sci 17, 341-348; Ma *et al.* 2001. Trends Plant Sci 6, 273-278; Ryan *et al.* 2009. Plant Physiol 149, 340-351; Ryan *et al.* 2011. J Exp Bot 62, 9-20; Sasaki *et al.* 2004 Plant J 37, 645-653; Stass *et al.* 2008. J Plant Nutr Soil Sci 171, 634-642; Tovkach *et al.* 2013. Plant Physiol 161, 880-892; Yokosho *et al.* 2010. Funct Plant Biol 37, 296-303.

Physiological and molecular characterization of pigeonpea (*Cajanus cajan*) for high phosphorus uptake through acid phosphatse activity

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Abstract
In our earlier work we identified high P uptake pigenopea genotypes, efficient in taking up more P under P deficient conditions. In this study we attempted to characterize high P uptake pigeonpea genotypes by measuring the activity of acid phosphatase(APase) and correlating it to the tissue P content. Further, we looked into the expression pattern of some of the important genes generally known to be highly expressed under P deficient conditions. We found that the high P uptake types have high acid phosphatase activity and high tissue P content. The expression of related genes like PAP-10, 12,15, 23 and 26 was also high under deficient conditions

INTRODUCTION

Availability of phosphorus (P) is a major constraint limiting the productivity of crops in acid soils owing to its adsorption and precipitation; as a result almost 85-90% of the P present in the soils is rendered unavailable to the plants. The concentration of P in the soil solution is much lower and ranges from 0.001 mg/L to 1 mg/L (Brady and Weil, 2002). It is also well established that 50-80 % of the total P in soil is organically bound to various substances and present mainly as phytic acid, this form of P is also not available to the plants. Plants have adapted several strategies to access the bound form of P. The P present in Pyhtic acid would be available to the plants only after it is hydrolyzed by enzymes like Acid phosphatase (APases). APases are the key enzymes involved in the acquisition of organically bound P from the soil. Purple acid phosphatases (PAPs) are a group of APases known to be involved in hydrolyzing the phytic acid. These phosphatases play a key role in phosphorous cycle by solubilising organic phosphates into available forms that support growth of crop plants (Wyszkowska and Wyszkowski, 2010). The major objective of this work was to characterize the identified high P uptake genotypes and to assess the relationship between APase activity and tissue P concentration and to examine the expression pattern of genes encoding PAPs.

MATERIAL AND METHODS

Pre-germinated seeds of the selected pigeonpea genotypes were grown in polythene bags filled with 2kg vermiculite and allowed to grow with only de-ionized water for 10 days. After 10 days all recommended nutrients were provided through half strength Hoagland nutrient solution for normal growth and development. After another 10 days, one set of plants were continued in Hoagland's nutrient solution with KH_2PO_4 as P source (normal), another set of plants with Phytic acid as P source (organic P), and the third set of plants without P [P (-)] for 20 days. The plants were maintained at a temperature of 32^0C and a relative humidity of 85 per cent with light intensity of 800 μ moles m^{-2} sec^{-1}. Observations on tissue P content, root and shoot APase activity and expression analysis of five important genes was carried at the end P stress (40 days after sowing).

RESULTS AND DISCUSSION

Plants have evolved numerous mechanisms to adapt to P deficient soils. Synthesis and secretion of APase from roots into the rhizosphere is one the efficient mechanisms to hydrolyze soil organic P (George *et al*., 2005; Tran *et al*., 2010). Genetic variability is also known to exist in pigeonpea both under sufficient and deficient P condition (Krishnappa, 2011). We observed increased activity of APase in roots of all the genotypes under P deficient conditions (Figure: A). There was a variation in the root P under P deficient conditions (Figure: B), this variability in P was related to the differences in the activity of APase, as reported by Arun (2012). In this study there was a considerable increase in root APase activity in high P uptake types like ICP3226 and BRG-2 under deficient P condition. A significant positive relationship between APase and P concentration observed (Figure:C) confirms the role of APases in acquiring P

Purple acid phosphatases (PAPs) are a group of APases that catalyze the hydrolysis of a wide range of phosphate esters in plants, fungi and animals. Hence emphasis of this study was to confirm the P uptake capacities of already identified contrasting pigeonpea genotypes, and to understand the molecular mechanisms involved in uptake of P and also to assess the involvement of Apases in P uptake.

Expression analysis of different PAP genes indicated that most of the reported genes are being expressed under deficient P conditions (Figure: D). The expression levels of all the genes were higher under P deficient conditions in high P uptake types like ICP-3226. The expression of PAP-10 was high in roots and expression levels correlated with enzyme activity. Similarly the expression levels of PAP-12 was high in high P uptake types, the expression levels of PAP15 was high in all the tissues, indicating its role in both acquisition and transport. Other related genes like PAP23 and PAP26 were highly expressed in all the tissues under P deficient conditions

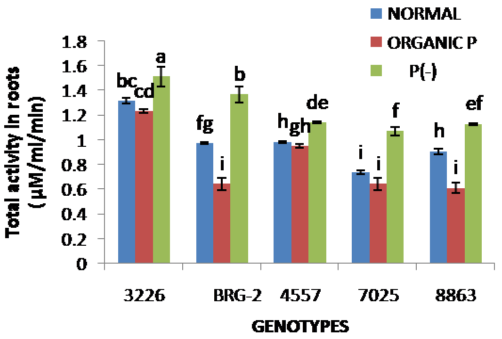

Figure: A. Variation in the activity of acid phosphatase in contrasting pigeonpea genotypes under different sources of P

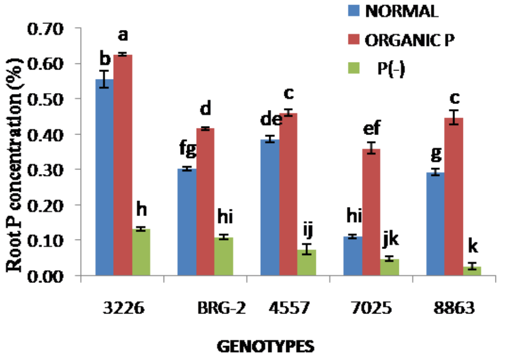

Figure: B. Tissue P content of contrasting Pigeonpea Genotypes under different sources of P

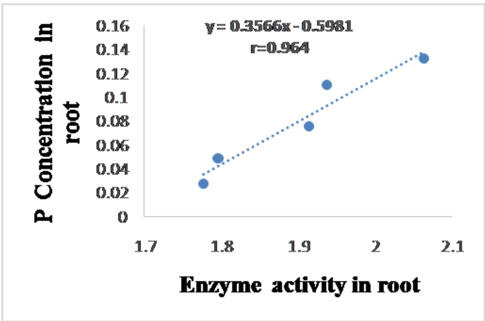

Figure: C Relationship between enzyme activity and P concentration in the roots

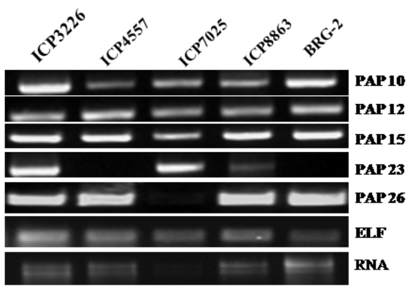

Figure: D. Expression pattern of PAP Genes in the roots of contrasting pigeonpea genotypes under P deficient conditions through RT-PCR

REFERENCES

Arun, N. 2012. Identification of pigeon pea genotypes for high phosphorus uptake through acid phosphatase activity: a biochemical approach, M.Sc (Agriculture) thesis submitted to the University of Agricultural Sciences Bangalore, India

Brady, N.C. and Weil, R.R. 2002. *Elements of the Nature and Properties of Soils.* Prentice Hall, New Jersey.

George, T.S., Richardson, A.E., Simpson, R.J., 2005, Behaviour of plant-derived extra- cellular phytase upon addition to soil.Soil Biol. Biochem.,**37**: 977–988.

Krishnappa.R, Umesh H.R, and Aftab Hussain I.S. 2011. Variations in phosphorus uptake and its utilization in pigeonpea genotypes grown under phosphorus deficiency Journal of Food Legumes 24(2): 96-100.

Tran, H.T., Hurley, B.A. and Plaxton, W.C., 2010b, Feeding hungry plants: The role of purple acid phosphatases in Phosphate nutrition. Plant Sci., **179**: 14-27.

Wyszkowska. J, Wyszkowski M.2010. Activity of soil dehydrogenases, urease, and acid and alkaline phosphatases in soil polluted with petroleum**.** J Toxicol Environ Health A.73(17-18)

Aluminium tolerance in tomato: Screening techniques and genetic control

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Abstract
A study was undertaken to infer gene governing Al tolerance in tomato. Tolerant genotype 'Pant Bahar' was crossed with sensitive genotype 'selection-18'. Al tolerance in the parents, F_1, F_2 and backcross generations was estimated by using the re-growth of the primary root after staining and scoring of fluorescent signals. The F_1 hybrids responded similarly to the tolerant parents, indicating dominance of Al-tolerance over sensitivity. The segregation ratios obtained for Al tolerance and sensitivity in the F_2 and back cross generations were 3:1 and 1:1, respectively. These results indicated that Al tolerance is a monogenic dominant trait that can be easily transferred to agronomic bases through backcross breeding technique.

INTRODUCTION
Aluminum (Al) is one of the most abundant metals in the earth's crust and is considered as the main abiotic stress that causes 25–80% yield losses in crop plants grown on soils containing excessive aluminium (Singh et al., 2011). The first visible symptom of Al toxicity is rapid inhibition of root growth which can occur within hours or even minutes of exposure to Al^{3+} (Kochian et al., 2005). Callose induction in roots has also been identified as a reliable physiological trait of Al toxicity under short-term exposure to aluminium (Singh et al., 2015). Some plant species have adaptive mechanisms to reduce the harmful effects of Al toxicity. The most important mechanism of Al tolerance is the secretion of anions of organic acids from the roots (Kochian et al., 2005).
The most reliable screening parameters are root re-growth after staining and callose accumulation (Singh et al., 2015) through hydroponic technique. Such technique has unconditional advantages over field screening. Different plant species exhibit distinct variation in tolerance and/or sensitivity to Al^{3+} and these variations are genetically controlled (Foy, 1988). Genetic variation for Al^{3+} tolerance exists among tomato genotypes (Singh et al., 2007). Several studies have demonstrated that Al-tolerance can be a simple (Singh and Choudhary, 2010, Singh et al., 2015) or a complex (Aniol, 1990) trait. Information on the genetics of Al tolerance in tomato is not available. The present study was, therefore, undertaken to determine the genetics of Al tolerance in tomato

MATERIALS AND METHODS
The parental lines such as 'Pant Bahar, and 'Selection -18' were selected on the basis of previous studies on the assessment of aluminium tolerance (Singh et al., 2007). Preliminary tests using 74, 100, 174 and 222 μM of $AlCl_3.6H_20$ in nutrient solution showed that 174 μM was the most efficient concentration to classify the parental genotypes analysed as tolerant and sensitive. The Al tolerance response of parental lines F_1's, F_2 s and backcross progeny was evaluated by root re-growth after staining and fluorescent signals (callose accumulation) under controlled greenhouse conditions. The root re-growth after staining has been routinely used in screening for aluminium tolerance in crop plants (Singh et al., 2007; Singh et al., 2012). The procedure as given by Polle et al. (1978), with partial modifications, was used in present study testing tomato seedlings for aluminum tolerance. Seeds were surface sterilized with 0.1% $HgCl_2$ for 2–3 minutes and rinsed thoroughly with distilled water and then transferred to filter paper in the growth chamber for germination. After 15 days, the seedlings were transferred to plastic containers in nutrient solution (4.0 mM $CaCl_2$, 6.5 mM KNO_3, 2.5 mM $MgCl_2$, 0.1 mM $(NH4)_2$ SO_4, 0.4 mM NH_4NO_3) that was adjusted to pH 4.5 with 0.1 M HCl or 0.1 M NaOH solutions. Seedlings were kept in the above nutrient solution for 2 days under continuous light and aeration. Thereafter, the seedlings were maintained for 24 h on the fresh nutrient solution containing 174 μ M Al concentrations, because this concentration gave best discrimination for tolerant and sensitivity in tomato. The root of seedlings were then placed in aerated distilled water and washed for 30 min to remove excessive aluminium on the root surface. The roots along with seedlings were then immersed in hematoxylin solution of 2 g/l and 0.02 g/l KIO_3 for 15–30 min. The seedlings of the parent genotypes, F_1, F_2 and backcross generations were then returned to the nutrient solution without Al for 48 h. The response of each plant was determined as re-growth of the primary root after root staining. Mean primary root re-growth <0.5 cm was classified as Al sensitive, while those with the mean root re-growth significantly >1.0 cm were considered tolerant. (Singh et al., 2015).
Evaluation of Al stress induced callose biosynthesis has been assessed by using aniline blue stain. The seedlings were evaluated to aluminium stress for 48 hours under conditions of 0 and 174μM Al in nutrient solution, having pH4.5, after which whole roots were transferred to fixative containing 10% formaldehyde, 5% glacial acetic acid and 10% ethanol (FAA) for 1 h. After this period, the root tip (first centimetre) was excised and washed in deionized water. The 1 cm segments were stained for 10 min in a solution of 0.1% water soluble aniline blue in

50 mM Glycine-NaOH buffer at pH 9.5 (Kauss, 1992). After staining, callose production was visualized under fluorescence microscope. Seedlings were visually scored as low fluorescent (1) or moderate fluorescent (2) and high fluorescent signals (3). Plants were considered tolerant, if scored low and medium fluorescent signals, and sensitive when high fluorescent signals. This was done because the tolerant and sensitive parents showed low, medium or high fluorescent signals.
The mode of inheritance was assessed by the chi-square ($\chi 2$) analysis of the observed ratios of tolerant and sensitive plants in the segregating populations derived from crosses for testing the goodness of fit into assumed phenotypic ratios.

RESULTS AND DISCUSSION

This is the first report on the genetics of tolerance to aluminium toxicity in tomato. The data recorded for root re-growth showed that the parents 'Pant Bahar had long root re-growth (3.99 cm and 2.68 cm, respectively) compared with sensitive genotype (Selection-18) which had short root re-growth of 0.37 cm under controlled nutrient solution study. The F_1 progeny of the cross 'Pant Bahar' x 'Selection 18' showed similar response to the tolerant parent. Reaction of F_2 plants to aluminium stress showed segregation with a 3 (tolerant): 1 (sensitive). It indicates that Al tolerance is controlled at a single locus in the Pant Bahar and its cross. The single locus control of Al tolerance was further confirmed from segregation pattern in backcross generations based on root re-growth after root staining and fluorescent signals (callose accumulation). The present results are in agreement with the previous results on other crops (Singh and Choudhary 2010; Singh et al. 2015). In contrast, complex resistance has also been reported in wheat (Carver and Ownby, 1995). Since aluminium tolerance of 'Pant Bahar' is monogenic, it could be easily transferred to high yielding genotypes. This study also allows developing Al^{3+} tolerant of tomato, investigating physiological and molecular mechanisms underlying Al^{3+} resistance.

REFERENCES

Aniol, A. 1990. Genetics of tolerance to aluminium in wheat (*Triticum aestivum* L. Thell). Plant Soil 123, 223-227.

Carver, B. F. and Ownby J. D. 1995. Acid soil tolerance in wheat. Advances in Agronomy 54, 117- 173.

Foy, C. D. 1988. Plant adaptation to acid, aluminum toxic soils. Communication Soil Science and plant Analysis 19: 959-987.

Kochian, L., M. A. Pineros, and O. A. Hoekenga, 2005: The physiology, genetics and molecular biology of plant aluminium resistance and toxicity. Plant Soil, 274:175-195.

Kauss, H., 1992: Callose and callose synthase. *In* Molecular Plant Pathology. Vol. 2. A Practical Approach. Eds. S.J. Gurr,M.J.McPherson and D.J. Bowles. Oxford University Press, New York, pp 1– 8.

Polle, E., Konzak C. F., and Kittrick J. A. 1978. Visual detection of aluminium tolerance levels in wheat by hematoxylin staining of seedlings roots. Crop Science, 18: 823-827.

Singh, D., and Choudhary, A. K. 2010: Inheritance pattern of aluminium tolerance in pea (*Pisum sativum* L). Plant Breeding, 129: 688-692.

Singh, D. Dikshit H.K and Kumar A. 2015. Aluminium tolerance in lentil with monogenic inheritance pattern. Plant Breeding, 134: 105–110

Singh, D., Dikshit, H.K., Singh, R. 2012. Variation of Aluminium tolerance in lentil. Plant Breeding 131: 751-761.

Singh, D, Rai A. K., Sureja A. K and Bhardwaj R. 2007: Effects of aluminium toxicity on tomato. Paper Presented in 2nd Indian Horticulture Congress held in ICAR Research Complex Barapani, Meghalaya, India from 18–21 April, 2007

Singh, D., Singh N. P., Chauhan S. K., and Singh P. 2011. Developing aluminium tolerant crop plants using biotechnological tools. Current Science, 100, 1807-1813.

A genome-wide association study of Aluminum tolerance in *Arabidopsis thaliana*

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Abstract
To identify genetic mechanisms associated with Al tolerance to adapt to acid soil stress, we performed a GWAS of root growth under Al stress in 196 A. thaliana accessions. The accessions divided into six subpopulations showed a broad variation of Al tolerance, where there were no significant differences in Al tolerance between the subpopulations, but tolerant/sensitive outliers were found in the subpopulations. GWAS identified over 100 loci associated with Al tolerance including signaling molecules and AtALMT1. Polymorphism in the AtALMT1 promoter was associated with the AtALMT1 expression. Our GWAS identified complex genetic architecture of Al tolerance.

INTRODUCTION

Al tolerance is a complex trait in *Arabidopsis thaliana*, which is regulated by various genes. For example, we have reported a series of Al tolerance genes such as STOP1 and STOP2 transcription factors that regulate other Al tolerance genes including AtALMT1, MATE and ALS3 (Iuchi, S. *et al.* 2007; Sawaki, S. *et al.* 2009; Kobayashi, Y. *et al*. 2014; Tokizawa, M. *et al.* 2015). Beside, various other signaling molecules are involved in the expression regulation of Al tolerance pathways, where genetic factors related to Al tolerance doesn't become yet clear (Kobayashi, Y. et al. 2013). To study these unknown factors, we performed GWAS (genome-wide association study), which is an approach to identify new genetic loci associated with target phenotypes across genome. Here, we carried out GWAS in advantageous Arabidopsis to identify novel genetic factors associated with Al tolerance.

MATERIAL AND METHODS

We used 196 natural *A. thaliana* accessions which were obtained from the seed stock centers: ABRC, NASC and RIKEN BRC. The primary root lengths of 5-day-old seedlings were measured for plants grown in a control solution without Al (pH 5.0) and in stress solutions with 5 μM AlCl3. The average relative root length (RRL; root length under stress conditions/root length under control conditions [%]) of five seedlings was determined for each line. The GWAS was performed in TASSEL 3.0 software using mixed linear model with 175,324 SNPs (Bradbury, P.J. et al. 2007). The STRUCTURE 2.3.4 software was used to estimate the genetic population structure with 1000 SNPs (Hubisz, M.J. et al. 2009). The SNP matrix obtained from public database (Atwell, S. et al. 2010; Cao, J. et al. 2011). TAIR database and AgriGO web-based tool were used in functional annotation and GO term enrichment analysis. The levels of gene transcripts were determined by real-time PCR as described previously (Kobayashi, Y. et al. 2014).

RESULTS AND DISCUSSION

The frequency distribution of RRL for Al tolerance in 196 *A. thaliana* accessions showed a normal distribution ranged from 6.6% to 70.3% with high broad sense heritability (Hb2 > 0.90). The population structure analysis inferred these accessions were classified into six subpopulations, which were corresponding to geographic regions: roughly at North/Western/Southern/Eastern Europe; Central Asia and North America. There were no significant differences in the mean of Al tolerance between the six subpopulations. However, there were eight accessions which showed markedly different in Al tolerance levels from the subpopulations, suggesting that any favorable/deleterious mutations in genetic background from each subpopulation would cause their variations. GWAS detected the most significant 21 association of SNPs ($P < 0.0001$) for Al tolerance across genome. The 15th-ranked SNP located in the promoter region of AtALMT1 encoding a malate transporter and it plays a critical role in Al tolerance by excluding of Al from rhizosphere. The RRL mean value and AtALMT1 expression among accessions associated with the tolerant/sensitive SNP alleles. Besides, according to the regression predict result, we focused on the top 140 SNPs ($P < 0.001$) explained 75% of variation of Al tolerance, where we identified 124 potential candidate genes located in linkage disequilibrium within the 10-kb windows. GO terms in molecular function such as "transcription factor activity," "transferase activity," "transporter activity," "protein binding" and "ATPase activity" were enriched in the potential candidate genes. In addition, some SNPs were linked to the genes involved in the various signaling pathways. Our GWAS focusing on root growth under the Al stress identified associations between SNPs and genes with various functions, which might explain the natural variation of Al tolerance in Arabidopsis and contribute to maintain root growth under acid soil stress.

REFERENCES

Iuchi, S. *et al.* 2007. Zinc finger protein STOP1 is critical for proton tolerance in Arabidopsis and coregulates a key gene in aluminum tolerance. Proc Natl Acad Sci U S A. 104:9900-9905.

Sawaki, S. *et al.* 2009. STOP1 regulates multiple genes that protect Arabidopsis from proton and aluminum toxicities. Plant Physiol. 148:969-980.

Kobayashi, Y. *et al.* 2014. STOP2 Activates Transcription of Several Genes for Al- and Low pH-Tolerance that Are Regulated by STOP1 in Arabidopsis. Mol Plant. 7:311-322.

Tokizawa, M. *et al.* 2015. SENSITIVE TO PROTON RHIZOTOXICITY1, CALMODULIN BINDING TRANSCRIPTION ACTIVATOR2, and other transcription factors are involved in ALUMINUM-ACTIVATED MALATE TRANSPORTER1 expression. Plant Physiol. 167:991-1003.

Kobayashi, Y. *et al.* 2013. Characterization of complex regulation of AtALMT1 expression in response to phytohormones and other inducers. Plant Physiol. 162:732-740.

Bradbury, P.J. *et al.* 2007. TASSEL: Software for association mapping of complex traits in diverse samples. Bioinformatics 23:2633-2635.

Hubisz, M.J. *et al.* 2009. Inferring weak population structure with the assistance of sample group information. Mol Ecol Resour. 9:1322-1332.

Atwell, S. *et al.* 2010. Genome-wide association study of 107 phenotypes in arabidopsis thaliana inbred lines. Nature 465:627-631.

Cao, J. *et al.* 2011. Whole-genome sequencing of multiple Arabidopsis thaliana populations. (2011) Nature Genetics 43:956-963.

Project presentation: Innovative wheat breeding – fast phenotyping and DNA marker assisted selection for improved phosphorus uptake efficiency and aluminium tolerance

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Abstract
In the comming years agriculture will face great challenges: increased demand for food, climate changes and the lack of suitable land. Acid soils present the most important limiting factor of agricultural production and low yield, especially in cereals is caused by aluminium toxicity and phosphorus deficiency. One of the solutions is breeding of new cultivars with increased tolerance to aluminium toxicity as well as better phosphorus uptake and use efficiency. The aims of this project are: i) to initialise the innovative wheat breeding pipeline through the development of fast phenotyping and genotyping protocol and expert training; and ii) to detect potentially prospective genotypes as the initial breeding material.

INTRODUCTION

Increased demand for food in the world caused by an increase in the population size will emerge together with the problems caused by the change of climate and deficiency of arable land suitable for modern agricultural production. Acid soils (with pH lower than 5.5) are one of the most important limiting factors of agricultural production and are very rampant with around 40% of arable and 50% of potentially arable land in the world (vonUexküll i Mutert, 1995) and on the EU level 16.7% of soils are very acid (pH value below 4.2) http://eusoils.jrc.ec.europa.eu/). Bogunović et al. (1996) estimated that in Republic of Croatia exists around 830 000 ha of acid soils. Low productivity of agricultural crops, especially cereals, on acid soils is due to aluminium (Al) toxicity and lack of nutrients, namely phosphorus (P) (Kochian et al., 2004). Modern agricultural production is based on high input of phosphorus fertilizers which are produced from phosphate rock and it is expected in near future (in the next 50 – 200 years) that the natural sources will be depleted (Jasinski, 2011). European Union is almost completely dependent on the import of raw phosphates (in year 2010 import was 1.4 milion tons) (http://ec.europa.eu/science-environment-policy). The most efficient solution for agricultural production on acid soils is breeding of new cultivars with increased tolerance to Al toxicity as well as better P uptake and use efficiency. Traditional breeding is long term and expensive process with uncertain outcome and simultaneous selection on several traits is very complex. The aim of this project is to create faster and more efficient protocols, that include rapid phenotyping and genotyping techniques and demand expert knowledge.

MATERIALS AND METHODS

To develop the innovative wheat breeding platform a scientific project was awarded, that will consist of two work packages: phenotyping and genotyping. For the phenotyping part, experiment will consist of measuring several root traits on seedlings of over 100 Croatian wheat cultivars and breeding lines that will be grown in transparent acrylic boxes on filter papers. Composition of the nutrient solutions representing acid soil conditions (toxic levels of Al and low levels of P) will be calculated using the GeoChem EZ software. Seedlings will be photographed using a single lens reflex camera and obtained photographs will be analysed using WinRhizo Pro software. Measured root traits would be: root length, root volume, root surface area, number and length of the seminal roots, root depth and the angle of seminal roots. Al tolerance and P uptake efficiency will be estimated by measuring relative root growth, dry weight of the roots and shoots and the concentrations of P in the shoots of wheat seedlings. Determined root traits will be correlated with the estimated Al tolerance and P efficiency. Genotyping part of the project will consist of two parts: genotype structure will be inferred with the use of 40 evenly distributed microsatellite markers, unrelated genotypes will be analysed using a 15K SNP array (Trait Genetics, Gatersleben, Germany) and association mapping approach for the detection of the genome regions correlated with root traits will be used.

RESULTS AND DISCUSSION

Expected outcomes

1. Screening of Al tolerance and P uptake and use efficiency within Croatian winter wheat germplasm, and identification of potentially Al tolerant and P efficient genotypes
2. Evaluation of the efficiency of analithical methods used in the breeding pipeline
3. Initialisation of the innovative and effective breeding pipeline and of the breeding programme
4. Expert training of young scientists

REFERENCES

Bogunovic, M., Vidacek, Z., Racz, Z., Husnjak, S., Sraka. M. 1996. Special purpose soil map of the Republic of Croatia, scale 1:300.000. Department of Soil Science, Faculty of Agriculture, University of Zagreb

Jasinski, S.M. 2011. Phosphate rock, U.S. Geological Survey Minerals Yearbook – 2010

Kochian, L.V., Hoekenga, O.A., Pineros, M.A. 2004. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. Annu. Rev. Plant Biol. 55:459–93 doi: 10.1146/annurev.arplant.55.031903.141655

von Uexküll, H.R., Mutert, E. 1995. Global extent, development and economic impact of acid soils. Plant Soil 171(1): 1–15

Grain yield and quality of maize hybrids grown on acid soil

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Abstract

Acid soils are limiting factor for achieving high maize yield, along with other factors of soil fertility, genotype and growing season characteristics. Less favorable factors are in connection with precipitation shortage and the higher air-temperatures. Many authors have shown different outspread of acid soil throughout the world. Acid soils are widespread in Croatia. It is estimated that there are about 1.6 million hectares of acid soils in Croatia and they represent about 30% of total agricultural land. Also, genotype of the same plant species can have different reaction to specific abiotic conditions with higher or lower grain yield. So, the aim of this research was to evaluate the impact of soil acidity, genotype and growing season on the maize grain yield and quality in terms of protein, oil and starch contents.

The field experiment in a randomized complete block design in four replicates was set up at the beginning of May 2011. on the acid soil in the east Croatia (45° 29' N and 18° 14' E). Based on chemical analyses, soil from experimental plot has very acid reaction (pH_{KCl} 4.77) and low levels of organic matter (1.75%) while high level of mobile phosphorus (33.67 mg 100 g $^{-1}$) and potassium (42.23 mg 100 g $^{-1}$) according to AL-method were found, due to prior rich mineral fertilization. Basic plot was 14 m^2 (two rows of 10 m length). Ten maize hybrids belonging to different FAO groups originating from Croatia were sown (H1 – Drava 404; H2 – OS 430; H3 – OSSK 444; H4 – OS499; H5 – OSSK 515; H6 – OS 5717; H7 – OSSK 552; H8 – OSSK 596; H9 – OSSK 602 and H10 – OSSK 617). Depending of FAO group hybrids from H1 to H7 were sown in planned plant density of 65683 plants per hectare and from H8 to H10 on 70863 plants per hectare. Usual management practice for maize was performed during vegetation period. Maize was manually harvested at the end of September 2011. After harvest, plants was enumerated and mass of cob weighed. From each basic plot ten cobs were used for determination of grain moisture and grain share in cob. Grain yields were calculated on 14% grain moisture basis. Protein, oil and starch in the maize grain were determined by Near Infrared spectroscopic method on Foss Tecator (Infratec 1241 Grain Analyzer). Statistical analyses were performed by the ANOVA and t-test using LSD at 0.05 and 0.01 probability levels.

Generally, weather characteristics, especially precipitation amounts and distribution and air temperatures, have important influence on maize growth and development. In temperate climate lower grain yield of spring crops are often in connection with less precipitation and the higher air temperatures, especially during summer. With this aspect 2011 growing season was less favorable for maize growth. Total precipitation during maize growing season (April to September) was only 257 mm or 43% lower in comparison with long term mean (453 mm). All months, especially April, August and September were with the lower rainfall. Only exception was July with some higher precipitation value. At the same time, mean air temperatures was for 1.5 °C higher compared to the long term mean (19.0 °C and 17.5 °C, respectively).

In this research, grain yield of maize was relatively low. Average grain yield was only 4.93 t ha^{-1} with variations among hybrids from 4.02 t ha^{-1} to 6.25 t ha^{-1}. Majority hybrids achieved grain yield of about 4 t ha^{-1}, what is quite below theirs potential. Main reason for low grain yields was great percentage of sterile plants which was in average 35%, what is consequences of unfavorable weather conditions during silking and pollination. Only four maize hybrids (H5, H6 and H1, H9) achieved grain yield above 5 t ha^{-1} and 6 t ha^{-1}, respectively. Higher yields of these hybrids could be attributed to somewhat smaller percent of sterile plants (20%) in comparison to average of experiment. Average grain moisture at harvest was 21.0% with high variations from 16.1% to 25.7%, what was expected because of different FAO groups.

Statistical analyses have shown significance in terms of protein, oil and starch content. The highest protein content (10.58%) was achieved by hybrids of earlier maturity group in comparison with last three hybrids in the research (8.70%) what was statistically proved. Average grain oil content was 3.26% with less pronounced variations among hybrids from 2.83% to 3.58%. Genotype effect on starch content was also significant with a realized average of 71.3%. Two hybrids (H2 and H3) was achieved only 69.7% starch content in maize grain while other two hybrids (H9 and H10) had 72.9% or 3.2% higher starch content. Based on this preliminary research we can assume that some hybrids can achieve better yield and grain quality on the same field conditions on acid soil.

The genetic diversity and aluminium toxicity profiles of selected Kenyan maize (*Zea mays* L.) lines for growth in acid soils

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Abstract
Maize (*Zea mays* L.) productivity is significantly reduced by aluminum toxicity on acidic soils. A total of eighty six diverse maize lines from Kenya and Brazil were genotyped with 1250 SNP markers to determine their genetic diversity. The SNPs classified the lines into 3 major groups reflecting their geographical origin and interbreeding. Forty of these lines derived either from CATETO or purely Kenyan were further screened for Al induced *Zm*MATE1 expression using Quantitative real-time PCR. The *Zm*MATE1 expression levels were as high as 16 folds in the CATETO lines, but as low as <2 folds in those with pure Kenyan origin including those with high Al tolerance under nutrient solution culture. This suggests a different gene responsible for their Al tolerance.

INTRODUCTION
Maize (*Zea mays* L.) is among the world's most important cereals and is a staple food for many people in developing countries. Its root growth is severely limited in Al toxic acid soils (pH < 5) owing to prevalence of rhizotoxic Al^{3+} species which inhibits cell expansion and division (Kochian et al., 2004). Approximately 50% of the world's potential arable lands is acidic (von Uexküll and Mutert, 1995). In Kenya 13.5 % of arable land is acidic where about 90 % of maize growing take place and grain yields are low (Kanyanjua et al., 2002; Muhammad and Underwood, 2004). Knowledge of genetic diversity is important to plant breeders for classification of germplasm and selection of parental combinations for hybrid production (Reif et al., 2005). There is, however, little information of the genetic diversity among elite Kenyan and Brazilian derived maize lines used for breeding to enhance Al tolerance. Many genetic studies on Al tolerance have evaluated the seminal root growth under nutrient solution culture as a screening technique (Magnavaca et al., 1987; Magalhaes et al., 2007). Other have used the quantification of root tip mRNAs (Maron et al., 2013). These techniques were adopted for this study.

RESULTS AND DISCUSSION
The SNPs classified the 86 maize lines into 3 major groups that reflect their geographical origin and interbreeding. Group I included majority of breeding lines from Kenya while group III included breeding lines mostly from Brazil. Group II included a mixture of breeding lines and interbreeds from both countries.

Aluminium toxicity tolerance of selected Kenyan maize breeding lines based on root growth in nutrient solution culture
Variance analysis using NSRL (Al) and RNRG phenotypic indices revealed significant genetic variability for Al tolerance among accessions. The Scott and Knott's test classified the 235 breeding lines into 10 groups of decreasing tolerances from left to right as depicted by the respective RNRG ranges (Fig 1).

Aluminium induced ZmMATE1 gene Expression
*Zm*MATE1 expression levels were as high as 16 folds in the accession SYN AL × R12C10 – 8 and as low as 0.54 folds in the accession MUL 891. The mean expression level of the gene among the 40 accessions examined was 2.64 folds. Two accessions, CATAL 237/167 × L3 – 5 and SYN AL × R12C10 – 8 that are single crosses between germplasm from Kenya and EMBRAPA exhibited the highest expression levels. Most of the inbred lines from Kenya including those that exhibited high Al tolerance under nutrient solution, exhibited exceptionally low activities of the gene (<2 fold) suggesting that a different gene from *Zm*MATE1 could be responsible for their Al tolerance.

CONCLUSION

This study found significant genetic variability among Kenyan and Brazilian maize which could be utilized in the breeding for Al tolerant cultivars for production in the Al toxic soils of Kenya and Brazil. Over 25 % of Kenyan maize accessions examined were Al tolerant, 40 % are moderately tolerant and about 35 % were Al sensitive. Most of the Kenyan maize lines including those that exhibited high root growth under Al stress had low activities of the *Zm*MATE1 (< 2 fold).

REFERENCE

Kochian, L.V., Hoekenga, O.A., Pineros, M.A. 2004. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. Annu Rev Plant Biol 55:459–493.

Magalhaes, J.V, Liu, J., Guimaraes, C.T, Lana, U.G.P, Alves, V.M.C. 2007. A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. Nature Genetics 39: 1156–1161.

Magnavaga, R., Gardener, C.O. and Clark. R.B. 1987. Comparison of maize populations for Aluminum tolerance in nutrient solution.Al tolerance in maize. Pp 201 – 212.

Maron, L.G., Guimarães, C.T., Kirst, M., Albert, P.S., Birchler, J.A., Bradburyf, P.J., Bucklerf, E.S., Coluccio, A.E., Danilova, T.V., Kudrna, D., Magalhaes, J.V., Piñeros, M. A., Schatz, M.C., Wing R.A. and Kochian L.V. 2013. Aluminum tolerance in maize is associated with higher MATE1 gene copy number. pnas.org/cgi/doi/10.1073/pnas.1220766110

Reif, J.C, Zhang, P., Dreisigacker, S., Warburton, M.L., van Ginkel, M., Hoisington, D. 2005. Wheat genetic diversity trends during domestication and breeding. TAG Theor Appl Genet 110:859 – 864.

Von Uexküll, H.R., Mutert, E., 1995. Global extent, development and economic-impact of acid soils. Plant soil journal 171(1):1–15.

Genetic effects of maize P efficiency traits in acid and non-acid soils of western Kenya

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Abstract
Low available phosphorus (P) still remains a major limitation to maize (*Zea mays* L.) productivity worldwide. This study investigated the inheritance of traits associated with phosphorus efficiency (PE) in maize and compared the genetic control of maize PE traits in acid and non-acid soils. For most of the traits, greater variation was accounted for dominance followed by epistatic and additive genetic effects in both acid and non-acid soils. The magnitude of both additive and non-additive gene effects were always greater in non-acid compared to acid soils pointing to the effects of soil acidity on gene action. The results suggested that the inheritance of major PE traits did not differ in acid and non-acid soils.

INTRODUCTION
Maize (*Zea mays* L.) is among the world's most important cereals and is a staple food for many people in developing countries (Awika, 2011). However, its yields are low in acid soils mainly because of Al toxicity and P deficiency (Kochian et al., 2005). Approximately 50% of the world's potential arable lands is acidic (von Uexküll and Mutert, 1995). In Kenya 13.5 % of arable land is acidic where about 90 % of maize growing take place (Muhammad and Underwood, 2004). Phosphorus starvation leads to stunted growth, thin and spindly stems with purpling of leaves. Overall, the inheritance of several important traits in maize evaluated under non-acid soils has been well documented (Furlani et al., 1998). However, information on the inheritance patterns of maize PE traits in acid soils especially those in Africa is still inadequate yet this information is extremely useful in developing a clear selection criteria for P efficiency in these soils. Many studies have used generation means analysis to estimate genetic effects from crosses between maize inbreds with different levels of tolerance to acid soils (Magnavaca et al., 1987b; Pandey et al., 2007; Vasquelez et al., 2008; Parentoni et al., 2010), consequently this methodology was adopted for this study.

RESULTS AND DISCUSSION
Comparison of gene actions on P efficiency traits under acid and non-acid soils
Under high P (36kgP/ha) acid soils, the mean value of the ratios a/m, d/m, and epist/m were 0.24, 1.98 and 0.8, respectively for grain yield (GYLD) indicating that dominance effects, followed by epistatic effects where more important than additive effects for the inheritance of GYLD. Under low P (6kgP/ha), similar results were reported with ratios a/m, d/m, and epist/m being increased at least 3.6 folds (0.81, 7.73 and 3.5). In non-acid soils, the ratios a/m, d/m, and epist/m were 0.41, 1.4 and 1.98 respectively with high P, while they were 0.44, 2.9, 1.97 with low P indicating that dominance effects, followed by epistatic effects where more important than additive effects for the inheritance of GYLD in non-acid soils. The mean additive effects did not differ significantly (0.51 t/ha vs. 0.43 t/ha) between acid and non-acid soils suggesting that estimates of pooled additive effects were not affected by soil acidity. These results imply the suitability of selecting for GYLD in low P conditions under acid soils because of increased magnitude of additive genetic effects or using either P level in non-acid soils since the magnitude of additivity did not differ significantly. These findings compare well with those of Parentoni et al. (2010) who reported the importance of dominance effects, followed by epistatic effects than additive effects for maize GYLD in acid soils. They also compare well with those of Vasqualez et al. (2008) who reported higher magnitude of pooled additivity in acid compared to non-acid soils. The pooled dominance effects for GYLD were significant for (83.3%) and for 100% crosses in acid and non-acid soils, respectively. The significant dominance effects averaged 4.4 tha^{-1} and ranged from 2.48-7.0 t ha^{-1} in acid soils whereas for non-acid soils, they averaged 5.6 tha^{-1} and ranged from 4.38 to 8.49 t ha^{-1}. Thus, contrary to the additive effects, the magnitudes of the dominance effects were significantly affected by soil acidity. These findings further compares well with those of other researchers (Gamble, 1962a; and Vasqualez et al., 2008) who reported on the importance of dominance genetic effects for the inheritance of GYLD in maize in non-acid soils. Epistatic effects for GYLD were detected in all the crosses in acid soils while in non-acid soils, only 33% of the crosses presented significant epistatic effects. The number of crosses with epistatic effects was greater in acid compared to non-acid soils. Besides, the magnitudes of these effects were also larger in acid than in non-acid soils. These results partially agree with those of Ceballos et al. (1998) who reported that epistasis was more important for grain yield in acid compared to non-acid soils.

For both shoot dry matter (SDM) and Root Length Density (RLD), the pooled dominance effects were significant for (83.3%) and for (100%) crosses in acid and non-acid soils, respectively. In both traits, the magnitude of these effects was greater than the average mean parameter in both acid and non-acid soils. Also, these effects were positive for all the crosses in both acid and non-acid soils. For SDM, significant dominance effects averaged 0.79 kg/plant and ranged from 0.54-1.04 kg/plant in acid soils, whereas for non-acid soil the dominance effects averaged 0.69 kg/plant and ranged from 0.46 -1.01 kg/plant. For RLD, the significant dominance effects averaged 19.96 cm/cm^3 and ranged from 13.39-29.9 cm/cm^3 in acid compared to non-acid soil where the dominance effects averaged 27.47cm/cm^3 and ranged from 17.1-44.16 cm/cm^3. Therefore, contrary to the additive effects, the magnitudes of the dominance effects were significantly affected by soil acidity for both SDM and RLD. Epistatic effects with high mean magnitude (1.43) was only detected in non-acid soils for RLD while in SDM, epistatic effects (aa and dd) were detected in both acid and non-acid soils (dd) suggesting that soil acidity significantly affected estimates of epistatic gene effects for these traits

CONCLUSION

Both additive and non-additive effects were detected in both acid and non-acids soils although this was more dependent on the trait studied and the level of available P. Dominance effects played a more important role than epistatic effects and the latter were more important than additive effects in the inheritance of majority of P efficiency traits studied in both acid and non-acid soils. The magnitude of both additive and non-additive gene effects were always greater in non-acid soils compared to acid soils pointing to the detrimental effects of soil acidity in the detection of gene actions in maize. The inheritance of grain yield and other P efficiency traits did not differ in acid and non-acid soils.

REFERENCE

Ceballos, H., Pandey, S., Narro, L and Pérez, J.C. 1998. Additive, dominant, and epistatic effects for maize grain yield in acid and non-acid soils. Theor Appl Genet 96:662-668.

Furlani, A.M.C., Lima, M., Nass, L.L. 1998. Combining ability effects for P-efficiency characters in maize grown in low P nutrient solution. Maydica 43: 169-174.

Kochian, L.V., Pineros, M.A. and Hoekenga, O.A. 2005. The physiology, genetics and molecular biology of plant aluminium resistance and toxicity. *Plant soil* 274: 175-195.

Magnavaca, R., Gardner, C.O. and Clark, R.B. 1987b. Inheritance of aluminum tolerance in maize. In: Gabelman, H.W and Loughman, B.C. (eds) Genetic Aspects of Plant Mineral Nu- trition. Martinus Nijhoff Publishers, Dordrecht, pp201-212.

Pandey, S., Narro, L., Friesen, D.K. and Waddington, S.R. 2007. Breeding maize for tolerance to soil acidity. Plant Breed Rev 28:59-100.

Parentoni, S.N., Souza, J.R., Alves, V.M.C., Gama, E.E.G., Coelho, A.M., Oliveira, A.C., Guimaraes, P.E.O., Guimaraes, C.T., Vasconcelos, M.J.V., Pacheco, C.A.P., Magalhães, J.V., Meirelles, W.F., Guimarães, L.J.M., Silva, A.R., Mendes, F.F., Schaffert, R.E. 2010. Inheritance and breeding strategies for phosphorus efficiency in tropical maize (*Zea mays L.*). *Maydica* 55: 1-15.

Velásquez, J.C., Pérez, C., Lopes de Souza Jr., Luis A. N., Shivaji, P. and Carlos D.L. 2008. Genetic effects for maize traits in acid and non-acid soils. Genetics and Molecular Biology, 31, (1) pp 89-97.

Von Uexkull, H.R. and Mutert, E. 1995. Global extent, development and economic impact of acid soils. Plant Soil. 171: 1-15.

Transcriptomic analysis of aluminum-tolerant phenotype of a cultured cell line of tobacco (*Nicotiana tabacum* L.)

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Abstract

Microarray analysis was performed, using a pair of cultured tobacco cell lines, SL (wild-type) and ALT301 (aluminum-tolerant) under normal growth condition. Compared to SL, enhanced expressions were observed in ALT301 in some genes related to energy metabolism such as sucrose synthase (SUS), pyruvate dehydrogenase kinase (PDHK; inhibitor of PDH activity), and lactate dehydrogenase (LDH), suggesting the shift of energy metabolism from [glycolysis-TCA cycle-oxidative phosphorylation] to [glycolysis-fermentation]. The enhanced expression was also observed in other genes related to antioxidant system such as superoxide dismutase (SOD) and peroxidase (POD) in ALT301. These results suggest that both the shift of energy metabolism pathway and the enhancement of antioxidant system contribute to the repression of reactive oxygen species (ROS) production under normal and Al stress conditions, which seems to be related to Al-tolerant phenotype in ALT301.

INTRODUCTION

Al is a most abundant metal and is a major growth-limiting factor in plants in acidic soils. The primary site of Al accumulation and toxicity is elongation zone of root apices and immediately causes elongation inhibition, then cell death. Cultured tobacco cell line SL at actively growing phase exhibits Al responses similar to those observed in root apex, such as callose accumulation, elongation inhibition, ROS production and cell death (Yamamoto et al., 2002; 2003; Abdel-Basset et al., 2010).

In order to elucidate possible Al tolerant mechanisms in plant cells, Al-tolerant cell line (ALT301) was previously selected from cultured cell line SL (Devi et al., 2001, 2003), and the responses to Al have been compared between these lines. Although both lines accumulate Al and produce callose to the same extents, ALT301 produces ROS much less than SL, which is the most distinguishable phenotype of ALT301 from SL.

Our previous metabolomic analysis suggested a constitutive shift of energy metabolism pathway in ALT301 from [glycolysis-TCA cycle-oxidative phosphorylation (a major site of ROS production)] to [glycolysis-fermentation], compared to that in SL (unpublished results).

In this study, we compared constitutively expressed genes between SL and ALT301, by performing microarray analysis, in order to elucidate the genes related to the acquisition of Al-tolerant phenotype in ALT301. We found that the changes of gene expression well support our previous results obtained by metabolomic analysis. The possible Al-tolerant mechanism in plant cells will be discussed.

MATERIALS AND METHODS

Growth conditions and Al treatment

Tobacco cells were cultured in a modified MS medium at 25°C as described previously (Yamamoto et al., 2002). The cells at logarithmic phase of growth were treated without or with several concentrations of $AlCl_3$ in a simple treatment medium containing 3 mM $CaCl_2$, 88 mM sucrose and 20 mM Mes (pH 5.0, adjusted with BTP) for 18 h at 25°C, as described previously (Abdel-Basset et al., 2010).

Microarray analysis of SL and ALT301 cells

Tobacco cells at the logarithmic phase of growth were used. The genome wide expression patterns were compared between SL and ALT301 cells, using Agilent’s tobacco microarray. Array spots with fluorescence signal intensities after subtracting the background were analyzed. Differentially expressed genes were selected based on a fold change of <0.5 or >2 and a probe-wise P value of <0.05, which was analyzed for the microarray data using GeneSpring software (Agilent Technologies).

Determination of SUS enzyme activity

SUS activity was measured in the direction of sucrose degradation as described previously (Zrenner et al., 1995). The production of UDP-glucose was coupled to the reduction of NAD in the presence of excess UDP-glucose dehydrogenase and the change in $A_{340\ nm}$ was followed.

RESULTS AND DISCUSSION

Transcriptomic analysis (microarray) of SL and ALT301 cells under normal growth condition

Both tobacco cell lines at the logarithmic phase of growth were compared the genome wide expression patterns. Compared to SL, enhanced expression was observed in some genes related to energy metabolism (SUS, LDH, PDC, PDHK) in ALT301. These results support our previous data of metabolomic analysis between two lines. Briefly, the metabolomic date indicated that lactate content was higher, while the contents of TCA cycle components were lower in ALT301 cells, compared to SL cells (our unpublished results). In addition, the genes coding antioxidant enzymes (SOD, POD) were more expressed in ALT301cells than that in SL cells.

Expression analyses of SUS

Sucrose is the primary translocatable carbohydrate in the majority of plant, and is broken down by invertase (INV) or sucrose synthase (SUS). Although SUS can catalyze both sucrose synthesis and degradation, the principal role of SUS in sucrose utilizing tissues is sucrose degradation. In this case, SUS catalyzes the degradation of sucrose into fructose and UDP-Glucose, providing carbon souse for glycolysis and for the synthesis of cell wall polymers and starch (Gang et al., 2007; Koch, 2004). SUS also provides UDP-glucose for an ATP-independent hexose phosphorylation so that the SUS pathway seems to be more advantageous than INV pathway, especially under ATP-limiting conditions such as hypoxia. Under the metabolic condition of ALT301 cells where the energy metabolism is supposed to be shifted from the mitochondrial pathway (efficient pathway for ATP production) to fermentation (much less efficient for ATP production) as described above. Since the microarray analysis indicated SUS to be one of the hyper-expressed genes in ALT301 than that in SL cells, the expressions of SUS was further examined at both gene expression and enzyme activity levels.

The expression levels of *SUS* were investigated by real-time RT-PCR in both lines. The expression level of *SUS* was approximately 4 times higher in ALT301 than that in SL. On the other hand, the specific activity of SUS enzyme was also approximately 1.5-fold higher in ALT301 than that in SL.

SUS gene expression under Al stress

After treatment without Al for 18 h, the expression levels of *SUS* were decreased in both lines. After treatment with Al, the expression level was only slightly increased in SL, but significantly increased in ALT301. After 18 h exposure to 100 μM $AlCl_3$, expression level was approximately 4 times higher in ALT301 than that in SL.

CONCLUSION

Our studies suggest that both the shift of energy metabolism pathway from oxidative phosphorylation to fermentation and the enhancement of antioxidant system seem to contribute to the repression of ROS production under normal and Al stress conditions, which seems to lead to Al-tolerant phenotype in ALT301. Furthermore, ALT301 seems to provide sugar for glycolysis via SUS pathway which is ATP-saving pathway.

REFERENCES

Yamamoto, Y., Kobayashi, Y., Devi, S.R., Rikiishi, R., Matsumoto, H. 2002. Aluminum toxicity is associated with mitochondrial dysfunction and the production of reactive oxygen species in plant cell. Plant Physiology. 128: 63-72

Yamamoto Y, Kobayashi Y, Devi SR, Rikiishi S, Matsumoto H. 2003. Oxidative stress triggered by aluminum in plant roots. Plant and Soil. 255: 239–243.

Abdel-Basset, R., Ozuka, S., Demiral, T., Furuichi, T., Sawatani, I., Baskin, T.I., Matsumoto, H., Yamamoto, Y. 2010. Aluminum reduces sugar uptake in tobacco cell cultures: A potential cause of inhibited elongation but not of toxicity. Journal of Experimental Botany. 61: 1597-1610.

Devi SR, Yamamoto Y, Matsumoto H. 2001. Isolation of aluminum tolerant cell lines of tobacco in a simple calcium medium and their responses to aluminum. Physiologia Plantarum. 112: 397–402.

Devi SR, Yamamoto Y, Matsumoto H. 2003. An intracellular mechanism of aluminum tolerance associated with high antioxidant status in cultured tobacco cells. Journal of Inorganic Biochemistry 97: 59–68.

Zrenner R., Salanoubat M., Willmitzer L., Sonnewald U. 1995. Evidence of the crucial role of sucrose synthase for sink strength using transgenic potato plants (*Solanum tuberosum* L.). The Plant Journal. 7(1): 97-107

G. Ren, R. Healey, H.T. Horner, M. James, R.W. Thornburg, Transient. 2007. Expression of starch metabolic genes in the developing nectaries of ornamental tobacco plants. Plant Science. 173: 621–637.

Koch K. 2004. Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. Current Opinion in Plant Biology. 7:235–246.

Section 04:

Aluminum toxicity, P deficiency, and other acid soil limitations with a focus on their amelioration and remediation

Chairpersons: Xiao Fang Zhu, Amy Whitley, Ivica Đalović

Mechanisms of aluminum toxicity and tolerance elucidated by use of cultured tobacco cell lines

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Abstract
Actively growing cells at root apices are sensitive to Al ion, and primary toxic events are cell elongation inhibition and cell death. These symptoms are also observed in cultured tobacco cells at actively growing phase. Thus cultured tobacco cell system is useful to study mechanisms of Al toxicity and tolerance at cellular level. Furthermore, Al-tolerant tobacco cell lines were successfully isolated from parental cell line. In this paper, we will review the responses to Al in cultured tobacco cell lines. Some of these responses were similarly observed in root apices.

INTRODUCTION

Aluminum (Al) toxicity in plants is observed in growing cells located at the elongation zone of root apex, and elongation inhibition and cell death are typical Al symptoms. The elucidation of Al toxicity mechanisms in growing cells should be useful to find out possible factors conferring Al-tolerant phenotype in plants. For this purpose, we have used cultured cell lines of tobacco (*Nicotiana tabacum* L.), such as SL and BY-2, and found that these cell lines exhibited responses to Al similar to those in plant roots (Yamamoto, Y. et al., 2002, 2003). In addition, SL was genetically stable so that Al-tolerant cell lines were successfully isolated from SL (Devi, S. R. et al., 2001, 2003). Comparative analyses between SL (parental line) and Al-tolerant cell lines were performed to find out factors involved in Al tolerant phenotype at cellular levels.
Using these lines, it was firstly found the major accumulation sites of Al to be the outer part of the cells including the cell wall and the plasma membrane. The accumulation of Al in nuclei seemed to be caused secondly after the Al-caused dysfunction of the plasma membrane. Other responses to Al found in tobacco cell system are described below.

RESULTS AND DISCUSSION

Al-induced elongation inhibition
The Al-induced elongation inhibition in tobacco cells were found to accompany both the decrease in water content and the decrease in soluble sugar content, suggesting that the inhibition of sugar uptake by Al causes an increase in osmotic potential which leads to a decrease in water uptake and eventually to cell elongation inhibition (Abdel-Basset et al., 2010).

Over-expression of sucrose transporter, NtSUT1, improves post-Al treatment growth
We investigated the effect of Al on sucrose transporter localized at the plasma membrane. Treatment of tobacco cells with Al decreased the expression level of *NtSUT1*. Furthermore, Al treatment decreased the degree of sucrose uptake via NtSUT1. Interestingly, the transgenic tobacco cell line over-expressing *NtSUT1* exhibited higher uptake rate of sucrose and higher growth rate during post-Al treatment culture, compared to its wild-type cell line (Sameeullah, M. et al., 2013). Furthermore, the over-expression of *NtSUT1*in tobacco also indicated better root growth than its wild-type line under Al stress. (Kariya, K. et al., unpublished results).

Al-induced cell death mechanisms involve mitochondria and vacuole
The Al-induced cell death process seems to involve two organelles. One is mitochondria where Al caused respiration inhibition together with the production of reactive oxygen species (ROS), and these symptoms were similarly observed at root apices of pea (*Pisum sativum* L.) (Yamamoto, Y. et al., 2002).
Another organelle was vacuole where the expression levels of vacuolar processing enzymes (VPE) were enhanced at the transcription level and the enzyme activity level (Kariya, K. et al., 2013). The increases in *VPE* gene expression and VPE activity were similarly observed in tobacco root under Al exposure (Kariya, K. et al., unpublished results). VPE was reported to be an essential factor to trigger tobacco mosaic virus (TMV)-induced hyper-sensitive cell death in tobacco leaves (Hatsugai, N. et al., 2004). The treatment of tobacco cells with a VPE inhibitor prevented Al-induced cell death to some degree. Thus, these results suggest that the increments of VPE gene expression and VPE activity under Al exposure are related to Al-induced cell death process in cultured tobacco cells and probably in root apices.

Metabolomic and transcriptomic analyses of cultured tobacco cell lines

We performed both metabolomic analyses (Yamamoto, Y. et al., our unpublished results) and transcriptomic analyses (Tsuchiya, Y. et al., our unpublished result) in order to find out the differences between SL (parental) and ALT301 (Al-tolerant) cell lines. The most striking feature of the Al-tolerant cell line was almost completely repression of ROS production under Al exposure (Yamamoto, Y., 2002). These analyses suggest that the shift of energy metabolism from the oxidative phosphorylation in mitochondria to fermentation in cytosol, as well as the enhancement of defense systems to oxidative stress, in the Al-tolerant cell line, suggesting that the repression of the ROS production in mitochondria by the shift of energy metabolism and by an increase in antioxidant system could be an avoidance system of ROS production under Al stress (Yamamoto, Y. , Tsuchiya, Y. et al., our unpublished results).

REFERENCES

Yamamoto, Y., Kobayashi, Y., Devi, S.R., Rikiishi, S., Matsumoto, H. 2002. Aluminum toxicity is associated with mitochondrial dysfunction and the production of reactive oxygen species in plant cell. *Plant Physiology* 128: 63-72

Yamamoto, Y., Kobayashi, Y., Devi, S.R., Rikiishi, S., Matsumoto, H. 2003. Oxidative stress triggered by aluminum in plant roots. *Plant and Soil.* 255: 239–243.

Devi, S.R., Yamamoto, Y., Matsumoto, H. 2001. Isolation of aluminum tolerant cell lines of tobacco in a simple calcium medium and their responses to aluminum. *Physiologia Plantarum* 112: 397–402.

Devi, S.R., Yamamoto, Y., Matsumoto, H. 2003. An intracellular mechanism of aluminum tolerance associated with high antioxidant status in cultured tobacco cells. *Journal of Inorganic Biochemistry* 97: 59–68.

Abdel-Basset, R., Ozuka, S., Demiral, T., Furuichi, T., Sawatani, I., Baskin, T.I., Matsumoto, H., Yamamoto, Y. 2010. Aluminum reduces sugar uptake in tobacco cell cultures: A potential cause of inhibited elongation but not of toxicity. *Journal of Experimental Botany* 61: 1597-1610.

Sameeullah, M., Sasaki, T. and Yamamoto, Y. 2013. Sucrose transporter NtSUT1 confers aluminum tolerance on cultured cells of tobacco (*Nicotiana tabacum* L.). *Soil Science and Plant Nutrition* 59: 756-770.

Kariya, K., Demiral, T., Sasaki, T., Tsuchiya, Y., Turkan, I., Sano, T., Hasezawa, S., Yamamoto, Y. 2013. A novel mechanism of aluminium-induced cell death involving vacuolar processing enzyme and vacuolar collapse in tobacco cell line BY-2. *Journal of Inorganic Biochemistry* 128: 196-201.

Hatsugai, N., Kuroyanagi, M., Yamada, K., Meshi, T., Tsuda, S., Kondo, M., Nishimura, M., Hara-Nishimura, I. 2004. A plant vacuolar protease, VPE, mediates virus-induced hypersensitive cell death. *Science*305: 855–858.

Border cells: sentinels for root protection against soil-borne stresses

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Abstract
Most plant species are programmed to release thousands of living cells in the rhizosphere, the so-called root-border cells (Hawes et al., 2000). As these cells separate from the root tip, they become specialized in the production of anti-microbial compounds including enzymes, phenolics and extracellular DNA (Brigham et al., 1995; Wen et al, 2007; 2009; Cannesan et al., 2011; Watson et al., 2015). Due to their very special position at the interface between roots and soil, root border cells are key elements that play a central role in root protection against biotic and abiotic stress.

For instance, border cells of pea - the most used plant model for border cell studies- are capable of inhibiting growth of the fungus *Nectria haematococca in vitro* (Gunawardena and Hawes 2002). They are also capable of repulsing the fungus thereby preventing infection of the root tip (Gunawardena et al., 2005). We found that pea root border cells are highly enriched in the phytoalexin pisatin and in arabinogalactan proteins (AGP) (Cannesan et al. 2011; 2012). We have assessed the impact of AGP produced by pea root cap and border cells on the behavior of zoospores of *Aphanomyces euteiches*, an oomycetous pathogen that causes root rot disease. AGP were shown to attract the zoospores, induce their encystment and prevent their subsequent germination *in vitro*. These data indicate that root AGP are involved in the control of pea root infection by *A. euteiches* and highlight a novel role for these proteoglycans in root-microbe interaction (Cannesan *et al*., 2012). In addition to these findings, we have also shown that root border cells from several plant species secrete large amounts of mucilage enriched in AGP (Durand et al., 2009; Cannesan et al., 2012; Koroney et al., submitted). Root border cells and mucilage are key elements in shaping soil-borne microbial communities surrounding the root tip (NGuema et al., 2013). Furthermore, data from Martha Hawes's laboratory (University of Arizona, USA) revealed that root border cells from pea were also shown to produce extracellular DNA (exDNA) (Wen *et al*., 2009). This exDNA-based matrix surrounding the root tip seems to function in root defense in a way similar to that of the recently characterized neutrophil extracellular traps (NET) in mammalian cells. We proposed a model termed "Root Extracellular Trap" or RET in which root exudates together with root border cells could function in root defense in a way similar to that of the NET (Driouich *et al*, 2013).

Although less studied root border cells were also reported to be involved in response to abiotic stress (Hawes et al., 2010; Cai et al. 2011). Soil acidification causes the release of highly toxic Al^{3+} in the rhizosphere responsible of devastating damages affecting crops. Roots are especially vulnerable to Al toxicity and the root tips accumulate large amounts of Al. Border cells from rice were shown to alleviate Al toxicity by means of their secreted mucilage (Cai et al. 2011). Exposure of root border cells to high Al concentration increased the thickness of the mucilage produced. The production of such mucilage appeared to be a key element in alleviating root stress by allowing extrusion of Al from the root apice (Cai et al., 2011). To conclude, these findings offer new perspectives to promote natural root protection against abiotic stress including soil acidification. One strategy would be to stimulate root border cells and/or mucilage production by using natural compounds (*e.g*. elicitors) to increase plant tolerance to abiotic stress.

REFERENCES

Brigham LA, Woo HH, Nicoll SM, Hawes MC. (1995) Differential Expression of Proteins and mRNAs from Border Cells and Root Tips of Pea. *Plant Physiology* 109: 457–463

Cai MZ, Wang FM, Li RF, Zhangb S, Wang N, Xu GD. (2011) Response and tolerance of root border cells to aluminum toxicity in soybean seedlings. *Journal of Inorganic Biochemistry* 105: 966–971

Cannesan MA, Gangneux C, Lanoux A, Giron D, Laval K, Hawes M, Driouich A, Vicré-Gibouin M. (2011) Association between border cell response and localized root infection by *Aphanomyces eutiches*. *Annals of Botany* 108: 459-469

Cannesan MA, Durand C, Burel C, Gangneux C, Lerouge P, Ishii T, Laval K, Follet-Gueye ML, Driouich A, Vicré-Gibouin M. (2012) Effect of arabinogalactan proteins from the root caps of Pisum sativum and Brassica napus on Aphanomyces euteiches zoospore chemotaxis and germination. *Plant Physiology* 159: 1658-1670

Driouich A, Follet-Gueye ML, Vicré-Gibouin M, Hawes M (2013) Root border cells and secretions as critical elements in plant host defense. *Current Opinion in Plant Biology* 16: 489-495

Hawes MC, Gunawardena U, Miyasaka S, Zhao X. (2000) The role of root border cells in plant defense. *Trends in Plant Science* 5: 128-133.

Nguema-Ona E, Vicré-Gibouin M, Cannesan MA, Driouich A (2013) Arabinogalactan proteins in root–microbe interactions. *Trends in Plant Science* 18: 440-449

Watson BS, Bedair MF, Urbanczyk-Wochniak E, Huhman DV, Yang DS, Allen SN, Li W, Tang Y. (2015) Integrated metabolomics and transcriptomics reveal enhanced specialized metabolism in *Medicago truncatula* root border cells. *Plant Physiology* 167:1699-716

Wen F, White GJ, VanEtten HD, Xiong Z, Hawes MC. (2009) Extracellular DNA is required for root tip resistance to fungal infection. *Plant Physiology* 151: 820–829

Wen F, Van Etten H. D, Tsaprailis G, Hawes MC. (2007) Extracellular proteins in pea root tip and border cell exudates. *Plant Physiology* 143: 773–783

A WRKY transcription factor regulates lateral root development and phosphate starvation-mediated responses in Arabidopsis

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Abstract
Plants have evolved well adaptive responses in root system architecture (via e.g. inhibition of primary root growth, increase of lateral root formation) to cope with Phosphate (Pi) limitations. Nevertheless, the molecular bases of root regulation in response to Pi starvation are poorly understood. Here, we present a WRKY transcription factor that is responsive to Pi starvation at both transcript level and protein level, that modulates lateral root development through auxin signals, and that regulates expression of Pi transporter genes during Pi starvation in Arabidopsis.

Phosphorus, a crucial component of major organic molecules (e.g. nucleic acid, ATP, etc.), is present in soils in the form of inorganic phosphate (Pi) that has low availability and poor mobility. To cope with Pi limitations, plants adapt their root system architecture through inhibition of primary root (PR) growth, increase in lateral root (LR) formation and growth, and production of root hairs. Whereas, the molecular mechanisms of plant adaptation to Pi starvation are poorly understood. Previously we identified a WRKY transcription factor, WRKYx, which is a potential regulator of LR development in Arabidopsis. Lack of WRKYx obviously promoted LR formation and growth, while constitutively overexpression of it (OE) significantly inhibited LR development but increased PR growth (versus WT; Figure 1A). GUS expression (driven by WRKYx native promoter) was detected in root stele, and was increased in the joint (LR base) between PR and LR (Figure 1B), suggesting a role of WRKYx in LR development.

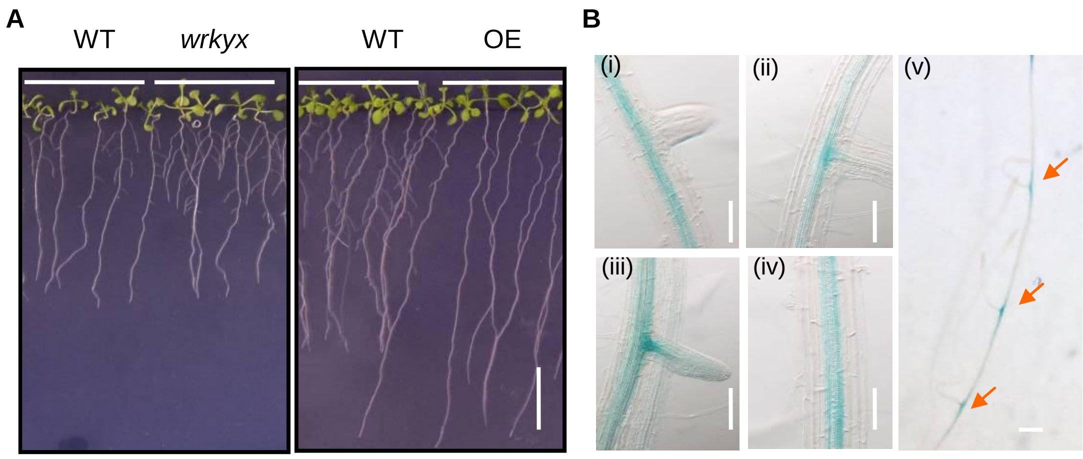

Figure 1. WRKYx as a potential regulator in LR development. (A) Root growth of wrkyx mutant and OE line versus wild type (WT) respectively; (B) GUS expression (driven by WRKYx native promoter) in root. Bars= 1cm in A, 100µm in B (i-iv), 1mm in B (v)

The repression of LR development in OE line can be well reversed by indole-3-acetic acid (IAA) and Pi starvation applications (Figure 2), rather than by other treatments (data not shown). Accordingly, many genes that are involved in phosphate starvation responses or in auxin responses had altered expression in OE line (versus in WT) revealed by microarray analysis (unpublished data). Furthermore, WRKYx directly regulated expression of two Pi transporter genes (PHT1;1 and PHT1;3; unpublished data). Although the transcript level of WRKYx was induced, the corresponding proteins were obviously degraded during Pi starvation (Figure 3). On the other hand, the expression of auxin reporter DR5:GUS was evidently attenuated in OE line (Figure 4), indicating WRKYx as a repressor in auxin responses or hoemostasis. Further study will uncover how WRKYx regulates LR development via affecting auxin signals. Thus, we present a potential regulator that connects the response to external phosphate starvation with internal auxin signaling in lateral root development.

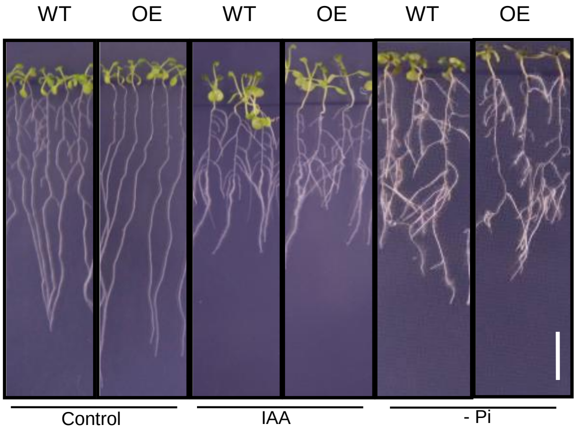

Figure 2. Restoration of LR development in OE line by IAA and Pi starvation applications Bar= 1cm

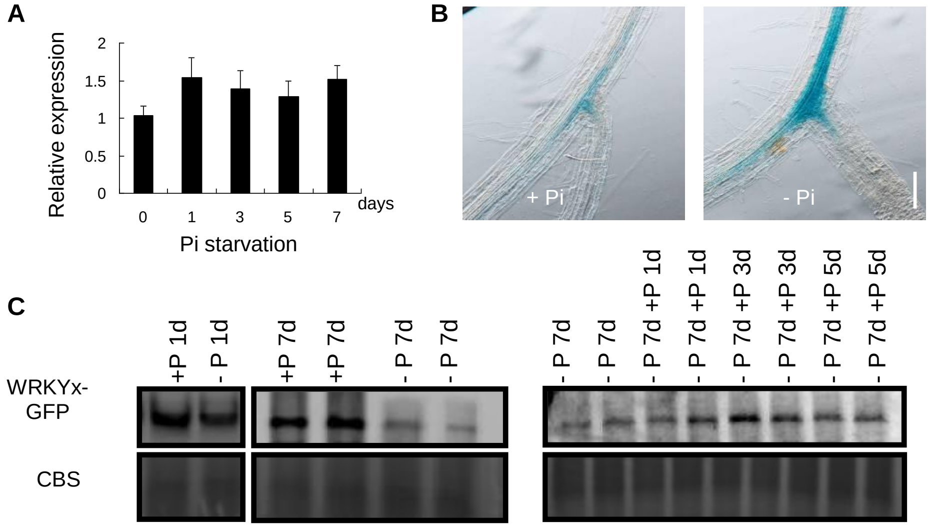

Figure 3. Response of WRKYx to Pi starvation at both transcript level and protein level. (A-B) Transcriptional response of WRKYx to Pi starvation revealed by qRT-PCR and GUS staining; (C) Response of WRKYx-GFP protein to Pi availability by western blot assay

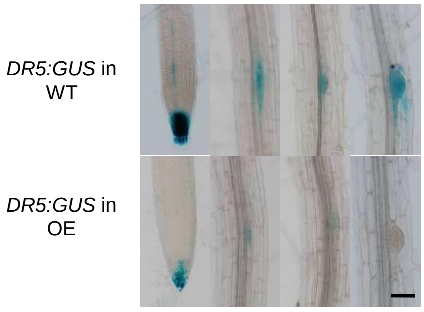

Figure 4. DR5:GUS expression in WT and OE roots

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AtEXPA12 is required for aluminum tolerance in Arabidopsis

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Abstract
Expansins are cell wall proteins that mediate acid-induced growth by catalyzing loosening of plant cell walls without lyses of wall polymers(Cosgrove 2015). Previous study showed Aluminum (Al) was the most potent inhibitor of expansin-mediated acid growth(Cosgrove 1989), which indicated expansions may play some role in Al tolerance in plants. In this study, we identified an Arabidopsis expansin gene *AtEXPA12*, which has higher expression in roots and could be greatly induced by Al stress, and its expression is not regulated by *STOP1*. The T-DNA mutants of *AtEXPA12* are more sensitive to Al stress, while *AtEXPA12* over-expression lines are significantly more Al tolerant than wild type. Interestingly, ectopic expression *AtEXPA12* in yeast also conferred increased Al tolerance. Overall, our study deepened our understanding of Al tolerance mechanism in plants and provided a useful genetics resource for enhancing Al tolerance in other important crops.

INTRODUCTION
Al toxicity is a major limiting factor for crop yields on acid soils in the tropics and subtropics. Therefore, developing Al-tolerant crops has been considered to be a key solution to increase crop productivity worldwide(Kochian, Piñeros et al. 2015). Plants have evolved complex mechanism to cope with Al stress internally and externally. Internal mechanism is achieved by transferring and accumulating Al in the vacuoles and chelation Al with organic acids(Ma 2007). External mechanism is widely used in many plants and is the best characterized mechanism of Al resistance. It involves the secretion of organic acids from the roots in response to Al stress, those organic acids can chelate toxic Al and prevent Al entering root cell (Sasaki, Yamamoto et al. 2004, Magalhaes, Liu et al. 2007, Liu, Magalhaes et al. 2009). Expect for the internal and external mechanisms, more and more studies showed a novel Al tolerance mechanisms involve modification of the properties of the root cell wall(Cosgrove 1989, Huang, Yamaji et al. 2009, Zhu, Shi et al. 2012), where the majority of root Al is accumulated(Kochian, Piñeros et al. 2015). In this study, we identified a novel cell wall protein from Arabidopsis a-expansins family named AtEXPA12, which may play important role in Arabidopsis Al tolerance by modifying cell wall structure under Al stress.

MATERIALS AND METHODS
Arabidopsis *AtEXPA12* T-DNA mutants were obtained from ABRC at Ohio State University. *AtEXPA12* Transgenic plants were generated using the Agrobacterium tumefaciens-mediated floral dip method(Clough and Bent 1998). For plant and yeast phenotype analysis, Real-time PCR experiments were performed as described by (Li, Liu et al. 2014).

RESULTS AND DISCUSSION
AtEXPA12 is up-regulated by Al
There are 4 subfamilies, total 36 genes in Arabidopsis genome(Cosgrove 2015). To find out which gene or genes could be involved in Al tolerance, we firstly examined all the genes' tissue expression pattern in Arabidopsis(Winter, Vinegar et al. 2007). Those expansin genes which have higher expression levels in roots were selected for further study. Real-time PCR results showed among all the selected genes, *AtEXPA12* is the only one which could be great induced by Al. The *AtEXPA12* is specifically induced by Al, not by other metals, including Cd(Cadmium)and La(Lanthanum), and also not by low pH. In the Al related C2H2-type zinc finger Transcription factor mutant *stop1*, AtEXPA12 could also be induced by Al, indicating that *AtEXPA12* is not regulated by *STOP1*

AtEXPA12 affects Al tolerance in Arabidopsis
To study the function of *AtEXPA12* in Arabidopsis, we obtained two independent T-DNA insertion lines of *AtEXPA12*. Real-time PCR analysis showed these two T-DNA lines have much lower *AtEXPA12* expression level than wild type. Phenotype analysis showed two mutants are more sensitive to Al stress than wild type. To confirm the function of AtEXPA12, we also generated *AtEXPA12* over-expression lines in wild type Arabidopsis driven by *35SMV* and *AtALMT1* promoter respectively. Four transgenic lines with high *AtEXPA12* expression level were chose for further study. All four lines have significantly longer root growth compared with wild type under Al stress, while no difference could be observed in normal situation. These results clearly indicated that *AtEXPA12* play important role in Al tolerance in Arabidopsis.

AtEXPA12 could enhance yeast Al tolerance

In order to further study *AtEXPA12*'s function, we also expressed *AtEXPA12* in yeast. In the time and concentration dependent yeast Al tolerance experiments, the results showed that *AtEXPA12* yeast transgenic line was much more tolerant to Al than wild type did. These results indicated plants and yeast may share some similar mechanism in Al tolerance.

CONCLUSION

Recently studies indicated that root cell wall modification may play important role in Al tolerance in plants. Expansins are cell wall protein and function in acid-dependent root growth. In this study, we identified a expansin gene *AtEXPA12*, which belongs to Arabidopsis a-expansins family. It has high expression in roots and very low expression in shoots. Quantitative RT-PCR analysis showed *AtEXPA12* could be greatly induced by Al, but was not regulated by STOP1. The mutants of *AtEXPA12* were more sensitive to Al than wild type did. Over-expression *AtEXPA12* in Arabidopsis could significantly enhance its Al tolerance. Surprisingly, ectopic expression *AtEXPA12* in yeast could confer increased Al tolerance. Our results showed expansin-mediated cell wall modification may play important role in plant Al tolerance.

REFERENCES

Clough, S. J. and A. F. Bent, 1998. "Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana." Plant J 16(6): 735-743.

Cosgrove, D., 1989. "Characterization of long-term extension of isolated cell walls from growing cucumber hypocotyls." Planta 177(1): 121-130.

Cosgrove, D. J., 2015. "Plant expansins: diversity and interactions with plant cell walls." Curr Opin Plant Biol 25: 162-172.

Huang, C. F., N. Yamaji, N. Mitani, M. Yano, Y. Nagamura and J. F. Ma, 2009. "A Bacterial-Type ABC Transporter Is Involved in Aluminum Tolerance in Rice." The Plant Cell 21(2): 655-667.

Kochian, L. V., M. A. Piñeros, J. Liu and J. V. Magalhaes, 2015. "Plant Adaptation to Acid Soils: The Molecular Basis for Crop Aluminum Resistance." Annual Review of Plant Biology 66(1): 571-598.

Li, J. Y., J. Liu, D. Dong, X. Jia, S. R. McCouch and L. V. Kochian, 2014. "Natural variation underlies alterations in Nramp aluminum transporter (NRAT1) expression and function that play a key role in rice aluminum tolerance." Proc Natl Acad Sci U S A 111(17): 6503-6508.

Liu, J., J. V. Magalhaes, J. Shaff and L. V. Kochian, 2009. "Aluminum-activated citrate and malate transporters from the MATE and ALMT families function independently to confer Arabidopsis aluminum tolerance." Plant J 57(3): 389-399.

Ma, J. F. (2007). "Syndrome of aluminum toxicity and diversity of aluminum resistance in higher plants." Int Rev Cytol 264: 225-252.

Magalhaes, J. V., J. Liu, C. T. Guimaraes, U. G. Lana, V. M. Alves, Y. H. Wang, R. E. Schaffert, O. A. Hoekenga, M. A. Pineros, J. E. Shaff, P. E. Klein, N. P. Carneiro, C. M. Coelho, H. N. Trick and L. V. Kochian, 2007. "A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum." Nat Genet 39(9): 1156-1161.

Sasaki, T., Y. Yamamoto, B. Ezaki, M. Katsuhara, S. J. Ahn, P. R. Ryan, E. Delhaize and H. Matsumoto, 2004. "A wheat gene encoding an aluminum-activated malate transporter." Plant J 37(5): 645-653.

Winter, D., B. Vinegar, H. Nahal, R. Ammar, G. V. Wilson and N. J. Provart, 2007. "An "Electronic Fluorescent Pictograph" Browser for Exploring and Analyzing Large-Scale Biological Data Sets." PLoS ONE 2(8): e718.

Zhu, X. F., Y. Z. Shi, G. J. Lei, S. C. Fry, B. C. Zhang, Y. H. Zhou, J. Braam, T. Jiang, X. Y. Xu, C. Z. Mao, Y. J. Pan, J. L. Yang, P. Wu and S. J. Zheng, 2012. "XTH31, encoding an in vitro XEH/XET-active enzyme, regulates aluminum sensitivity by modulating in vivo XET action, cell wall xyloglucan content, and aluminum binding capacity in Arabidopsis." Plant Cell 24(11): 4731-4747.

Metabolite profiling of shoot extract, root extracts and root exudates of rice and soybean under phosphorus deficiency

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Abstract
We applied a metabolite profiling technique to investigate root exudates of rice and soybean under phosphorus (P) deficiency. Rice and soybean were grown in a culture solution at P concentrations of 0 (P0) and 8 (P8) mg P L^{-1}. Shoot extracts, root extracts, and root exudates were obtained and their metabolites were determined by capillary electrophoresis/time-of-flight mass spectrometry. Concentrations of several metabolites including amino acids and organic acids in root exudates were higher at P0 than at P8. These findings suggest that rice and soybean roots actively release metabolites in response to P deficiency.

INTRODUCTION

The low phosphorus (P) tolerance of plants is mainly determined by the ability of plants to acquire P from soil and use P efficiently. Organic acids and phosphatase are P acquisition metabolites among various kinds of metabolites in root exudates. The roles of other metabolites in low-P tolerance are not yet clarified. It is necessary to detect changes of metabolites in root exudates under P deficiency. Metabolomics is the unbiased identification and quantification of all metabolites in a biological material. The capillary electrophoresis/time-of-flight mass spectrometry (CE-TOF MS) is a useful analytical method of separating and detecting a wide range of ionic metabolites, such as amino acids, organic acids, sugar phosphates, and nucleotides. The purpose of the present study is to apply metabolite profiling using CE-TOF MS to the analysis of root exudates and to clarify the composition of rice and soybean root exudates under P deficiency.

MATERIALS AND METHODS

Rice (*Oryza sativa* cv. Nipponbare) and soybean (*Glycine max* cv. Suzuyutaka) were grown in a culture solution at P concentrations of 0 (P0) and 8 (P8) mg P L^{-1} for 1, 5, 10, and 15 days after transplanting. Frozen shoot and root were homogenized with Zirconia beads and their extracts were obtained. Plants were bound with urethane foam and were placed at top of a paper cup containing 100 mL of sterile deionized water. Cups were aerated and placed in a dark room for 12 hours. The root exudate solution was stored at -20˚C. Frozen root exudates were freeze-dried.
Metabolites in shoot extract, root extract and root exudates were determined by capillary electrophoresis/time-of-flight mass spectrometry (CE-TOF MS). Shoot dry weight and shoot P concentration of rice and soybean were determined.

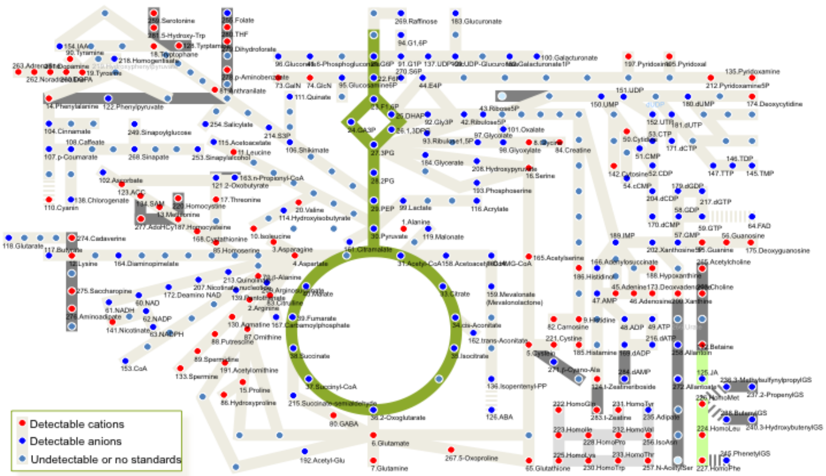

Fig.1. Detectable ions in shoot extract, root extract and root exudates with CE-TOF MS

RESULTS AND DISCUSSION

The shoot P concentration and dry weight of rice and soybean plants grown at P0 were lower than those at P8. Eighty, 90, and 65 metabolites were identified in shoot extracts, root extracts, and root exudates of rice, respectively. Sixty-three to eighty-four percent of the metabolites were exuded to the rhizosphere. More than 33% of the metabolites in the root exudates of rice showed higher concentration at low P than at high P. On the other hand, only 14% of the metabolites in the root extracts of rice showed lower concentration at low P than at high P. One hundred and eight, 116, and 79 metabolites were identified in the shoot extract, root extract, and root exudates of soybean, respectively. The concentrations of several metabolites including amino acids and organic acids in root exudates of soybean were higher at P0 than at P8, irrespective of the P concentration in the shoot or root extract. Concentration of L-asparagine, L-proline, L-serine and shikimic acid were higher in both P0 rice plants and P0 soybean plant. These findings suggest that rice and soybean roots actively release metabolites in response to P deficiency.

Table 1. Change in metabolite concentration of shoot extract, root extract and root exudate of soybean under phosphorus deficiency on 1, 5, 10 and 15 days after transplanting

Plant part	Response	Days after transplanting			
		1	5	10	15
Shoot extract	Increased	**9**	**26**	**20**	**30**
	Decreased	**3**	**10**	**17**	**27**
	Unchanged	93	76	64	55
	Total	105	112	101	112
Root extract	Increased	**16**	**33**	**22**	**25**
	Decreased	**7**	**9**	**25**	**44**
	Unchanged	86	75	63	61
	Total	109	117	110	130
Root exudate	Increased	**1**	**10**	**4**	**10**
	Decreased	**7**	**8**	**10**	**14**
	Unchanged	67	68	62	63
	Total	75	86	76	87

REFERENCES

Tawaraya, K., Horie, R., Saito, A., Shinano, T., Wagatsuma, T., Saito, K., and Oikawa, A. 2013. Metabolite Profiling of Shoot Extracts, Root Extracts, and Root Exudates of Rice Plant under Phosphorus Deficiency. *J Plant Nutr,* 36, 1138-1159.

Tawaraya, K., Horie, R., Shinano, T., Wagatsuma, T., Saito, K., and Oikawa, A. 2014. Metabolite profiling of soybean root exudates under phosphorus deficiency. *Soil Science and Plant Nutrition*, 60, 679-694.

PARVUS affects aluminum sensitivity by modulating the biosynthesis of glucuronoxylan and aluminum-binding capacity in Arabidopsis

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Abstract
Here we have found a mutant *parvus*, which loss α-D-glucuronic acid (GlcA) side chains in glucuronoxylan (GX), was more Al resistant than wild type (WT) plants, with less Al in the root and cell wall, which was not correlated with pectin content. However, the decreased cell wall Al accumulation was found to be in hemicellulose fraction. While the hemicellulose content did not change, the GlcA level of xylan was increased by Al treatment. Taken together, we conclude that modulation of the GlcA level of xylan influences the Al sensitivity in *Arabidopsis* by affecting its cell wall Al binding capacity.

INTRODUCTION
Aluminum (Al) toxicity is the major limiting factor for crop production (von Uexkull and Mutert, 1995). When the soil pH is below 5, Al is solubilized in the soil water and absorbed by plant roots (Vardar and Ünal, 2007). Al ions at micromolar level inhibit root elongation through structural and functional damage presumably due to Al ion interaction with the cell wall. Cell wall pectin and hemicellulose have been regarded as the major Al binding sites either as their negatively charged carboxylic groups have a high affinity for Al^{3+} or as the decreased *O*-acetyl-substituent in xloglucan (Blamey et al., 1990; Chang et al., 1999; Yang et al., 2011; Zhu et al., 2012; Zhu et al., 2014). As xylan also can bind Al (Zhu et al., 2012), we studied if and to what extent the degree of GlcA in xylan affects Al binding and changes Al sensitivity of the plant. For this purpose, mutant which losses tetrasaccharide primer sequence at the reducing end of glucuronoxylan (GX) and an absence of glucuronic acid side chains in GX was utilized (Lee et al., 2007).

MATERIALS AND METHODS
The Columbia ecotype (Columbia-0) of Arabidopsis (*Arabidopsis thaliana*) (WT) and the GX content reduced mutant *parvus* were used in this study. For short time treatments (24 h), 0.5mM $CaCl_2$ solution (pH 4.5) was used as Control, while for long time treatments (7 d), the nutrient solution (pH 4.5) was used as Control and different concentrations of Al was directly added for dose treatments. Cell wall and total RNA extraction with or without treatments were based on Zhu et al (2012). The measurement of root and cell wall Al content were according to Zhu et al (2013). Generation of *PARVUS*-GUS Transgenic Lines was according to Zhu et al (2014).

RESULTS AND DISCUSSION
In plants, the earliest and most distinct symptom of Al toxicity is the inhibition of root growth (Kochian, 2005).When grown on agar medium containing 50 μM Al^{3+} for 7 days, the root growth of Arabidopsis wild-type Col-0 (WT) was inhibited by 50%, but less so in *parvus* (12%), indicating that a lack of GX results in more Al resistant roots. The Al content of the root of the *parvus* mutant was less than WT, indicating that an decrease in GX content lead to observable alleviation effect of Al toxicity.
The expression pattern of *PARVUS* mRNA measured by quantitative real-time PCR (RT-qPCR). Results showed that the *PARVUS* transcripts were accumulated in roots as well as leaves, stems and flowers, with the highest levels in roots and stems, and relatively lower levels in leaves and flowers. The in vivo tissue-specific localization of *PARVUS* was further investigated by a promotor GUS staining approach. A DNA fragment consisting of 2.3-kb located upstream of the *PARVUS* coding region was used to drive the expression of GUS reporter gene and transformed into Arabidopsis WT plants (Zhu et al., 2012). GUS staining was observed in both roots and shoots except the hypocotyl. In shoots, p*PARVUS*:GUS was constitutively expressed in the young leaves whereas the activity was greatly reduced in fully expanded leaves. In roots, it was predominantly expressed in the root tips, including the elongation zone.
To examine whether Al stress affects the transcriptional level of *PARVUS*, dose-response and time course experiments were conducted. RT-qPCR analysis revealed that the expression level of *PARVUS* in WT was substantially induced even at an Al concentration as low as 5 μM and up-regulated by 50 μM A^{l3+} treatment even within 1 h, suggesting that *PARVUS* expression is very sensitive to Al stress. Moreover, Al treatment strengthen GUS expression, further confirming its response to Al.
Since xylan is the major Al binding site in Arabidopsis (Zhu et al., 2012) and mutant *parvus* exhibited reduced

level in GX content (Lee et al., 2007), we first measured Al content in the root cell walls and found that less Al was accumulated in *parvus*. The Al adsorption kinetics of whole crude wall preparations further demonstrated that the root cell walls of *parvus* adsorbed significantly less Al than that of WT, then we analyzed the non-methylated GlcA residue (X_4G) and a methylated GlcA residue (X_4M) content in root cell walls after Al treatment. β-endoxylanase digestion and MALDI-TOF analysis indicated that the ratio of X_4G/X_4M significantly increased under Al stress, consistent with a significant accumulation of Al in cell wall, indicating that the accumulation of cell wall Al may be depend on the ratio of X_4G/X_4M, as the reduction of the cell wall Al was observed in *parvus*, which possess a lower ratio of X_4G/X_4M (Lee et al., 2007), further prove the assumption above.

Besides xylan, pectic polysaccharides and other polysaccharide in hemicellulose, such as xyloglucan also can bind Al in Arabidopsis (Yang et al., 2011; Zhu et al., 2012). Therefore, we also measured uronic acid content as well as Al retention in pectin. As expected, there was no difference in the hot water extracted pectin between WT and *parvus* as measured by the uronic acid content of the pectin extract. Despite this increase in the pectin content in the WT, there was no difference in Al binding capacity between WT and *parvus* in the extractable pectin. Hemicelluloses were extracted from depectinated root cell walls combining a mild alkali extract (4% w/v) and a strong alkali extract (24% w/v KOH). While no difference in hemicellulose content was observed between WT and *parvus* under normal growth and Al treatment conditions. Therefore the lower Al content in the hemicellulose in the *parvus* compared to WT plants also is not attributed to the change of hemicellulose content.

In conclusions, our study demonstrated that Al stress increased the ratio of X_4G/X_4M, thus more Al was bound to the cell wall hemicellulose. In other words, plants with a lower XG content (lower X_4G/X_4M ratio) might result in less Al accumulation in the cell wall, thus becoming more Al resistant.

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REFERENCES

Vardar F, ünal Meral (2007) Aluminum toxicity and resistance in higher plants. Advances in Molecular Biology 1: 1-12

von Uexküll HR, Mutert E (1995) Global extent, development and economic impact of acid soils. In RA Date, NJ Grundon, GE Raymet, ME Probert, eds, Plant-Soil Interactions at Low pH: Principles and Management. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 5-19

Blamey FPC, Edmeades DC, Wheeler DM (1990) Role of cationexchange capacity in differential aluminium tolerance of Lotus species. J Plant Nutr 13 :729-744

Chang YC, Yamamoto Y, Matsumoto H (1999) Accumulation of aluminium in the cell wall pectin in cultured tobacco (*Nicotiana tabacum* L.) cells treated with a combination of aluminium and iron. Plant Cell Environ 22: 1009-1017

Lee C, Zhong R, Richardson EA, Himmelsbach DS, McPhail BT, Ye, ZH (2007) The *PARVUS* gene is expressed in cells undergoing secondary wall thickening and is essential for glucuronoxylan biosynthesis. Plant Cell Physiol 48: 1659-1672

Yang JL, Zhu XF, Peng YX, Zheng C, Li GX, Liu Y, Zheng SJ. 2011. Cell wall hemicellulose contributes significantly to Al adsorption and root growth in Arabidopsis. Plant Physiol 155:1885-1892.

Zhu XF, Shi YZ, Lei GJ, Fry SC, Zhang BC, Zhou YH, Braam J, Jiang T, Xu XY, Mao CZ, Pan YJ, Yang JL, Wu P, Zheng SJ. *XTH31*, encoding an in-vitro XEH/XET-active enzyme, controls Al sensitivity by modulating in-vivo XET action, cell wall xyloglucan content and Al binding capacity in Arabidopsis. Plant Cell 24: 4731-4747

Zhu XF, Sun Y, Zhang BC, Wan JX, Liu Y, Wang ZW, Shi YZ, Zhou YH, Zheng SJ (2014) *TRICHOME BIREFRINGENCE-LIKE27* Affects Aluminum Sensitivity by Modulating the *O*-Acetylation of Xyloglucan and Aluminum-Binding Capacity in Arabidopsis. Plant Physiol. 166:181-189.

A field survey of aluminium toxicity in New Zealand upland soils varying in parent material and climate

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Abstract
Soil acidity and associated aluminium (Al) toxicity severely limit the establishment and growth of legumes in New Zealand upland pastures. A survey of 10 soils, differing in location, soil order, parent material and climate, showed soil pH ranged from 4.9-6.4, with exchangeable Al concentrations ($CaCl_2$ extractable) of 0.5-23.3 mg Al/kg. At all sites exchangeable soil Al was measured (0-50+ cm) at concentrations well above the current toxicity threshold for legumes. Exchangeable Al was extremely variable at any given pH value. Factors driving the variability in exchangeable Al concentrations were unclear and require further research.

INTRODUCTION
In New Zealand most high country (upland) soils are acidic, low fertility and have Al toxicity (Moir and Moot, 2014). Pasture legumes play a critical role in these grazed pasture systems, providing a high quality feed source for stock, and critically, fix atmospheric N (N_2). However, many pasture legumes are sensitive to acid soils and aluminium toxicity, which reduce the yield and persistence of legumes (Scott *et al*, 2008). However it is often uneconomic for farmers to apply lime across extensive hill and high country areas with challenging topography (Moir and Moot, 2014). The first step in the remediation process is to identify soils which are most susceptible to Al toxicity.

MATERIALS AND METHODS
Soils were collected from New Zealand sites with known acidity and Al issues. Sites differed in location, seasonal/annual climate, relief and soil order (Table 1). At each site bulk soil (0-15 cm horizon) was collected along a 40 m transect. Soil core samples were also taken at 0-7.5 cm and 7.5-15 cm depth across the area and bulked. Also, a soil profile was dug and samples were collected down the profile to a maximum depth of 75 cm, or shallower where gravels prevented sampling. Soils samples were air-dried and 2 mm sieved. Soil pH was measured using a soil water ratio of 2.5:1 (Blackmore *et al.*, 1987) and exchangeable Al was measured using 0.02M $CaCl_2$ extraction (Edmeades *et al.*, 1983) followed by ICP-OES.

Table 1- Site information for 10 soils sampled across New Zealand

Soil Code	Location	Mean Annual rainfall (mm)	Soil Order	Parent material	Topography	Elevation (m asl)
AR	Taupo	1000-1300	Pumice	Pumice (rhyolitic)	Gentle slope	495
PK	Gisborne	1600-2000	Pumice	Pumice (rhyolitic)	15° slope	495
WT	Waikato	1600-2000	Andesitic ash	Ash (andesitic)	20° slope	84
MO	Marlborough	1000-1300	Allophanic Brown	Sedimentary (colluvium)	Valley terrace	914
GM	Tekapo	800-850	Brown	Sedimentary (moraine)	Flat-rolling	765
OM	Omarama	500-600	Recent	Sedimentary (alluvial)	Terrace flats	489
BD	Omarama	1000-1200	Brown	Sedimentary (alluvial)	Valley floor	545
LP	Tarras	700-800	Pallic	Sedimentary (loess)	Downs	530
GF	Hawea	600-800	Dense brown	Sedimentary	25-30° hill slope	786
MG	Hawea	600-800	Brown soil	Sedimentary	20-25° hill slope	564

RESULTS AND DISCUSSION
Results
The exchangeable Al concentrations showed a curvilinear decline from 23.3 mg/kg to 0.5 mg/kg across the pH range of 4.9 to 6.4 (Figure 1; R^2 = 0.65). The coefficient of variation reflected considerable variation in soil Al values for the same pH value. The threshold for plant aluminium toxicity (3 mg/kg) occurred for pH values below 5.7. However, some samples had a value above the Al threshold up to a pH of 5.9. Equally there were individual values below the threshold at a pH of 5.2. The MO soil (Allophanic brown) was the most toxic in Al in the top 15 cm (pH of 5.1) and an Al concentration of 18.9 mg/kg (Figure 1). However, the dense brown soil (GF) peaked at 20.2 mg/kg Al at a depth of 20 cm and a pH of 5.0. The lowest Al value was measured at 0.5mg/kg in both the AR (pumice soil) at 30-50 cm at a pH of 6.2 and 6.4 and in the WT (ash soil) from 40-50 cm at a pH of 5.9.

Discussion

This research clearly shows that across different New Zealand soil orders (pumice, ash, brown, recent and pallic soils) and differing geographical locations, Al toxicity currently represents a significant issue for New Zealand farmers. Concentrations of exchangeable Al were measured above the toxicity threshold of 3 mg/kg at all of the 10 sites. The depth and the soil pH at which Al peaked in each soil varied between soils. For several soils high Al was found in the top 15 cm of the profile (pasture root zone) while in others it was measured at depths >15cm. The large variability in soil Al concentrations at a single pH supports the results of Moir and Moot (2014) and extends the data to include new information on several previously unstudied soil orders. As yet, the mechanisms driving the aforementioned variability remain unexplained.

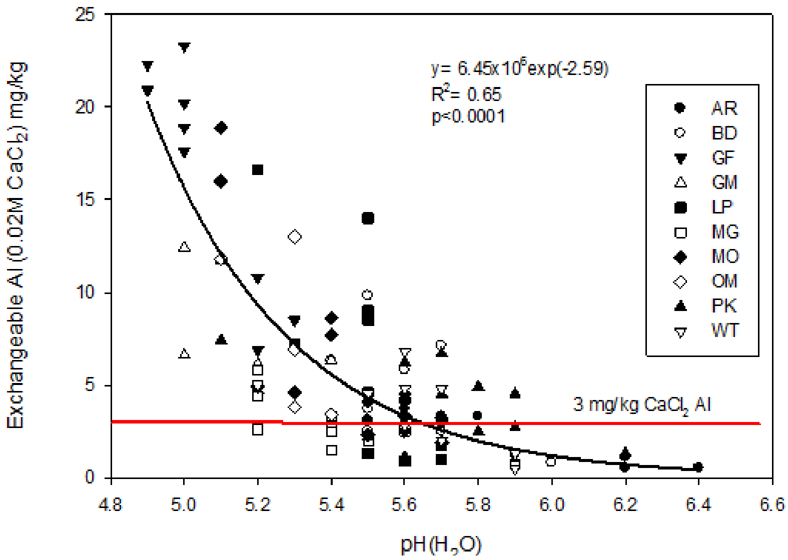

Figure 1. The relationship between soil pH (H_2O) and exchangeable soil Al for 10 New Zealand soils

There is a wide range of mean annual rainfalls across these sites, but all soils had high Al concentrations somewhere in their profiles. There was no apparent relationship between soil Al concentrations and annual rainfall. Interestingly, the MG and GF sites were close in location, experience the same mean annual rainfall and yet the GF site was much more acidic and showed higher exchangeable Al concentrations than the MG site. This result remains unexplained and further research is required to determine the key factors driving Al concentrations. These data also indicated that high Al concentrations followed the sequence of brown soils > pallic soils ≥ recent soils > ash soils ≥ pumice soils. However, this study has limited sample numbers and therefore a more extensive data analysis is currently underway to confirm this result. These findings support other work showing high Al on New Zealand brown soils (Hochman *et al*, 1992).

CONCLUSIONS

This study confirms that Al toxicity is not restricted to specific soil orders, or rainfall regimes, but rather extends across many soil orders and climatic zones in the high country of New Zealand. These soils are widespread in terms of their geographical location and yet the majority of the soils at the 10 sites had Al present at concentrations toxic to pasture legumes. Soils with the same pH varied considerably in exchangeable Al concentrations. Extensive further research is required to determine the key factors driving exchangeable Al concentrations in New Zealand soils. This field soil survey represents initial work contributing to a larger research program examining soil pH and Al toxicity in New Zealand high and hill country soils.

REFERENCES

Blackmore, L.C., Searle, P.L., Daly, B.K., 1987. Methods of chemical analysis of soils. New Zealand Soil Science Bureau, vol 80, Lower Hutt.

Edmeades, D.C., Smart, C.E., Wheeler, D.M., 1983. Aluminium toxicity in New Zealand soils: preliminary results on the development of diagnostic criteria. New Zealand Journal of Agricultural Research, 26: 257-268.

Hochman, Z., Edmeades, D.C., White, E., 1992. Changes in effective cation exchange capacity and exchangeable aluminium with soil pH in lime- amended field soils. Australian Journal of Soil Research. 30:177-187.

Moir, J.L., Moot, D.J., 2014. Medium term soil pH and exchangeable aluminium response to liming in three high country locations. Proceedings of the New Zealand Grassland Association 76:41-46.

Scott, B.J., Ewing,M.A., Williams,R., Humpheries, A.W and Coombes, N.E., 2008. Tolerance of aluminium toxicity in annual Medicago species and lucerne. Australian Journal of Experimental Agriculture 48: 499-511.

Aluminium sensitivity and phosphorus response of twelve forage legumes grown in an acid upland New Zealand soil

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Abstract
Soil acidity, associated Al toxicity and low P fertility are key factors driving low legume production and persistence in New Zealand upland pastures. Twelve novel legume species were grown for 42 weeks in glasshouse conditions in a typical acidic high country soil, with incremental rates of lime and P. Lime increased the yield of annual and perennial species up to a point, beyond which yield decreased. All responded to P. Lotus and tagasaste yielded highest under acidic conditions. The optimum pH, corresponding soil exchangeable Al and Olsen P level for maximum yield has been identified for these 12 legume species.

INTRODUCTION
Soil acidity, aluminum (Al) toxicity and low fertility are major issues in New Zealand high country (uplands; Moir & Moot, 2014). Many pasture legumes, critical for quality stock feed and nitrogen (N) inputs, are very sensitive to Al toxicity and low P status (Moir et al, 2000). As a result, most legumes fail to establish, grow and persist in these environments (Scott et al, 2008). Legumes tolerant of high soil Al and low P are urgently required, but such information is scarce in literature.

MATERIALS AND METHODS
Bulk soil (0-15 cm) was collected from a high country farm with known Al issues in North Canterbury, New Zealand. Soil pH (H_2O) was 5.0, Olsen P 9 mg/L an exchangeable Al (0.02M $CaCl_2$) 13.9 mg/kg. Twelve legume species were grown; Arrowleaf clover (*Trifolium vesiculosum*; West Coast Seed (Aus) cv. 'Cefalu'), Balansa clover (*Trifolium michelianum*; Seed Mark cv. 'Bolta'), Caucasian clover (*Trifolium aumbiguum*; PGG Wrightsons cv. 'Endura 3'), Gland clover (*Trifolium glanduliferum*; Kiwi Seed Co. cv. 'Prima'), Lotus (*Lotus pedunculatus*; Tai Tapu, Selwyn district cv. 'Maku'), Lucerne (*Medicago sativa*; Seed Force cv. 'Force 4'), Persian clover (*Trifolium resupinatum*; Specialty Seeds cv. 'Enrich'), Falcata lucerne (*Medicago falcate*; Kiwi Seed Co.), Subterranean clover (*Trifolium subterraneum*; Kiwi Seed Co. cv. 'Mt Barker'), Strawberry clover (*Trifolium fragiferum*; Gentos cv. 'Lucila'), Tagasaste (*Chamaecytisus proleferus*; Selwyn district) and White clover (*Trifolium repens*; Grasslands cv. 'Nomad'). Either $CaCO_3$ (0, 2, 5, 8 or 15 T/ha) or P (0, 10, 30, 60, 100, 250,500 or 1500 mgP/kg) was mixed into the soil and 600 g added to pots. All pots received basal nutrient solution throughout the experiment (no N), with P treated pots receiving basal lime of 5 T/ha. Pots also received species-specific inoculum soon after seed germination. Shoots were harvested at 4-5 week intervals for 42 weeks, dried at 70ºC for 48 hours, weighed, fine ground, acid digested and analyzed for elemental concentration by ICP-OES (Varian 720-ES ICP-OES; Varian Inc., Victoria, Australia). Soils were analyzed for pH, exchangeable Al and Olsen P ststus at the completion of the experiment.

RESULTS AND DISCUSSION
Results
Of the annual species, persian clover was the highest yielding (mean 8.5 g DM pot^{-1}) and Lotus was the highest yielding perennial (mean 9.6 g DM/pot, Table 1). All annual species increased in yield to a maximum point, at either 2 or 5 t lime/ha, then declined with further lime applications. At 0 t lime/ha (pH = 5.0) tagasaste and lotus were the highest yielding species at 8.2 and 7.5 g DM/pot. There was also a strong ($P < 0.001$) species by lime rate interaction. Phosphorus uptake by the plants was strongly affected by lime rate. The average concentration of P in the plant tissue increased ($P < 0.001$) from 0.184% at 0 t lime/ha up to 0.226% at 5 t lime/ha, then decreased to 0.182% at 15 t lime/ha across all species (Table 1). For P treatment soils, all species increased in yield with increasing P rates. At 0 t lime/ha gland and white clovers had the highest herbage molybdenum (Mo) concentration (0.25 and 0.21 ppm) while lotus and tagasaste had very low Mo (0.02 and 0.03 ppm). Soil Al was 13.9, 5.0, 1.7, <0.5, <0.5 at pHs 5.0, 5.4, 5.8, 6.7 and 7.5 (lime rates 0, 2, 5, 8 and 15 T/ha). At high lime rates, boron (B) concentrations dropped by 70% across all species, indicating potential lime induced B deficiencies.

Table 1. Values of mean shoot yield, P concentration, P uptake, Mo and B concentrations by 12 pasture legume species grown in glasshouse conditions in a NZ high country soil supplied with increasing rates of lime.

Species		Mean yield (g DM/Pot)	Mean Shoot P Concentration (% P)	Mean Shoot P Uptake (mg P/Pot)	Mean Shoot Mo Conc (ppm)	Mean Shoot B Conc (ppm)
Arrowleaf clover		2.16	0.156	3.8	0.30	6.37
Balansa clover		0.64	0.183	1.3	0.60	10.68
Caucasian clover		4.23	0.249	10.1	2.27	13.94
Gland clover		0.24	0.107	4.6	0.23	6.77
Lotus		9.62	0.233	22.5	0.49	17.09
Lucerne		5.86	0.194	12.1	0.71	16.31
Persian clover		6.12	0.191	12.1	0.92	14.77
Falcata lucerne		3.92	0.256	10.4	0.58	14.73
Subterranean clover		1.54	0.193	3.2	0.54	9.30
Strawberry clover		4.39	0.196	9.2	0.85	13.64
Tagasaste		7.05	0.203	13.2	0.59	8.32
White clover		3.94	0.243	9.6	1.27	16.14
Grand Mean		4.41	0.200	9.1	0.78	12.38
Species	SEM	0.142	0.0032	0.34	0.086	0.666
	LSD(5%)	0.395	0.0089	0.94	0.241	1.858
P	Species	***	***	***	***	***
	L Rate	***	***	***	***	***
	Sp*L Rate	***	***	***	***	***

*** Significant at < 0.001 level; L = Lime; Sp = Species

Discussion

Soil pH had a large effect on the yield of both perennial and annual species ($P > 0.001$). The increase in yield up to pH 6.0 was strongly driven by increasing P availability and decreased Al in the soil, directly resulting from lime inputs. Increased Mo availability was apparent from increased herbage Mo, resulting from increased lime inputs, in agreement with Hochman *et al* 1992. With the control soil at a pH of 5.0, lime addition to the soil reduced the exchangeable Al levels in the soil, while increasing the P and Mo availability to the plants up to a pH of 6.0, in accordance with Wheeler and O'Conner, 1998. Yield increases with lime inputs (increasing soil pH) were different between species ($P < 0.001$). Strawberry clover, lucerne and falcata lucerne had very significant increases in yield from the control compared to other species such as tagasaste and lotus, which only had moderate increases in yield of 34% and 46% with lime addition. Some researchers have suggested that these differences are driven by species adapting to acidic environments, potentially making them more suitable for acidic high country soils (Maxwell *et al*, 2014). Species exhibiting very large lime responses are those which are particularly sensitive to low soil pH, in terms of Al toxicity, and P and Mo deficiency. In many cases, significant improvements in yields were achieved here with low inputs of lime, for example gland clover, lotus, persian clover and tagasaste all produced around 90% of maximum yield with just 2 t lime/ha on this soil.

CONCLUSIONS

This study confirms that pasture legume species vary considerably in terms of sensitivity to soil Al. The optimum pH ranges for plant growth were identified for 12 legume species. Yields were driven primarily by P availability and exchangeable Al levels in the soil, which were in turn driven by soil pH. This experiment is part of larger research program examining soil pH and Al toxicity in New Zealand high and hill country soils.

REFERENCES

Hochman, Z., Edmeades, D.C., White, E. (1992). Changes in effective cation exchange capacity and exchangeable aluminium with soil pH in lime- amended field soils. *Australian Journal of Soil Research.* 30:177-187.

Maxwell, T.M.R., Moir, J.L, Edwards, G.R. (2014). Sulphur and lime response of four adventive annual clovers grown in a New Zealand high country soil under glasshouse conditions. *New Zealand Journal of Agricultural Research* 55: 47-62.

Moir, J.L., Moot, D.J. (2014). Medium term soil pH and exchangeable aluminium response to liming in three high country locations. *Proceedings of the New Zealand Grassland Association* 76:41-46.

Moir, J.L.; Scotter, D.R.; Hedley, M.J.; MacKay, A.D. 2000. A climate-driven, soil fertility dependent, pasture production model. *New Zealand Journal of Agricultural Research* 43: 491-500.

Scott, B.J., Ewing, M.A., Williams, R., Humpheries, A.W and Coombes, N.E. (2008). Tolerance of aluminium toxicity in annual *Medicago* species and lucerne. *Australian Journal of Experimental Agriculture* 48: 499-511.

Wheeler, D.M., O'Connor, M.B. 1998. Why do pastures respond to lime? *Proceedings of the New Zealand Grassland Association* 60: 57-61.

Amelioration of aluminum toxicity for rice cultivation in an acid sulfate soil using plant-growth promoting bacteria, ground magnesium limestone and basalt

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Abstract
A study was conducted in laboratory and field conditions to ameliorate aluminum (Al) toxicity using plant-growth promoting bacteria (PGPB), ground magnesium limestone (GML) and ground basalt. Five-day-old rice seedlings were inoculated by PGPB and grown under pH 4.0 for 21 days at various Al concentrations. Results showed that Al severely affected the plant growth with ruptured roots and cell collapse. However, no root damage was observed in the PGPB inoculated seedlings. Field study showed highest rice yield in the PGPB and GML treatments. The Al toxicity was reduced by PGPB via production of organic acids that chelated the Al ion and polysaccharides that increased pH.

INTRODUCTION

In acidic soils, Al^{3+} limits the growth of roots either by inhibition of cell division or cell elongation or both (Marschner, 1991). Al toxicity can be reduced by neutralizing the acidity using calcareous amendments. Studies have shown that higher number of plant-growth promoting bacteria (PGPB)is associated with rice rhizosphere (Ma 2001) and the bacteria have the potential to produce large amounts of organic acids (Naher et al., 2009) resulted in phosphorus binding through chelation, and this may also be a possible mechanism for reducing Al toxicity of roots. The performance of PGPB in plant growth promotion is better with a consortium of bacterial strains than individual strains (Panhwar, 2012). Addition of these potential PGPB would enhance the growth of rice on soils with high Al content. In order to fully utilize soil for food production studies are now focusing on rice production in less fertile acidic soils with Al toxicity. Hence, the present study aimed to determine the effects of PGPB and ground magnesium limestone (GML) and basalt application on rice growth in high Al containing acid sulfate soils and to explain the possible mechanisms involved.

MATERIALS AND METHODS

Laboratory experiments were conducted at Universiti Putra Malaysia, while the field study was conducted on acid sulfate soil in Semarak, Kelantan, Malaysia. The PGBP isolates *Bacillus* sp. PSB16, *Stenotrophomonas maltophila* Sb16, *Burkholderia thailandensis* ASB7 and *Burkholderia seminalis* ASB21 inoculums were applied to MR219 rice seedlings. Plants were grown for 21 days in growth chamber. Organic acids were determined from plant growth medium using high performance liquid chromatography (HPLC) and plant root morphology was analyzed by using a root scanner. The field study comprised six treatments, control, biofertilizer (Biof) (consisting of PGPB), GML, basalt, biofertilizer+GML, and biofertilizer+basalt at 4 t ha^{-1} was applied.

RESULTS AND DISCUSSION

Laboratory Study - Effect of Al on Growth of Rice Seedlings Inoculated with PGPB

It was observed that high Al concentration severely affected growth of the rice seedlings. Plant height and root volume were significantly decreased with increased in Al concentrations (Fig. 1a–b). In contrast, the PGPB inoculated plants showed good growth with roots affected by the high Al concentrations. The PGPB has great potential to enhance plant growth in acid soil (Ngoc Son et al., 2006).

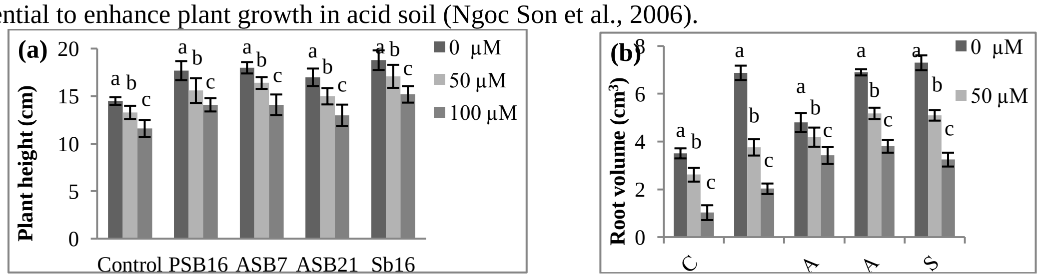

Figure 1. Effects of Al on the growth of rice seedlings inoculated with PGPB: (a) plant height; (b) root volume
Means within treatments with the same letters are not significantly different at $p > 0.05$.
Bars indicate standard error, *n* = 4.

Effects of Al on the Release of Organic Acids

It was observed that organic acids released by the rice roots with or without PGPB varied with Al concentrations (Figure 2a–c). Rice roots without PGPB secreted lower amounts of organic acids compared to those inoculated with the bacteria. Organic acids released was enhanced by PGPB inoculation at high concentrations of Al. Among the organic acids, higher amounts of malic and citric acids were released by the bacteria compared to oxalic acid. The amount of malic acid was found to be high, particularly at 100 µM Al concentration. The PGPB was able to produce organic acids to chelate the Al and solubilize the insoluble P, thus, providing the nutrient to the plants (Panhwar et al., 2011).

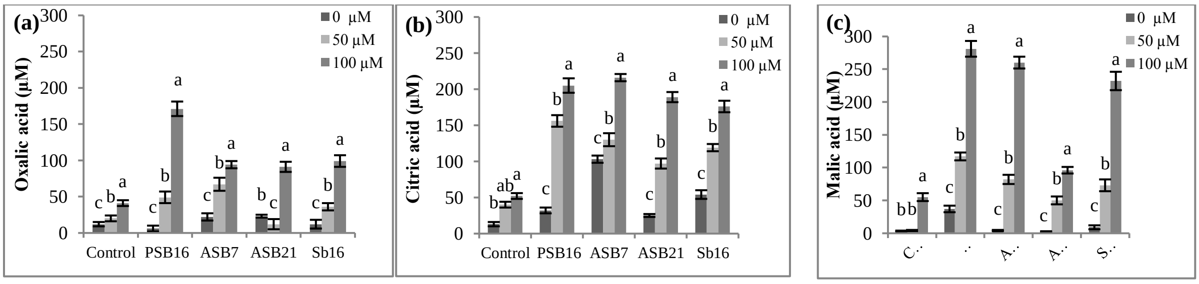

Figure 2. Effects of Al on the release of organic acids by rice plant and or PGPB: (a) oxalic; (b) citric; and (c) malic acid. Means within treatments with the same letters are not significantly different at $p > 0.05$.
Bars indicate standard error, $n = 4$.

Field study showed that the biofertilizer, basalt and GML applications improved the growth and yield of rice. Unamended control plants were severely affected with low growth and yield. The highest grain yield of 6.82 t ha^{-1} was obtained in biofertilizer in combination with GML treatment (Table 1). This is consistent with earlier study of Panhwar et al. (2014).

Table 1. Effects of biofertilizer, GML and basalt on growth and yield of rice on acid sulfate soil

Treatments	**Root Length (cm)**	**Tillers plant^{-1}**	**Number of Panicle plant^{-1}**	**Size of Panicle $^{-1}$**	**Fertile Spikelets panicle^{-1}**	**Number of Unfilled Grains (%)**	**Weight of 1000 grain (g)**	**Grain Yield t·ha^{-1}**	**Harvest Index**
Control	19.66d	9c	7c	17.83e	61.01e	26.21a	17.02d	2.93d	0.40e
Biofertilizer	32. 41a	20a	14ab	20.10c	119.51b	16.12f	21.40c	5.39b	0.35d
[a]GML	21.34b	19a	15a	22.60b	116.81b	18.31d	22.33b	5.36b	0.45b
Basalt	20.13c	16b	16a	18.33d	98.85d	20.45b	20.01c	3.47c	0.41c
Biof + basalt	22.30b	19a	15a	23.00b	107.32c	19.24c	23.68a	5.33c	0.47b
Biof + GML	32.58a	21a	15a	24.23a	129.03a	17.82e	22.55b	6.82a	0.55a

Means within the same column followed by the same letters are not significantly different at $p > 0.05$

CONCLUSIONS

The study showed a possible method of alleviating aluminum toxicity effect to rice plant grown in acid sulfate soil. Application of plant-growth promoting bacteria as biofertilizer in combination with GML improved growth and yield of rice through chelation of Al ions by the organic acids produced by the bacteria. Application of biofertilizer provide a promising potential for rice cultivation in acid sulfate soils.

REFERENCES

Ma, J.F., Ryan, P.R., Delhaize, E. 2001. Aluminum tolerance in plants and the complexing role of organic acids. Trends of Plant Science. 6: 273–278.

Marschner, H. 1991. Mechanisms of adaptation of plants to acid soil. Plant and Soil. 134:1–20.

Ngoc Son, T., Diep, C.N., Giang, T.T.M. 2006. Effect of bradyrhizobia and phosphate solubilizing bacteria application on soybean in rotational system in the mekong delta. Omonrice. 14: 46-57.

Panhwar, Q.A., U.A. Naher, J. Shamshuddin, O. Radziah, M.A. Latif and M.I. Razi. 2014. Biochemical and molecular characterization of potential phosphate-solubilizing bacteria in acid sulfate soils and their beneficial effects on rice growth. PLOS ONE. 9(10): 1-14.

Panhwar, Q.A.; Radziah, O.; Zaharah, A.R.; Shariah, M.; Mohd Razi, I. Isolation and characterization of phosphorus solubilizing bacteria from Aerobic rice. Afr. J. Biotechnol. 2012, 11, 2711–2719.

Crop production on acid soils: overcoming soil acidity and aluminum toxicity

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Abstract
Soil acidity and aluminum (Al) toxicity in acid soils is a significant limitation to crop production worldwide. The high concentration of Al in the acid soil limits root development, reducing water and nutrient uptake. Decades of studies have resulted in a significant progress in revealing the mechanism of Al tolerance in plants. Several key genes have been identified and the Al tolerance was validated to be related with gene sequence variations in some plants. Elucidating the genetic and molecular mechanisms underlying Al tolerance is expected to accelerate the development of Al-tolerant genotypes. This review assesses the literature on aluminum toxicity, as well as crop-yield improvement–strategies for adaption to combined aluminium toxicity and drought stress. The emphasis is on the role of breeding to improve acid soil and aluminum tolerance.

INTRODUCTION

Soil acidity and aluminum (Al) toxicity in acid soils is a significant limitation to crop production worldwide, as approximately 50% of the world's potentially arable soil is acidic (Uexküll and Mutert, 1995). Soil acidity influences many chemical and biological reactions that control plant nutrient availability and toxicity of some elements, and is a serious limitation for crop production in many regions of the world. Crops growing on acid soils yield less than their potential because of the poorly developed root system that limits nutrient and water uptake (Maron et al., 2008). Toxicity of acid soils is mainly caused by low pH, thus agronomic practices to overcome this problem are primarily based on increasing soil pH. Lime increases soil pH, improves crop growth and decreases extractable Al^{3+}. Crop species and genotypes within species differ significantly in relation to their tolerance to soil acidity. Plant species have evolved to variable levels of tolerance to aluminum enabling breeding of high Al-tolerant cultivars. Improving practices plant tolerance to acid soil through breeding is still the best solution to cope with Al toxicity. This review assesses the literature on aluminium toxicity, as well as crop-yield improvement–strategies for adaption to combined aluminium toxicity and drought stress. The emphasis is on the role of breeding to improve acid soil and aluminum tolerance.

ALUMINUM TOXICITY

Aluminum toxicity is widely considered to be the most important limiting factor to plant growth in acid soils. In most plant species there is considerable genotypic variation for the ability to withstand Al toxicity. Plants have evolved different mechanisms to overcome Al stress, either by preventing Al_3^+ from entering the root („exclusion“ mechanisms) or by being able to neutralize toxic Al^{3+} absorbed by the root system (true „tolerance“ mechanisms (Kochian et al., 2015). The decrease in root growth is one of the initial and most evident symptoms of Al toxicity at micromolar (μM) concentrations in plants (Rengel and Zhang, 2003), inducing reduced capacity for water and nutrient uptake. Although progress has been made in the characterization of rooting characteristics, root phenotyping is still a bottleneck for the breeding of cultivars with improved nutrient acquisition.

LIMING AS A SOIL ACIDITY MANAGEMENT STRATEGY

Liming is the most widely used long-term method of soil acidity amelioration, and its success is well documented (Scott et al., 2001; Kovačević et al., 2005; Jovanović et al., 2006; Lončarić et al., 2006). Application of lime at an appropriate rate brings several chemical and biological changes in the soil, which are beneficial or helpful in improving crop yields on acid soils. Adequate liming eliminates soil acidity and toxicity of Al, Mn, and H; improves soil structure (aeration); improves availabilities of Ca, P, Mo, and Mg, pH, and N_2 fixation; and reduces the availabilities of Mn, Zn, Cu, and Fe and leaching loss of cations. For several crops, liming results in some chemical changes in the soil such as, increase in pH, effective cation exchange capacity (ECEC), and exchangeable Ca, decrease in toxic elements for example Al^{3+} and Mn^{2+} and changes in the proportion of basic cations in CEC sites. Over-liming, however, can significantly reduce the bioavailability of micronutrients (Zn, Cu, Fe, Mn and B), which decrease with increasing pH (Fageria and Baligar, 2008). Although soil acidity can be an important yield-limiting factor but little research has focused on within-field variation of soil pH and crop response to lime application.

CROP-YIELD IMPROVEMENT–STRATEGIES FOR ADAPTION TO COMBINED ALUMINUM TOXICITY AND DROUGHT STRESS

Present knowledge suggests that Al toxicity decreases drought resistance primarily by reducing the use of subsoil water and nutrients, and crops yield less under combined stresses (Yang et al. 2013). The most direct method of evaluating the interaction of Al and drought stresses is by measuring economic yield (grain or forage) under field conditions. Deleterious effects of subsoil soil-acidity on crop yield will thus be influenced by the extent to which a plant depends on the subsoil for supply of water and nutrients, especially when the topsoil dries out (Tang et al. 2001, 2003). A possible breeding strategy for developing crops for superior adaptation to combined stress conditions of soil acidity and drought could involve screening germplasm under well watered and drought stressed conditions on an acid soil and make selections based on superior performance (yield) under both conditions. Both Al toxicity and water stress should be considered in breeding for better adaptation to acid soils.

BREEDING TO IMPROVE ACID SOIL AND ALUMINUM TOLERANCE

Decades of studies have resulted in a significant progress in revealing the mechanism of Al tolerance in plants. Several key genes have been identified and the Al tolerance was validated to be related with gene sequence variations in some plants (Krill et al., 2010). The genetic variation for Al tolerance indicates it is a complex trait, involving many genes and physiological processes. Breeding programmes include field testing of a large number of varieties. Progress in breeding could be accelerated if quick screening techniques could be used during the selection process (Samac and Tesfaye, 2003). The development of maize hybrids for high yielding and tolerant to acid soils requires the widely genetic variability of inbred lines as a candidate of superior parental lines.

CONCLUSIONS

In order to improve crop yields and fertility of cropland a combination of improved genetics, sustainable management practices and amending of acid soils should be used. Recent advances in understanding of the physiology and molecular biology of Al^{3+} tolerance mechanisms have led to the identification of many major Al^{3+} tolerance genes. With advances in molecular techniques, such as marker-assisted selection (MAS), breeding for acid soil tolerance becomes more effective.

REFERENCES

Fageria, N. K., Baligar, V. C. 2008. Ameliorating Soil Acidity of Tropical Oxisols by Liming For Sustainable Crop Production. Advances in Agronomy Journal, 99: 345-399.

Jovanović, Ž., Đalović, I., Komljenović, I., Kovačević, V., Cvijović, M. 2006. Influences of liming on vertisol properties and yields of the field crops. Cereal Research Communications 34 (1): 517–520

Kochian, L. V., Piṅeros, A. M., Liu, J., Magalhaes, V. J. 2015. Plant Adaptation to Acid Soils: The Molecular Basis for Crop Aluminum Resistance. Annu. Rev. Plant Biol. 66: 23.1–23.28.

Krill, M. A., Kirst, M., Kochian, V. L., Buckler, S. E., Hoekenga, A. O. 2010. Association and linkage analysis of aluminum tolerance genes in maize. PloS One 5: 1–11.

Kovačević, V., Lončarić, Z., Šimić, D., Šimić, B. 2005. Influences of liming on soil fertility in the Eastern Croatia. Plant nutrition for food security, human and envirinmental protection. Fifteenth International Plant Nutrition Colloquimu. C. J. Li et al. (ed.). Tsinghua University Press. Beijing. China. Pp. 958-959.

Lončarić, Z., Rastija, D., Karalić, K., Popović, B. 2006. Mineral fertilization and liming impact on maize and wheat yield. Cereal Research Communications. 34 (1): 717–720.

Maron, L. G., Kirst, M., Mao, C., Milner, M. J., Menossi, M., Kochian, L. V. 2008. Transcriptional profiling of aluminum toxicity and tolerance responses in maize roots. New Phytol. 179: 116–128.

Rengel, Z., Zhang, W. H. 2003. Role of dynamics of intracellular calcium in aluminium-toxicity syndrome. New Phytol. 159: 295–314.

Samac, A. D., Tesfaye, M. 2003. Plant improvement for tolerance to aluminum in acid soils – a review. Plant Cell, Tissue and Organ Culture 75: 189–207.

Scott, B. J., Fisher, J. A., Cullins, B. R. 2001. Aluminnum tolerance and lime increase wheat yield on the acid soils of central and southern new south Wales. Aust. J. Exp. Agric. 41: 523–532.

Tang, C., Diatloff, E., Rengel, Z., McGann, B. 2001. Growth response to subsurface soil acidity of wheat genotypes differing in aluminium tolerance. Plant Soil 236: 1–10.

Tang, C., Asseng, S., Diatloff, E., Rengel, Z. 2003. Modelling yield losses of aluminium-resistant and aluminium-sensitive wheat due to subsurface soil acidity: effects of rainfall, liming and nitrogen application. Plant Soil 254:349–360

Yang, Z. Bao., Rao, M. I., Horst, J. W. 2013. Interaction of aluminium and drought stress on root growth and crop yield on acid soils. Plant and Soil

von Uexküll, H. R. Mutert, E. 1995. Global extent, development and economic impact of acid soils. Plant and Soil, 171: 1-15.

Sub-soil acidity in red and lateritic soils of India

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Abstract
Sub-soil acidity in red and lateritic soils of India has been studied and in series control section of Malappuram soils, 36.9 per cent have very strongly acid reaction, followed by strongly acid (27.1 %), extremely acid (16.7 %), moderately acid (6.5 %) and slightly acid (6.03 %). Slightly acid or neutral soils are best suited for crop production, as most of the soil nutrients are available to plants at this pH range, the pH has to be corrected by liming on surface and gypsum application on subsurface.

INTRODUCTION
The acid soils in India are mostly found in the Himalayan region, Eastern and North-Eastern region, peninsular India and coastal plains of different agro-climatic situations (Bhattacharyya *et al*., 2015). The states which have large areas under degraded acid soils include Arunachal Pradesh, Chhattisgarh, Kerala, Assam, Manipur, Nagaland, Mizoram, Meghalaya, Uttarakhand, Madhya Pradesh and Jharkhand. The degraded area under acid soils in Chhattisgarh and Arunachal Pradesh is predominantly under forests (Roy and Bhadra, 2014). Alluvial acid soils are found in West Bengal, Bihar, Assam and parts of Orissa. Marshy acid soils are distributed across Assam, Kerala, West Bengal, coastal Orissa, southeast coast of Tamil Nadu, and Tarai regions of Uttrakhand, Bihar and West Bengal. Acid sulphate soils are unique to the Kuttanad area of Kerala, with its reported presence at Sundarbans in West Bengal and Goa (Maji *et al*., 2010). In India, red and lateritic soils cover an area of about 91 million ha (28 % of TGA) mainly in the states of Kerala, Tamil Nadu, Andhra Pradesh, Maharashtra, Goa, Orissa, West Bengal, Sikkim, north eastern parts of Andaman and Nicobar and Pondicherry, representing semi arid, moist through sub humid, humid/ perhumid to coastal and Island ecosystems. The soils are developed as a result of transformation, translocation and illuviation leading to the release and dispersion of iron and aluminium hydroxides. The soils are slightly to moderately acidic, low to moderate cation exchange capacity and organic carbon content due to variation in soil forming factors and processes, of which the dominating are rainfall, drainage, translocation and oxidation (Sehgal *et al*., 1993). The red and lateritic soils occur in the tropical and subtropical conditions where rainfall ranges from 600 to 4000 mm and mean annual temperature between 22 and 30 ^{o}C with narrow difference in mean summer and mean winter soil temperatures. The precipitation however varies significantly and exceeds the potential evapo- transpiration in almost all the areas from June to September except in the semi arid zone (Sehgal *et al.*, 1998).

Table. 1 Major characteristic of red and lateritic soils in sub-soils (25-100 cm) in different agro-ecological regions (AER) of India

Pedon ***	Parent material	OC (%)	pH		CEC	Mineralogy
			KCl	Water		
1	Granite	0.62	6.0	5.3	15.4	Mixed
2	Weathered granite gneiss	0.32	5	5.1	8.7	Mixed
3	Granite gneiss	0.36	-	5.5	14.9	Mixed
4	Ferruginous sand stone	0.42	3.8	4.8	8.2	Mixed
5	Old alluvium	0.14	4.3	5.1	7.8	Mixed
6	Gneiss	1.70	-	5.3	-	Mixed
7	Alluvium	0.62	-	5.5	6.6	Mixed
8	Sand stone	0.22	-	5.6	3.4	Mixed
9	Laterite	0.49	-	4.8	4.6	Kaolinitic
10	Laterite	0.59	-	5.6	4.2	Kaolinitic
11	Laterite	0.54	4.5	5.6	7	Kaolinitic
12	Sandstone and shales	0.21	-	5.6	-	Mixed
13	Marine deposits	0.50	-	5.3	-	Mixed

***1.Medek, A.P.; 2, 3 Bangalore, Karnataka; 4. Puri, Odissa; 5. Bankura, West Bengal; 6. Meghalaya; 7. Nagaland; 8. Pondicherry; 9, 10. Trivandrum and Calicut, Kerala; 11. South Kanara, Karnataka; 12, 13. Andaman and Nicobar (Sehgal *et al.*, 1993)

With this background a case study was conducted in Malappuram district of Kerala state to know the extent of subsoil acidity. Climate of the area is hot humid tropical monsoonic type. Mean annual rainfall is about 2551 mm. Rainfall is received mainly South West monsoon and very little from North East monsoon. The mean annual temperature of the area is 27.3 ºC. Mean annual soil temperature is 28.3 ºC, mean annual summer soil temperature is 29 ºC and mean annual winter soil temperature is 27.5 ºC (IMD, 1999). At present, coconut and rubber based cropping systems are the major ones (30.2 % & 8.2 %) followed by arecanut and paddy based (5.6 % & 4.9 %) systems (Anil Kumar *et al.*, 2014b).

MATERIALS AND METHODS

A field survey was undertaken to assess the extent of area in Malappuram district, which is having appreciable amount of subsoil acidity. Soil profiles were excavated at close intervals along transects and studied covering all the landforms and land use as per the guidelines given in the USDA Soil Survey Manual (Soil Survey Staff, 2004). Soil samples were collected horizon-wise from typifying pedons of the soil series for laboratory analysis. Soil pH was determined in both 1:2.5 ratio soil water suspension and also in 1.0 N KCl method, extractable acidity was determined by both $BaCl_2$-TEA and 1.0 N KCl method following Jackson (1973).

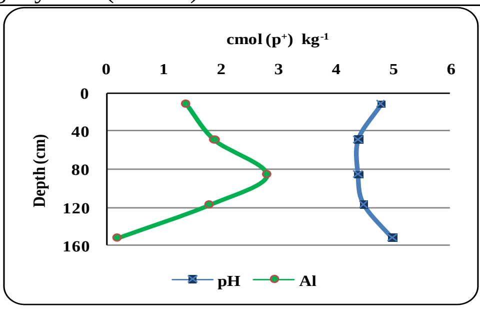
RESULTS AND DISCUSSION

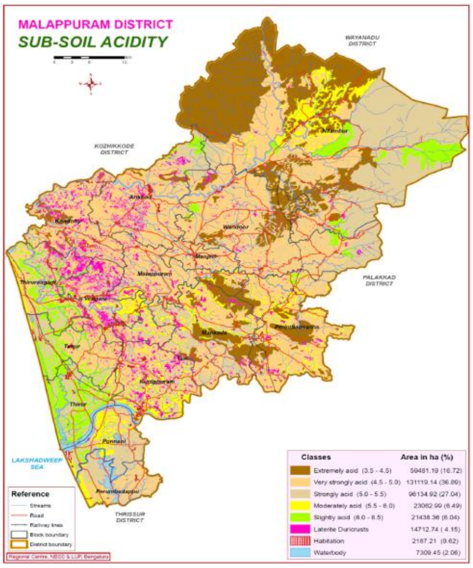
Subsoil acidity status in hot humid tropical soils of Malappuram

Soil reaction of series control section of the area (Table 2) revealed that 36.9 per cent of area have very strongly acid reaction on subsurface followed by strongly acid (27.1 %), extremely acid (16.7 %), moderately acid (6.5 %) and slightly acid (6.03 %). Slightly acid or neutral soils are best suited for crop production, as most of the soil nutrients are available to plants at the pH range. High rainfall, received in a shorter spell, is the major constraint associated with multi nutrient deficiencies of primary, secondary and micro nutrients for crop production in the area because of strongly, very strongly or extremely acidic soils. It was in accordance with Ananthanarayana and Ravindra (1998), who studied the soils of Karnataka and concluded that the severity of soil acidity may increase because of poor management and heavy rainfall.

Table 2.

Soil reaction classes	Malappuram district	
	Area (ha)	%
Extremely acid (3.5-4.5)	59481.2	16.73
Very strongly acid (4.5-5.0)	131119.1	36.88
Strongly acid (5.0-5.5)	96134.9	27.05
Moderately acid (5.5-6.0)	23063.0	6.49
Slightly acid (6.0-6.5)	21438.4	6.03





Subsoil acidity and aluminium toxicity:

The pH of the soil water suspension in most of the profile studied decreasing with depth. The same time the Al concentration of subsurface soil got increased wherever the pH of the subsurface goes below 4.5. The higher concentration of Al in the subsurface cause damage to root growth and most of the plants susceptible to the pH range and finally leads to poor growth and development.

Source of subsoil acidity:

Development of subsoil acidity here is linked to hot humid tropical climate with heavy rainfall, removal of bases and laterization, but in plantations this is linked to excess cation absorption by plant roots and use of ammoniacal nitrogen fertilizers (Tang, 2004) and removal alkaline materials as farm produce of hay and grain (www.ruralsolutions.sa.gov.au)

Consequences of subsoil acidity:

Plant species and varieties differ in their reaction to subsoil acidity and can make un-even crop and pasture, yellowing of leaves, poor nodulation in legumes and stunted root growth (Sumner *et al*., 1986) and it can aggravate Ca and Mg deficiencies also in tropical soils (Anil Kumar *et al.*, 2014a).

Subsoil acidity reclamation:

A treatment with dolomite or lime can reclaim moderately and strongly acid soils, while liming can reclaim moderately acid soils. Since lime is less mobile, there is less chance to reclaim the acidity in the subsurface and deep placement of lime got advantage upto 30 cm (Doss *et al.*, 1979). So application of gypsum along with lime can be recommended to manage the subsurface acidity. The recommendation of lime and gypsum can be made based on the soil clay content, Al concentration and gravel percentage as per the guidelines given by Lopes (1986) and Sousa *et al.* (1989). Amending subsoil acidity by surface applications of gypsum, lime and composts gave good results by improving Ca over Al below plough layer (Liu and Hue, 2001). Compared with phoshogypsum, alkaline slag was more effective in decreasing soil acidity and soluble Al and Mn, and increasing exchangeable Ca and Mg in the Ultisol profile in ameliorating soil acidity in the surface soil and subsoil (Li *et al.*, 2015).

CONCLUSIONS

As majority of soils reported high subsoil acidity, a treatment with dolomite or lime can reclaim moderately and strongly acid soils, while liming can reclaim moderately acid soils. Since lime is less mobile, there is less chance to reclaim the acidity in the subsurface. So application of gypsum along with lime can be recommended to manage the subsurface acidity with compost or basic slag.

REFERENCES

Ananthanarayana. R. and Ravindra, M.R. 1998. Soil Acidity and Liming in Kamataka. Technical Bulletin, Department of Soil Science and Agricultural Chemistry, UAS, Bangalore.

Anil Kumar, K.S., Nair, K.M., Krishnan, P., Naidu, L.G.K. and Sarkar Dipak 2014a. Climatic and terrain influence on acidity, exchangeable bases and cation exchange capacity in soils of coffee plantations of Karnataka, *Journal of Plantation Crops* 42 (1): 41-50.

Anil Kumar, K. S., Nair, K.M. Thayalan, S., Ramesh Kumar, S.C. Hegde, Rajendra, Srinivas, S. Niranjana, K.V., Naidu, L.G.K., Sarkar, Dipak and Singh, S.K. 2014b *"Land Resources of Malappuram District, Kerala for Land Use Planning"*, NBSS Publication 1083 National Bureau of Soil Survey and Land Use Planning, Nagpur, India 134 p.

Bhattacharyya, Ranjan Ghosh, B.N., Mishra, P.K., Mandal, Biswapati Ch. Srinivasa Rao, Sarkar, Dibyendu Das, K. Anil, K.S., Lalitha, M. Hati, Kuntal and Franzluebbers Alan 2015. Overcoming Soil Degradation in India: *Sustainability*, 7, 3528-3570.

Doss, B.D., Dumas, W.T. and Lund, Z.F. 1979. Depth of lime incorporation for correction of subsoil acidity. *Agronomy Journal*, 71, 541-544.

Indian Meteorological Department (IMD), 1999. Climatological Tables, 1951-1980, IMD, Pune, Controller of Publications, New Delhi, 782p.

Jackson, M.L., 1973, Soil Chemical Analysis. *Prentice Hall Of India (Pvt) Ltd*., New Delhi.

Lopes, A.S. 1986. Calagem e gesso agrícola. Encontro técnico sobre gesso agrícola. Fosfértil/Petrofértil, Belo Horizonte, MG. 58 p. (mimeo).

Li, Jiu-yand Xuu, Liu, Zhao-dong, Zhao, Wen-zhi, Masud, M. M. and Xu Ren-kov, 2015. Alkaline slag is more effective than phosphogypsum in the amelioration of subsoil acidity in the Ultisol profile, Soil and Tillage Research, 149, 21-32.

Liu, J. and Hue, Nguyen, 2001. Amending subsoil acidity by surface applications of gypsum, lime and composts, Commun. *Soil Sci. Plant Anal.* 32 (13 & 14), 2117-2132.

Maji, A. K. Obi Reddy, G.P. Sarkar, Dipak 2010. Degraded and Wastelands of India: Status and Spatial Distribution. ICAR, NARS joint publication. pp.158.

Raghu Nandan, K.R., Vidyadharan, K.T. Srinivasan, J., Balakrishnan S. and Abbas, M.H. 1993. Distribution and classification of lateritic soils in parts of Goa, Kerala and Karnataka. In Ed. Seghal, J., V.A.K. sarma, R.K. Batta, K.S.Gajbhiye, S.R. Nagabhushana, and K.R. Venugopal. 1993. Red and Lateritic soils of India-Resource Appraisal and Management., NBSS Publ. 37 NBSS&LUP, Nagpur, India, 346P.

Roy Bidhan and Bhadra Sanjib. 2014. Effects of Toxic Levels of Aluminium on Seedling Parameters of Rice under Hydroponic Culture. *Rice Science*, 21(4): 217−223

Seghal, J., Sarma, V.A.K., Batta, R.K., Gajbhiye, K.S., Nagabhushana, S.R. and Venugopal. K.R. 1993. Red and Lateritic soils of India-Resource Appraisal and Management., NBSS Publ. 37 NBSS&LUP, Nagpur, India, 346P.

Seghal, J., W.E. Blum, and K.S.Gajbhiye. 1998. Red and Lateritic soils vol. 2 Red and Lateritic soils of the World. Oxford and IBH publishing Co. New Delhi.113p

Soil Survey Staff 2004. *Soil Survey Manual*, Agric. Handbook no. 18, (USDA: Washington, D.C) Published by Scientific Publishers, P.O. Box 91, Jodhpur, India.

Sousa, D.M.G. de and Ritchey. K.D. 1986. Correção de acidez subsuperficial: uso de gesso no solo do cerrado, p. 91- 113. In: Decher, A.R. and Q.A. C. Carmelo de (ed.). Simpósio avançado de quimico e fertilidade do solo. Campinas, SP, Fundação Cargill, p. 91-113.

Sumner, M.E., Shahandeh, H., Bouton, J. and Hammel, 1986. J. Amelioration of an acid soil profile through deep liming and surface application of gypsum. *Soil Science Society of America Journal*, 50, 1254-1258

Tang, C. 2004. Causes and management of subsoil acidity *SuperSoil 2004*: 3rd Australian New Zealand Soils Conference, 5 – 9 December 2004, University of Sydney, Australia.Published on CDROM. Website www.regional.org.au/au/asssi/

Phytotoxic effect of aluminium and manganese in barley

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Abstract
In order to determine the cause of the characteristic symptoms of the toxicity of manganese in barley that is grown on acid soils, pot experiment in the open field was set. The experiment with winter barley (*Hordeum vulgare sp.*) was set on a plot with three levels of pH (1M KCl): I), 3.68-3.52; II) 3.87-3.77; III) 4.32-4.13. The concentration of available Mn=93.9-174.7 mg/kg, mobile Al=2.2-34.5 mg/100g. The first symptoms of the toxicity of Mn (dark spots) occur in the phase of the first fully developed leaves, when the ratio of Fe/Mn in the leaf is <0.5. The most intense symptoms at low and medium Al damage of the root system occur, when the ratio of Fe/Mn <0.2.

INTRODUCTION

Among the researchers prevails general agreement that Al-toxicity is a major factor of the limited production of crops on the highly acidic soils. Top of the root is a critical point of the Al-toxicity (*Ryan et al., 1993; Sivaguru i Horst, 1998; Zhang and Rengel, 1999*). Unlike the toxicity of Al, analysis of plant material provides a starting point for finding excess Mn. *Rengel, 2000;* state that the plant through the roots certainly uptake Mn^{2+} cations, but toxic concentrations of manganese in the plant depend on the plant species (varieties, hybrids), site conditions, ranging over a wide range. Among cereals, barley is the most sensitive on increased levels of Al and Mn in the soil solution (*Scott et al., 1997*). Barley, alfalfa and some varieties of soya beans can tolerate levels of Mn at leaves from 300 to 500 mg/kg (*Owen, 2002*). Unlike Al, symptoms of Mn toxicity in natural conditions occur rarely. In order to determine the cause of the characteristic symptoms of Mn toxicity on young plants of barley, on acid soils study was conducted on soils with three levels of acidity.

MATERIALS AND METHODS

Soils for the experiment were taken in early spring in the crop of barley within the irregular oasis of small area, in the depth *(0 to 15 cm*) close to the plants that are lagging behind in growth with varying amounts of the onset of symptoms of chlorosis and necrosis: I) no symptoms of chlorosis, pH/KCl 4.32-4.13; II) with symptoms of chlorosis, pH/KCl 3.87-3.77 III) strong symptoms of chlorosis and necrosis, pH / KCl 3.68-3.52. The experiment was set up in a form of a Latin square (3x3) in vegetation pots with a perforated base (r = 12.5 cm, h = 12 cm) in the open field. The winter barley was sown, 50 seeds per pot, without fertilization. Observation of plants within the experimental field was done daily, with monitoring climate data. The experiment was stopped when the symptoms of chlorosis and necrosis affected about 50% of the leaf surface of barley on some of the tested combinations of soil acidity. Methods for chemical analysis of soil: Mobile Al - method by *Sokolov*, available Mn, extraction with 1M CH_3COONH_4 (*pH 7.0*), reading on the AAS,; Analyses of plant material of root and leaf in wet burned sample (*conc* $H_2SO_4 + H_2O_2 + 450\ °C$): Nitrogen *(% N*): *Kjeldahl* method, Phosphorus *(%* P_2O_5), *vanadt - molybdate method*; *Mn, Zn, Fe,* reading on the AAS.

RESULTS AND DISCUSSION

In all variants of soil, 28 days after sprouting, the symptoms of lagging behind the rise of barley were identified, spots on the leaves, chlorosis and necrosis of different intensity (Table 1, Pic. 1). With the decrease of pH and increase of the concentration of Al^{3+} the reduction of the root system increased. I) At a level of 5.5 mg Al^{3+} /100g of soil, root is grown throughout the whole vegetation pots (Pic.1a). The ratio of roots mass and part of the plant above-ground is 1:1.1. Barley has reached the phase of the third leaf. Chlorosis affected 25% of the leaf mass, spots are of the middle intensity and the concentration of Mn in the leaf is 554 mg/kg. II). At a concentration of 16.2 mg Al/100g of soil, root system is callous, without branched lateral secondary roots, grown through 1/2 of soil in containers (Pic. 1b). The ratio of roots mass and part of the plant above-ground is 1:1.9. Barley is at the stage of 3 leaves. Chlorosis affected 50% of the leaf mass, spot has strong intensity (Pic.1b), the concentration of leaf Mn is 642 mg/kg. In this variant, the first symptoms of toxicity of Mn were noticed. Dark brown dots appeared at the top of the first mature leaf. At the next new leaf, there were dark spots observed, but they have been intensified in the old leaf, from top to bottom with the occurrence of chlorosis and necrosis. III) At a concentration of 31.4 mg Al/100g of soil, root system consisted only of a few primary thickened roots of 0.5 cm length (Pic.1c). The ratio of the root mass and above-ground part of the plant 1:3.9. Barley has reached phase of 2 leaves. Chlorosis affected 20% of the leaf, symptoms of spots are sporadically present. The leaves were "cowed" solid, wrapped inside and they look like elongated, similar to the symptoms that occur with drought (Pic.1c). The concentration of Mn in the leaf is 308 mg/kg.

Table 1. Results of chemical analysis of soil (Al, Mn) and the parts of barley plants

	Soil			Above-ground part of the plant								Intensity of spots on Leaf	% root damage	growing phase*
	pH 1M KCl	mg/100g	mg/kg	%					mg/kg		Fe/Mn			
		Al^{3+}	Mn	N	P_2O_5	K	Ca	Mg	Mn	Fe				
I	4,15	5,5	147,8	3,7	1,4	4,9	0,81	0,31	554	114	0,21	Medium	5	3-4
II	3,85	16,2	172,1	4,0	1,6	6,5	0,34	0,22	642	103	0,16	High	40	3
III	3,56	31,4	104,3	3,7	1,5	4,1	0,18	0,15	308	139	0,47	Low	95	2
$Lsd_{0,05}$		*1,6*	*18,8*	*0.49*	*0.15*	*1.02*	*0.09*	*0.03*	*165*	*27*				
$Lsd_{0,01}$		*2,7*	*25,5*	*0.67*	*0.20*	*1.38*	*0.13*	*0.04*	*224*	*36*				

* no. of developed leaves

a) pH 4,15; 5,5 mg Al/100g Fe/Mn 0,21 | b) pH 3,85; 16,2 mg Al/100g Fe/Mn 0,16 | c) pH 3,56; 31,4 mg Al/100g Fe/Mn 0,47

Pic 1. The intensity of the appearance of toxicity of manganese in the form of dark brown dots on the leaves of barley plants and degree of damage to the root system, depending on the concentration of mobile Al in the soil, and the relation of Fe/Mn in the above-ground part of the plant. (Predic, orig.)

Characteristic symptoms of lack of phosphorus (which often accompany Al toxicity) were not observed in this experiment, which can be explained by the phase of plant development in which the experiment was interrupted (3th leaf). By this stage, plants have used the reserves of phosphorus from seeds. Unlike phosphorus, calcium and magnesium content has significantly decreased in the aboveground part of the plant by increasing mobile Al in the soil and the reduction of the root system (tab.1.). Depending on the level of mobile Al in the soil and the degree of damage to the root system in the development stage of 2-3 leaves of barley in the above the ground parts of plants reduces the concentration of Ca and Mg, and Mn concentration increases (Table 1.). Interaction of Fe/Mn is well known, so that the induced chlorosis by the excess of manganese, at the same time is a result of secondary lack of iron. *Foy (1984)* states that the barley plant, at the value of the relation of Fe/Mn of 0.25, may lead to mild, and in the relation of 0.11, to severe toxicity of manganese. The results obtained in this experiment confirm this thesis.

CONCLUSIONS

The first symptoms of the toxicity of Mn in the form of dark brown spots may appear in the highest part of the first formed leaf of barley and intensified towards the base of the leaf with the appearance of chlorosis and necrosis. The appearance and intensity of symptoms of Mn toxicity in the developed second-third leaf of barley in tested soils with very acid reaction, several factors have a decisive role: content of available Mn in the soil; the concentration of mobile Al in the soil i.e. the degree of damage to the root system of plants; climatic conditions (temperature and humidity), the concentration of biogenic elements in the above-ground parts of plants of barley Ca, Mg, Mn, Fe and relation of Fe/Mn in the aboveground part of the plant. Researches should be continued.

REFERENCES

Foy, C.D. 1984. Physiological efects of hydrogen, aluminum and manganese toxicities in acid soils. Iz. F. Adams (Ed), Soil Acidity and Liming, 2-nd Edition, Agronomy 12, Madison, Wsconsin, 57–97.

Owen, P.C. 2002. Plant analysis Handbook, Nutrient Content of Plant, The University of Georgia, College of Agricultural and Enviromental Sciences

Rengel, Z., 2000. Aptake and transport manganese in plants, in A.Sigel and H. Sigel: Metal ions in Biological Systems. Marcel Dekker, New York, p. 57-87

Ryan, P.R., Di Tomaso, J.M., and Kochian, L.V. 1993. Aluminum toxicity in roots: an investigation of spatial sensitivity and the role of the root cap. Journal of Experimental Botany 44: 437-446.

Sivagura, M., and Horst, W.J. 1998. The distal part of the transition zone is the most aluminum-sensitive apical root zone of mainz. Plant Physiology 116: 155-163.

Zhang, W.-H., Rengel, Z. 1999. Aluminium induces an increase in cytoplasmic calcium in intact wheat root apical cells, Aust. J. Plant Physiol, 26: 401 – 409.

The mechanisms of high Al tolerance in *Rhodotorula taiwanensis* RS1

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Abstract
We isolated a red yeast, which could tolerate Al^{3+} concentrations as high as 200 mmol L^{-1}. This red yeast was identified as *Rhodotorula* sp. and designated as *Rhodotorula taiwanensis* RS1. The result showed that no or little secretion of organic acids was observed in RS1 growth media, and the thickness of the cell walls significantly increased as Al^{3+} concentration increase. The result suggest that thickening of the cell wall may play an important role in the high tolerance of RS1 to Al, and Al-induced intracellular citrate could play an important role in detoxification of Al in *R. taiwanensis* RS1.

INTRODUCTION
The high concentration of aluminum (Al) in acidic soils is often regarded as an important toxic factor for plants (Kochian et al. 2004), and also for soil microorganisms (Dorea and Clarke 2008). There have been many studies on the mechanisms of resistance by plants to Al stress (Kochian et al. 2004). Since soils are always populated by microorganisms, Al tolerance by microorganisms from these soils has also attracted interest and a number of microorganisms exhibiting strong tolerance to high levels of Al^{3+} have been identified (Kawai et al. 2000). It is therefore important to understand the mechanisms that allow these organisms to survive under extremely high concentrations of Al^{3+}. In the present study, a strain of red yeast with strong Al tolerance was isolated from acidic soils, and its Al-tolerant mechanisms were studied. This information extends our understanding of the mechanisms that allow this yeast to thrive in high Al^{3+} conditions.

MATERIALS AND METHODS
The Al-tolerant microorganisms were collected from acidic oil-tea fields in the Yingtan Red Soil Ecological Experiment Station (28°14'N, 117°03'E), China, according to the method of (Kanazawa and Kunito 1996). Glucose medium (GM) was used to study the effects of a range Al^{3+} concentrations on the isolated microorganism, as described by Zhang et al. (2002). The organic acids were excreted and determined by HPLC and GC-MS as described by Nishiumi et al. (2010). Cell was fixed, dehydrated, embedded and examined in the TEM. Cell wall was isolated according to the method of Bahmed et al. (2003). The cells and cell walls were digested with concentrated $HNO_3/HClO_4$ (4:1 v/v), and their Al contents determined by ICP-AES. 2-DE was carried out according to the methods of Li et al. (2010), and MDH activity was measured as described by Pines et al. (1997).

RESULT AND DISCUSSION
An Al-tolerant microorganism, which could survive in the GBM (pH 3.5) containing 10 mmol L^{-1} Al^{3+}, was isolated and purified from the acidic soil of oil-tea fields (pH 4.3). Phylogenic analyses suggested that this Al-tolerant microorganism belonged to the genus *Rhodotorula*, and this *Rhodotorula* isolate was designated as RS1 in this study. The lower concentrations of Al^{3+} (1.0 and 10 mmol L^{-1}) did not significantly affect the growth of RS1 but growth was markedly inhibited at the higher concentrations (100 and 200 mmol L^{-1}).TEM observations showed that treatment of RS1 with a range of concentrations of Al^{3+} (10, 100 and 200 mmol L^{-1}) conspicuously increased the cell wall thickness compared with controls without Al. In the treatment of 100 mmol L^{-1} Al^{3+} for 24 h, the overall concentration of Al incorporated into whole RS1 cells was 77.9 ± 9.4 μg g^{-1} wet weight, while that of Al in cell wall was 60.7 ± 5.4 μg g^{-1} wet weight. These results showed that 78.0% of total Al was present in the cell walls. It is well known that cell wall is the first protective barrier and possess significant adsorption capacity for metal ions. Binding of heavy metal ions to the cell wall is considered to be an important detoxification mechanism in fungal systems (Garciatoledo et al. 1985). In the yeast RS1 studied, an important mechanism of Al tolerance is dependent on accelerated growth of cell walls that prevents the toxic Al^{3+} ions from reaching the interior of the cells.Using 2-DE technology, 29 proteins decreased in abundance and four proteins increased in abundance were detected in the treatment of 100 mmol L^{-1} Al^{3+}. Through analysis, we found that level of malate dehydrogenase (MDH) was significantly increased under Al stress in RS1 cells. The activity of MDH was enhanced under Al stress, indicating that the interconversion and turnover between malate and oxaloacetate was improved in yeast RS1. The increase in the activity of MDH correlated with the observed increase in protein level revealed by proteomic analysis in RS1 cells.In plants, the Al-stimulated increase in organic acids complexed with Al ions is the best documented and characterized Al-tolerant mechanism, either internally or externally. Unfortunately, no or little organic acids were detected in the medium of RS1 grown in

the treatments of 0, 1 and 100 mmol L^{-1} Al^{3+}, it seems that the high Al-tolerance of RS1 was not due to secretion of Al^{3+} chelators.In the cell, MDH can convert oxaloacetate into malate and citrate; therefore, we tested whether the levels of intracellular malate and citrate were increased in RS1 cells with increased MDH activity. The citrate content in RS1 was significantly higher than the malate content under control conditions, and Al stress led to an increased citrate content but not to increased malate, suggesting that the increase in MDH activity in RS1 cells exposed to high Al concentration provides more substrate oxaloacetate for citrate synthesis. It is generally believed that forming an Al-citrate complex is easier than forming an Al-malate complex, and citrate is the most frequently employed organic acid for relieving Al toxicity among organic acids. Therefore, formation of Al-citrate complexes within cells is considered as an internal Al-tolerance mechanism in certain highly Al-tolerant plants (Ma et al. 2001) and in yeast (Anoop et al. 2003).

CONCLUSION

In conclusion, *Rhodotorula* sp. RS1, isolated from the acidic oil-tea field soils (pH 4.3), was able to tolerate Al^{3+} concentration up to 200 mmol L^{-1}. We suggest that RS1 cells have evolved a range of regulatory mechanisms to adapt Al toxicity. One strategy employed by RS1 cells is to block entry of Al into the cell interior by reducing the capacity of the cell surface to adsorb Al ions and increasing the thickness of the cell wall. Another strategy is to chelate Al ion by forming stable, non-phytotoxic complexes with internal citrate, where increased amounts of citrate are related to increased MDH amount and activity.

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REFERENCES

Anoop VM, Basu U, McCammon MT, McAlister-Henn L, Taylor GJ, 2003. Modulation of citrate metabolism alters aluminum tolerance in yeast and transgenic canola overexpressing a mitochondrial citrate synthase. Plant Physiology 132 (4):2205-2217

Dorea CC, Clarke BA, 2008. Effect of aluminium on microbial respiration. Water Air and Soil Pollution 189 (1-4):353-358.

Garciatoledo A, Babich H, Stotzky G, 1985. Training of *Rhizopus-Stolonifer* and *Cunninghamella-Blakesleeana* to Copper - Cotolerance to Cadmium, Cobalt, Nickel, and Lead. Can J Microbiol 31 (5):485-492

Kanazawa S, Kunito T, 1996. Preparation of pH 3.0 agar plate, enumeration of acid-tolerant, and Al-resistant microorganisms in acid soils. Soil Science and Plant Nutrition 42 (1):165-173

Kawai F, Zhang DM, Sugimoto M, 2000 Isolation and characterization of acid- and Al-tolerant microorganisms. Fems Microbiology Letters 189 (2):143-147

Kochian LV, Hoekenga OA, Pineros MA, 2004. How do crop plants tolerate acid soils? - Mechanisms of aluminum tolerance and phosphorous efficiency. Annu Rev Plant Biol 55:459-493

Li BQ, Lai TF, Qin GZ, Tian SP, 2010. Ambient pH stress inhibits spore germination of *Penicillium expansum* by impairing protein synthesis and folding: A Proteomic-Based Study. Journal of Proteome Research 9 (1):298-307

Ma JF, Ryan PR, Delhaize E, 2001. Aluminium tolerance in plants and the complexing role of organic acids. Trends Plant Sci 6 (6):273-278.

Nishiumi S, Shinohara M, Ikeda A, Yoshie T, Hatano N, Kakuyama S, Mizuno S, Sanuki T, Kutsumi H, Fukusaki E, Azuma T, Takenawa T, Yoshida M, 2010. Serum metabolomics as a novel diagnostic approach for pancreatic cancer. Metabolomics 6 (4):518-528

Pines O, Shemesh S, Battat E, Goldberg I, 1997. Overexpression of cytosolic malate dehydrogenase (MDH2) causes overproduction of specific organic acids in *Saccharomyces cerevisiae*. Appl Microbiol Biot 48 (2):248-255

Chemical and physical characteristics of a sewage sludge from the company of water and sewage sludge south treatment station of Brasilia city

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Abstract
Some chemical and physical characteristics from a representative sample of a sewage sludge obtained from the Company of Water and Sewage Sludge South Treatment Station of Brasilia (CAESB ETSouth), were determined. Total contents of Copper, Fe, Mn Zn, Se, Mo, Cd, As, Ni, Li, Al, Cr, Co, Pb, except Hg were determined according to the official methods published by Brazil's Ministry of Agriculture. The results were compared with those of safe levels in urban waste compost permitted in the USA, Italy, France, Netherlands and by those established by the United States Environmental Protection Agency (USEPA) and substrates in Brazil.

INTRODUCTION
The Federal District where is located Brasilia-Brazil's capital, produces daily 400 m^3 sewage sludge. This residue is a viable option as substrate for the growth of ornamental plants in gardens due to low cost as compared with cattle and chicken manures. However, information is lacking on adequate use of sewage sludge in cerrado soils which not only reduce the yields but also causes nutritional imbalances. In addition, this residue may have heavy metals. The aim of this work was to determine some chemical and physical characteristics from a representative sample of a sewage sludge obtained from CAESB ET South. Total content of Cu, Fe, Mn, Zn, Se, Mo, Cd, As, Ni, Li, Al, Cr, Co, Hg and Pb were compared with those of safe levels safe in urban waste compost permitted in the USA, Italy, France, Netherlands and by those established by the United States Environmental Protection Agency (USEPA, 1993) and substrates in Brazil.

MATERIAL AND METHOD
Sewage Sludge were collected during the dry season (July, 2010), to cover a wide range of chemical composition (Khiel and Porta, 1980), from CAESB ETSouth, located in Brasilia-Brasil. The total number of subsamples was 9 which were mixed to have one representative sample. Copper, Fe, Mn Zn, Se, Mo, Cd, As, Ni, Li, Al, Cr, Co and Pb were extracted by aqua regia, a mixture of HCl + HNO_3 1:3 using a microwave digestion. The total content were evaluated according to Brasil (2007) and determined by inductive coupled plasma atomic emission spectrophotometer (ICP-AES). pH, N, C, OM (organic mater), Na, K, Ca, Mg, Al, S, eletric conductivity, humidity, ash, residue and chemical oxigen demand (COD), were determined according to Khiel and Porta (1980). For Hg determination, sodium borohydrated was used as reductor in a hydrated generator attached to an atomic absorption Shimadzu AA670 G (cold vapor technique). B was determined by azomethine H method (Table 1).Total content of heavy metals were compared with those of safe levels in urban waste compost permitted in the USA, Italy, France Netherlands, the standards for the use or disposal of sewage sludge (USEPA, 1993), Code of Federal Regulations 40 CFR Part.503 (Data not showed) and for substrates in Brazil.

RESULTS AND DISCUSSION
Table 1 shows some chemical and physical characteristics of a sewage sludge from the Company of Water and Sewage Sludge South Treatment Station of Brasilia city. Due to the principles of the analytical process, the pH and electric conductivity are often classified as physical methods although their impacts is mostly chemical. Therefore they might be seen as physico-chemical parameters. The biostability and the low salinity of the sewage sludge are assured by the values of chemical oxygen demand (COD) and the eletric conductivity (EC). However the relation C/N indicates that it is not completely humified. The concentration of P, Na, Ca, Mg and S are low. Total content of Cu, Fe, Mn, Zn, Se, Mo, Cd, As, Ni, Li, Al, Cr, Co, Hg and Pb detected in the representative sample of sewage sludge are under those permitted in the USA, Italy, France Netherlands and for substrates in Brazil, as also with those of safe levels established by the United States Environmental Protection Agency USEPA, 1993, Code of Federal Regulations 40 CFR Part.503 (Data not showed) and for substrates in Brazil (Table 2). Even the highest levels of heavy metals found in the sample sewage sludge were lower than safe levels above said, and even Malavolta (1994) reported that heavy metals, micronutrients or not, contained in the commercial fertilizers and amendments, as well as those present in certain by products if used to the recommended rates, do not raise their content neither in the soil, nor in the crops, to harmful levels, in the short,

medium or long term. However, some concerns about potential public health hazards related to pathogens present in the compost need to be evaluated (Epstein and Epstein, 1989).

Tabela 1. Chemical and physical characteristics of a sewage sludge derived from the Company of Water and Sewage Sludge, South Treatment Station of Brasilia (CAESB EtSouth).

Cu	Fe	Mn	Zn	Se	Mo	Cd	As	Ni	Li	Al	Cr	Co	B
mg kg^{-1}													
146	23,518	121	724	< 0.1	< 0.01	2,1	< 0.01	18,3	3.12	43,715	48	1.83	24

pH H_2O	OM*	C/N	N	C	P	Na	K	Ca	Mg	S	Hg	Pb
(1:50)	(g kg^{-1})		g kg^{-1}								µg kg^{-1}	
7.2	632	7	52.2	366.6	35.8	0.05	4.1	13.6	6.7	9.3	726	218

EC*	Humidity	Ash	Residue	COD**
(dS m^{-1})	(g kg^{-1})			(mg g^{-1})
1.35	890	360	34,2	628

EC - Eletric conductivity.
COD - Chemical oxigen demand.

Table 2. Safe levels of heavy metals in urban waste compost permitted in the USA, Italy, France, Netherlands and for substrates in Brazil*****.

Element	Country						
	USA*	Netherlands*	USA*	Italy*	Spain*	França*	Brasil**
	mg kg^{-1}						
Cu	100	630	200	422	200	250	-
Ni	-	110	-	-	0.76	190	175
Mn	1500	1650	500	875	700	1000	-
B	-	60	-	-	3	60	-
Hg	-	5	-	-	-	4	2,50
Pb	-	900	-	6-5	9	600	300
Cd	-	6	100	8	0.04	7	8
Cr	-	220	-	215	2	270	500

*Source: Adaptad from Xin-Tao et al.,1992.
** Source: BRASIL. Ministério da Agricultura, Agropecuária e Abastecimento (MAPA). Instrução Normativa n. 27, de 5 de junho de 2006.
(-) Data not available.

REFERENCES

Brasil. Ministério da Agricultura, Agropecuária e Abastecimento. Instrução Normativa n. 27, de 5 de junho de 2006. Dispõe sobre a importação ou comercialização, para produção, de fertilizantes, corretivos, inoculantes e biofertilizantes. Diário Oficial (da) República Federativa do Brasil, Brasília, DF, n. 110, p. 15, 9 jun. 2006.

Brasil. Ministério da Agricultura, Agropecuária e Abastecimento. Instrução Normativa SDA n. 17, de 21 de maio de 2007. Aprova os métodos analíticos oficiais para análise de substratos e condicionadores de solos, na forma do Anexo da presente Instrução Normativa. Diário Oficial (da) República Federativa do Brasil, Brasília, DF, n. 99, p. 8, Seção I, 24 maio 2007.

Epstein, E., Epstein, J. I. Public health issues and composting. BioCycle 30(8):50-53, 1989.

Kiehl, E.J., Porta, A. Análise de lixo e composto. Piracicaba: ESALQ, 1980. 55p.

Malavolta, E. *Fertilizantes e seu impacto ambiental:* micronutrientes e metais pesados, mitos, mistificação e fatos. São Paulo: Produquímica Indústria e Comércio, 1994. 153p.

United States Environmental Protection Agency – USEPA. Standards for the use or disposal of sewage sludge. Washington: EPA, v. 48: p. 9387-9415, 1993. (Code of Federal Regulations 40 CFR Part.503).

An aluminum-induced cell death mechanism involving vacuolar processing enzyme in tobacco

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Abstract
Aluminum (Al) ion causes cell death, but the mechanism has not been fully elucidated yet. In plants, vacuolar processing enzyme (VPE) localized in vacuole is reported to be necessary to induce hypersensitive cell death in tobacco after infection of tobacco mosaic virus (TMV). In this study, we examined Al-induced cell death process in seedlings of tobacco (*Nicotiana tabacum* L, cv. Bright Yellow), focusing on VPE. Exposure of roots to Al for 24 h under hydroponic culture caused root elongation inhibition, cell death and the increases in both the expression of *VPE* genes and VPE activity. Time course experiment during Al exposure indicated that cell death and the enhancement of *VPE* expression were detected simultaneously. These results indicate the involvement of VPE during cell death process in root apices under Al stress, suggesting VPE to be a factor leading to cell death.

INTRODUCTION
Aluminum is a major component of soil elements, and free Al ion is the main factor causing the inhibition of plant growth in acidic soils (von Uexktill et al., 1995). Many suggestions have been proposed to enlighten the toxic effect of Al in plants. Aluminum accumulates at root apical meristem and rapidly inhibits both primary root and root hair growth, and later causes cell death (Ryan et al., 1993). The mechanisms of Al-induced cell death have not been fully elucidated yet. The enhancement of reactive oxygen species (ROS) production by Al has been commonly observed at root apex in various plant species and also in actively growing cultured cells of tobacco (Yamamoto et al., 2002). Some responses to Al in cultured tobacco cells, such as ROS production and callose secretion, are similarly observed during hypersensitive responses (HR) to TMV in tobacco plant (Allan et al., 2001).

VPE is the vacuole-localized cysteine protease caspase-1 like activity. VPE is involved in the maturation of various proteins in seed and leaf, and is also involved in cell death processes induced by TMV in tobacco (Hatsugai et al., 2004). The involvement of VPE was also reported in *Arabidopsis* under heat shock (Li et al., 2012). Thus, VPE seems to be a key factor causing various types of cell death in plant (Hatsugai et al., 2015).

Therefore, we have investigated a possible involvement of *VPE* genes in cell death mechanisms caused by Al in plant. Firstly, we investigated an involvement of VPE during Al responses in cultured tobacco cells (Kariya et al., 2013). In cell line BY-2, Al caused the increases in *VPE1a* and *VPE1b* expression, then VPE activity and a loss of plasma membrane integrity (a marker of cell death) simultaneously. Furthermore, caspase-1 inhibitor (Ac-YVAD-CHO) decreased the degree of Al-induced cell death. Taken together, we proposed a novel cell death process under Al stress: the exposure of cells to Al causes the enhancement of *VPE* expression and then VPE activity which leads to a loss of integrity of the plasma membrane and eventually cell death. Based on these previous results, in this study, we examined if VPE is involved in Al-induced cell death process in roots of tobacco.

MATERIALS AND METHODS
Seeds of tobacco were germinated on the net floating on the surface of Ruakura medium (a low phosphate nutrient medium) (Snowden et al., 1995) at pH 5.0 at 25 ˚C with a 16 h photoperiod for one week. Then, seedlings were treated in the absence (control) or presence of $AlCl_3$ (50 μM, unless otherwise described) for up to 72 h in Ruakura medium (pH 4.5). Then root part was used for investigations of Al responses as follows: The adsorption of Al was detected by hematoxylin staining (Ono et al., 1995). Dead cells were detected by staining using propidium iodide (PI) and Evans blue (EB), while live cells were detected with fluorescein diacetate (FDA). For *VPE* expression analysis, root apices of 5 mm were used. Four *VPE* genes, *VPE1a*, *VPE1b*, *VPE2* and *VPE3*, were reported in tobacco (Hatsugai et al., 2004). Expression levels of these *VPE* genes were investigated by real time RT-PCR. Cell-free extracts were prepared from root apices of 10 mm and were used for the assay of VPE activity which was measured using fluorescent substrate (z-ANN-MCA) as described previously (Kariya et al., 2013). The fluorescent of MCA produced by VPE activity was measured with a fluorescence microplate reader.

RESULTS
Root elongation inhibition and cell death under Al exposure
Tobacco seedlings were treated with or without Al. Root elongation was inhibited by 60% after one day and 80%

after 3 days of 50 μM Al exposure. Hematoxylin staininig indicated that the adsorption of Al at root apices started from 3 h of the exposure time. EB staining also revealed similar pattern of dead cells at root apices after treatment for one day and 3 days. On the other hand, FDA staining indicated no live cells after one day exposure to Al. Within the 24 h exposure time, apparently all the cells of a root apex seemed to be alive until 6 h, and some cells started to die from 9 h, then live cells were not detected after 12 h.

Enhancement of VPE gene expression under Al exposure

The *VPE* gene expression in control and Al-treated roots was examined every 3 h after a start of Al exposure. The increase in both the *VPE1a* and *VPE1b* were detected after 9 h exposure to Al. On the other hand, the gene expression of both the *VPE2* and *VPE3* increased after 24 h exposure.

Increase in VPE activity under Al exposure

VPE enzyme activity was examined in control and Al-treated roots after one day treatment. VPE activity increased with an increase in a Al concentration (about 1.5 fold at 50 μM Al and 2 fold at 100 μM Al, over the control level).

DISCUSSION

Al treatment (50μM Al for 24 h) caused root elongation inhibition in tobacco. At root apices, Al caused cell death as well as the increases in both *VPE* expression and VPE activity. During exposure to Al, the increase in *VPE* expression (*VPE1a* and *VPE1b*) and cell death started simultaneously after 9 h exposure to Al. Taken together, Al seems to cause the increase in *VPE* expression, which leads to an increase in VPE activity and then cell death. The elucidation of details of the process leading to cell death will be our future research. In addition, it is worth to mention that both BY-2 tobacco cells and root apices of tobacco (cv. Bright Yellow) similarly respond to Al. Based on this, we are now constructing *VPE* suppression lines of BY-2 cells, in order to obtain the evidence indicating the VPE to be a determining factor for Al-induced cell death in actively growing plant cells.

REFERENCES

von Uexktill H. R. and Mutert E. 1995 Global extent, development and economic impact of acid soils. Plant and Soil. 171: 1-15.

Ryan P. R., Ditomaso J. M. and Kochian L. V. 1993 Aluminium toxicity in roots: An investigation of spatial sensitivity and the role of the root cap. J. Exp. Bot. 44: 437-446.

Yamamoto Y., Kobayashi Y., Devi S.R., Rikiishi R., Matsumoto H. 2002. Aluminum toxicity is associated with mitochondrial dysfunction and the production of reactive oxygen species in plant cell. Plant Physiol. 128: 63-72.

Allan AC, Lapidot M, Culver JN, Fluhr R. 2001. An early tobacco mosaic virus-induced oxidative burst in tobacco indicates extracellular perception of the virus coat protein. Plant Physiol. 126(1):97-108.

Hatsugai N., Kuroyanagi M., Yamada K., Meshi T., Tsuda S., Kondo M., Nishimura M., Hara-Nishimura I. 2004. A plant vacuolar protease, VPE, mediates virus-induced hypersensitive cell death. Science. 305: 855–858.

Li Z., Yue H., Xing D. 2012. MAP kinase 6-mediated activation of vacuolar processing enzyme modulates heat shock-induced programmed cell death in Arabidopsis. New Phytol. 195: 85–96.

Hatsugai N, Yamada K, Goto-Yamada S, Hara-Nishimura I. 2015 Vacuolar processing enzyme in plant programmed cell death. Front Plant Sci. 6:234.

Kariya K., Demiral T., Sasaki T., Tsuchiya Y., Turkan I., Sano T., Hasezawa S., Yamamoto Y. 2013. A novel mechanism of aluminium-induced cell death involving vacuolar processing enzyme and vacuolar collapse in tobacco cell line BY-2. J. Inorg. Biochemi. 128: 196-201.

Snowden K. C., Richards K. D., Gardner R. C. 1995. Aluminum-induced genes -induction by toxic metals, low calcium, and wounding and pattern of expression in root Tips-. Plant Physiol. 107: 341–348.

Ono K., Yamamoto Y., Hachiya A., Matsumoto H. 1995. Synergistic inhibition of growth by aluminum and iron of tobacco (Nicotiana tabacum L.) cells in suspension culture. Plant Cell Physiol. 36: 115-125.

Liming effects on improving acid soil properties

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Abstract
In order to evaluate the residual effect of liming on soil pH and phosphorus and potassium availability, soil samples from field trial was analyzed five years after application of liming material. Trial was conducted on the acid stagnosol with low phosphorus and potassium availability, in 2008 in the central Croatia. Granulated dolomite (trade name Fertdolomite) containing 24.0% CaO and 16.0% MgO was applied in the six rates: 0, 5, 10, 20, 30 and 40 t ha^{-1}. Liming gradually raised soil pH value up to 1.5 pH units. Increased pH considerably improved soil phosphorus status (AL-method) from low to very high, and at the highest rate, phosphorus content increased by 24.8 mg 100 g^{-1} soil compared to the control. Impact of liming was less pronounced on potassium as its availability was improved from class of very low to low soil supply. Soil organic matter contents were independent on applied treatments.

INTRODUCTION
Soil acidity is limiting factors of soil fertility and crop productions worldwide, as well as in Croatia. Acid soils in Croatia cover an area about 32 % compared to the total area of agricultural land (Mesić et al., 2009). Poor soil fertility in Croatia is often linked to low levels of available phosphorus (Kovačević et al., 1993). Liming and ameliorative phosphorus fertilization are useful management practice for alleviation of crops yield reduction on such soils (Andrić et al., 2012; Kovačević and Rastija, 2010; Rastija et al., 2007; Kovačević and Lončarić, 2014). It is widely known that liming reduces the availability of most heavy metals and enhances the accessibility of phosphorus and soil base cations. Through the alteration of soil pH, liming affect the solubility and availability of plant nutrients and improves field crops growth and development. The objective of this research was to determine the residual effect of liming on improving acid soil properties.

MATERIAL AND METHODS
The liming field trial in a randomized complete block design in four replication was set up in the 2008, on the stagnosol with pH_{KCl} below 5 and poor phosphorus and potassium availability, in the central Croatia (45°30' N, 17°11' E). Granulated dolomite, trade name Fertdolomite containing 24.0% CaO and 16.0% MgO, enriched with 3.0% N, 2.5% P_2O_5 and 3.0% K_2O was applied in the amounts (t ha^{-1}) as follows: 0, 10, 20, 30 and 40. In the following years subsequent effects of Fertdolomite was evaluated while crop rotation was: maize (2008) - spring barley (2009) - maize (2010) – maize (2011) – winter wheat (2012) – maize (2013) – wheat (2014). The results of grain yields, soil and crop management and weather characteristics were elaborated in previous studies (Kovačević et al., 2015a, 2015b, Rastija et al., 2015). Soil samples for analysis were taken from each basic plot in 2013, after maize harvesting. Soil pH was determined according to ISO (1994), soil organic matter (SOM) content by sulfocromic oxidation (ISO, 1998) and plant available phosphorus and potassium by ammonium-lactate extraction (Egner et al. 1960). The data were statistically analyzed by ANOVA and treatment means were compared using t-test and LSD at 0.05 probability level.

RESULTS AND DISCUSSION
Fertdolomite application significantly affected soil pH as well as plant available P and K in surface soil layer, while effect of liming wasn’t statistically proved for soil organic matter contents (Table 1). The soil pH is one of the main indicators of soil fertility as it significantly affects the availability of nutrients. Five years after liming the soil pH gradually raised from 5.36 by 5 t ha^{-1} Fertdolomite to the 6.48 at the highest rate. However, regarding favorable soil pH for most field crops, rate of 20 t ha^{-1} was adequate.

Increasing soil pH by liming considerably improved P availability, particularly by higher rates, and contents of plant available P were extremely increased compared to the control (no liming) close to three times, four times and five times, for the treatment 20, 30 and 40 t ha^{-1}, respectively. As affected by liming, soil-P status was changed from low to very high (above 30.0 mg 100 g^{-1} soil) supplies with raising amounts of added Fertdolomite. It is well known that moderate pH increase enhance phosphorus availability, but excessive amount of liming materials could lead to phosphorus reduces and nutritional unbalances in soil. However, this was not the case in this experiment, although 40 t ha^{-1} of liming material can be considered as a relatively high liming rate. On the other hand, effect of liming on potassium was less manifested, and its availability was improved from class of very low (<12 mg K_2O 100 g^{-1}) to low (12.1-19.0) K supply (Table 1).

Table 1. Impact of liming on the soil properties five years after application

Liming t ha^{-1}	pH(H_2O)	pH(KCl)	P_2O_5 mg 100 g^{-1} soil	K_2O mg 100 g^{-1} soil	SOM %
0	5.69 c	4.98 e	6.54 d	9.60 c	2.13
5	6.31 b	5.36 d	8.00 d	10.05 c	2.31
10	6.51 b	5.69 c	9.78 d	9.74 c	2.21
20	6.79 ab	6.16 b	18.75 c	10.71 bc	2.27
30	6.88 a	6.26 ab	25.23 b	12.08 ab	2.19
40	7.04 a	6.48 a	31.13 a	12.69 a	2.39
Mean	6.54	5.82	16.85	10.81	2.25
$LSD_{0.05}$	0.34	0.28	2.51	1.76	ns

values followed by the same letter are not significantly different at P≤0.05 level; ns- non significant

Considerable effect of liming on grain yields of the field crops in rotation was found in the interaction with growing season characteristics (Kovačević et al., 2015a, 2015b; Rastija et al., 2015). Few years after application lower yields of maize and barley were achieved on the highest rate due to overliming (Table 2). Only in the two last years of trial (2013 and 2014) yields on the higher liming rates were also significantly higher implying positive prolonged effect of liming. From the point of satisfactory yield achieving, it can be concluded that lower amounts of Fertdolomite, like 10 and 20 t ha^{-1} was an adequate.

Table 1. Grain yields (t ha^{-1}) of field crops in rotation (2008-2014)

Liming t ha^{-1}	2008 Maize	2009 Barley	2010 Maize	2011 Maize	2012 Wheat	2013 Maize	2014 Wheat
0	13.59	4.22	12.38	10.84	7.47	10.04	4.71
5	13.66	5.14	13.60	11.85	7.77	10.31	4.91
10	13.44	4.81	13.90	11.66	7.41	10.65	5.17
20	13.30	4.54	13.59	10.70	7.01	11.29	5.40
30	13.32	4.52	13.30	9.50	7.06	11.68	5.67
40	12.69	4.53	12.57	9.36	6.96	11.78	6.10
Mean	13.33	4.62	13.22	10.65	7.28	10.96	5.33
$LSD_{0.05}$	0.42	0.38	0.40	0.70	0.38	0.80	0.51

values followed by the same letter are not significantly different at P≤0.05 level; ns- non significant

CONCLUSION

Liming significantly improved acid soil properties especially regarding pH value and phosphorus availability. Five years after application, soil pH was raised by 1.5 pH units at the highest liming rate, while soil phosphorus supply was changed from low to very high, implying prolonged effectiveness of liming. At the highest rate, phosphorus content increased by 24.8 mg 100 g^{-1} soil compared to the control. Still, liming less affected soil potassium status, whilst soil organic matter contents were independent on applied treatments.

REFERENCES

Egner, H., Riehm, H., Domingo, W.R. (1960): Untersuchungen uber die chemische Bodenanalyse als Grundlage fur die Beurteilung des Nahrstoffzustandes der Boden II. Chemische Extractionsmethoden zu Phosphor- und Kaliumbestimmung. K. Lantbr. Hogsk. Annlr. W.R., 26, p.199-215.

ISO (1994): Soil quality. Determination of pH , ISO 10390:1994.

ISO (1998): Soil quality. Determination of organic carbon by sulfochromic oxidation. ISO 14235:1998.

Kovacevic V., Loncaric Z. (2014): Using of carbocalk for improvement of soil fertility. Technologica Acta 7 (1): 1-8.

Kovacevic V., Rastija M. (2010): Impacts of liming by dolomite on the maize and barley grain yields. Poljoprivreda/Agriculture, 16(2): 3-8.

Kovacevic V., Rastija M., Iljkic D., Brkic I., Kovacevic J. (2015a): Response of maize and barley to liming with fertdolomite. Poljoprivreda / Agriculture 21 (in press).

Kovacevic V., Rastija M., Sudar R., Iljkic D., Varga I. (2015b): Response of maize and wheat to fertdolomite application. Columella (in press).

Rastija M., Kovacevic V., Drezner G., Iljkic D., Varga I. (2015): Response of winter wheat to liming with fertdolomite. In: Proc. 26th Intern. Scientific-Expert Conf. of Agriculture and Food Industry. 27-30 Sept. 2015, Sarajevo (in press).

Rastija M., Banaj Đ., Marković M. (2007). Influences of liming on nutritional status of soil. Cereal Research Communications. 35, 2; 981-984.

Mesić, M., Husnjak, S., Bašić, F., Kisić, F., Gašpar, I. (2009.): Suvišna kiselost tla kao negativni ĉimbenik razvitka poljoprivrede u Hrvatskoj (Excessive Soil Acidity as a Negative Factor for Development of Croatian Agriculture). Proceedings of 44th Croatian&4th International Symposium on Agriculture. February 16 - 20, 2009, Opatija, Croatia. 9-18.

The effect of acid soils liming on alfalfa mineral composition change

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Abstract

Soil acidification is a slow, continuous natural process resulting in acid soils being common in areas where soil development continued for long, geological periods of time and under climatic conditions which rainfall exceeeds evapotranspiration. Soil acidification process may be accelerated by intensive agricultural production with fertilization as one of the main factors of intensification. The process of soil acidifications is aided by water leaching base cations to lower horizons and acid soils are widespread in the eastern Croatia. Consequently, in conditions of excessive soil acidity, numerous negative effects are present like hydrogen ions toxicity, toxicity of aluminum and manganese ions, phosphorus and molybdenum deficiency, reduction of microbiological activity and increased heavy metals availability. These unfavorable chemical properties of acid soils represent one of the main factors limiting the field crops yield.

Alfalfa or lucerne (Medicago sativa L.) is the most widely used fodder legume in temperate regions. Soil acidity is one of the limiting factors affecting the production and sustainability of pastures and crops in many parts of the world. In Croatia, soil acidity is main limiting factor of field crops yield. Correcting soil pH by liming can influence plant nutrient availability and plant yield and quality. Alfalfa belongs to crops requiring a pH in the range of 6.5 to 6.8 or higher. Therefore the aim of the research was to determine the influence of liming, organic and mineral fertilization on alfalfa mineral composition change and above ground mass response, as well as impact on soil chemical properties.

The pot experiment was conducted in field conditions, in eastern part of Croatia with two different acid soils of heavy and light textural class from Donji Miholjac and Magadenovac site. Acid soils were set up in plastic pots of 20 L volume with 10 liming and organic fertilization treatments in four repetitions according to random block design using alfalfa as the indicator plant. Lime material was added in two rates: 7,5 and 15 t/ha of Mischkalk lime material (65 % CaO, ENV = 82,5) as well as organic fertilization: 20 and 40 t/ha cattle manure and mineral fertilization 100:200:300 kg/ha $N:P_2O_5:K_2O$. During each year, first cutting of alfalfa were sampled at the beginning of flowering stage by cutting whole above ground mass and analysed to determine mineral composition. Also, soil was sampled and analysed in a first year of investigation (2014.) and in second investigation year (2015.).

A higher concentration of all investigated elements in the leaf and stalk was found in the first year, except of calcium, which concentration has increased in the second year because of the liming extension effect. However, in the second year was achieved higher total production of dry matter of alfalfa above ground mass. Liming significantly impacted on the increase in the number (8-14 %), height (4-8 %) of alfalfa plants, and leaf dry matter yield (13-28 %), the stem (19-30%), and the total above ground mass (17 -29 %) and root mass (20-34 %).

A higher concentration of all investigated microelements in the leaf and stalk was found in the soil which had a lower initial pH and higher concentrations of the same elements. Increasing doses of liming and fertilizer doses resulted with concentration increase of N, P, K and Ca in the leaf and stem of alfalfa, while liming reduced the concentration of Mg in the leaf. At the same time, increasing the doses of organic fertilizer impacted on the concentration increase of Fe, Mn, Zn and Cu in the leaf and stem of alfalfa, while liming resulted with the opposite effect where increment of lime rates reduced the concentrations of all investigated microelements in the leaf and in the stalk.

Liming doses increment also resulted with positive effect on the concentrations increment of N, P, K and Ca in alfalfa root, and the with opposite effect on the concentration of Mg and microelements. At the same time, the impact of organic fertilization was smaller and slightly different on the root composition where organic fertilization significantly impacted on the concentration of N, K, Mn, Zn and Cu.

So, liming of two acid soils positively impacted on alfalfa yield components and increased plant number and hight, as well as yield of above ground and root mass and also leaf and stalk dry matter yield. Moreover, liming and organic fertilization rates increment, increased concentrations of macroelements in alfalfa leaf and stalk, but decreased Mg leaf concentrations. Increment of organic fertilizer rates increased concentrations of micronutrients in alfalfa leaf and stalk, while liming showed the opposite effect and decreased micronutrients concentrations in alfalfa leaf and stalk by increament of lime rates.

Early vigour in wheat improves phosphate uptake from P-fixing soils

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Abstract
Quantitative trait loci (QTL) for early shoot biomass were identified in wheat grown on a soil high in total phosphorus (P) but low in plant-available P. Two populations were screened: recombinant-inbred lines (RILs) from Chuan-Mai 18/Vigour 18 and doubled-haploid lines (DHLs) from Kukri/Janz. Seven QTLs for shoot biomass were identified in the RILs with the largest on chromosome 7A accounting for 7.4% of the phenotypic variance. RILs from the upper tail had larger embryos than RILs from the lower tail and additional trials on soil with non-limiting P indicated that early vigour and an increased capacity to access P contributed to the biomass results. The influence of early vigour on P nutrition was examined further with advanced vigour lines (AVLs). The AVLs accumulated more shoot biomass, maintained lower shoot P concentrations and showed greater P acquisition efficiency than Vigour 18. Nine QTLs for shoot biomass were identified in the Kukri/Janz population. Two on chromosomes 4B and 4D accounted for 24.8% of the variance. Candidates underlying these QTLs are the *Rht* genes. We confirmed the influence of these *DELLA* genes using near-isogenic lines with different *Rht-B1* alleles. Dwarf and semi-dwarf alleles reduced shoot and root biomass at high and low P but not the efficiency of P acquisition (PAE). We conclude that early vigour contributed to the distributions in both populations. Early vigour can increase plant growth at sub-optimal P and some sources can also improve PAE.

INTRODUCTION

The adoption of more P-efficient crops and pastures in agriculture is one strategy to reduce wastage and minimise environmental contamination (Veneklaas *et al.*, 2012). Phosphorus efficiency can been defined in different ways depending on the spatial and temporal scale being considered (e.g. farm, plant, grain or cell). P efficiency in plants usually target two fundamental processes: the absorption of P from the soil, which relates to P-acquisition efficiency or P-uptake efficiency, and the conversion of absorbed P into harvestable product (biomass, grain, fruit) which relates to P-ultilization efficiency. P-efficient crops would ideally utilise all or most of the P applied each season (Helyar, 1998) but this rarely occurs because P can be sequestered into sparingly-soluble fractions in the soil which are unavailable to plants. (e.g. Al:P and Fe:P compounds in acid soils) (Richardson *et al.*, 2011) (Simpson et al., 2011). Plants which can readily absorb P from the soluble pool and access more of the sparingly-soluble P should maintain high yields with reduced inputs.

Plants respond to reduced P availability by changing biomass allocation between roots and shoots, by altering root structure and physiology and by modifying cellular metabolism (Lopez-Bucio *et al.*, 2002; Plaxton and Tran, 2011; Vance *et al.*, 2003) (Beebe *et al.*, 2006; Gamuyao *et al.*, 2012; Rose *et al.*, 2013; Shimizu *et al.*, 2008; Wissuwa *et al.*, 2002; Yang *et al.*, 2011; Zhu *et al.*, 2005). Most P absorbed by wheat occurs pre-anthesis and early biomass remains a useful surrogate for P uptake and efficiency screens. We previously screened 190 genotypes of wheat for shoot biomass in a low pH soil that was high in total P but low in plant-available P. Two cultivars Kukri and Vigour 18 consistently accumulated greater shoot and root biomass and absorbed more P in low and high P treatments than two other genotypes, Janz and Chuan-Mai 18 (Liao *et al.* 2008). Kukri and Vigour 18 also showed greater P acquisition efficiency (PAE) than Janz and Chuan-Mai 18. PAE is defined as the ratio of shoot biomass at low and high P. This study investigated the genetics of PAE in segregating populations of wheat.

MATERIAL AND METHODS

Two segregating populations were screened for early shoot biomass (~5 leaf stage in pots) and P acquisition efficiency (PAE) on a ferrosol that was high in total P but low in plant-available P. PAE is defined as the ratio of shoot biomass at low and high P. The populations were recombinant-inbred lines (RILs) from Chuan-Mai 18/Vigour 18 and doubled-haploid lines (DHLs) from Kukri/Janz. Advanced vigour lines (AVLs; Zhang *et al* 2015) with superior early vigour as well as near-isogenic lines (NILs) that vary in *DELLA* genes (*Rht-B1*) were also tested.

RESULTS AND DISCUSSION

Seven QTLs for shoot biomass were identified in the RILs with the largest on chromosome 7A accounting for 7.4% of the phenotypic variance. RILs from the upper tail had larger embryos than RILs from the lower tail (data not shown). Tail lines were then grown in non-limiting P and the results indicated that early vigour and the capacity to access P contributed to the initial distribution. The influence of early vigour on P uptake was tested further with AVLs with greater early vigour. In similar trials, the AVLs accumulated more shoot biomass, maintained lower shoot P concentrations and showed greater PAE than Vigour 18 (Fig 1A,B).

Nine QTLs for shoot biomass were identified in the DHLs. Two on chromosomes 4B and 4D accounted for 24.8% of variance. Candidates underlying these QTLs are the *Rht-B1* genes. We confirmed the influence of these genes using near-isogenic lines (NILs) in a Maringa background that have different *Rht-B1* alleles. $\mathrm{NIL_{TALL}}$ is the wild-type *Rht-B1a* allele, $\mathrm{NIL_{SD}}$ is the *Rht-B1b* semi-dwarf allele and $\mathrm{NIL_{DWF}}$ is the *Rht-B1c* dwarf allele. The *Rht-B1b* and *Rht-B1c* alleles affected shoot biomass at high and low P but not the PAE (Fig 1C,D).

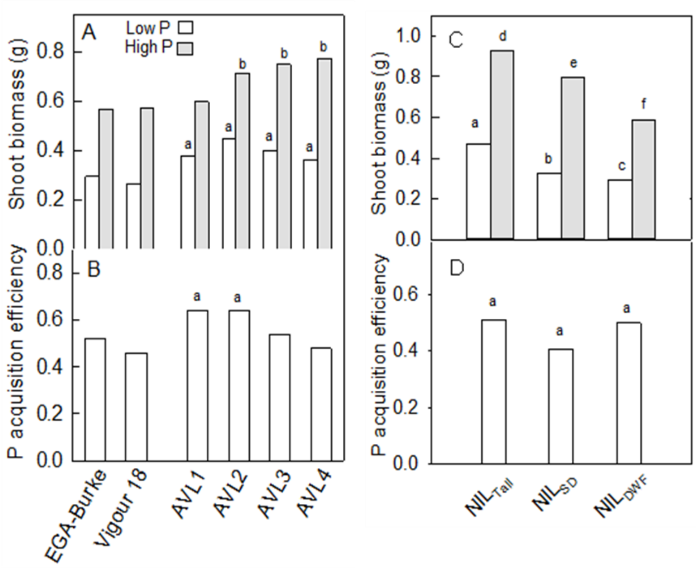

Figure 1. Shoot biomass of the Advanced Vigour Lines AVLs and NILs differing in dwarfing alleles (A) Shoot biomass (5 leaf stage) of plants grown in soil with low or high P. LSD (P<0.05) for the low P treatment is 0.063 and for high P is 0.088 (n=4). AVLs labelled with "a" in low P and "b" in high P are greater than Vigour 18 and EGA-Burke (an elite cultivar control). (B) P-acquisition efficiency (PAE) from data in A. AVLs with "a" are significantly greater than Vigour 18 and EGA-Burke. (C) Biomass of NILs grown in low or high P. Genotypes and P treatment were significant ($P_{0.01}$) in a two-way ANOVAR but the interaction was not. Different letters are significantly different (P<0.05). (D) PAE from the data in C. Data are mean and SE (n=6). Different letters are significantly different (P<0.05).

The study demonstrates that early vigour contributed to the variation in biomass in both segregating populations screened. Early vigour can increase plant growth at sub-optimal P and some sources can also improve the efficiency of P acquisition. We conclude that "early vigour" is a blanket term to describe a general phenotype which can arise via different metabolic and physiological pathways. For example, early vigour in Vigour 18 and the AVLs is positively correlated with embryo size and independent of *Rht* genes. By contrast, vigour in the Kukri/Janz population is unrelated to embryo size but linked, in part, with *Rht-B1* genes. Not surprisingly these diverse sources of vigour affect other aspects of plant biology in different ways, including P nutrition. The AVLs showed altered root structure (data not shown) and greater PAE than less vigorous controls. Different *Rht-B1* genes could affect biomass accumulation as well but without altering shoot P concentrations or PAE. Therefore while both sources of vigour increased overall plant size only the AVL material showed concomitant increases in PAE. This outcome highlights the value of alternative dwarfing genes to reduce plant height without affecting early vigour.

REFERENCES

Beebe et al. 2006. Crop Science 46, 413-423; Gamuyao et al. 2012. Nature 488, 535-539; Helyar KR. 1998. Field Crops Research 56, 187-195; Lopez-Bucio et al. 2002. Plant Physiology 129, 244-256; Plaxton WC, Tran HT. 2011. Plant Physiology 156, 1006-1015; Richardson et al. 2011. Plant and Soil 349, 121-156; Rose et al. 2013. Frontiers in Plant Science 4; Shimizu et al. 2008. Theoretical and Applied Genetics 117, 987-996; Simpson et al. 2011. Plant and Soil 349, 89-120; Vance et al. 2003. New Phytologist 157, 423-447; Veneklaas et al. 2012. New Phytologist 195, 306-320; Wissuwa et al. 2002. Theoretical and Applied Genetics 105, 890-897; Yang et al. 2011. Plant and Soil 339, 97-111; Zhang et al 2015. Journal of Experimental Botany 66, 1215–1226; Zhu et al. 2005. Theoretical and Applied Genetics 111, 688-695.

Some new strategies for amelioration of soil acidity

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Abstract
Some new strategies were developed in recent years to ameliorate acidic soils from tropical and subtropical regions of southern China. Biochars derived from oxygen-limited pyrolysis of crop residues contain alkaline substances of carbonate and organic anions and have alkaline pH, and thus can be used to correct soil acidity. Application of biochars also improved acidic soil fertility through increasing soil CEC and exchangeable Ca^{2+}, Mg^{2+} and K^+ and thus increased crop yields. Uptake of nitrate by maize and wheat induced alkalization in rhizosphere of the plants and decreased rhizosphere soil acidity. When the biochars were applied combined with nitrate, the synergistic effects of the biochars and the rhizosphere alkalization were observed and enhanced their ameliorating effects on the acidity of strongly acidic soils.

INTRODUCTION
There are large areas of acidic soils distributed in tropical and subtropical regions of southern China. In recent decades, the sharp increase in the application of ammonium-based fertilizers in cropping systems in these regions has greatly accelerated soil acidification (Guo et al., 2010), which enhanced Al toxicity to plants and led to reduction in crop yields. Application of mineral lime is a common agricultural practice to correct soil acidity. However, because of the lack of liming amendments in many areas and their high costs, attention has been given to alternatives. In this article, some new methods for amelioration of soil acidity developed in recent years were described.

RESULTS AND DISCUSSION
Crop straw biochars
In the partial or total absence of oxygen, thermal decomposition of plant-derived biomass produced a solid carbon-rich material referred as biochar. Biochar is commonly alkaline and thus can be used as an amendment to neutralize soil acidity and increase soil pH (Chan et al., 2007; Novak et al., 2009). The ameliorating effects of biochars generated from nine crop residues were highly correlated with their alkalinity (Yuan and Xu, 2011). Therefore, the alkalinity of biochars is a key factor determining their ameliorating effects on soil acidity. Our results indicated that carbonate and organic anions were the main forms of alkalis in crop straw biochars (Yuan et al., 2011). Carbonate content in biochars increased with pyrolysis temperature, while the contents of organic anions changed oppositely. 500 °C was suggested as the optimum pyrolysis temperature for producing biochar amendments from crop straws for acid soils. At this temperature, both organic anions and carbonates can contribute significantly to the alkalinity of the biochars (Yuan et al., 2011). The ameliorating effects of biochars on soil acidity were also confirmed under field condition in an Ultisol located in Anhui Province, China (119°8′E, 31°6′N). The results in Table 1 showed that the application of both biochars generated from the straws of canola and peanut increased soil pH and exchangeable Ca^{2+}, Mg^{2+} and K^+ and decreased soil exchangeable acidity, especially at application rate of 7500 kg/ha. The yield of canola seeds was increased greatly by the biochars (Table 1). Compared with control, the yield increased by 91% for treatment of canola straw biochar and by 67% for the treatment of peanut straw biochar at application rate of 7500 kg/ha.

Table 1. Effects of biochars on soil acidity of an Ultisol and yield of canola seeds in Anhui Province, China

Treatment	Application rate(kg/ha)	Soil pH	Exchangeable acidity (mmol/kg)	Exchangeable base cations (mmol/kg)				Yield (kg/ha)
				Ca^{2+}	Mg^{2+}	K^+	Na^+	
Control	--	4.16	56.0	23.3	5.0	4.4	2.7	1055
CS biochar	7500	4.54	41.6	35.5	8.4	6.1	2.7	2013
PS biochar	3750	4.28	48.7	23.7	5.6	4.8	2.9	1585
PS biochar	7500	4.40	44.8	36.5	9.3	5.3	2.9	1765

CS biochar: canola straw biochar; PS biochar: peanut straw biochar

Rhizosphere alkalization induced by plant roots due to nitrate uptake
Plants have an ability to change rhizosphere soil pH through ion uptake by their roots. Changes in rhizosphere pH are mainly based on the theory of anion and cation balance. When plant uptake of one type of charge exceeds the other, the plant maintains electro-neutrality by extrusion of H^+ or OH^-, which leads to acidification or

alkalization of the rhizosphere soil. A biological method was suggested to ameliorate soil acidity in a semi-arid region of Australia based on the root-induced alkalization of rhizosphere due to nitrate uptake by wheat crops (Tang et al., 2011; Conyers et al., 2011). The applicability of the method for an acidic Ultisol from subtropical region of China was examined with pot experiments (Masud et al., 2014). The growth of maize with application of calcium nitrate reduced exchangeable acidity and increased the pH in the Ultisol rhizosphere, compared with bulk soil. The release of hydroxyl ions from maize roots due to nitrate absorption by the plant was responsible for pH increase in rhizosphere soil (Masud et al., 2014). In recent time, maize and wheat varieties, tolerant, less sensitive, or sensitive to Al, were grown with $Ca(NO_3)_2$ to study the effect of rhizosphere alkalization on soil acidity with pot experiments. Our results, from two crops of maize and one of wheat, showed that application of nitrate increased soil pH in all varieties and the effects were greater in Al-tolerant varieties than in other varieties. The greater biomass produced by Al-tolerant varieties resulted in larger amounts of OH^- being released from plant roots and, in turn, greater bio-amelioration on soil acidity.

Crop straw biochars in combined with rhizosphere alkalization

Plants can not grow well in strongly acidic soils even nitrate is applied. The combination of crop straw biochars with rihzosphere alkalization induced by plant roots provides a new strategy for amelioration of soil acidity. The amelioration of biochars on soil acidity promoted growth of plants and uptake of nitrate by the plants. Subsequently, more OH^- were released from plant roots and thus enhanced alkalization of plant rhizosphere. The results from pot experiments indicated that the biochars from the straws of canola and peanut and nitrate applied separately increased soil pH and decrease soil exchangeable acidity (Table 2). When the biochars were applied with nitrate, the increase in soil pH was not only greater than that for the treatments with biochars and nitrate applied separately, but also greater than the sum of pH increase induced by the biochars and uptake of nitrate by maize roots (Table 2). This suggested that there were the synergistic effects of the biochars combined with the rhizosphere alkalization of maize induced by nitrate uptake on amelioration of soil acidity.

Table 2. Effects of crop straw biochars combined with rhizosphere alkalization of maize induced by uptake of nitrate on the pH and exchangeable acidity of an Ultisol from Guangdong Province, China

Treatment	Soil pH	Increase of soil pH	Exchangeable acidity(mmol/kg)
Control	4.58	--	26.69
200 mg N/kg	4.78	0.20	13.48
1% Canola straw biochar	4.85	0.27	15.40
1% Peanut straw biochar	5.16	0.58	6.0
200 mg N/kg + 1% Canola straw biochar	5.29	0.71	5.70
200 mg N/kg + 1% peanut straw biochar	5.46	0.88	4.87

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REFERENCES

Chan, K.Y., van Zwiete, L., Meazaros, I., Downie, A., Joseph, S. 2007. Agronomic values of greenwaste biochar as a soil amendment. Australian Journal of Soil Research. 45: 629-634.

Conyers, M.K., Tang, C., Poile, G.J., Liu, D.L., Chen, D.L., Nuruzzaman, Z. 2011. A combination of biological activity and the nitrate form of nitrogen can be used to ameliorate subsurface soil acidity under dryland wheat farming. Plant and Soil. 348: 155-166.

Guo, J.H., Liu, X.J., Zhang, Y., Shen, J.L., Han, W.X., Zhang, W.F., Christie, P., Goulding, K.W.T., Vitousek, P.M., Zhang, F.S. 2010. Significant acidification in major Chinese croplands. Science. 327: 1008-1010.

Masud, M.M., Guo, D., Li, J.Y., Xu, R.K. 2014. Hydroxyl release by maize (Zea mays L.) roots under acidic condition due to nitrate absorption as related with amelioration of an acidic Ultisol. Journal of Soils and Sediments. 14: 845-853.

Novak, J.M., Busscher, W.J., Laird, D.L., Ahmedna, M., Watts, D.W., Niandou, M.A.S. 2009. Impact of biochar amendment on fertility of a southeastern coastal plain soil. Soil Science. 174: 105-112.

Tang, C., Conyers, M.K., Nuruzzaman, M., Poile, G.J., Liu, D.L. 2011. Biological amelioration of subsoil acidity through managing nitrate uptake by wheat crops. Plant and Soil 338: 383-397.

Yuan, J.H., Xu, R.K. 2011. The amelioration effects of low temperature biochar generated from nine crop residues on an acidic Ultisol. Soil Use and Management. 27: 110-115.

Yuan, J.H., Xu, R.K., Zhang, H. 2011. The forms of alkalis in the biochar produced from crop residues at different temperatures. Bioresource Technology. 102: 3488-3497.

Section 05:

Sustainable utilization and management of agricultural, forestry and natural ecosystems on acid soils

Chairpersons: Prakash Nagabovanalli, Jusop Shamshuddin, Kazuyuki Inubushi

Acid sulfate soils in Southeast Asia and their utilization for rice cultivation

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Abstract
Some years ago sea level rose a few meters above the present level in Southeast Asia, inundating the lowland coastal plains that resulted in the formation of pyrite (FeS_2). This pyrite was oxidized, releasing high acidity. Rice grown on the soils produce low yield due the stress of low pH and Al and/or Fe toxicity. Rice can be grown sustainably after ameliorating the soils using lime or basalt in combination with bio-fertilizer having phosphorus-solubilizing bacteria (PSB). Besides increasing soil pH, the bacteria releases organic acids that chelate Al and Fe, rendering them inactive. Rice root itself is able to excrete some organic acids under stress, further reducing Al and Fe concentration.

INTRODUCTION
Pyritization and generation of acidity
About 4300 before present a large part of the coastal lowlands in Southeast Asia was inundated with seawater due to the rise in sea level in the region (Shamshuddin et al., 2014). It was during this period of the geological history that the affected areas were pyritized (Figure 1), which in the end formed acid sulfate soils. The soils are defined by low pH of < 3.5 and the presence of jarosite in the sulfuric horizon. Rice grown on these soils is subjected to the stress of H^+ as well as Al^{3+} and/or Fe^{2+} toxicity, resulting in very low yield. However, with proper agronomic practices, using appropriate amendments, rice can be grown sustainably on these soils (Panhwar et al., 2014a). The objectives of this paper are: 1) to explain the formation and chemical properties of acid sulfate soils in Southeast Asia; and 2) to discuss innovative soil management practices for sustainable rice production on the soils.

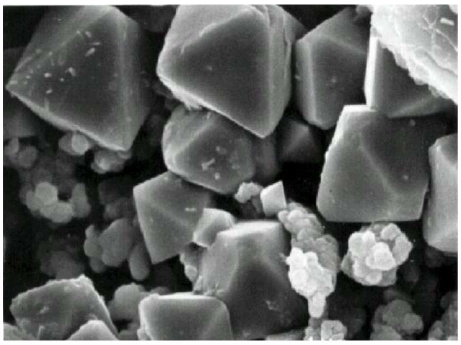

Figure 1. Pyrite found in the coastal sediments of Southeast Asia

When the areas are opened up for rice cultivation, the pyrite is oxidized, releasing acidity that dissolves silicates. Finally, high amount of Al and/or Fe are present in the soils that affect rice growth. The growth of rice is also affected by low available P as well as low exchangeable Ca and Mg; hence, its yield is usually below the national average without proper amelioration.

RESULTS AND DISCUSION
Effects of pH, Al and/or Fe on rice growth
Under the condition of low pH, roots growth is curtailed due to the absence of roots hairs. Additionally, in the presence of high amount of Al and/or Fe in the water, root cells shrink or even rupture. Our study showed that rice root length and root surface area linearly decreased with increasing concentration of Al and/or Fe (Shamshuddin et al., 2014). Water pH in the paddy fields should be preferably increased to a level above 5 in order to precipitate Al as inert Al-hydroxides by application of amendments, such as lime or basalt.

How rice protects itself against Al^{3+} and/or Fe^{2+} toxicity
Even at pH below 5, rice is able to grow and produce reasonable yield due to its ability to defend itself against Al and/or Fe toxicity. Under the stress of high amount of Al and/or Fe, rice roots excrete organic acids that

subsequently chelate Al and Fe, rendering them inactive (Shamshuddin et al., 2014). Hence, the availability of Al and/or Fe in the water that affect rice growth is substantially reduced.

Improving soil productivity for rice cultivation

Normal agronomic practice to ameliorate the infertility of acid sulfate soils is application of ground magnesium limestone (GML) that increases soil pH and supplying Ca and Mg (Figure 2). However, a more superior amendment is basalt, which not only increases soil pH and supplies Ca and Mg, but also increases P and K in the soils. Recent studies indicated that bio-fertilizer fortified with phosphate-solubilizing bacteria improved soil productivity, which was translated into increased rice yield (Panhwar et al., 2014b).

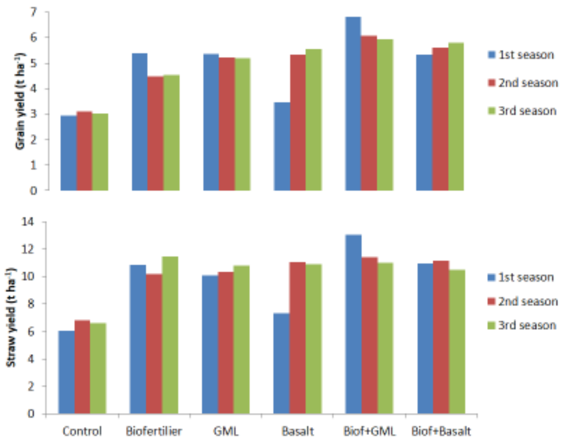

Figure 2. Effects of applying various amendments on rice yield

Three potential PSB living in acid sulfate soils of Malaysia were isolated, which were found to release organic acids that inactivated Al and Fe via the process of chelation (Panhwar et al., 2014b). Additionally, the bacteria produced a chemical that increased water pH significantly. Figure 2 shows that GML and bio-fertilizer application improved grain and straw yield significantly and the ameliorative effects can last at least three consecutive seasons. Due to its low rate of dissolution, basalt application did not give impressive result in the first season. But with time, more basalt dissolved; hence, the grain and straw yield increased. It is believed that the best option is to apply basalt in combination with bio-fertilizer having PSB. The PSB are expected to help increase the rate of basalt disintegration/dissolution. Furthermore, Si so released by basalt in the form of silicic acid can be taken up by rice that helps prevent rice blast, a disease that affects rice production severely in Southeast Asia.

CONCLUSION

Rice grown on acid sulfate soils in Southeast Asia is subjected to the stress of low pH and Al^{3+} and/or Fe^{2+} toxicity that result in low yield. With proper soil management using GML or basalt in combination or bio-fertilizer containing phosphate-solubilizing bacteria, the soils can be used for sustainable rice production.

REFERENCES

Panhwar, Q.A., U.A. Naher, O. Radziah, J. Shamshuddin and M.I. Razi. 2014a. Bio-fertilizer, ground magnesium limestone and basalt applications may favourably alter the chemical properties of a Malaysian acid sulfate soil and improve rice growth. Pedosphere. 24(6): 827-835.

Panhwar, Q.A., U.A. Naher, J. Shamshuddin, O. Radziah, M.A. Latif and M.I. Razi. 2014b. Biochemical and molecular characterization of potential phosphate-solubilizing bacteria in acid sulfate soils and their beneficial effects on rice growth. PLOS ONE. 9(10): 1-14.

Shamshuddin, J. A. Elisa Azura, M.A.R.S. Shazana, C.I. Fauziah, Q.A. Panhwar and U.A.Naher. 2014. Properties and management of acid sulfate soils in Southeast Asia for sustainable cultivation of rice, oil palm and cocoa. Advances in Agronomy. 124: 91-142.

Soil acidity and toxicity problems attributed by volcanic eruption: their management to support crop growth

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Abstract

Sinabung volcano is one of the 127 Indonesia's active volcanoes and is currently being erupted, resulting serious problems on agriculture. The objective of the study was to assess soil acidity and toxicity problems as affected by ashfall depositions of Sinabung volcano and to provide the strategic management to restore soil productivity. Result showed ashfall depositions resulted severe soil acidity and Al and S toxicity accompanied by formation of surface encrustation that limit water infiltration and root penetration. The benefit of volcanic ashfall deposition is the release of new nutrients (Ca, Mg, K, Na, P, Si and S) for crops. The strategic management to restore soil productivity include: lime application, selection of acid tolerant crops, physically break up encrustation in the soil surface, and incorporation of dry ashfall into underlying soils to release new nutrients.

INTRODUCTION

Sinabung volcano in Indonesia has erupted intermittently since July 2010 to July 2013. Unusual continuous daily eruption has taken place from September 2013 to the present (Mei 2015). This is the longest period of the eruption history ever recorded in Indonesia having 127 active volcanoes. Hot ashfall depositions cover many areas killing and damaging many crops. Currently the areas around the volcano face the problems of various limiting factors for crop growth in addition to the decrease of environmental services. The objective of the study was to assess soil acidity and nutrient problems as affected by ashfall deposition of Sinabung volcano and to provide the strategic management to restore soil productivity.

MATERIALS AND METHODS

Sampling sites were designed to cover different environmental conditions including elevations, land uses, landforms, ashfall deposition thicknesses, and distance from the eruption center. Very fresh ashfall deposition (SNF) was collected within an hour after eruption to determine soluble salts (readily released nutrients for crops) once in contact with water. Soil mini pits (SN1, SN2 and SN3) and profile pits (SN5, SN6 and SN7) were made and both the ashfall deposits and the underlying soil horizons were sampled for mineralogical, chemical and physical analyses.

RESULTS AND DISCUSSION

The pH values for the new fresh ashfall (collected within an hour after deposition) were significantly higher (pH 6.1) than the older ashfall deposition (pH values 3.4-4.8) (Table 1). It seems that the extreme acidification of ashfall is attributed to cumulative effect of prolonged continuous acid input from eruptions. The condensed acid aerosols (e.g. H_2SO_4 and HCl) on ashfall particulates increased acidity. Sulfate is produced by SO_2 gas eruption when reacting with water (Kiyosu and Kurahasi, 1983) in the atmosphere. Previous studies using a scanning electron microscope of airborne ash collected from volcanic plumes showed the presence of liquid coatings on many particles, indicating sulfuric acid (Casadevall et al., 1984; Rose et al., 1980). This sulfuric acid is considered generating extreme low pH of the older ashfall deposition in the present study. Encrustation formation was observed on soil surfaces receiving mud ashfall but not on soils receiving dry ashfall. This suggested that rainfall (water) in the atmosphere at the time of violent eruption played a key role in the formation of encrustation. Encrustation in the soil surfaces limited water infiltration and root penetration. The XRD analysis indicated (data not shown) the presence of gypsum and jarosite minerals in a high amount for the mud-ashfall, while in dry ashfall gypsum was very small and that jorosite was absent. Gypsum is considered as the cementing agent in promoting hard setting (encrustation) in the soil surfaces.

Despite the volcanic ashfall deposition damaged the agricultural land, it provided the benefit in soil nutrient enrichment by releasing new cations (the order of Ca > Na > Mg > K) in addition to P, S and Si (Table 1). This finding agrees well with the previous study that showed the volcanic ashfall from Merapi volcano contains various new macronutrients (Ca > Na > K > Mg > P > S) and micronutrients (Zn, Fe, Mn, Cu, and Co) for crops (Anda and Sarwani, 2012). The effect of ashfall depositions has dramatically changed the chemical properties of the underlying soils by decreasing soil pH and cation exchanged capacity (CEC) and by increasing exchangeable Ca, Al, and available S.

Tabel 1. Chemical properties of volcanic ashfall and their effect on underlying soil properties

Profile or mini pit	Depth	P_2O_5 Bray 1	pH H_2O	Soluble S	CEC	Exchangeable cations Ca	Mg	K	Na	Sum cation	Exch. Al
	cm	mg kg^{-1}		mg kg^{-1}	----------	----------	cmol$_c$ kg^{-1}	----------	----------	----------	----------
New fresh ashfall was collected within an hour after deposition (Novemeber 15, 2014)											
SNF		69.5	6.1	150	3.14	2.56	0.27	0.16	0.30	3.29	0.00
Older ashfall deposition overlying initial soil surfaces at different land uses											
SN 1/I	0-2	15.5	3.5	1710	4.39	12.45	0.13	0.05	0.23	12.86	5.19
SN 1/II	2-12	8.5	3.4	2478	5.53	20.06	0.13	0.12	0.16	20.47	5.70
SN 6/0	0 - 5	6.6	3.3	3881	4.94	18.93	0.27	0.04	0.28	19.52	6.93
SN1, Initial soil underlying ashfall of 5-12 cm deposition under a coffee plantation land use											
Ap	12 - 21	87.7	3.7	4515	13.46	7.55	1.43	0.07	0.11	9.16	23.57
Bw1	21 - 32	1.8	4.7	1758	16.57	6.85	2.49	0.22	0.26	9.82	0.67
SN2, deposition of 2 cm under vegetable land use											
SN2-Ap	0 -23	15.2	4.7	1835	21.34	8.91	0.81	0.15	0.14	10.01	1.80
SN2-Bw1	23 - 50	1.7	5.4	1796	20.32	13.79	1.23	0.03	0.14	15.19	0.00
SN6, Initial soil underlying ashfall of 5 cm deposition under citrus and avocado plantation land uses											
A1p	5 - 22	3.3	4.2	2747	13.69	3.56	0.73	0.19	0.25	4.73	13.06
A2	22 - 48	2.0	4.8	2248	12.11	5.78	2.37	0.31	0.34	8.80	1.11
Bw1	48 - 84	0.8	5.6	444	11.17	4.84	1.32	0.02	0.19	6.37	0.19
Bw2	84 - 120	0.6	6.0	186	11.44	3.39	0.49	0.04	0.10	4.02	0.06
SN7, Soil without ashfall deposition as a baseline											
Ap	0 - 21	3.3	5.2	51	17.27	4.60	1.78	1.23	0.20	7.81	0.56
Bw1	21 - 54	1.8	5.4	113	15.13	0.97	0.71	1.85	0.08	3.61	0.51
Bw2	54 - 93	1.0	5.8	70	18.78	2.84	0.77	0.45	0.05	4.11	0.04
BC	93 - 110	1.1	5.9	50	16.59	4.43	1.83	0.08	0.22	6.56	0.00
Encrustation on soil surfaces resulted from mud ashfall deposition											
SNC	1-2	47.4	2.9	8261	6.16	42.45	1.47	0.02	0.03	43.97	49.84

Strategic management of volcanic ashfall deposition to restore soil productivity include: (i) lime application to suppress Al and S toxicities and increase soil pH and cation exchange capacity, (ii) selection of crop tolerant to low pH and high Al and soluble S in order to reduce involved cost of restoration, (iii) physically break up the encrustation (< 2 cm thick) and incorporate it into underlying soil at the time of tillage to allow water infiltration and root penetration, (iv) application of N and K fertilizers, and (v) incorporation of dry ashfall (if thickness < 5 cm) into initial underlying soils to take advantage of new nutrients being released from volcanic ashfall.

CONCLUSIONS

Ashfall has resulted soil degradation by physically forming surface encrustation limiting water infiltration and root penetration, and chemically producing high acidity (very low pH values), Al and S contents. Ashfall also released new elements that naturally enrich soil exchangeable cations (Ca, Mg, Na, K) in addition to S and P as potential new sources of plant nutrients. Ashfall deposition has significantly changed the underlying soil properties by decreasing pH, P retention, and CEC values and by increasing exchangeable Ca, Al, available P and S.

REFERENCES

Anda, M., Sarwani, M., 2012. Mineralogy, chemical composition, and dissolution of fresh ash eruption: new potential source of nutrients. Soil Sci. Soc. Am. J. 76, 733-747.

Casadevall, T.J., Rose Jr. W.I., Fuller, W.H., Hunt, W.H., Hart, M.A., Moyers, J.L., Woods, D.C., Chuan, R.L., Friend, J.P., 1984. Sulfur dioxide and particles in quiescent volcanic plumes from Poas, Arenal, and Colima volcanos, Costa Rica and Mexico. Journal of geophysical Research 89, 9633-9641.

Rose Jr. W.I., Chuan, R.L., Cadle, R.D., Woods, D.C., 1980. Small particles in volcanic eruption clouds. American Journal of Science 280, 671-696.

Phosphorus fertility of low pH soil in upland rice field in Uganda

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Abstract
In West Africa, soil fertility is generally low, especially for upland rice. JICA (Japan International Cooperation Agency) started long-term fertilizer experiment (LTFE) for NERICA (New Rice for Africa; *Oryza sativa* x *O. glaberima*) since 2004 in Uganda to elucidate the effect of the fertilizer management there. We investigated chemical properties of soils taken from the LTFE and analyzed with yield data of harvested rice, and found that soils are acidic, and nitrogen and phosphorus contents are main factors for NERICA. It suggested that fertilizer management in the field affect NERICA growth from the viewpoint of availability of N and P.

INTRODUCTION
Acid soil has low fertility and limited crop productivity even in Africa. Together with shortage of available water, upland rice could be useful in acid soil in Africa. It was about 10 years ago when NERICA (New Rice for Africa; *Oryza sativa* x *O. glaberima*) was advenced, aiming a new rice Green Revolution in sub-Saharan Africa, then JICA (Japan International Cooperation Agency) started long-term experiment (LTE) for NERICA since 2004 in National Crops Resources Research Institute (NaCRRI), Namulonge in Central Uganda (Haneishi et al., 2013) Treatments are total 8 as T1: Control (no fertilizer), T2: N as 60 kg/ha/crop (same unit for other treatments onward), T3: N/P 60/30, T4: P/K 30/30, T5: N/K 60/30, T6: N/P/K 60/30/30, T7: N/P/K/Compost 60/30/30/30/500. T8: Compost 500, with urea, DAP (Diammonium phosphate), potassium chloride and rice straw compost. The LTE was conducted in duplicate by randomized block design. It is important to examine the effect of long-term fertilizer experiment on soil physicochemical properties to understand nutrient dynamics and best way of soil managements.

MATERIALS AND METHODS
We took surface soil in the LITE at NaCRRI after 9 years with 18 crop seasons in December 14, 2013 when it was ripening stage of NERICA and analyzed its soil pH(H_2O), electric conductivity (EC), total organic C (TC) and nitrogen (TN) contents and also 4 different types of available soil phosphorus (water soluble P, Truog P, Bray 2 P and Olsen P). Average yield of NERICA was examined to correlate with soil chemical properties and treatments.

RESULTS AND DISCUSSION
Soil physico-chemical properties
Soil physical condition was rather hard, as indicted by more solid phase (47-56%) than liquid (22-30%) and gas (18-29%) phases. Soil pH was in the range of 5.1-6.3, and average as 5.7. Lowest was in P/K and N/P/K, while highest in control, compost and N/K, indicating fertilization effect to reduce pH and compost medicated acid condition. Soil EC was in the range of 2.8-8.7 mS/m, and average as 5.6 mS/m. Lowest was in control and N/K, while highest in N/P, N/P/K. Nitrate contents are similar as soil EC. Soil C contents were about 1.3-1.5 % within the narrow range and similar in soil total nitrogen (TN) as 0.12-0.16 %, indicating fairly well stabilized soil condition between organic matter input such as stubble and root and organic matter decomposition. Available soil phosphorus was observed as Bray 2 P => Truog P > Olsen P > water soluble P in this order. Especially, P applied treatments as N/P/K/compost, N/P and N/P/K had a tendency that P content was high, while in control, N, and P/K, they were low.

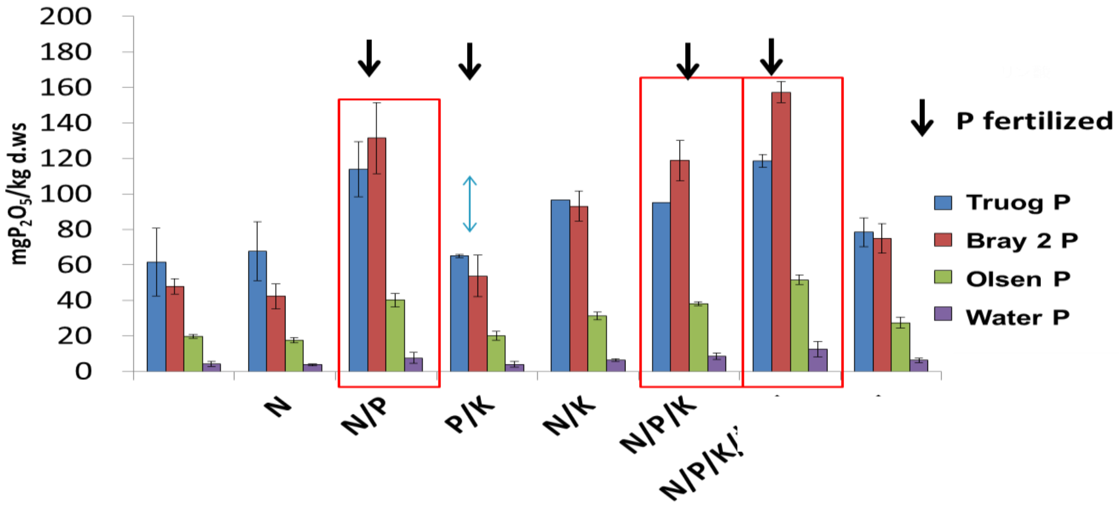

Fig. 1 Effect of long-term fertilizer application on available P in LTE, NaCRRI, Uganda

Correlation of soil properties and rice yield

Rice yield was varied widely due to rain, but average yield showed high correlation with soil EC, nitrate-N and available P contents. Among various P forms, Bray 2 showed highest correlation coefficient (Fig. 2), indicating Fe-bound P would be important under this experimental conditions.

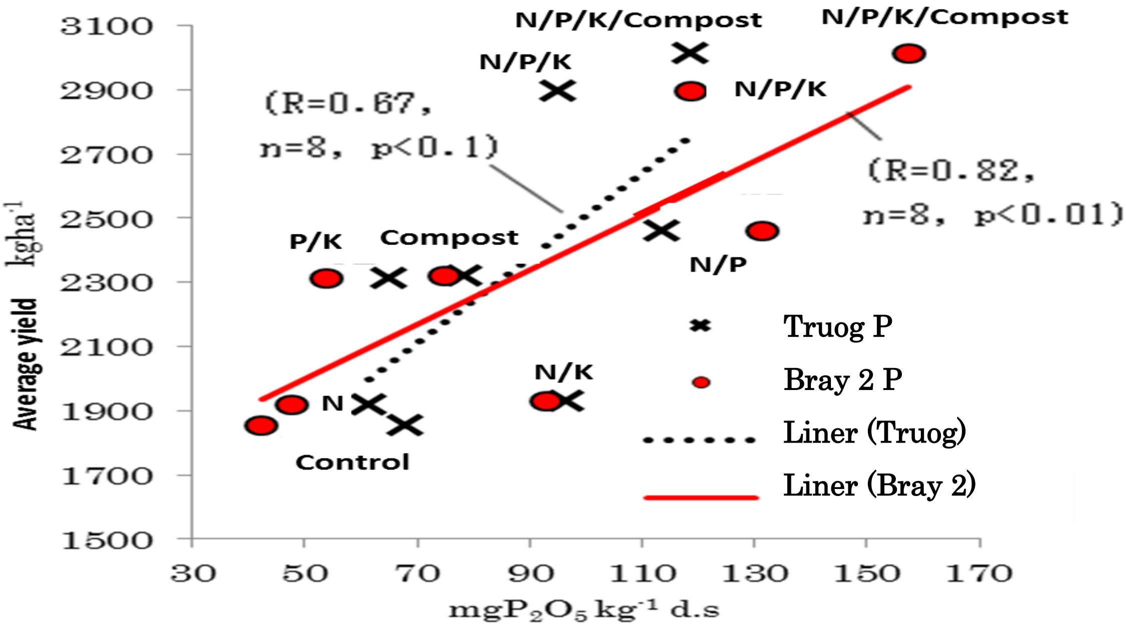

Fig. 2 Correlation between available P and average rice yield in LTE, NaCCRI, Uganda

CONCLUSION

Soil in NERICA long-term experiment in NaCRRI, Uganda was acidic and low available P. Among various P forms, Bray 2 showed highest correlation coefficient with average rice yield.

REFERENCES

Haneishi, Y., Maruyama, A., Miyamoto, K., Matsumoto, S., Okello, S., Asea, G., Tsuboi, T., Takagaki, M. and Kikuchi, M. 2013. Introduction of NERICA into an upland farming system and its impacts on farmer's income: A case study of Namulonge in Central Uganda. Tropical Agriculture and Development. 57(2). 61-73.

The effects of liming of a dystric cambisols in a period of a 10 years

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Abstract
A field trial with liming and fertilization of Dystric Cambisols at Lika area in mountain part of Croatia was set up in 1998. The trial involved 12 different combinations of liming, mineral and organic fertilization in 4 replications. The crops included in the crop rotation are barley, potato, maize, rye and wheat. Two rates of limestone were applied (10 and 20 t ha^{-1}). Marked acidity of the soil in this region is one of the main causes of poor soil fertility and relatively low yields of grown crops. Mineral and organic fertilizers very often are not applied in the correct manner, while liming, as the key soil-improvement measure, is generally not practiced. The differences and changes in soil pH and in other soil chemical properties have been monitored in a period of 10 years after liming.

INTRODUCTION
Excessive soil acidity is a limiting factor that strongly determines the effectiveness of all plant growing practices at numerous agricultural farms in Croatia. Today, liming in Croatia is still not represented at the rate that would ensure "sustainability" in soil management. In another words, the loss of calcium and magnesium as well as their crop absorption is basically greater than the quantity added to the soil. In the long-term, this approach is not good and it should be changed. For a successful management of the farms that are operating on different agricultural soils liming is, together with tillage and fertilization, a key factor. In a last few years application of lime in Croatia is intensified, but there is still a need for the correction of excessive soil acidity at numerous agricultural farms. The fact that soil analyses are not carried out always before the application of lime we see as a problem, and such practice can cause negative consequences, because doses of liming materials in such cases are not determined in a proper way. This paper presents the information about the changes in soil acidity obtained in a Mountain Region of Croatia in a field trial, after liming. Our intention was to get the information about the change of basic chemical properties of Distryc Cambisols after application of lime, in a field conditions.

MATERIALS AND METHODS
This study was carried out on Dystric Cambisols near Gospic, at Lika area, in mountain part of Croatia (N: 44°36' E: 15° 20'). The trial was established in 1998 with various combinations of conventional NPK fertilizers, two different limestone (CaCO3) rates and one rate of solid farmyard manure (FYM) (Table 1). The experimental design was a randomized block with twelve treatments and four replications. The treatments are listed in Table1.

Table 1. Treatments and applied amounts of nutrients, limestone and solid farmyard manure

Treatment	Applied materials
Control	-
$CaCO_3$ I	(10 t ha^{-1})*
$CaCO_3$ II	(20 t ha^{-1})*
N1P1K1	70 kg N ha^{-1} + 90 kg P ha^{-1} + 70 kg K ha^{-1}
N2P2K2	105 kg N ha^{-1} + 135 kg P ha^{-1} + 105 kg K ha^{-1}
FYM	(30 t ha^{-1})**
N1P1K1 + FYM	70-90-70 kg ha^{-1} + (30 t ha^{-1})**
N2P2K2 + FYM	105-135-105 kg ha^{-1} + (30 t ha^{-1})**
N1P1K1 + $CaCO_3$ – I	70-90-70 kg ha^{-1} + (10 t ha^{-1})*
N2P2K2 + $CaCO_3$ – II	105-135-105 kg ha^{-1} + (20 t ha^{-1})*
N1P1K1 + FYM+ $CaCO_3$ – I	70-90-70 kg ha^{-1} + (30 t ha^{-1})** + (10 t ha^{-1})*
N2P2K2 + FYM + $CaCO_3$ – II	105-135-105 kg ha^{-1} + (30 t ha^{-1})** + (20 t ha^{-1})*

- = two rates of limestone were applied only in 1998;
- ** = FYM was applied in 1998 and 2006, and mineral fertilizers each year

Soil sampling was conducted before the establishment of the experiment, and each year after tha harvest of the grown crop. Composite samples per parcel were taken from one depth (0-30 cm). Soil samples for physical and chemical analysis were air dried, milled, sieved and homogenized. Texture was determined by sieving and sedimentation method according to ISO 1127 (modificated). The soil pH was determined in 1:2.5 (w/v) soil suspension in 1 M KCl. Plant available phosphorus and potassium were extracted by ammonium lactate (AL) solution (Egner et al., 1960).

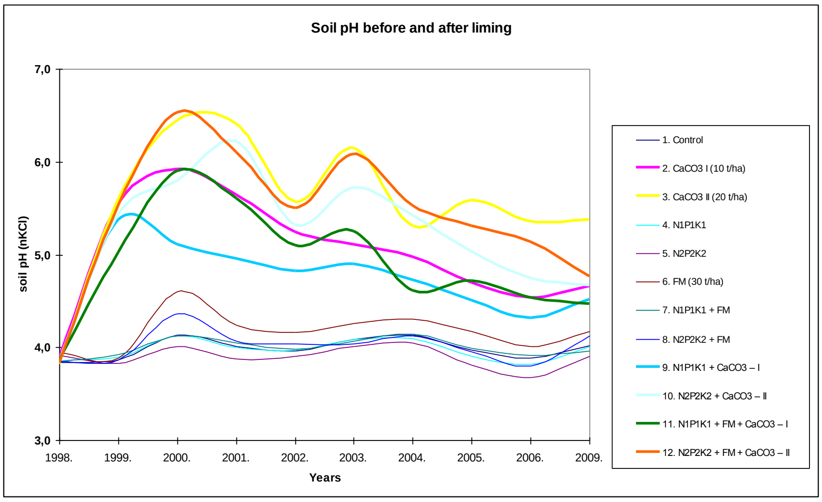
RESULTS AND DISCUSSION

Soil texture is clay loam (Table 2). The results of the changes in soil pH are presented in a graph 1.

Table 2. Texture of Dystric Cambisols from experimental field

Depth (cm)	Texture class	Particle size distribution, %			
		Coarse sand	Fine sand	Silt	Clay
0-30	Clay loam	4.4	39.8	20.6	35.2

Figure 1. The pH of a Dystric Cambisols before and after liming



Compared to the control treatment there was a significant increase of soil reaction in a treatments with lower, and especially with higher rate of limestone. Positive and significant influence on soil reaction was also recorded in treatments with combination of limestone, mineral and organic fertilizers. Soil reaction slightly decreased over the years in treatments with combination of limestone, mineral and organic fertilizers. The negative trend of soil pH at the treatments with applied limestone started already second year after application.

CONCLUSION

Application of 10 and 20 t ha^{-1} of limestone for the correction of the excessive acidity of Dystric Cambisols has been efficient in terms of soil pH changes. Application of a lower rate (10 t ha^{-1}), and a higher rate (20 t ha^{-1}) of limestone significantly increased soil reaction – a lower dose for near 2 pH units and a higher dose for approximately 2.5 pH units. Second year after application of the limestone, pH values of soil treated with the limestone started to decrease, but in a period of research they didn’t reach the original soil pH.

REFERENCES

ISO 11277: 2004. Soil quality - Determination of particle size distribution in mineral soil material - Method by sieving and sedimentation

Kisić I., Bašić F., Mesić M., Butorac A., Vađić Ž., 2004. The Effect of Fertilization and Liming on Some Soil Chemical Properties of Eutric Gleysol. Agriculturea Conspectus Scientificus, 69: 2-3. 43-49

Lončarić Z., Popović B., Karalić K., Rastija D., Engler M., 2007. Phosphorus fertilization and liming impact on soil properties. Cereal Research Communications, 35: 2-1. 733-736.

Mesić M., 2001. Correction of excessive soil acidity with different liming materials. Agriculturea Conspectus Scientificus, 66: 2. 75-93

Mesić M., Kisić I., Bašić F., Butorac A., Zgorelec Ž., Gašpar I., 2007. Losses of Ca, Mg and SO42-S with drainage water at fertilisation with different nitrogen rates. Agriculturea Conspectus Scientificus, 72: 1. 53-58

Rastija D., Loncaric Z., Karalic K., Bensa A., 2008. Liming and Fertilization Impact on Nutrient Status in Acid Soil. Cereal Research Communications, 36: 1-Suppl 5. 339-342.

Tree species for biological recultivation by afforestation of open coal pit mines

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Abstract
Mining waste disposal sites of open pit mines are extremely unfavorable substrates for biological recultivation by afforestation. All the characteristics, starting from the physical properties that range from inert sterile sands to heavy clays of Pliocene, followed by chemical characteristics and microbiological inactivity, are problems that must be taken into consideration when choosing species for the afforestation and landscaping. The paper presents the results of pedological analysis in Kolubara coal basin and the results of afforestation with some dendroflora species.

INTRODUCTION
Kolubara basin is one of the largest lignite basins in the Republic of Serbia, formed at the beginning of upper Miocene, on the southern border of the Pannonian depression. Morphologically is characterized by mild relief, with small absolute and relative altitudes. These areas are represented by sedimentary formations of Devonian and Carbon, Middle and Upper Permian, Lower and Middle Triassic, Jurassic, Lower and Upper Cretaceous, Neogene and Quaternary. Knowledge of geologic column, especially layers above the coal is of great importance, because in the process of coal exploitation and disposal of tailings all mentioned materials appear as substrates for reclamation. This, of course, relates to a process of non selective disposal of overburden.
The largest deposits of coal were formed in the periods of intense orogenesis and the existence of widespread wetland forests where a large amount of biomass due to anaerobic conditions of marshy bottom remains unaltered and represent a basic raw material for creation of coal. Paleopalinological analysis of pollen and spores samples indicates that the area of today's Kolubara lignite basin was located within the swampy region.
During the process of surface mining, the original image of the landscapes has drastically changed. As a part of these processes, the natural soils completely disappear. In Serbia, mostly, it is still in the use unselective waste disposal with or even without technical reclamation, creating in that way very difficult conditions for the successful reclamation process. Overburden is removed and then disposed next to the pit. The most productive layers of the original natural soils are buried dipper, and over them unproductive layers of overburden.

MATERIAL AND METHODS
The samples of soil have been analyzed in accredited laboratory of the Institute for forestry Belgrade (SRPS ISO/IEC 17025: 2006, LOB 510.01). The standard analyzes and the preparation of samples was applied, by the following methods: Active and substitutional acidity - Electrometrically LUP504.07; Total humus - Method by Tjurin LUP504.04; Total nitrogen (N) - Method by Kjeldlahlu LUP 504.33; Easily accessible phosphorus and potassium - AL method LUP504.03; Content of free carbonate - Volumetric method LUP504.05; Textural composition of soil - Sedimentation method LUP504.02; Preparation of samples for the analysis of heavy metals - Destruction in nitric acid and H_2O_2. On the experimental fields by standard methods, breast height diameter and total height of trees planted in the process of biological recultivation by afforestation is determined.

RESULTS AND DISCUSSION
Mine soils of the studied area show a wide variability of properties as a result of the diverse starting characteristics of the deposited material. It is characteristic that they have very deep solum. As a consequence of the method of disposal, they present a mosaic of various lithological layers, different in texture and mineralogical composition. Composition, physical and chemical properties of the newly formed substrate depends on mineralogical composition of the overburden and the geological profile above coal layer.

Physical properties
The main characteristic is determined by the textural classes from “sandy loam” to “clay”. The adsorption complex belongs to the “distric soils”. The reaction of the soil solution is from “very strong acidified” to “strongly acidic”. According to the content of the total humus and organic matter the soils belong to the “very poor” to “poor” humus content. In recent deposits, in some cases, humus content is below the limit of detection. Content of total nitrogen is generally low, depending on the humus content, and in some cases it is below the limit of detection. Easily accessible forms of phosphorus for the plants are low and often below detection limit. Supply with potassium is “poor” to “very good” (Drazic et al., 2005).

Chemical properties

The chemical properties of the soils are characterized by a pH in a range from 4.1 to 4.7 of the soil solution in the water, i.e. from 3.2-4.7 in KCl. Among many soil properties that affect on tree growth (topografic position, texture, drainage, etc.), soil pH is one of the most important. Soil pH approximately indicate the chemical status of the soil and also the potential possibilities of plant growth. Though most tree species can, more or less successfully, grow in a wide range of pH values, many of them prefer certain soil pH ranges where these species have the best growth potential, vitality and decorativeness. But, the tree species will often grow on soils outside the certain ranges. The adsorption capacity is low, as well as the total content of humus is extremely low, especially in the surface layer with the light texture. As a result of higher content of clay in texture composition, the adsorption capacity is significantly higher in the deeper layer of the analyzed soil. The soil has a very low content of humus, with a low content of total nitrogen, and especially in the deeper layers where the amount of this element is below detectable limits. The surface layer of soil is poorly provided with potassium easily accessible to the plants, while in deeper layers easily available forms of potassium are on the border between medium and low. Amounts of the phosphorus available to the plants are below detectable limits throughout the depth of the analyzed soil.

Table 1. Chemical properties

Values	Adsorptive complex				Y_1 cm^3	pH		Humus	N	Available	
	T	S	T-S	V		H_2O	KCl	%		P_2O_5	K_2O
	equiv. centimol/1kg			%						mg/100 gr	
Min	14.02	6,90	7.12	23,14	11.392	4.1	3.2	< LD	< LD	< LD	4,8
Max	44,70	22,04	22.66	49,08	36.256	5,5	4,7	1,0	0,1	2,6	25,4

Microbiological characteristics

The number of different groups of microorganisms depends on the chemical nature of soil organic matter. If the organic matter in the soil is characterized by a wide C/N ratio, i.e. with wide lignin/protein ratio, the dominant physiological group will represent oligonitrophyls (free fixing bacteria). They are able to compensate the deficiency of nitrogen in the organic matter which they decompose, by using atmospheric nitrogen. If the organic matter in the soil is rich in protein then the dominant physiological group of microorganism will be ammonifiers, because the proteins are their primary energy supply. In acid soils fungal organisms are always predominate over actinomycetes, which are often entirely absent in acid tailings soil. Mineralogy microorganisms use the same plant assimilative as well as Cormophytae. They indicate trophicity of the micro-environment and high amounts of plant assimilative. In most recent deposits, the number of saprophytes is negligible, because organic matter - which is their energetic material - is completely lacking. By increasing the organic residue in the soil, gradually the number of saprophytic microorganisms also increases.

Development characteristics of tree species in forest plantations

At the age of 10 years, on the deposol of lighter mechanical composition, *Alnus glutinosa* L.Gaertn. attains the greatest values of diameter (12.3 cm), *Larix europaea* L. (11.7 cm), *Pinus strobus* L. and *Pseudotsuga menziesii* Mirbel.Franco (8.6), *Pinus sylvestris* L. (8.3 cm) and *Betula verrucosa* Ehrh. (8.1 cm). *Pinus nigra* Arn. has the smallest diameter (7.3 cm). On the more heavily-textured deposol, the state is somewhat different. *A. glutinosa* also attains the largest diameter (10.0 cm), then *P. strobus* (9.2 cm), *P. menziesii* (9.1 cm). *L. europaea*. is in the fourth position (8.6 cm), and finally *P. sylvestris* L. and *P. nigra* with equal diameters (6.7 cm).

As for tree height, on the lighter-textured deposol, the greatest values are attained by larch (10.80 m), then *A.glutinosa* (10.00 m) and *B. verrucosa* (9.40 m). *P. sylvestris* (7.10 m), *P. strobus* (6.85 m), P. menziesii (6.50 m) and *P. nigra*. (4.60 m) have considerably lower heights. On the more heavily-textured deposol, *L. europaea* and *A. glutinosa* have identical heights (10.00 m), then *P. strobus* (8.00 m), *P. menziesii* (6.55 m), *P. nigra* (4.85 m) and *P. sylvestris* L. (4.50 m). As it can be seen, *A. glutinosa* on both deposol types attains the same height. On the more heavily textured deposol, *P. strobus* attains a notably superior height. *P. nigra* and *P. menziesii* attain a slightly greater height on this soil type (Drazic, 2000).

All the implemented tree species in afforestation have very good dynamics of diameter, height and volume development, but there are differences among the species on the same deposols, and the differences in the development of each species on different deposols. Monitoring of their development makes it possible for each type of deposol to make the optimal selection of species for afforestation, to achieve the highest productivity effects, vitality, decorativeness and other functional values (Drazic, 2006).

Acknowledgements

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REFERENCES

Drazic, D. 2006: Open-cast coal mining: from destruction to art. Book of abstracts from V Europaean Ecological Conference on Ecological Restoration, 78. Greifswald, Germany.

Dražić, D. 2000. Polyfunctional evaluation of the landscape and ecosystems formed by the recultivation of minespoil banks of opencast mines in the Kolubara basin. Monograph. ISBN 86-7650-000-2. (In Serbian), Belgrade. Publisher: Savezni sekretarijat za rad, zdravstvo i socijalno staranje - Sektor za životnu sredinu, Beograd. 1-261.

Dražić, D., Miletić, Z., Veselinović, M., Nešić, N. 2005: Plantations of some pine species on deposols of the Kolubara lignite basin. Zemljište i biljka, Beograd. Vol. 54, No.2: 151-166.

Assessment of sediment yield in the Tronosa river basin of Montenegro

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Abstract
Soil erosion is one of the physical indicators of land degradation occurrence and is a growing problem to environment. We studied erosion processes in the Tronosa watershed. The most common soil types in the watershed are: Dystric Cambisols, Kalkomelanosol, Eutric Cambisols, Fluvisols and Colluvial Fluvisols. According to our analysis, Q_{max} was 216 m^3s^{-1} and there is a possibility for large flood waves to appear in the basin. Net soil losses were calculated on 8465 m^3year^{-1}, specific 286 m^3km^{-2} $year^{-1}$. This study shows that IntErO model is a useful tool for calculation of sediment yield in this Region.

INTRODUCTION

Soil erosion caused by water is a natural process that occurs when the force of raindrops or running water on the soil surface exceeds the cohesive forces that bind the soil together. In general, vegetation cover protects the soil from the effects of these erosive forces. However, land management activities such as ploughing, burning or heavy grazing may disturb this protective layer, exposing the underlying soil. The erodibility of the underlying soil depends on its type, location and degree of exposure to erosive influences (Meyer & Wischmeier 1969).
Information on sediment yield and runoff is needed for erosion risk assessment and models that have proved to be good tools to understand these processes, quantifying sediment yield and runoff. IntErO model (Spalevic, 2011) was chosen for this study for the following reasons:
(1) The model has been widely used in Montenegro and applied in some river basins in the Region but also worldwide (Bosnia & Herzegovina, Brazil, Iran, Macedonia, Saudi Arabia and Serbia). The model was earlier validated for simulating the erosion processes over the Polimlje Regionin Montenegro (Spalevic, 2011).
(2) To continue the use of IntErO model that can be applied without modifications for Montenegro with the idea of using the research results for preparing later an overall picture on soil erosion process in the Region.
The new detailed report about the state of the runoff and sediment yield in this format may be used further in watershed management sector of Montenegro, illustrating the possibility of modelling the sediment yield with such approach in Croatia.

MATERIAL AND METHODS

The study is conducted in the Tronosa Watershed of Montenegro, a right-hand tributary of the river Lim located in the upper part of the Polimlje region of Montenegro, between the towns Berane and Bijelo Polje. We processed the data related to the characteristics of the studied area: physical-geographical parameters, geological features, soils and climate characteristics, including the research on state of vegetation, needed to calculate sediment yield and runoff. For defining the bedrock permeability we used the data base of the Institute of Geology of Montenegro including the Geological map of Montenegro (Zivaljevic, 1989); Data on soil characteristics: Soils of Montenegro (Fustic and Djuretic, 2000). Data on the volume of the torrent rain, hb; Average annual air temperature, t0; Average annual precipitation, Hyear; we received from the Institute of Hydro-meteorology and Seismology of Montenegro: Meteorological stations Berane & Bijelo Polje.
We drew on the earlier filed work of the Institute of Forestry of Montenegro in Podgorica who analysed the status of the plant cover, the type of land use of all the Montenegrin forests including those in the study area. All those data were further processed using the IntErO model (Spalevic, 2011) based on the Erosion potential method – EPM (Gavrilovic, 1972) and used to characterise the watershed. The analytical equation is as follows:

$$W_{year} = T \cdot H_{year} \cdot \pi \cdot \sqrt{Z^3} \cdot F$$

where W_{year} is the total annual erosion in m^3year^{-1}; T is the temperature coefficient; H_{year} is the average yearly precipitation in mm; Z is the erosion coefficient.

The erosion coefficient, Z, was calculated as follows:

$$Z = Y \cdot X \cdot (\phi + \sqrt{I})$$

where, Y is Soil erodibility coefficient; X is Soil protection coefficient; ϕ is Erosion development coefficient (tables for Y, X and ϕ coefficients available at Gavrilovic, 1972). F is the watershed area in km^2.

The actual sediment yield was calculated as follows:

$$G_{year} = W_{year} \cdot R_u$$

where, G_{year} is the sediment yield in m^3year^{-1}; W_{year} is the total annual erosion in m^3year^{-1}; R_u is sediment delivery ratio. The actual sediment yield was calculated as follows:

$$R_u = \frac{\left(\sqrt{O \cdot D}\right)}{0.2 \cdot (L + 10)}$$

where, O is perimeter of the watershed in km; D is the average difference of elevation of the watershed in km; L is length of the catchment in km.

RESULTS AND DISCUSSION

The surface of Tronosa watershed is calculated on 29 km^2. H_{min} on its inflow to Lim is 622 m asl, over the Godusa to the hills, where the H_{max} is 1476 m asl. The average slope gradient in the river basin, I_{sr}, is calculated as 31%, indicating that in the Tronosa watershed very steep slopes prevail. The average river basin altitude, H_{sr}, is calculated as 953 m; the average elevation difference of the river basin, D, on 331 m. The absolute maximum air temperature is 37.8°C and minimum of -28.3ºC. The amount of torrential rain, hb, is calculated on 157 mm. The average annual air temperature, t0, is 8.9 °C. The average annual precipitation, Hyear, is 983 mm. The temperature coefficient of the region, T, we calculated on 0.99. The study area belongs to the Durmitor geotectonic unit of the inner Dinarides of Northern and North-eastern Montenegro (Frankl et al., 2015). The geological structure of that part of Montenegro consists mainly of Paleozoic clastic, carbonate and silicate volcanic rocks and sediments of the Triassic, Jurassic, Cretaceous-Paleogene and Neogene sediments (Zivaljevic, 1989). Our analysis shows that the structure of the river basin, according to bedrock permeability, is the following: f0, poor water permeability rocks, 76%; fpp, medium permeable rocks, 4%; very permeable products from rocks 20%. The coefficient of the region's permeability, S1, according to the analysis if geological substrate is calculated on 0.87.

Based on the previous results of pedological research (Fustic and Djuretic, 2000; Spalevic, 2011), and our own research, the most common soil types in the watershed are: Dystric Cambisols (82%), Kalkomelanosol (8%), Eutric Cambisols (6%), Fluvisols and Colluvial Fluvisols (4%). For the purposes of calculating the peak discharge (Q_{max}), we analysed vegetative cover. According to our analysis, the coefficient fs, (portion under forest) is 0.54; ft (grass) is 0.31 and fg (bare land) is 0.15 and the coefficient of the river basin planning, Xa, is 0.51. The coefficient of the vegetation cover is calculated as 0.72. The upper part of the river basin, above the villages Godusa and Bosniak, is covered with mixed forests of fir and spruce (*Piceetum – Abietis montanum*). Below this band there are small forest areas covered by the mixed forests of fir and beech (*Abieti fagetum montanum*). In the middle part of the basin dominated forest form are coppice beech forests (*Fagetum montanum*). Thickets of Sessile oak and Turkish oak (*Quercetum petraeae-cerridis*) are positioned in the lower part of the basin towards the village Srdjevac. Are near river banks is covered with hygrophilic forest (*Alnetea glutinosae, Salicetea herbacea*). Characteristic of those forest are high level of degradation, especially near settlements and roads. Calculated peak discharge, Q_{max}, for the Tronosa Watershed was 216 m^3s^{-1} (the incidence of 100 years); 193 m^3s^{-1} (the incidence of 50 years); 175 m^3s^{-1} (the incidence of 25 years); 125 m^3s^{-1} (the incidence of 10 years); 103 m^3s^{-1} (the incidence of 5 years). There is a possibility for large flood waves to appear in the studied basin. Real soil losses, G_{year}, were calculated on 8465 m^3year^{-1}, specific 286 m^3km^{-2} $year^{-1}$. The value of the Z coefficient, 0.476 indicate that, according to the classification system of Professor Gavrilovic, the river basin belongs in „Destruction Category III". The strength of the erosion process is medium; the type: surface erosion. The IntErO model was earlier validated for simulating the processes of soil erosion and sediment transport over the Polimlje River Basin in North of Montenegro (Spalevic, 2011) and in this study was also successfully applied, providing valuable estimates of the spatial distribution of soil erosion risk at drainage basin scale, allowing a valid risk assessment of soil erosion processes.

REFERENCES

Frankl, A., Lenaerts, T., Radusinović, S., Spalevic, V., Nyssen, J., 2015. The regional geomorphology of Montenegro mapped using Land Surface Parameters. Zeitschrift für Geomorphologie, in press.

Fustic, B. and Djuretic, G., 2000. Soils of Montenegro. Biotechnical institute, University of Montenegro.

Meyer, L.D. & Wischmeier, W.H., 1969. Mathematical simulation of the process of soil erosion by water. Transactions of the American Society of Agricultural Engineers 12, 754-758.

Spalevic, V., 2011. Impact of land use on runoff and soil erosion in Polimlje. Doctoral thesis, Faculty of Agriculture of the University of Belgrade, Serbia, p 1-260.

Zivaljevic, M., 1989. Geological map of SR Montenegro [Tumac Geoloske karte SR Crne Gore], 1:200 000; Special editions [Posebna izdanja] Geoloskog glasnika, Knjiga VIII, Titograd.

The analysis of the best practices to soil protection in the EU

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Abstract
A fundamental point considered in best practice cases is that soil formation is an extremely slow process. Thus, once a soil is sealed and its functions or most of them, in the best of circumstances, are gone, they are effectively lost for ever (Siebielec et al., 2010). In the European Union (EU) about 1,000 km² are annually subject to land take for housing, industry, roads or recreational purposes. Soil sealing means an irreversible loss of soil and its biological functions and loss of biodiversity, directly by habitats loss or indirectly due to fragmentation of the landscape (Prokop et al., 2011). It is the most intense form of land take and is essentially an irreversible process. The main aim of this work is to present a framework of analysis which might be used for policy evaluation purposes and to discuss what should be further required for a useful completion of the dataset with the aim of making it the subject of our empirical analyses. The absence of specific data, allows only indirect observations and analysis, thus identify, measure and monitor the elements that make up this issue ground is one of the objectives of this work.

INTRODUCTION

The permanent covering of soil (e.g. with roads or concrete) is the only intentional threat to soil. It affects 9% of the area of the EU and is made worse by urban and industrial sprawl and transport networks. It disrupts gas, water and energy flows and leads to irreversible loss of fertile soil. The evaluation of the soil strategy protection in the context of this work assumes the objective of analyzing the economic behavior and classify them through the selected indicators, so check in time and find the best practices the process of indicators useful to apply them in the territory, even if the common behaviors differ from those pursued in model. The evaluation on soil protection should be relative the contribution of economic science, but is often ignored. The paradox is that, while it is true that, as a social science, it might identify quantitative and qualitative variables to measure the efficiency of the systems. As remarked in some official reports and scientific works showed that 21.3% of the Italian territory is at risk of desertification; and that the percentage rises to 41.1% in the central and southern regions. Authors such as Burgio and Vieri (2010), stressed what has just been said, the soil's ability to retain Italian waters was reduced by 30%, which significantly increases the geological risk and the threat of catastrophic events.

However, it must be said that over the last years policy protect soils has become a prominent issue and an autonomous field of investigation. We have identified the loss of soil and in the policies of structural support Community two useful indicators to assess "*best practices*".

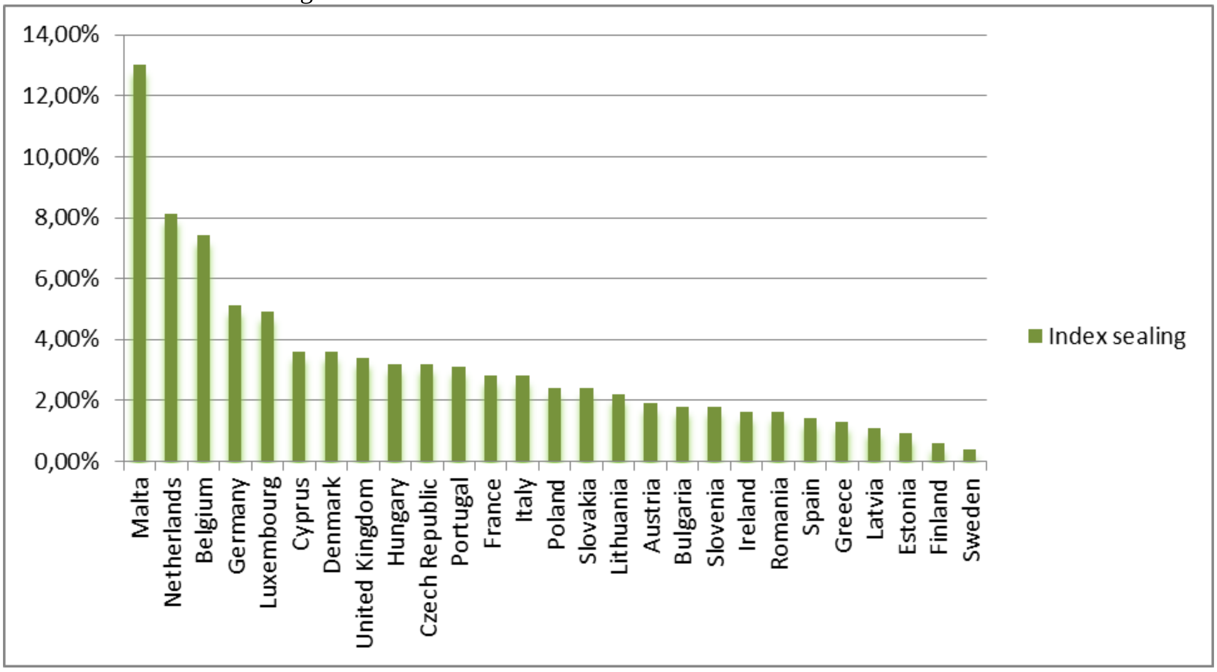
Nevertheless, unlike that of resources such air and water, for it the resource-soil there isn't a Community legislation specifically aimed about protection and a system of scientific data on the state of the soil at across Europe (Bosco et al. 2014).

New Regulations and more effective policies to disciplinary the consumption of soil would be necessary also as regards all Member States. The only instruments used by Community policies have been make efforts aimed at land consolidation policy and the set-aside of land, with results not excellent. Adopt best practices, you have to define what you're doing, to identify some possibilities, thus make certain you understand exactly what a particular best practice, will require an analysis in social aspect and some theoretical issues concerning the policy decisions and actions that guide the choose economic. The use of such indicators, properly assessed through consider the consequences, would verify the situation of the degradation processes and the impact that certain practices have achieved.

RESULTS AND DISCUSSION

Every year the catastrophic events, are the result of equilibrium that been compromised, and that, in mostly cases, are the result of human intervention. Policies of soil protection is limited largely to political nature of emergency, as a result of catastrophic events, or policies of pure containment of soil erosion phenomena, or political development plans in a specific area or sector. The use of such indicators, adequately assessed through a weighted system, would verify the situation of the degradation processes and the impact that certain practices have achieved. In any case, would be opportune a comparative analysis between the evaluation of current projects researches and the awareness political of the importance of soil and the value of his protection (Bosco C., de Rigo D., Dewitte O., Poesen J., Panagos P., 2014).How it can be observed in the table 1, the results show the index of soil sealing between the Members State, high even for State Members considered "virtuous" from the ecological point of view. The results, are related to the total area, but do not expose the relationship between arable land and inhabited areas.

Table 1: Index of soil sealing between Members State



Source: own calculation on Umweltbundesamt, 2010

CONCLUSION

The measures implemented, in many cases are a results of soil erosion and a dynamic process that are not directly related to the land consumption. It is need a specific measures in all members State to address specific issue. The role of the CAP in promoting sound soil/land management practices, has not led to significant changes in the consumption of the soil. More specifically, through the data collected and analysis SWOT, could be possible achieve the level of economic that makes evident the social and economic benefit of soil conservation. It would be interesting, make a tool available to the institutions and citizens, which is able to evaluate the trends related to consumption of soil for different territories, which realizes a "dashboard" of indicators able to verify and to orient decisions.

REFERENCES

Burgio, G., Vieri, S., 2011. Politica Agricola Comune (PAC) e la gestione dei disastri ambientali. Il ruolo dell'agricoltura. Il Progetto AGRI 2010 . Casa editrice e commissionaria La Sapienza, Roma.

Bosco, C., de Rigo, D., Dewitte, O., Poesen, J., Panagos, P., 2015. Modelling soil erosion at European scale: towards harmonization and reproducibility, www.nat-hazards-earth-syst-sci.net/15/225/2015/.

Siebielec, G., Lazar, S., Kaufmann, C., Jaensch, S., 2010. Handbook for measures enhancing soil function performance and compensating soil loss during urbanization process. Urban SMS - Soil Management Strategy project, pp 37.

Dazzi, C., 2008. La salinizzazione. Il suolo, la radice della vita. APAT, Roma.

Agarwala, A. N., Singh, S. P., 1966. L'economia dei Paesi sottosviluppati. Feltrinelli, Milano.

Aru, A., 1991."Il suolo, parte fondamentale dell'ecosistema per l'agricoltura" in Accademia Nazionale di Agricoltura, Agricoltura e ambiente. Edagricole, Bologna.

Basili, M., 2007."Lo sviluppo sostenibile: principi, strumenti e applicazioni", in Franzini M., Mercato e politiche per l'ambiente. Carocci, Roma.

Prokop G., Jobstmann H., Schönbauer A., 2011. Overview on best practices for limiting soil sealing and mitigating its effects in EU-27 (Environment Agency Austria). Technical Report - 2011-50, ISBN: 978-92-79-20669-6. http://ec.europa.eu/environment/soil/sealing.htm.

Yield and nitrogen uptake of sunflower as influenced by nitrogen sources and rates

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Abstract
A greenhouse experiment was conducted to evaluate the shoot dry weight (SDW) and achene yield, and nitrogen (N) concentration in leaves and achene as influenced by N sources. The N sources was urea and ammonium sulfate (AS), and the N rates were 0, 50, 100, and 200 mg kg^{-1}. SDW and achene yield and N concentration in leaves and achene were significantly influenced by N sources. The maximum SDW and achene yield were 200 mg N Kg^{-1} of urea, and 147 and 100 mg N kg^{-1} of AS application. Regardless of the N source, there was a significant linear effect with increasing N rates.

INTRODUCTION
Nitrogen (N) is one of the most production limiting nutrient in crop yield in all agroecological regions of the world (Fageria et al. 2011). The main reasons of N deficiency in annual crops are its low recovery efficiency. For the efficient management of N in the cropping systems, adequate rate, appropriate source and timing of application during crop growth cycle play an important role (Fageria et al. 2014). Ammonium sulfate and urea are the main sources of N for annual crop yield in developing countries (Fageria et al. 2011). The objective of this research was to evaluate ammonium sulfate and urea in sunflower achene yield and N concentration in the leaves and achene in an Organic soil of Brazil.

MATERIAL AND METHODS
The greenhouse experiment was conducted at the National Soybean Research Center of EMBRAPA to evaluate ammonium sulfate and urea as sources of N in sunflower yield and nutritional status under greenhouse conditions. The N rate applied was 0, 50, 100, and 200 mg kg^{-1} of soil. The soil used was an Organic soil having following chemical and physical characteristics before the application of N treatments: pH in $CaCl_2$ was 7.0, calcium (Ca) 9.5 cmolkg^{-1}, magnesium (Mg) 1.5 cmolkg^{-1}, aluminum (Al) 0.0 cmolkg^{-1}, phosphorus (P) 30.6 mg kg^{-1}, potassium (K) 145.0 mg kg^{-1}, and organic matter (OM) content 25.1 g kg^{-1}. Experiments were conducted in plastic pots with 3 kg of soil in each pot. At the time of sowing, each pot received 5 mg Zn kg^{-1}, and 100 mg K kg^{-1} of soil. Completely experimental design with three replicates was used. Cultivar shown was BRS 323 and there were two plants in each pot. Pots were watered every day to maintain soil moisture at about field capacity during growth cycle. Achene, aerial part, and diagnostic leaves were dried in an oven at 65±5^{o}C to a constant weight. Dried material was grounded and N concentration (grain and leaves) was determined by the micro-Kjeldahl method (Malavolta et al. 1997). Data were analyzed by analysis of variance (ANOVA), and regression analysis was performed. Appropriate regression model was selected on the basis of R^2.

RESULTS AND DISCUSSION
Nitrogen source × N rates interaction for shoot dry weight (SDW) and achene yield were significant, indicating variability between urea and ammonium sulfate (AS) for SDW and achene yield. Hence, values of these characteristics are presented at two N sources at different N rates (Figure 1). SDW yield increase significantly in a linear (urea) and quadratic (AS) fashion. Based on regression equation, maximum SDW yield estimated was obtained with 200 mg N kg^{-1} (urea) and 147 mg N kg^{-1} (AS), while the achene yield, the urea application was similar and maximum yield estimated with AS application was 100 mg N kg^{-1} (Figure 1). There was significant linear effect in the N concentration in the leaves ($p \leq 0.05$) and interaction source × rates in N concentration in achene that varied from 44.3 to 46.2 g kg^{-1} and 26.2 to 33.1 g kg^{-1} under urea, and under SA from 44.3 to 46.5 g kg^{-1} and 21.0 to 37.8 g kg^{-1}, respectively (Figure 2). On average, the values of N concentration in the leaves were similar 44.8 g kg^{-1} (urea) and 44.9 g kg^{-1} (AS), while in the achene were 23.7 g kg^{-1} (urea) and 23.1 g kg^{-1} (AS) (Figure 2). The values of N concentration in the leaves are within the considered appropriate for sunflower cultivation in tropical and subtropical conditions (Malavolta et al. 1997).

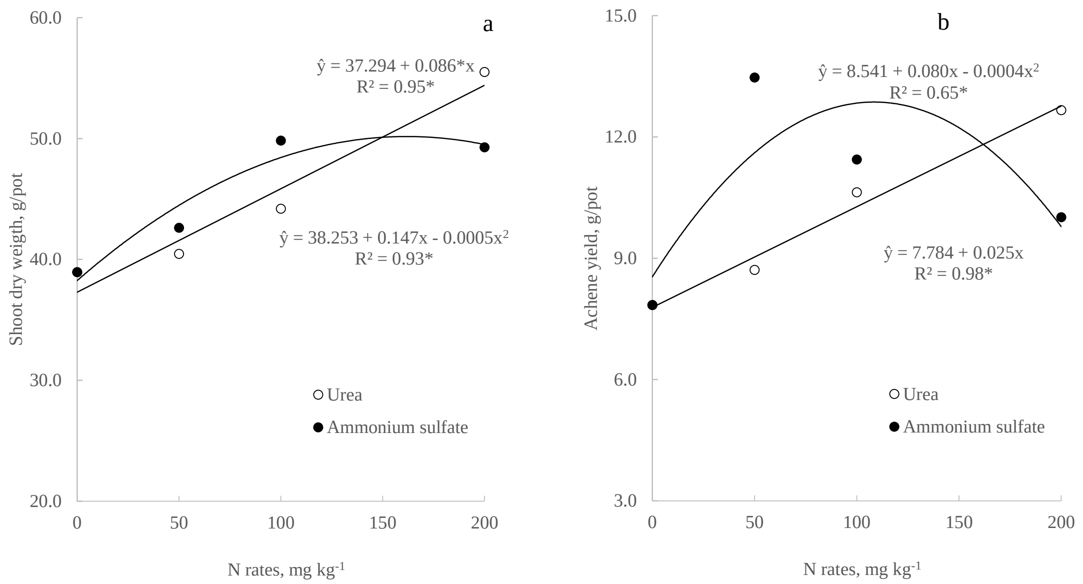

Figure 1. Relationship between N application rate by urea and ammonium sulfate and shoot dry weight (a), and achene (b) yield of sunflower. *significant at 5% probability level

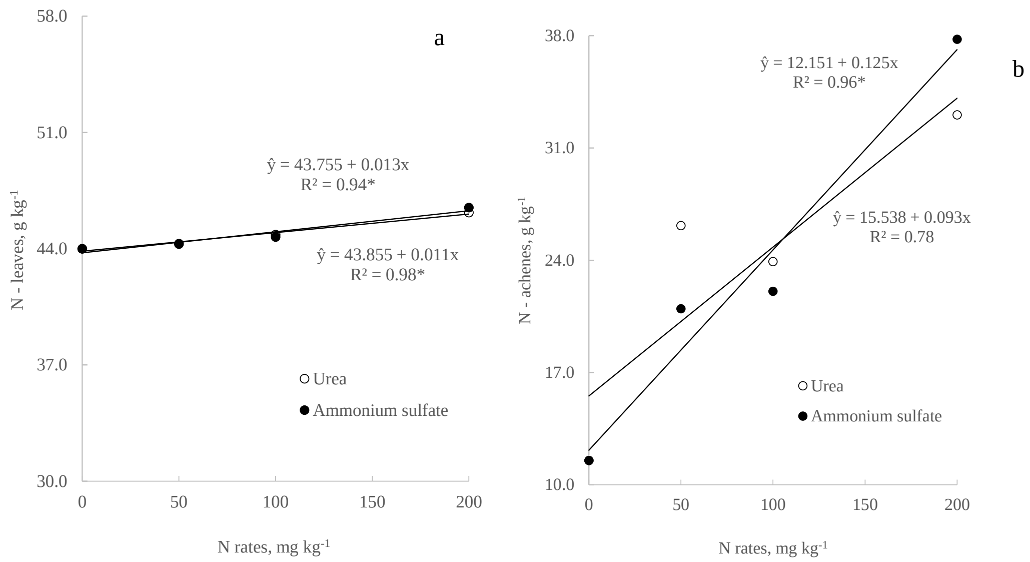

Figure 2. Relationship between N application rate by urea and ammonium sulfate and N concentration in leaves and achene of sunflower. *significant at 5% probability level.

REFERENCES

Fageria, N.K., Moreira A, Coelho A. M., 2011. Yield and yield components pf upland rice as influenced by nitrogen sources. Journal of Plant Nutrition 34: 361-370.

Fageria, N.K., Moreira A., L., Moraes A. C., Moraes, M. F., 2014. Nitrogen uptake and use efficiency in upland rice under two nitrogen sources. Communications in Soil Science and Plant Analysis 45: 461-469.

Malavolta, E., Vitti G. C, Oliveira S. A., 1997. Evaluation of nutritional status of plants; principles and applications. Piracicaba: Potafos.

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Sulfur efficiency application on soybean in two types of Oxisols

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Abstract
To assess the effect of Sulphur on soybean yield and to establish critical levels of $S\text{-}SO_4^{2-}$ available in Typic Haplorthox and Typic Eutrorthox soils, respectively. The experimental design was randomized blocks with five S rates. The maximum estimated yields on average for the two years were obtained with application of 49.9 and 63.0 kg ha^{-1} in the Typic Haplorthox and Typic Eutrorthox soils, with $S\text{-}SO_4^{2-}$ concentrations in the 0–20 cm depth of 16.9, and 19.3. In turn, at the 21–40 cm depth, the S concentrations were 49.5, and 74.2 kg ha^{-1}.

INTRODUCTION

The adoption of varieties having high productive potential, besides needing suitable management techniques, requires application of fertilizers and soil correctives to increase the yields of various crops. However, the use of concentrated fertilizers can cause symptoms of deficiency of some nutrients, such as sulfur (S). In the case of soybean, Brazil's leading agricultural export (Fageria et al. 2011), fertilization with this element is of great importance because of this crop's higher demand than grasses (Jamal et al. 2010) – 6.0 kg of S for each 1.0 t ha^{-1} of soybean produced, and the fact that 90% of tropical soils, especially those in Cerrado regions, have sub-critical concentrations. For soybean, the definitions of the sufficient levels of $S\text{-}SO_4^{2-}$ in the soil in tropical and subtropical conditions were established based on only a few experiments (TPS 2011). These studies show, as expected, that crops grown in soils with low OM and clay concentrations are more likely to respond to application of sulfate fertilizers. The objective of this study was to determine the relationship of the S rates and concentration of $S\text{-}SO_4^{2-}$ available in the soil at depths of 0–20 cm and 21–40 cm with the productivity of soybean in two types of soil (Typic Haplorthox and Typic Eutrorthox) in the subtropical region of Brazil.

MATERIALS AND METHODS

Two experiments were conducted under field conditions in two consecutive years, in the municipalities of Londrina (23º18'36" LS and 51º09'46" LW), in a Typic Eutrorthox, and Ponta Grossa (25º05'42" LS and 50º10'43" LW), in a Typic Haplorthox, located in the Paraná State, Brazil. Six months before sowing, the 0–20 cm layer of the fields was fertilized by broadcast with incorporation with dolomitic limestone. The experimental design was randomized blocks with five S rates (0, 25, 50, 75 and 100 kg ha^{-1}) and four replicates, applied in the form of elementary S with 98% purity. After physiological ripening in both years, the plants in the five central rows of each plot were harvested to determine the grain yield (kg ha^{-1}) and 100-seed weight. Then soil samples from each treatment were collected at the 0–20 cm and 21–40 cm depths to determine the available $S\text{-}SO_4^{2}$. The data on soybean yield, 100-grain weight and concentrations of $S\text{-}SO_4^{2-}$ available in the two soil types were tested for normality and then submitted to analysis of variance and F-test for statistical significance of treatments effects. The regression analysis were performed at the 5% probability level.

RESULTS AND DISCUSSION

In the first planting year, before application of the treatments at the 0–20 cm depth, the concentrations of $S\text{-}SO_4^{2-}$ available in the two soils were above the sufficiency level of 10 mg kg^{-1} recommended by TPS (2011) for soybean grown in tropical soils with clay content above 400 g kg^{-1}. Even considering the average uptake and exportation of 15 kg of S for each metric ton of grain produced, in the control plot (without S) after the first and second growing years the concentration of available $S\text{-}SO_4^{2-}$ remained above the adequate, while at the 21–40 cm depth it was at or slightly below the sufficiency concentration of 35 mg kg^{-1}. Even with application of S only before the first planting, the concentrations of $S\text{-}SO_4^{2-}$ in the 0–20 cm and 21–40 layers of both soil types cm remained above 10 mg kg^{-1} and 35 mg kg^{-1}, respectively. At the 21–40 cm depth, the $S\text{-}SO_4^{2-}$ concentrations were always higher than those in the 0–20 cm depth. The presence of high OM content in the surface layer reduces the adsorption of $S\text{-}SO_4^{2-}$ by oxides, increasing the quantity of negative charges with the elevation of the pH with the release of the adsorbed sulfates (Raij 2011). The concentrations of available $S\text{-}SO_4^{-2}$ (0-20 and 21-40 cm) were significantly influenced by the S rates, with a linear increase in the areas sampled ($\hat{y} = 11.50 + 0.114x$, $R^2 = 0.68$ and $\hat{y} = 28.76 + 0.169x$, $R^2 = 0.88$). Even with broadcast application, at the 21–40 cm depth the angular coefficient was greater than at the 0–20 cm depth, indicating the presence of vertical movement of the element in the soil profile. The soybean yields from the two soil types were significantly increased with the application of S (Figure 1). On average over the two years, the application of 49.9 kg ha^{-1} of S in the Typic Haplorthox and 63.0 kg ha^{-1} of S in the typic Eutrorthox resulted in the highest estimated yields, corresponding to 3,031.6 kg ha^{-1} and 2,925.7 kg ha^{-1}, respectively, while on average for the two soils the rate was 56.4 kg ha^{-1} of S for estimated productivity of 3,022.6 kg ha^{-1}. Considering that at the 0–20 cm and 21–40 cm depths, the critical available S-SO_4^{2-} levels are 10 mg kg^{-1} and 35 mg kg^{-1} for tropical and subtropical soils with more than 400 g kg^{-1} of clay (TPS 2011), the concentrations of 16.9 mg kg^{-1}(typic Haplorthox), 19.3 mg kg^{-1} (typic Eutrorthox) in the 0–20 cm layer in both years to obtain maximum estimated potential yield (Figure 2) are well above that indicated as adequate (TPS 2011). The same result was observed in the 21–40 cm layer, with higher estimated yields obtained with S-SO_4^{2-} concentrations of 49.5 and 74.2 mg kg^{-1}, respectively. Regardless of the soil type, cultivar and year of evaluation, the 100-grain weight was not influenced by the treatments.

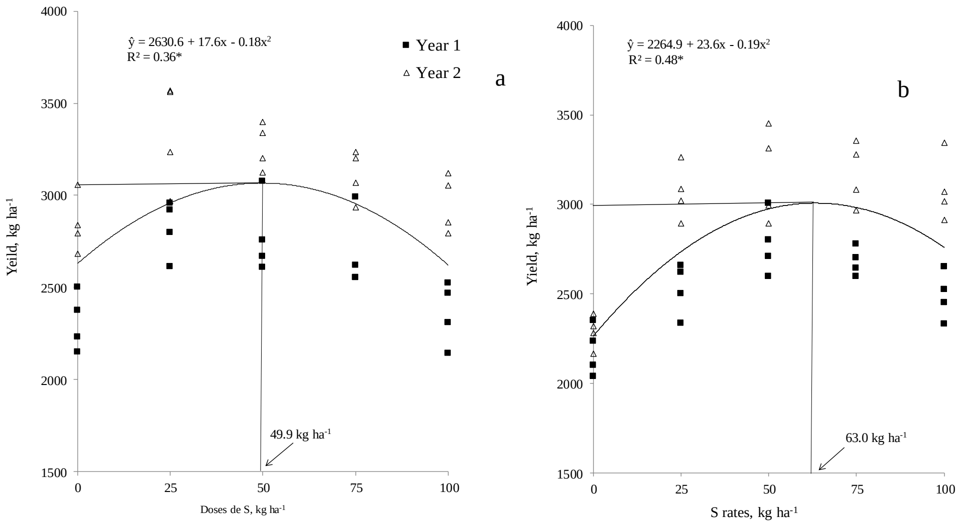

Figure 1. Soybean grain yield in a Typic Haplorthox (a) and Typic Eutrorthox (b) in two years of harvest and S rates applied (kg ha^{-1}). * Significant at 5% probability.

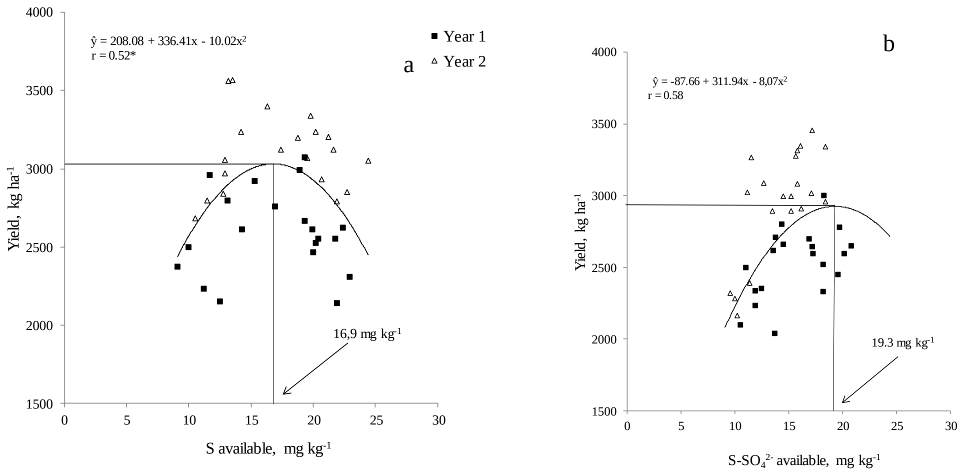

Figure 2. Soybean grain yield in a Typic Haplorthox (a) and Typic Eutrorthox (b) in two years of harvest in response of concentrations of S-SO_4^{2-} (mg kg^{-1}) applied in the soil. *Significant at 5% probability.

REFERENCES

Fageria, N. K., Moreira A., Castro, C., 2011. Response of soybean to phosphorus fertilization in Brazilian Oxisol. Communications in Soil Science and Plant Analysis, 42: 2716–2723.

Raij, B. van., 2011. Soil fertility and management of nutrients. Piracicaba, Brazil, INPI.

TPS (Tecnologia de Produção de Soja)., 2011. Technology of soybean yield in central region of Brazil. 2012 and 2013. Londrina, Brazil, Embrapa Soja.

Innovative ways for acid soils Dobrogea remediation

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Abstract

This paper presents innovative ways for the recovery of the soil fertility through using, beside of chemical fertilizers, the ecological fertilizers that are rich in nutrients and organic matter and that can improve the quality of the Dobrogea acid soils. The main methods used were: potentiometric determination of the soil pH and of the policompozit fertilizer and determination of the humus from soil, through wet combustion method - Walklay-Black. The registered results showed a slight acid soil pH and slight alkaline pH of the fertilizer used for remediation, also a highly contain of humus in soil, values for the local agricultural crops.

INTRODUCTION

Short characterization of Dobrogea soils

Soil is the latest natural formation on the surface of the lithosphere, in which are conducted without interruption physico-chemical and biological processes, being constantly under the action of living matter: microflora, flora, fauna, microfauna. It is structured into levels, presenting a succession of layers (horizons) that have formed and are permanently formed by turning rocks and organic materials, under the joint action of physical, chemical and biological factors, in contact area of the atmosphere with the lithosphere (Simpson, T.W. 1984). Corresponding to the geological conditions, terrain, hydrological and climatic, pedogenetical factors on Romanian territory determine a wide range of soil types; in Dobrogea are described the central and south areas - dominant are chernozem with steppe vegetation, hydromorphic soils (with excess moisture - especially in Danube Delta, along the rivers and near lakes), halomorphic soils (with excess salts, especially in coastal regions). The soils of Dobrogea show a variety of genetic and environmental conditions, and hence a wide range of suitability for different crops. In general, fertility and production potential of these soils, decrease from south to north, the mountainous area and delta area. A distinctive feature of the Dobrogea climate climate is common priority of the drought which reduced the background and form amounts of rainfalls across Romania. Both high temperatures and rainfall failure, and high intensity and high frequency of the wind, lead to the need for use irrigation in Dobrogea agricultural domain. There are various soils representative for Dobrogea area. Except for the north-western (with mountains) and north-eastern (where is the Danube delta), the most parts of the Dobrogea area have more and less fertile soils; few regions presents very good soils for agriculture like: mollic soils (especially typical chernozems), clay-illuvial soils, cambic soils, hydromorphic soils (gley-soils), hallomorphic soils, undeveloped soils, etc (Seceleanu, I., 2000). Physico-chemical characteristics of the soils from Dobrogea are influenced by parental material texture and depth of groundwater. Have a favorable aerohydric regim, the content in hummus varies between 2 and 3% degree of saturation in the database: 80-100% and the pH is between 6 and 8.5. Soil pH is a very important element, which is required to be known and watched as it occurs in many physical and chemical mechanisms and biological properties of soil. Because the soil contains water, it has been divided according to the three main categories pH: acidic, neutral and alkaline. Due to the specific conditions for cultivation of sunflower and corn crops and taking into consideration the fact that the Dobrogea soils have a slight acid pH, necessitate quality remediation. In this scope, according with the presented paper, it was made an experiment regarding the application of a policompozit fertilizer, made by organic wastes (algae biomass, sewage sludge and manure), which contributed to the pedological modification, creating proper conditions for the mentioned crops (Borcean A., 2004).

MATERIALS AND METHODS

The experiment took place between 2014-2015, on a surface of 5000m^2, in the south are of Dobrogea, Agigea City, Constanta County, Romania. The soil that was used at the experiment was collected from 3 different lots. Daily there were determined the following parameters: temperature, pH, the content of humus and the determination of soil soluble salts from aqueous extract.

Determination of pH through potentiometric method; apparatus pH- meter multichannel WTW

Determination of soil soluble salts from aqueous extract

Aqueous extract 1:5 obtained by filtering a mixture of soil/water in which the ratio between the mass of soil

sample in g and the volume in square cm is 1:5.
Stir the mixture soil/water for 15 minutes either by hand or using a rotary mechanical shaker.
Soil-water mixture is filtered through a glass funnel in which is inserted a filter consisting in a small porosity filter paper which is placed under a filter paper of good quality. The filtrate is caught in a glass vessel with a capacity of 200-250 cm^3; the first portion (about 5 cm^3) of the filtrate is discarded. During filtration filter funnel will be covered with a watch glass. After filtering flask with aqueous extract obtained is agitated for mixing and closed with rubber stopper (Ştirban M., 1995). Measure the electrical conductivity (specific electrical conductivity) of the aqueous extract at room temperature; determined by calculating the value of electrical conductivity at 25^0C temperature with the correction (k) for the geometry of the cell, then calculate the total content of soluble mineral salts, by multiplying the amount calculated for electrical conductivity by a factor F (determined experimentally).

Titrimetric determination of humus by Walklay and Black method
Methods that are used to determine the humus can be grouped into direct and indirect methods. The simplest direct method of determination is to determine loss on slow calcination in presence of air, a method that does not give satisfactory results for all soils.
Indirect methods are based on the determination of carbon and nitrogen content, which by multiplication with certain transformation coefficients give the humus content in soil.
The most common method of determining soil humus is the dosage of organic carbon, which is based on the oxidation of organic matter in soil. Humus determination by Walklay and Black method consists in soil organic matter oxidation with potassium dichromate in sulfuric acid and titration of the excess of potassium dichromate with a solution of Mohr salt.

RESULTS AND DISCUSSION
The registered results highlighted the following values presented in table 1.

Table 1. Agrochemical analysis

no.	Soil type	Culture	Humidity at harvest (%)	pH	Salts mg/100 g soil	Humus (%)
1	Unfertilized soil with low pH	Corn	13,67	6.80	48.69	1.34
		Sunflower	15,70	6.72	47.54	1.39
2	Remediated soil with policompozit fertilizer	Corn	15,13	7.80	63.20	1.15
		Sunflower	15,14	7.83	62.32	1.44

It can be notice high values of the soil pH (from 6,72 to 7,83) by applying the remediation process with policompozit fertilizers to both crops of sunflower and corn and also it can be notice a high content of soil soluble salts (from 47,54 to 63,20 mg/100 g soil).

CONCLUSION
For remediation of Dobrogea slight acid soils we recommend the controlled application of the organic policompozit fertilizer on sunflower and corn crops.
Through the modification brought to the soil it could be noticed improvement of the quality and height of the plants.

REFERENCES
Borcean I., Borcean A., 2004. Cultura şi protecţia integrată a cerealelor, leguminoaselor şi plantelor tehnice, Ed. de Vest, Timişoara.
Kelly, W.D., Martenrns, D.C., Reneau, Jr. R.B and Simpson, T.W. 1984. Agricultural Use Of Sewage Sludge: A Literature Review. Virginia Water Resources Reesearch Center. Virginia Polytecnic Institute and State University.Bulletin 143. 46pp
Muntean L.S., Ştirban M., 1995. Ecologie, agroecosisteme şi protecţia mediului. Ed. Dacia, Cluj-Napoca.
Păltineanu, Cr., Mihăilescu, I.Fl., Seceleanu, I., 2000, Dobrogea – Condiţiile pedoclimatice, Consumul şi necesarul apei de irigaţie ale principalelor culturi agricole; Ed. Ex Ponto, Constanţa, 258 p.
Soils are named based on pedogenetical horizons redefined and based on identifiable features, measurable in the field (5 new types of soil) 1973.

Influence of low soil pH on growth and development of *Gladiolus hybridum* L. in greenhouse

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Abstract

Aim of this study was to evaluate influence of corms size (4, 5, 6 cm) of two gladiolus varieties (Oscar and Amsterdam) at different soil pH values (5, 07 and 6, 5) on growth and development of gladiolus in greenhouse. Results showed significantly better development of gladiolus plants grown from largest corms of both investigated varieties. Generally, better development of gladiolus plants was recorded on soil with higher pH value. As regard to varieties, Oscar showed slightly better development on acid soil. It can be concluded that greater corm size and slightly acid soil are more suitable for gladiolus production.

INTRODUCTION

Gladiolus are very decorative flower species that belongs to family *Iridaceae*. Upright stems, thin leaves in the shape of a sword and a long beautiful flowers make gladiolus a perfect flower for every garden and an essential element of big floral arrangements (Parađiković, 2014). It is considered that all today's varieties of gladiolus originate from six species of gladiolus native to South Africa (Lewis et al., 1972; Pfleger and Gould, 2002). Breeding of gladiolus species provided width range of colors and early, mid- and late-blooming varieties that made gladiolus available throughout season for cut flower production. Possibility of growing gladiolus as a cut flower spread their production from traditional growers such as the Netherlands, Germany and France to countries with low cost production China, Kenya and Ethopia as well as to today major producing countries United States (Florida and California), Australia, Japan, Italy, Poland, Iran, India, Brazil, Malaysia and Singapore (Memon et al., 2009.). Production of quality gladiolus flowers is among other affected by corm size (Uddin et al., 2002; Sharma and Gupta, 2003.), planting depth (Peanav et al., 2005; Ahmed et al., 2010.), cultivar (Saleem et al., 2013; Sloan and Harkness, 2005) and fertilizer management (Pant, 2005; Butt, 2005). Growing them under controlled conditions in greenhouse the soil properties will have a key role in plant development and flower quality. The mobility of nutrients is determined by soil pH why is important to maintain a stable pH over the life of the crop (Pennisi and Thomas, 2015.). Thus, the objective of this study was to evaluate growth and development of two gladiolus varieties (Oscar and Amsterdam) under influence of corm size (4, 5, 6 cm) and soil type (A and B) in greenhouse.

MATERIAL AND METHODS

Experiment was conducted in greenhouse of DP Orhideja Magadenovac. This research was conducted on two types of soil (A and B) which samples were taken and analyzed for common soil chemical properties in laboratory. The soil pH (ISO 10390, 1994), the content of organic matter in the soil by dichromate method (ISO 14235, 1994), the concentration of AL- available phosphorus and potassium (Egner et al., 1960). Two varieties of gladiolus Oscar and Amsterdam were used. Gladiolus corms from both varieties were measured before planting and sort in 3 groups: 4 (4, 0 - 4, 9 cm), 5 (5, 0 - 5, 9 cm) and 6 (6, 0 - 6, 9 cm). Each group from each variety were planted on each soil type in four repetitions. In order to determine the effect of soil type and size of corms, at the emergence of spikes, plant height of all plants were measured.

RESULTS AND DISCUSSION

Soil type

Results of chemical properties for soil type A were as follows: actual acidity pH_{H2O} 5,07, OM content 23,1 gkg^{-1}, AL-P_2O_5 concentration 20,10 $mg100g^{-1}$ soil, AL-K_2O concentration 26,18 $mg100g^{-1}$ soil, content of Ca 2400 $mgkg^{-1}$, Mg 196 $mgkg^{-1}$, Fe 415 $mgkg^{-1}$, Mn 88 $mgkg^{-1}$, Zn 1,80 $mgkg^{-1}$, Na 60 $mgkg^{-1}$. Based on the result of soil analysis fertilization recommendation was: N 87 kg/ha, P_2O_5 70 kg/ha, K_2O 130 kg/ha, $CaCO_3$ 10 t/ha.

Results of chemical properties for soil type B were as follows: actual acidity pH_{H2O} 6, 52, organic matter content 38,6 gkg^{-1}, AL-P_2O_5 concentration 39,90 $mg100g^{-1}$ soil, AL-K_2O concentration 65,80 $mg100g^{-1}$ soil, content of Ca 1958 $mgkg^{-1}$, Mg 2186 $mgkg^{-1}$, Fe 14230 $mgkg^{-1}$, Mn 1092 $mgkg^{-1}$, Zn 61,68 $mgkg^{-1}$, Na 77,9 $mgkg^{-1}$. Too high content of P_2O_5 and K_2O are result of fertilization with chicken manure so the recommendation was more frequent irrigation. Statistical analysis showed significantly higher plant height of both investigated varieties of gladiolus grown on soil type B. Average plant height of Oscar variety grown on soil type A was 54,55 cm, while grown on soil type B was 70,85 cm. As regards Amsterdam variety average plant height of plants grown on soil type A was 23,12 cm, while grown on soil type B was 44,82 cm. Plant height of Oscar variety was 29,15 % higher at plants grown on soil type B, while plant height of Amsterdam variety was 48,42 % higher at plants grown on soil type B. This indicates that Oscar variety have slightly better varietal characteristics to adapt to low pH.

Corm size

Result showed statistically significant difference in plant height of variety Oscar between plants grown from corms belonging to group 4 and corms belonging to group 6 in favor of group 6 on soil type A. The highest average plant height was recorded on plants that belonged to group 6 and was 56,76 cm, while lowest average plant height was recorded on gladiolus plants grown from corms that belong to group 4 and was 52,81 cm. There were no significant difference between plants grown on soil type B regardless of corm size. Amsterdam variety had significantly higher plants grown on soil type A from corms that belonged to group 5 and 6 in comparison with plants that were grown from corms that belonged to group 4. The highest average plant height was recorded on plants that belong to group 6 and was 26 cm, while lowest average plant height was recorded on plant from group 4 and was 19,53 cm. Likewise at variety Oscar, Amsterdam showed no significant difference between plants grown from different sizes of corms on soil type B. Mohanty et al. (1994) reported in their research that plants grown from larger corm were taller than once grown from medium and small corms. Similar, Sarkar et al. (2014.) have noted in their research that large size corm produced the highest plant height associating that with higher food material storage reserves in larger corm than smaller ones. Opposite results were obtain in the research of Memon et al. (2009) were plant height of all three investigated gladiolus varieties (Traderhorn, White Friendship, Peter Pears) was under significant influence of corm size but in favor of small corms. This opposite results are probably outcome of other factors affecting plant height of gladiolus plants not just the corm size.

CONCLUSION

Plant height of both investigated varieties of gladiolus were under significant influence of soil type achieving greater plant height on soil with greater pH value (6,5). Variety Oscar showed better development on soil with lower pH value in comparison with Amsterdam. Larger corm size resulted in greater plant height of both varieties but just on soil with lower pH value (5,07). It can be concluded that slightly acid soil are more suitable for gladiolus production and if it is unavoidable for the production to be on soil with low pH value it’s recommended to use lager corms.

REFERENCES

Ahmed, J., Ahmad, M., Bashir, T., Yaqoob, A., Jillani, M. S., Saeed, M., 2010. Effect of plant spacing on vegetative and reproductive growth of gladiolus cultivars. Sarhad Journal of Agriculture. 26(4): 539-543.

Lewis, G. J., Obermeyer, A. A., Barnard, T. T. ,1972. Gladiolus: a revision of the South African species. Journal South African Botany Suppl., 10.

Egner, H., Riehm, H., Domingo, W. R., 1960. Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nahrstoffzustandes der Boden, II: Chemische Extractionsmetoden zu Phosphorund Kaliumbestimmung. Kungliga Lantbrukshügskolans Annaler, 26: 199-215.

International Standard Organisation, [ISO 10390: 1994 (E)] (1994c); [ISO 14235: 1994 (E)] Soil quality – determination of pH; Determination of organic carbon by sulfochromic oxidation

Memon, N., Qasim, M., Jaskani, M. J., Ahmad, R., Anwar, R., 2009. Effect of various corm sizes on the vegetative, floral and corm yield attributes of gladiolus. Pakistan Journal of Agricultural Sciences, Vol. 46(1): 13-19.

Mohanty, C. R., Das, R. C., Maharana, T.,1994. Effect of corm size and growth regulators on growth, flowering and corm production in gladiolus (Gladiolus grandiflorus L.). In: J. Prakash and K.R. Bhandary (Eds.). Floriculture-Technology, trades and trends. Oxford & IBH Pub. Co. Pvt. Ltd., India. 209–211.

Pant, S. S., 2005. Effect of different doses of nitrogen and phosphorus on the corm and cormel development of gladiolus (Gladiolus sp.) cv. American beauty. Journal of the Institute of Agriculture and Animal Science 26: 153-157.

Parađiković, N., 2014. Floriculture basics – textbook, Faculty of agriculture Osijek.

Pennisi, B. V., Thomas, P. A., 2015. Essential pH Management in Greenhouse Crops. Bulletin 1256. The University of Georgia, Fort Valley State University.

Pflegr, F. L. and Gould, S. L. 2002., Gladiolus Diseases. Commun. & Educ. Technol. Serv. Univ. Minnesota Ext. Service

Rana, P., Kumar, J., Kumar, M., 2005. Response of Ga3, Plant Spacing and Planting Depth on Growth, Flowering and Corm Production in Gladiolus. Journal of Ornamental Horticulture. 8(1): 41-44.

Saleem, M., Ahmad, I., Khan, M. A., 2013. Cultivar Effects on Growth, Yield and Cormel Production of Gladiolus (Gladiolus grandiflorus L.). Journal of ornamental and horticultural plants. 3 (1): 39-48.

Sarkar, M. A. H., Hossain, M. I., Uddin, A. F. M. J., Uddin, M. A. N., Sarkar, M. D., 2014. Vegetative, floral and yield attributes of gladiolus in response to gibberellic acid and corm size. Scientia Agriculturae. 7 (3): 142-146.

Sharma, J. R., Gupta, R. B. 2003. Effect of corm size and spacing on growth, flowering and corm production in gladiolus. Journal of Ornamental Horticulture. 6(4): 352-356.

Sloan, R. C. and Harkness, S. S. 2005. Gladiolus cultivar evaluation. Ann. Rep. North Mississippi Res. Ext. Cent., Mississippi Agric. Fores. Exp. St. Inf. Bul. 419: 306-308.

Uddin, F., Rahman, M., Rabbani, G., Mannan, A. 2002. Effect of corm size and depth of planting on the growth and flowering of gladiolus. Pakistan journal of biological sciences. 5(5): 553-555.

Economic aspect of liming in eastern Croatia

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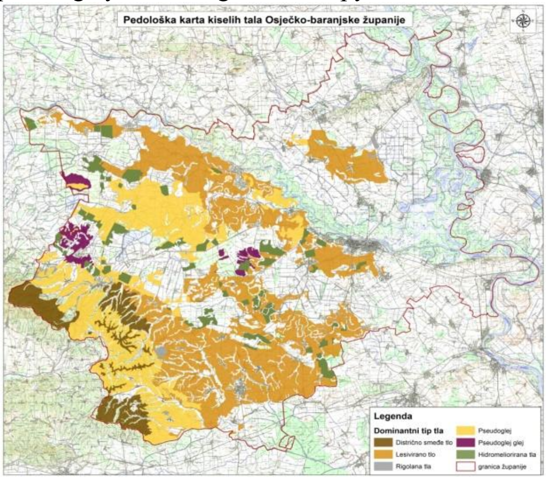
Abstract
Eastern Croatia (County of Osijek-Baranya and Vukovar-Srijem) is Croatian granary. Acid soils are significant problem, participating with 27% of total agricultural soils. If we compare the counties, in the OB County is even ⅘ of acid soils (80.7%) and in VS County remaining ⅕ of acid soils (19.3%). Liming is usual recommendation with a purpose to increase acid soil fertility. As a result of really low price, the most cost-effective liming material in eastern Croatia is sugar factory lime or carbocalc although there is other different liming material on Croatian market. According to field experiment with liming and fertilization, the best impact on yield was achieved by using 20 t of carbocalc and 200:150:300 kg ha^{-1} of N-P_2O_5-K_2O fertilizers.

INTRODUCTION

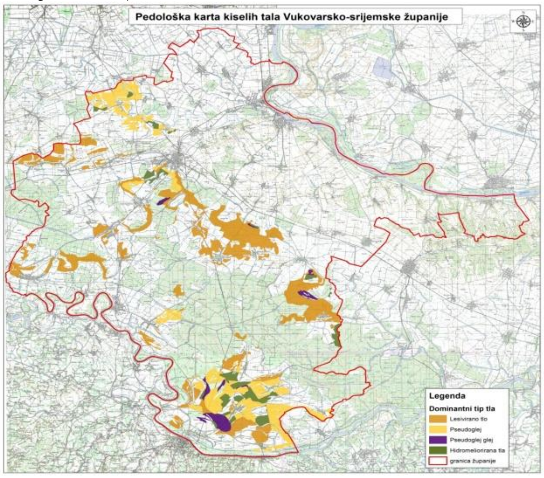
East part of Croatia is the most important agricultural part of Croatia, particularly its two counties: Osijek-Baranja (OB) and Vukovar-Srijem (VS). OB county with 4,155 km^2 occupies 7.3% of the national territorial space and participates 8.6% in the total agricultural population in Croatia. Importance of OB county in Croatian agricultural production is in the fact that OB county participates almost in ¼ of sown agricultural land of the Republic of Croatia (24%, ie. 199,358 ha from 830,888 ha sown in Croatia in 2007). The largest share of the county in the total Croatian area is under sunflower (up to 54%), sugar beet (41%), wheat (35%) and soybeans (32%). In the sown structure of OB county the most common crops are wheat (35.8%) and corn (31.3%), followed by soybeans (8.7%), sugar beet (8.3%), sunflower (6.5%) and barley (6.2%) and the very small surfaces of oats (1.5%) and rape (1%). The remaining of 0.5% occupy potatoes, tobacco, cabbage, and beans. According to the Croatian Agricultural Census (Central Bureau of Statistics, 2003), VS County in 2003 used 121,078 hectares, ie. 11.2% of the area at the national level. Households in the VS County used 2.5 times more mineral than organic fertilizers (Lončarić, 2015).

RESULTS AND DISCUSSION

In OB and VS counties there is the total is about ¼ of acid soils, or 27,53%. According to soil usage, 72% of acid soils are agricultural soils, and 28% are under the forests. However, geographic distribution of acid soils is interesting because it is significantly more acid soils in OB than in VS county. In OB county, acid soils makes up 35.4% of agricultural soils in OB County. On the other hand, in VS county has 4.2 times less acid soils, or only 14.2% of total agricultural soils (Graph 1 and 2). If we compare the counties, in the OB County is even ⅘ acid soils (80.7%) and in VS remaining ⅕ acid soils (19.3%). The most common types of soils are luvisol and pseudogley which together occupy ¾ areas of acid soils (Rastija, 2015).



Graph 1. Map of acid soils in OB county

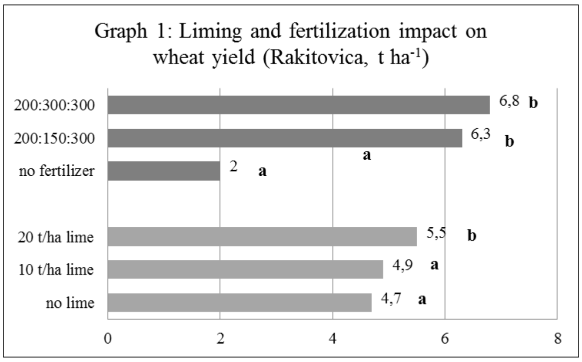


Graph 2. Map of acid soils in VS county

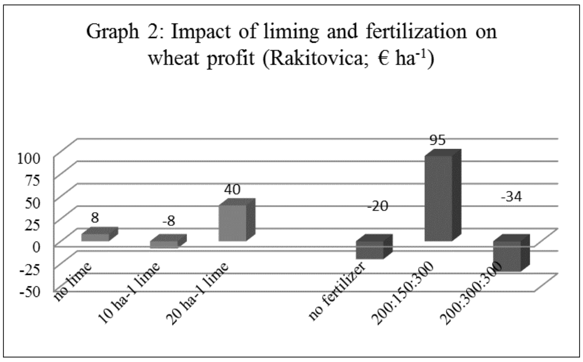
Soil acidity in Croatia is one of the most important limiting factors for achieving high and stable yields (Kovačević et al., 1993; Lončarić et al., 2006; Petrač et al., 2007). Liming is usual recommendation with a view to increase soil fertility. Liming is agro technical measure when the product containing Ca and/or Mg is applied into the acid soil with the aim of neutralizing the acidity and reaching the target pH, or optimum acidity for the cultivation of certain plant species (Lončarić, 2015). As a result of really low price, the most cost-effective liming material in Croatia is sugar factory lime. Other materials available on market are: quick lime, hydrated lime, crushed dolomitic limestone with 69% Ca and granulated dolomite with added NPK.

According to Lončarić et al. (2009) who researched profitability of different liming material in agriculture, they concluded the following: financially, the most effective material for liming is carbocalc, followed by crushed dolomitic limestone and granulated dolomite with NPK; increasing of soil acidity results in more expensive fertilization for the same yield; liming of the least acid soils (pH_{KCl}=4,5) is the most profitable by using ocarbocalc on fertile and medium fertile soils; on poor soils the best financial results are obtained with granulated dolomite with added NPK; increasing of pH from 3,5 to 4,5 decreases the difference between profitability of liming with carbocalc and crushed dolomitic limestone on fertile and medium fertile soils; profitability of liming with granulated dolomite is increased by soil fertility degradation.

Impact of liming and fertilization on wheat yield and profit was researched by Lončarić et al., (2012). Field trial were set up on Rakitovica site (Eastern Croatia) with low pH_{KCl} (4,02). 0, 10 and 20 t ha^{-1} of carbocalc were applied (total Ca content 344 g kg^{-1} and P 4,6 g kg^{-1}) and fertilized (no mineral fertilization, 200:150:300 and 200:300:300 kg ha^{-1} N-P_2O_5-K_2O). Besides costs for fertilizers and liming, production cost structure is formed of other raw material costs (seed, pesticides), mechanization costs according of applied fertilizers, seed harvesting, processing and drying, insurance and soil analysis costs. Relative fertilization costs were from 7% (only liming) to 55% of total costs for double liming & double phosphorus fertilization (costs of fertilizers along with costs of mechanized fertilizer incorporation). If the costs calculation is based on computer model for optimal fertilization, it would implicates even lower costs and higher profitability of wheat production. However, there were confirmed significant differences (P<0,001) among costs with increasing levels of fertilization and liming.



Graph 1. Impact of liming and fertilization on yield



Graph 2. Impact of liming and fertilization on profit

Yield data (Graph 1) showed important fertilization and doubled liming (20 t ha^{-1}) impact on wheat yield. According to profit analysis (Graph 2), the highest profit is achieved on fertilized (95€) and double limed treatment (40 €), what is in accordance with *Quaggio et al., 1995*, who reported highest profitability on level of 12 t lime per ha. Yield level is significantly increased by fertilization and liming compared to control treatment.

CONCLUSION

Acid soils are significant problem in Eastern Croatia, Croatian granary. Liming is usual recommendation with a purpose to increase acid soil fertility. As a result of really low price, the most cost-effective liming material in Eastern Croatia is sugar factory lime. The best impact on wheat profit was achieved by using 20 t of carbocalc and 200:150:300 kg ha^{-1} of N-P_2O_5-K_2O fertilizers. Costs calculation based on computer model for optimal fertilization implicates lower costs and higher profitability of wheat production.

REFERENCES

Kovačević, V., Bertić, B., Grgić, D. (1993): Response of maize, barley, wheat and soybean to liming on acid soils. Rastlinna Vyroba 39. 41-52.

Lončarić, R., Lončarić, Z., Vukobratović, Ž. 2009. Liming impact on cost and profit management in crop production. Proceedings of the 7th international Symposium on PSILPH (Liao H. et al. (eds)). Guangzgou. China. 221-222.

Lončarić, R., Lončarić, Z., Zmaić, K, Sudarić, T. 2012. Impact of liming and fertilization on winter wheat profitability. Proceedings of the 8th International Symposium on PSILPH (Prakash et al. (eds)). Bangalore. India. 222-223.

Lončarić, Z. 2015. Plodnost i opterećenost tala u pograničnome području. Faculty of agriculture. Osijek.

Lončarić, Z. 2015. In: Kalcizacija tala u pograničnome području (Lončarić, Z., ed.). Faculty of agriculture. Osijek.

Lončarić, Z., Rastija, D., Karalić, K., Popović, B. 2006. Mineral fertilization and liming impact on maize and wheat yield. Cereal Research Communications 34. 717-720.

Petrač, B., Lončarić, R., Zmaić, K. (2007): Economic effect of liming in maize, sunflower and winter barley production. Cereal Research Communication 35. 925-928.

Rastija, D. 2015. In: Kalcizacija tala u pograničnome području (Lončarić, Z., ed.). Faculty of agriculture. Osijek.

Quaggio, J. A., Teofilo Sobrigno, J., Dechen, A. R. 1995. Response to liming of Valencia orange tree on Rangpur lime: effects of soil acidity on plant growth and yield. Proceedings of the International Society of Citriculture. Vol 2. 628-632.

Calculation model: economic effectiveness of organic fertilizers application

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Abstract
Soil acidity in Croatia is one of the most important limiting factors for achieving high and stable yields. In eastern Croatia there is 27% of acid soils. Organic fertilizing is common measure usually applied together with liming. The aim of developing a computer model was to determine the cost-effectiveness of using solid stock manure from various aspects: the type of fertilizer, plot distance, the concentration of nitrogen and other nutrients in the manure, the market price of manure, and machinery costs (tractor, trailer, loader and plow). Manure use profitability depends on manure quality (N %), organic manure prices, trailer capacity, distance from economic yard to field plot and type of organic fertilizer. The contribution of the model to the decision support system in stock manure management is to precisely calculate profitability of manure use, which can affect the reduction of production costs.

INTRODUCTION

Soil acidity in Croatia is one of the most important limiting factors for achieving high and stable yields. Soil acidity factor is the restrictions that largely determines the efficiency of plant cultivation activities on many farms in Croatia. As part of a comprehensive problem-solving management of agricultural soils, application of lime material occupies a key position, together with fertilization and tillage (Mesić et al., 2009). The most important agricultural area of Croatia is north-east part of Croatia, i.e. Osijek-Baranja and Vukovar-Srijem counties. Those two counties participates in used agricultural land by 21% in Croatia. According to Rastija (2015) those two counties have 27% of acid soils.

Organic fertilizing is common measure usually applied together with liming. Fertilizing with stock manure is a common agro-technical measure in order to increase soil fertility. Farm manure is occasionally not disposed in a proper way and often farmers consider it as undesirable by-product. Furthermore, stock manure is quite inexpensive fertilizer, even when farmers are forced to buy it to increase soil fertility. Moreira et al. (2015) claims that the appropriate application of lime and cattle manure corrects soil acidity, improves physical and biological properties, increases soil fertility, and reduces the use of chemical and/or synthetic fertilizers. The maximum grain yield was obtained with the application of 10 Mg ha^{-1} of lime and 80 Mg ha^{-1} of cattle manure. Liming significantly increased pH index. Also, Mbutia et al. (2015) concluded that organic fertilization is often associated with greater microbial biomass and activity that are linked to improvements in soil quality.

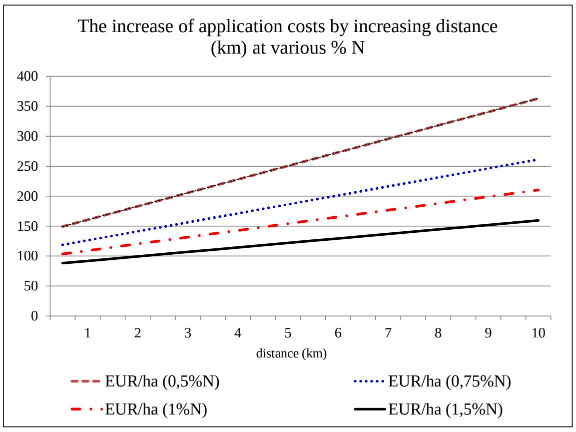
Many studies speaks about favorable effect of organic fertilizers to soil productivity and microbial activity. For example, Zhang et al. (2015) conducted study aimed to evaluate the effects of chemical fertilizer (NPK), NPK with livestock manure (NPK+M), NPK with straw (NPK+S), and NPK with green manure (NPK+G) on soil enzyme activities and microbial characteristics of albic paddy soil. The results showed that NPK+M and NPK+S significantly increased rice yield, with NPK+M being approximately 24% greater than NPK. Meanwhile, the bacterial community of NPK+M treatment was the most distinct. Fertilization with organic fertilizer (manure or compost) could be very important in vegetable production, not just because of nutrient applied, but also because of impact on soil fertility and additional yield increase (Zmaić et al., 2013).

The effect of low and high doses of mineral and organic fertilization on the quality of top yield and root mass of alfalfa and effect of mineral and organic fertilization on formation of quality of soil organic matter in dry condition were studied in a field trial. It was found dry mass yield of alfalfa fertilized with manure was to 15.9% higher as compared to dry mass yield obtained from alfalfa fertilized with mineral fertilizer. Humus content in the soil after manure fertilization was from 10.9 to 41.9% higher as compared to humus content after mineral one (Vasileva and Kostov, 2015). Tao et al. (2015) investigate the impacts of organic-supplementation of a chemical fertilizer for improving soil biological activity. The total microbial community size increased with application of chemical fertilizer ($P< 0.05$), and more so when chemical fertilizer was supplemented with organic amendments ($P < 0.05$). Enzymatic activity was greater in soils receiving fertilizer with organic supplement ($P < 0.05$). Sharath and Ghosh (2015) investigate the effect of inorganic fertilizers and organic manures on plant and soil. The soil pH in organic manures treated plots was improved and was near to neutral whereas inorganic fertilizers applied plots were acidic. However, fertilizing with stock manure is measure which often includes inadequately analyzed additional costs of agricultural production. The aim of developing a computer model was to determine the cost-effectiveness of using solid stock manure from various aspects: the type of fertilizer, plot distance, the concentration of nitrogen and other nutrients in the manure, the market price of manure, and machinery costs (tractor, trailer, loader and plow).

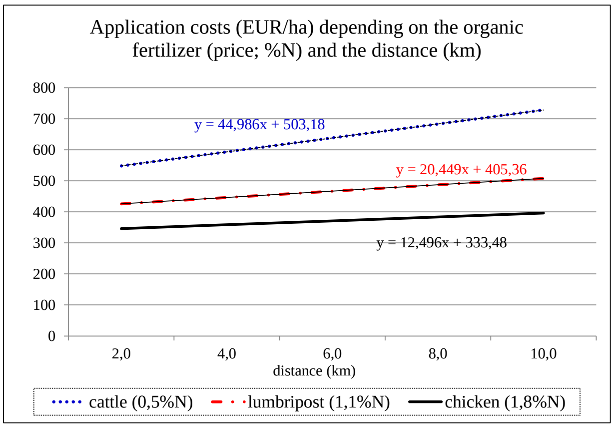
RESULTS AND DISCUSSION

The manure prices often are determined and fertilization is performed without accurate information on concentrations of nutrients. If the increase in nitrogen concentration (e.g. 0.3 to 2.0 %), is followed by an increasing of fertilizer prices (e.g. 50-200 HRK t^{-1}), then the price of required amount (equivalent to 170 kg N ha^{-1}) of the most expensive and the most concentrated fertilizer decreased 2.1 times. However, if the price of fertilizer was the same for manure with concentrations from 0.3 to 0.7 % N (expected range of concentrations depending on the proportion of litter and storage methods), the cost of equivalent fertilization of 0.7 % N would be 47 % of the costs by concentration of 0.3 %.

Furthermore, expectedly, the increase of application costs is higher by distancing from economic yard and, again, the nutrients concentration is a very significant factor. Thus, the curve of increasing costs by distancing from 0.5 to 10 km is much steeper with the 0.5 % N in manure (slope coefficient about 11), but with 1.5 % N slope coefficient is about 3, depending on used machines (Graph 1).



Graph 1. Application costs & distance (various N %)



Graph 2. Application costs & fertilizer type & distance

The machinery cost are decreasing proportionally to the trailer volume, and by using a trailer of 5.5 t instead of 3 t, the costs are decreased by 42% (application at the distance of 1 km), 51% at 3 km, and 61% at 7 km. The importance of optimal organic fertilization plan illustrates an example of farm with two manures of different concentrations of N (0.3 % and 0.7 %), which should be delivered and applied to the plots approximately equal fertility, distanced 1 and 6 km from the economic yard. Optimal decision implies application of 0.7 % N to the more distanced plots because in the opposite case the costs of machinery were 24 % higher (Graph 2).

CONCLUSION

The contribution of the model to the decision support system in stock manure management is to precisely calculate profitability of manure use, which can affect the reduction of production costs. Manure use profitability depends on manure quality (N %), organic manure prices, trailer capacity, distance from economic yard to field and type of organic fertilizer.

REFERENCES

Alam, M.N. 2015. Socio-economic and technical efficiency level of organic rice farming with system of rice intensification: A case study in morowali regency Indonesia. Americal Journal of Applied Sciences. 12 (4). 290-294.

Mbuthia, L.W., Acosta-Martínez, V., DeBryun, J., Schaeffer, S., Tyler, D., Odoi, E., Mpheshea, M., Walker, F., Eash, N. 2015. Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: Implications for soil quality. Soil biology ans biochemistry. 89. 24-34.

Mesić, M., Husnjak, S., Baši, F., Kisić, I., Gašpar, I. 2009. Suvišna kiselost tla kao negativni čimbenik razvitka poljoprivrede u Hrvatskoj. 44. hrvatsko i 4. međunarodno savjetovanje agronoma. Poljoprivredni fakultet u Osijeku (Marić, S. ed).

Moreira, A. , Sfredo, G.J., Moraes, L.A.C., Fageria, N.K. 2015. Lime and Cattle Manure in Soil Fertility and Soybean Grain Yield Cultivated in Tropical Soil (Article)

Rastija, D. 2015. In: Kalcizacija tala u pograničnome području (Lončarić, Z., ed.). Faculty of agriculture. Osijek.

Sharath, A.A., Ghosh, S.N. 2015. Effect of organic and inorganic nutrition on plant and soil of Karonda orchard (Carissa carandas), a semi-wild fruit in India. Special paper of the Geological Society of America. 1074. 73-76.

Tao, R.a, Liang, Y.b, Wakelin, S.A.c, Chu, G. 2015. Supplementing chemical fertilizer with an organic component increases soil biological function and quality. Applied soil ecology 96. 42-51.

Vasileva, V. , Kostov, O. 2015. Effect of mineral and organic fertilization on alfalfa forage and soil fertility. Emirates journal of food and agriculture. 27 (9). 678-686.

Zhang, Q, Zhou, W, Liang, G, Wang, X, Sun, J, He, P, Li, L. 2015. Effects of different organic manures on the biochemical and microbial characteristics of albic paddy soil in a short-term experiment. PLoS One. 10 (4). Article number e0124096.

Zmaić, K., Loncarić, R., Sudarić, T. 2013. May compost lead to increased horticultural production on mixed farms? Acta horticulture 1013. 239-245.

The economic value of cattle manure

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Abstract
The aim of this paper is to determine economic value of cattle manure. Manure contains the nutrients that are necessary for plants growing, and organic matter especially important for maintaining soil fertility. It is usually underestimated and not often used when compared with mineral fertilizer. Application of the farmyard manure affects the higher sustainability of the farm. Manure is a by-product which does not have well known market values. Therefore, it is necessary to estimate the value of calculating its relative purchase price or cost of replacement. The estimation is based on the determination of the quantity and value of basic nutrients in the cattle manure and mineral fertilizers.

INTRODUCTION

Modern day technologies have brought impressive production and economic results. But the success of this technologies is responsible for degradation of the soil and pollution of the soil and water with nitrogen from mineral fertilizers (Goodstein, 2003.).
Apart from the production of sufficient food quantities, modern agricultural production should produce high-quality food, but also to preserve the environment, primarily by preserving the soil as a natural, renewable, but also exhaustible resource. This present the need for better management of soil and prevention of further degradation wherein the organic fertilizers are irreplaceable as an agrotechnical measure (Vukobratović et al., 2008).
Cattle manure, obtained as a by-product of livestock production, is used as a raw material for plant production. Manure is completely spent during the production, wherein its value is transferred into obtained products. Considering that cattle manure does not have constant market circulation, it also does not have market price, but because of the great value and the quantity, it is necessary to determine the value (Karić, 2002).
Estimating the value of basic nutrients in cattle manure (N, P_2O_5, K_2O) is performed by determining the purchase price of these elements in mineral fertilizers (which can be replaced with manure from the point of inputting of nutrient elements in the soil, and which have known market price). Then, quality of the manure is defined (Table 1, Column 3), and equivalent amount of mineral fertilizers which can be used as a replacement for the certain amount of cattle manure is calculated (Table 1, Column 6).

RESULTS AND DISCUSSION

Calculation of the equivalent value of nutrients

Cattle manure is a by-product of high economic importance for crop production, but with hardly predictable content of nutritive elements (Cvjetković et al., 2014). Nutrients in manure are mainly in organic form and are released gradually, comparatively with the decomposition of organic matter. One ton of cattle manure contains 4-6 kg N, 2-4 kg P_2O_5 and 5-10 kg K_2O (Lončarić et al., 2015), therefore it is necessary to select fertilizers that can replace N, P_2O_5 and K_2O in the manure.
Utilization of nitrogen from cattle manure is at least 30% lower when compared to mineral fertilizers, while there is no important difference considering final P_2O_5 and K_2O balance in soil (Table 1, Column 4).

Table 1. The process of calculating of the relative purchase price

Ingredients	Nutrients	kg in 1 ton of manure	Utilization (%)	Content substitutes (%)	Substitutes an equivalent amount (kg)	The market price of (kn/kg)	The relative purchase price (kn/t)
1	*2*	*3*	*4*	*5*	*6 (3*4/5)*	*7*	*8 (6*7)*
CAN	N	5	70	27	12.96	2.49	32.27
Superphosphate	P_2O_5	3,6	100	19	18.95	2.85	54.01
Potassium salt	K_2O	5	100	60	8.33	4.18	34.82
Total					40.24	-	121.10

The data in Table 1 show that at the cost of 121.10 kn/t, it is possible to enrich the soil with same amount of nutrients that can be found in 1 ton manure of assumed quality. Thus established equivalent value of the fertilizers represent the relative purchase price of 1 ton of cattle manure on the place of his production.

Besides the above mentioned nutritive elements, manure contains significant amounts of organic matter. According to Cvjetković et al. (2014), contents of dry organic matter, especially important for maintaining fertility of the soil, is 25%, or 250 kg/t. Therefore, the assessment of manure only on the basis of nutritive elements values (121.10 kn), would make the actual value significantly undervalued.

Calculation of the relative purchase value of farmyard manure

Assessment of the value of organic matter in cattle manure can also be made by calculating the value of its replacement, that is based on the purchase value of organic matter that can be used to enrich the soil in some other way, for example, by using green fertilization.

Considering that the resulting organic matter from these sources also does not have market price (as it is not a consumer product), its assessment could be made on the basis of determining the cost of its production (cost price), which is 0.88 kn/kg according to Očić et al. (2009). The costs are determined on the basis of committed investment capital and labour in the production of organic matter by crops sown for green fertilization in a specific area (used seeds and fertilizers, human and mechanization labour required to perform the operations and the corresponding amount of interest on invested funds).

Table 2. The relative purchase price of cattle manure

Elements of assessment	The equivalent amount in 1 ton cattle manure (kg)	The market price (kn/kg)	The relative purchase price (kn/t)
Organic matter	250.00	0,88	220.00
CAN	12.96	2.49	32.27
Superphosphate	18.95	2.85	54.01
Potassium salt	8.33	4.18	34.82
Total			340.10

Determined relative purchase price of cattle manure as non-market products can express its real value because it is derived from market prices and thus classified in a certain system of objective, market values. The amount of these costs will depend on the distance of plots from place where manure is disposed, way of loading and unloading, the type and capacity of transport vehicles etc. Increase of distance between plot and the place of production (purchase) of manure will increase the transport costs per measure unit. For that amount, relative purchase price will descend. Direct costs of fertilization are much higher on less fertile soils due to the high need for nutrients (Lončarić et al., 2014.). According to Gogić (2009) if the manure is carried more than 10 km, its use in plant production would not be economically justified.

CONCLUSION

Cattle is a by-product of livestock production, and it is used as reproduction material for plant production. There is no general market price, but cattle manure has a great value that is estimated with the process of replacing the basic nutrition elements with the equivalent amount taken from mineral fertilizers.

The calculated relative purchase cost of solid cattle manure is 340.10 kn/t at the production site.

Using the method of relative purchase cost, it is possible to determine the relative purchase price for other materials used in agricultural production that does not have general market price, as long as they have possibility of substitution in the production process.

REFERENCES

Cvjetković, S., Gantner, R., Spajić, R., Kundih, K., Bukvić, G., Stanisavljević, A. 2014. Sadržaj biljnih hraniva u goveđem stajskom gnoju i svinjskoj gnojovci. http://sa.agr.hr/pdf/2014/sa2014_p0503.pdf (20.8.2015.)

Goodstein, E.S. 2003. Ekonomika i okoliš. Mate. Zagreb

Gogić, P. 2009. Teorija troškova s kalkulacijama. Poljoprivredni fakultet. Beograd-Zemun

Karić, M. 2002. Kalkulacije u poljoprivredi. Poljoprivredni fakultet u Osijeku. Osijek

Lončarić, R., Kanisek, J., Lončarić, Z. 2014. Difference between mineral and organic-mineral fertilization from economic point of view. http://sa.agr.hr/pdf/2014/sa2014_p0110.pdf (22.8.2015.)

Lončarić, Z., Parađiković, N., Popović, B., Lončarić, R., Kanisek, J. 2015. Gnojidba povrća, organska gnojiva i kompostiranje. Poljoprivredni fakultet u Osijeku. Osijek

Očić, V., Leto, J., Grgić, Z. 2009. Ekonomičnost različitih načina sjetve travnjaka. Mljekarstvo 59 (4). 330-335.

Vukobratović, M., Lončarić, Z., Vukobratović, Ž., Dadaček, N. 2008. Promjene kemijskih svojstava stajskih gnojiva pri kompostiranju. Poljoprivreda / Agriculture. 14(2). 28-37.

Positive legal regulations allowing the establishment and support of alley cropping in the Republic of Croatia

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Abstract
This paper provides a brief overview of the current positive legal regulations in the Republic of Croatia related to the agroforestry system of alley cropping production and discusses the possibilities and establishing this kind of production on the Croatian territory, especially its eastern and agriculturally most developed part - Slavonia. The paper also reviews the agricultural stimulations to farmers for establishing short rotation coppice with specific recommendations for individual tree species.

INTRODUCTION
Agroforestry, the integration of trees, crops and/or livestock on the same area of land, has been identified by the International Assessment of Agricultural Knowledge, Science and Technology for Development (IAASTD) as a ‘win–win’ multifunctional land-use approach that balances the production of commodities (food, feed, fuel, fibre, etc.) with non-commodity outputs such as environmental protection and cultural and landscape amenities (IAASTD, 2008). Agroforestry across Europe includes both traditional systems that are an essential part of cultural heritage and modern alley cropping systems that combine high productivity with protection of the environment. Agroforestry meets the need identified by the Commission for “*resource efficiency with a view to smart, sustainable and inclusive growth for EU agriculture”* (Council Regulation 2011/0280, page 2). This is recognised within the proposed reforms with Article 24 identified within Annex V (Council Regulation 2011/0282, page 109) as a measure of particular relevance to *“Restoring, preserving and enhancing ecosystems dependent on agriculture and forestry” and “Promoting resource efficiency and supporting the shift towards a low carbon and climate resilient economy in agriculture, food and forestry sectors.”*

RESULTS AND DISCUSSION
Croatian model of Common Agricultural Policy
In many European countries there are support schemes for establishing short rotation coppice. Some of them are called greening - green payments, which is introduced in Croatia since 2015 as well.
The Croatian Government adopted the 2014 *Decision to accept the reforms of the Common Agricultural Policy (CAP) - Croatian model of direct payments in the programming and financial period 2015-2020*. The new model of agricultural stimulations came into force on 1 January 2015. In particular, especially interesting is the introduction of a new compulsory form of agricultural stimulation - green payments (greening), which aims to encourage the sustainable development of agriculture and involves the application of the diversity of crops, maintenance of permanent grassland and maintenance of ecologically important areas on the farm economy. Short rotation crops (woody cultures for bio-mass) are defined in Decision as environmentally significant areas if the surfaces do not use mineral fertilizers and/or pesticides. Cultures that are classified as short rotation coppice (SCR) according to the *Regulation on the implementation of direct payments and individual measures of state support to agriculture in 2014* include the following types of wood: alder (Alnus glutinosa), birch (Betula sp.), hornbeam (Carpinus sp.), Chestnut (Castanea sp), ash (Fraxinus sp.), poplar (Populus sp.), acacia (Robinia pseudoacacia) and willow (Salix sp.) with a cycle of cutting every 20 years (NN 27/14) (Frištek and Karadža, 2014).
Farmers who are the main beneficiaries of payments shall implement three measures of "green payment "on all its particles, not only to those that have applied for support. Maintenance of ecologically important areas (5% of arable land) must implement farms that have more than 15 ha of arable land (excluded are perennial crops, permanent grassland and pastures).

Reasons for direct agricultural stimulations to alley cropping
In eastern Croatia acid soils cover around 30 % of the area out of which 72 % are agricultural soils and 28 % are forest soils. These areas could be used for agroforestry practice such as alley cropping by planting SRC that are tolerant to acidity (i.e. black locust). Together with tree species crops could be intercropped for food production. Some trees play an important role in improvement of soil quality as they can be N-fixing, increasing organic matter content, improving biodiversity, etc. Such species have characteristics that make them ideal for alley cropping systems on poor soils where the intention is soil improvement. For example, in terms of acidity black

locust can be grown on soils with a wide pH range and it has been show that it tolerates extremely acidic soils. In addition, it is also N-fixing specie and from this year (2015) it is on a list of species that are considered for governmental subsidies for SRC plantations in Croatia so the benefits from planting black locust are numerous.

CONCLUSION

The alley cropping systems with trees and arable crops present an opportunity for farmers in achieving their goals of diversifying farm incomes, increasing biodiversity and possible energy production from biomass. Agroforestry is inexpensive and can diversify the landscape of eastern Croatia counties, it can be applied to all scales, for the benefit of all.

REFERENCES

Recommendations for changes to CAP reform proposals (draft Council Regulation 2011/0280 and 2011/0282) to increase support for agroforestry in Europe, 2012. Paper produced by the CAP reform working group of the European Agroforestry Federation (EURAF).

Fištrek, Ž., Karadža, N., 2014. Strategija za održivi uzgoj i korištenje biomase kultura kratkih ophodnji u Osječko-baranjskoj i Vukovarsko-srijemskoj županiji. Energy Institute Hrvoje Požar, Zagreb, Croatia, page 9.

Pravilnik o provedbi izravne potpore poljoprivredi i IAKS mjera ruralnog razvoja (NN 35/15, 53/15, 69/15)

Zakon o poljoprivredi (NN 30/15)

Zakon o potpori poljoprivredi i ruralnom razvoju (NN 80/13, 41/14, 107/14)

Biomass and nutrient retraction capacity of forest plantation trees grown on tropical acid soil

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Abstract
Plantation forests in Malaysia largely occupy soils of low fertility that are limiting in nutrients especially the major elements. For the trees planted on these kinds of soils, one of the strategies to reduce nutrient loss is to capture back as much as possible nutrients in the old leaves before they abscised. In the present study, foliar nutrient concentrations were measured in leaves collected at different stages of development. The leaves were from *Acacia mangium* and *Khaya ivorensis* trees of similar age and planted on the same site having soil developed from shale with pH range 4.42-4.60. Data collected showed that nitrogen, phosphorus and potassium, the major plant nutrients, were withdrawn back into the trees well before leaf yellowing. Out of these three nutrients, P portrayed the main limitation in this plantation as it demonstrated the highest percentage of retraction by both species. *A. mangium* grew at much faster rate than *K. ivorensis* at four magnitudes higher in biomass production.

INTRODUCTION

Retranslocation or withdrawal of nutrients from plant tissue into the perennial plant part prior to senescence is one of the mechanisms for conserving nutrients by perennial tree species. It occurs primarily in foliage and fine roots. The retranslocated nutrients are important for the production of new tissues, and the process is driven by shoot growth rather than by nutrient supply (Nambiar and Fife, 1991). Generally, the percentage of nutrient retracted is greater on nutrient poor sites. The extent of this movement is also governed by the mobility of elements, the tree species and nutrient status of the soil. K is the most mobile element, followed by N and P. Mg usually experience little retranslocation as it has very low mobility. By the tree species, coniferous conserves nutrients better than deciduous (Cole and Rapp, 1981). For example, larch seems particularly good at nutrient resorption, recovering as much as 80 % of the N in the needles before they fall off (Raven et al., 1998).

Forest tree planting in Malaysia usually occupies unproductive sites with poor inherent soil fertility. These kinds of soils are exhausted in weatherable minerals including the major plant nutrients, in addition to low pH value of highly weathered soils. For the trees grown on these soils, capturing back as much as possible nutrients in the old leaves before leaf fall is one of the strategies to compensate nutrient needed for the development of young leaves. This paper describes a study carried out to estimate the extent of nutrient retraction by two forest tree species established as plantation forests on tropical acid soil. The species studies were *Acacia mangium* and *Khaya ivorensis*. The former species is a nitrogen fixer, originated from Northestern Australia and Papua New Guinea while the latter species is indigenous to the coastal area of West African rainforests. Soil belongs to Batu Anam series (close to Aquic Kandiudults) developed over iron poor shales. Internal drainage was rather poor with silt and clay totaled up to 60 %. The soil was acidic with low level of plant nutrients.

RESULTS AND DISCUSSION

Tree biomass

The total amount of biomass produced across the range of plantation ages is presented as the sum of dry matter yield of above and below ground plant parts (foliage, branches, stem and roots). The rate of biomass production for *A. mangium* is far higher than *K. ivorensis*, though the yield patterns do not differ to a great extent. The biomass increase slowly in the first six months after field planting, then there is an increase in the rate of biomass production and rapid increase occurred after eighteen months in the field. Foliage constituted a major part of the whole tree biomass in the early growth while wood formation significantly increased the contribution of biomass by stem at later growth stage (Wan Rasidah, 1995). Regression analysis showed that the increment for *A. mangium* is best described by the exponential function with R^2 of 0.98 while *K. ivorensis* fits the power function better ($R^2 = 0.99$).

Dynamics of nutrients

Significant variations in foliar nutrient concentration were observed between the species. Except for Mg, concentrations of other nutrients are much higher in *A. mangium* compared with *K. ivorensis*. Biomass yields for *A. mangium* were four times higher than *K.* ivorensis. This indicates that fast growing tree species require higher nutrient input and it seems that *A. mangium* will compete aggressively for nutrients under mix planting system.

Approximately half of the nutrients in *A. mangium* leaves disappeared when leaves abscised. The most important reduction was for leaf-P (reduced six times), a symptom of hungry response. This could hardly be due to leaching as nutrient levels in intact yellow leaves were almost similar to the fresh fallen leaves (Wan Rasidah 1995). *K. ivorensis* showed only slight reduction in nutrient concentration in leaf litter. The increase in litter Mg content for this species could probably be due to small reduction in leaf mass during senescence.

From the difference in nutrient levels in intact green leaves and freshly fallen leaves, the amount of nutrient retracted was calculated (Wan Rasidah et al., 1998). Efficient retranslocation of P by both species was not surprising as the soil at the study site was highly deficient in extractable P (less than 2 $\mu g\ g^{-1}$). *A. mangium* withdrawn back almost 80% P while *K. ivorensis* retracted about 25% P prior to leaf senescence. Since the soil hardly supplied enough P, the trees have to strategize for their P intake for growth requirement. *A. mangium* probably needed much higher P for its biological nitrogen fixation processes. According to Nambiar and Fife (1991), retranslocation efficiency is increased by high soil fertility and rapid nutrient uptake and growth. However, Salifu and Timmer (2001) found that retranslocation of P and K were relatively independent of soil fertility. The process is also driven by shoot growth rather than by nutrient supply in the soil, which is very applicable to *A. mangium* that regularly shed its leaves and produces many new shoots.

A. mangium also retranslocated much higher N compared with *K. ivorensis* even though it is capable of fixing the atmospheric N. Perhaps the hypothesis that retranslocation is governed by plant nutrient reserves (Salifu & Timmer 2001) is applicable in this case. The former species has much higher N concentration in its green leaves than the latter species. K was also retranslocated but no distinct differences were noted between the two species studied.

CONCLUSION

The findings of this study indicate that foliar nutrient analysis is essential in understanding the way nutrients are conserved and recycled in forest trees. The overall results showed high demand for P, and to some extent N and K by *A. mangium* and *K. ivorensis* trees.

REFERENCES

Cole and Rapp 1981. Elemental cycling in forest ecosystems. In: Dynamic Properties of Forest Ecosystems. 341-409. Cambridge University Press, London.

Nambiar, E. K. S. and Fife, D. N. 1991. Nutrient retranslocation in temperate conifers. Tree Physiology 9. 185-207.

Raven, P. H., Evert, R. F. & Eichhorn, S. E. 1998. Biology of Plants. Freeman/Worth, New York.

Salifu, K. F. and Timmer, V. R. 2001. Nutrient retranslocation response of *Picea mariana* seedlings to nitrogen supply. Soil Science Society of America Journal 65. 905-913.

Wan Rasidah K. 1995. Nitrogen Budget in an *Acacia mangium* Willd. Plantation in Peninsular Malaysia. Ph.D Thesis, University of Ghent. 144 pp.

Wan Rasidah, K., Van Cleemput, O. and Zaharah, A. R. 2001. Microbial respiration and nitrogen release pattern of decomposing *A. mangium* leaf litter from Kemasul Forest Reserve. Journal of Tropical Forest Science 13(1). 1-12.

Wan Rasidah, K., Van Cleemput, O. and Zaharah, A. R. 1999. Soil nitrogen dynamics in the early establishment phase of a forest plantation in Peninsular Malaysia. Journal of Tropical Forest Science 11(1). 100-110.

Wan Rasidah, K., Van Cleemput, O. and Zaharah, A. R. 1999. Nutrient retranslocations during the early growth of two exotic plantation species. In: Soils of Tropical Forest Ecosystems. Characteristics, Ecology and Management. 133-136. Springer-Verlag Berlin.

Zulkefli, M. 1989. Compensatory Forest Plantation in Peninsular Malaysia: its scope and potential. Paper presented at the Persidangan Perhutanan Malaysia Kesepuluh, 24-29 July 1989, Pahang, Malaysia. 16pp.

Determining most suitable areas for alley cropping systems by using GIS modelling

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Abstract

Growing demand for energy production from biomass can result with a substantial increase in price of food. To avoid such scenario careful planning of planting short rotation coppice (SRC) for biomass production is necessary. The great potential of SRC plantations is on poor soils that are not suitable for agricultural production. In order not to exclude food production from such areas completely, developing intercropping systems such as agroforestry systems is possible. One of such systems is alley cropping, where fast growing trees are intercropped with agricultural crops, in addition to food and energy production it can also mitigate negative environmental impact of agricultural land use in main agricultural region of Croatia. A modelling method by using GIS (Geographic Information System) is possible to determine which areas are most suitable for such systems that would produce food crops and biomass for energy from same plot/area. At the same time, on these sites, alley cropping systems will reduce potential risk of soil erosion, contribute to groundwater protection and increase landscape diversity. In these areas environmental benefits could justify the support by subsidies.

Acid soils can have negative impact on plat growth, especially if pH is below 5 there is a serious threat of aluminium and manganese toxicity, reduction or unavailability of nutrients (Ca, Mg, P and Mo). In main agricultural region of Croatia acid soils cover around 30% of the area out of which 72% are agricultural soils and 28 are forest soils. These areas could be used for planting SRC that are tolerant to acidity, such as black locust (*Robinia Pseudoacacia*). Together with black locust, crops could be intercropped for food production. In Croatia black locust is not indigenous species (North American specie) however it has adopted very well and it has spread to a large area. Numerous research and data show that *Robinia* in its non-native area is found on a wider range of soil conditions than in its native area. Therefore, in terms of acidity it is grown on soils with a wide pH range and it has been shown that it tolerates extremely acidic soils. Previous research showed that black locust cannot increase the pH level but it can have a positive impact on the increase of organic matter and nutrients such as P and K. These characteristics make it ideal specie for alley cropping systems on poor soils where the intention is soil improvement. From this year (2015) black locust is on a list of species that are considered for governmental subsidies for SRC plantations in Croatia which makes the planting of alley cropping systems with black locust more attractive to farmers.

Our Investigation included 222 soil samples from top soils (0-30 cm) of eastern Croatia. Out of these 222 samples more than half (132) had pH below 6,9 and are considered acidic, out of these 132 samples four had pH below 5 and therefore are considered very acidic and fifty samples had pH below 6 and are moderately acidic. All of these samples are from agricultural fields where crops are grown, such low pH can be a problem from the aspect of trace element toxicity and nutrient availability, thus application of liming is necessary to increase pH. Results showed that organic matter and nutrient content also varied a lot, acidic soils tend to have lower organic matter content and significantly lower available P concentrations. Therefore, main reason for using black locust on acid soils is not to increase the soil pH, as it cannot do that, but to improve these soils in terms of organic matter content and availability of nutrients.

Each sampling site has been recorded by GPS and coordinates were later uploaded in ArcGIS software. Based on the coordinate points each field/location has been drawn as a polygon. GIS tools were used to standardise, integrate and query data on soil type, soil nutrients (P and K), soil pH, organic matter, climate and land cover. The soil data was derived from the map of Croatian soil types, data on soil nutrients, pH, organic matter and land cover are from our study and data on climate and land cover from Croatian Bureau of Statistics. By using ArcGIS modelling tools such as interpolation and kriging we have determined most suitable areas for alley cropping systems. Areas where negative environmental issues exist were considered as target regions that were defined by overlaying agricultural plots and data of particular variable (pH, organic matter, nutrients P and K).

Our study was based only on the data of 222 agricultural fields that are used for food production. In addition to these sites, areas of poor soils that at the moment are not being used for agricultural production, due to its low fertility values, should be also included in the survey to obtain the total area in eastern Croatia that is suitable for alley cropping and production of energy from biomass. This approach can be also used for the assessment of soil erosion or other negative environmental impacts. For further studies more data should be collected as larger data pool with more variables makes the GIS modelling more accurate.

The intercropping systems with trees and arable crops provide an opportunity for achieving policy goals of diversifying farm incomes, increasing biodiversity, energy production from biomass, tree planting at marginal areas that makes the landscape more attractive.

Keywords: acid soil, agriculture, energy production, food production

An overview of mechanism of amelioration of acid soils by biochar application

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Abstract

Acid soils often require lime and fertilizer application to overcome nutrient deficiencies and metal toxicity to increase soil productivity. The search for new technology to mitigate climate change has lead to number of innovative ideas, among which the pyrolysis of biomass residue seems to hold considerable potential as it act as soil amendment and sequester carbon for longer time (Lehmann, 2007). Pyrolysis is thermo – chemical decomposition of organic material in absence of oxygen where in biomass acids are converted into bio- oil and alkalinity is inherited by solid biochar (Laird et al, 2010). Liming potential of biochar though focused in many studies, (Chan et al., 2008), yet the mechanisms involved remain unaddressed. An integrated overview of the probable mechanisms responsible for amelioration is presented and discussed in this paper. The liming potential of biochar can be attributed to their alkalinity, proton consumption capacity and base cation concentration (Chintala et al, 2014).

The recalcitrant nature of biochar increases its potential value as a soil amendment for the longer time. Controlled carbonization, converts biomass organic carbon into stable C pools, which are assumed to persist in the environment over centuries (Glaser et al, 1998). Variation in feedstock type and pyrolysis condition induces a broad spectrum in the observed rate of reactivity and thus microbial and chemical stability of biochar (Spoaks, 2010).

The observed changes in pH of soil incubated with biochar can be ascribed to the release of alkaline compounds, which neutralized soil acidity and thus elevate the soil pH. Further liming effect of biochar could be divided into two phases: a rapid neutralization of acidity by association of H+ ion with organic anion and a later phase associated with less soluble $CaCO_3$ which react slowly with H^+ giving long lasting liming effect. The liming potential of biochar varies depending upon feedstock, pyrolysis temperature and residence time (Wan et al, 2014). Combined application of biochar with other materials results in synergic effect. The alkaline compounds of biochar can be divided into Inorganic carbonates and organic anions. As the pyrolysis temperature increases contribution of organic anion to alkalinity decreases and while that of inorganic carbonates increases.

Apart from increase in soil pH, the incorporation of biochar can increase base saturation of soil as ash in biochar rapidly releases free bases such as Ca^{2+}, Mg^{2+} and K^+ to the soil solution (Houben et al, 2013). The increase of soil pH due to the application of biochar makes the soil surface more negative, high charge density per unit surface of biochar (Liang et al, 2006) and slow oxidation of biochar surface (Glaser et al, 2001) results increased CEC of biochar amended soil.

Alleviation of phytotoxicity of Al in wheat (Qian et al. 2013), radish (Chan et al. 2007), tomato (Hossain et al. 2010), and in other crops shows the ability of biochar to reduce metal toxicity. Not only Al, biochar is also known to reduce Fe and Mn toxicity too (Butnan et al. 2015). The mechanism of alleviation involves liming effect and adsorption properties of biochar. Qian and Chen (2014) studied the effect of aging on adsorption property of biochar.

Phosphorus solubility with biochar application was more complex as it is affected by altering soil pH, changes of Fe and Al oxides and direct P contribution from biochar (Xu et al, 2013). Biochar application not only improves P availability but also other essential and beneficial nutrients that are helpful in improving crop productivity (Novak et al, 2009). Despite all listed benefits of biochar, its use is not popular. Lack of proper pyrolyser, uniform pyrolysis temperature, high rates of application to get desired benefits, availability of the feedstock material, varied biochar effect on soil and crop depending upon soil type, difficulties in application and lack of information on its effect on long term application limit its widespread usage in agriculture.

REFERENCES

Butnan, S., Jonathan, L, D., Banyong, T., Michael, J, A., Patma, V., 2015. Biochar characteristics and application rates affecting corn growth and properties of soil contrasting in texture and mineralogy. Geoderma, 237-238: 105-116

Chan, K,Y., Van Zwieten, L., Meszaros, I., Downie, A., Joseph, S., 2007. agronomic value of greenwaste biochar as a soil amendment. Australian Journal of Soil Research, 45: 629-634

Chan, K,Y., Van Zwieten, L., Meszaros, I., Downie, A., Joseph, S., 2008. Using poultry litter biochar as soil amendments. Australian Journal of Soil Research, 46: 437-444.

Chintala, R., Javier, M., Thomas, E, S., Douglas, D. M., James, L. J., 2014. Effect of biochar on chemical properties of acidic soil. Archives of Agronomy and Soil Science, 60(3): 393- 404.

Glaser , B., Haumaier, L., Guggenberger, G., Zech, W., 1998. Black carbonin soil: the use of benzenecarboxylic acids as specific markers. Organic Geochemistry, 29: 811-819.

Glaser , B., Haumaier, L., Guggenberger, G., Zech, W., 2001. The terra preta phenomenon – a model for sustainable agriculture in the humid tropics. Naturwissenschaften, 88: 37- 41.

Houben, D., Sonnet, P., Cornelis, J.T., (2013): Biochar from Miscanthus: a potential silicon fertilizer. Plant Soil.

Hossain , M. K., Strezov, V., Chan, K, Y., Nelson, P, F., 2010. Agronomic properties of waste water sludge biochar and bioavailability of metals in production of cherry tomato. Chemosphere, 78: 1167-1171.

Lehmann, J., 2007. Bio- energy in the black. Frontiers in Ecology and the Environment, 5: 381- 387.

Laird, D.A., Flemig, P., Davis, D.D., Horton, R., Wang,B. And Karlen, D.L., 2010. Impact of biochar amendment on the quality of typical Midwestern agricultural soil. Geoderma, 158: 443-449.

Liang, B. L., Solomon, J., Kinyangi, D., Grossman, J., O Neill, J., Skjemstad, B., Petersen, F, J., Neves, J., 2006. Black carbon increases cation exchange capacity in soil. Soil Science Society of America Journal, 70: 1719-1730.

Novak M, J., Warren, J, B., Laird, D, L., Ahmedna, M., Watts, D, W., Mohamed, A,S, N., 2009. Impact of Biochar Amendment on Fertility of a Southeastern Coastal Plain Soil. Soil Science. 174(2): 105-112.

Qian, L., Chen, B., Hu, D., 2013. Effective alleviation of aluminum phytotoxicity by manure derived biochar. Environmental Science And Technology.47: 2737-2745.

Qian, L., Chen, B., 2014. Interaction of aluminum with biochars and oxidized biochars: implications for the biochar aging process. Journal of Agricultural and Food Chemistry. 62(2): 373-380.

Spoaks, K. A., 2010. Rreview of the stability of biochar in soils: predictability of O:C molar ratios. Carbon Management. 1(2): 289-303.

Wan, Q., Yuan, J.H., Xu, K.R. And Li, X.H., 2014. Pyrolysis temperature influences ameliorating effects of biochar on acidic soil. Environmental science and pollution research. 21: 2486- 2495.

Xu,G., Wei,L. L., Sun, J, N., Shao, H, B., Chang, S.X., 2013. What is more important for enhancing nutrient bioavailability with biochar application into a sandy soil: Direct or indirect mechanism?. Ecological Engineering. 52: 119– 124.

Section 06:

Soil Acidity Effects on the Food Chain (Food Quality, Nutrition and Human Health)

Chairpersons: Zdenko Lončarić, Bal Ram Singh, Tihomir Florijančić

Mineral bio-fortification of food crops on acid soils and impact on human health

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Abstract
Acid soils occupy about 3495 million ha or 30% of the total global land area and they occur both in temperate and tropical regions. Among the various soil related constraints associated with acid soils for crop production, deficiency of several essential trace elements (e.g. Se and Zn) is quite prevalent in these soils. Micronutrient deficiencies are common worldwide and about half of the world's population suffers with deficiencies of micronutrients, for example selenium (Se) and zinc (Zn), because of low dietary intake of these minerals and consumption of less diversified food. Biofortification of staple food crops with micronutrients by using agronomic and genetic breeding tools is a cost-effective and sustainable approach. Agronomic approaches either with application of mineral fertilizers and/or improving the mobilization of mineral elements in the soil have proved successful in enhancing the mineral content in food and feed crops. Similarly, breeding approach with increased abilities to acquire mineral elements and accumulate them in edible tissues has also given good results. Deficiency of Se on both acid and other soils is widely spread in temperate and tropical regions and the need for bio-fortification of both food and feed crops with Se but also with Zn is felt. Examples are provided where consumption of bio-fortified food elevated the level of minerals in blood leading to positive health effects.

INTRODUCTION

Acid soils occupy about 3495 million ha or 30% of the total global land area and they occur both in temperate and tropical regions. Only 178 m ha of these lands are under arable and 33 m ha are under perennial tropical crops (von Uexkull and Mutert, 1995). In the temperate region of northern hemisphere, they are managed by intensive use of lime and phosphate fertilizers while in tropical regions they are poorly managed and suffer from a numbers of soil related constraints for good quality food production for human consumption. Among the various soil related problems associated with acid soils, deficiency of several essential trace elements (e.g. Se and Zn) is quite prevalent in these soils.

Micronutrient malnutrition affects over 2 billion people in the developing world. Iron (Fe) deficiency alone affects >47% of all preschool aged children globally, often leading to impaired physical growth, mental development, and learning capacity. Zinc (Zn) deficiency, like iron, is considered to affect billions of people, hampering growth and development, and destroying immune systems (Cakmak et al. 2010). However, it can avoided through dietary diversification, mineral supplementation, food fortification and/or increasing mineral concentrations in edible crops (biofortification). However, mineral supplementation and food fortification have not always been successful (White and Broadley, 2009). Therefore, biofortification of crops through the application of mineral fertilizers, combined with breeding varieties with an increased ability to acquire mineral elements, is advocated as an immediate strategy not only to increase mineral concentrations in edible crops but also to improve yields on infertile soils (Graham *et al*., 2007; Pfeiffer & McClafferty, 2007 Cakmak et al., 2010). This presentation provides an overview of the agronomic and genetic approaches used for biofortification of food and feed crops to enhance the concentration of desired minerals for improving human and animal health and how to enhance bioaccessibility of minerals by either increasing the concentration of promoter substances (e.g. ascorbate) or reducing the concentration of antinutrients (e.g. phytate).

RESULTS AND DISCUSSION

Agronomic approaches either with application of mineral fertilizers and/or improving the mobilization of mineral elements in the soil have proved successful in enhancing the mineral content in food and feed crops. Similarly, breeding approach with increased abilities to acquire mineral elements and accumulate them in edible tissues has also given good results. It has also been shown that nitrogen (N) fertilization and its status of plants can affect positively the root uptake and the deposition of micronutrients in seed. Field trials conducted in Balkan countries and elsewhere show positive biofortification of Zn and Se in the edible parts of winter wheat and maize crops and that foliar application of Zn and Se fertilizers was more effective than their soil application. Results also suggest that making full use of Zn fertilizers can provide an immediate and effective option to increase grain Zn concentration, and productivity in particular, under soil conditions with severe Zn deficiency (Cakmak et al. 2010). Severe deficiency of Se in cows and sheep was observed in all Balkan countries and the need for bio-fortification of both food and feed crops with Se but also with Zn was felt. The consumption of bio-fortified food elevated the level of minerals in blood leading to positive health effects.

CONCLUSION

Micronutrient malnutrition associated on acid or other soils can be rectified by using agronomic and breeding approaches of bioforitication and consumption of bio-fortified food elevated the level of minerals in blood leading to positive health effects. Furthermore, the e bio-accessibility of minerals can be enhanced by either increasing the concentration of promoter substances (e.g. ascorbate) or reducing the concentration of antinutrients (e.g. phytate).

REFERENCES

Cakmak, I. W. H. Pfeiffer, and McClafferty., 2010. Biofortification of Durum Wheat with Zinc and Iron. Cereal Chem. 87:10–20.

Graham RD, Welch RM, Saunders DA, Ortiz-Monasterio I, Bouis HE, Bonierbale M, de Haan S, Burgos G, Thiele G, Liria R et al. 2007. Nutritious subsistence food systems. *Advances in Agronomy* 92: 1–74.

Pfeiffer WH, McClafferty B. 2007. HarvestPlus: breeding crops for better nutrition. *Crop Science* 47: S88–S105.

Von Uexkull, H.R. and E. Mutert. 1995. Global extent and development and economic impact of acid soils. Plant and Soil. 175:1-15.

White P. J. and Martin R. Broadley 2009 Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine *New Phytologist* 182: 49–84

Heavy metals concentrations in soil, plants and fallow deer (*Dama dama* L.) tissues

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Abstract
Game is suitable bioindicator of environmental pollution by heavy metals. At the same time concentrations of heavy metals and essential elements in the game is an important indicator of the quality of game meat. Balanced mineral nutrition is required for adequate growth, reproduction and health. It is a complex process that has been studied mostly in domestic animals while in the game is poorly researched. Heavy metals usually have targeted physiological or toxic effects and they are stored or implanted into living tissues.

Selenium is an essential micronutrient for all animals which has attracted much attention in past few years, because of its antioxidant and immune stimulatory activity. A variety of data shows protective action of selenium against the effects of heavy metals in the organism.

The aim of this study was to determine the concentration of heavy metals (Cd, Pb, Hg and As) and essential elements (Fe and Se) in deer tissues (muscle, kidney, liver, adipose tissue and spleen) which resides in the natural habitat, and then after anthropogenic activity enrichment supplemental feed with the selenium, to determine the interaction of selenium with heavy metals in the tissues. Analysis of habitat included analyzes of soil, tree leaves, grasses and fodders. The research area is characterized by acid soils that are medium humus to humus, poor with potassium and phosphorus and medium provided with iron and deficient with selenium. Supplemental nutrition with the addition of selenium (0.5 mg/kg) carried out for 60 days in the second year of research. The concentration of heavy metals (Cd, Pb, Hg and As) and essential elements (Fe and Se) in the soil, ground flora, fodder for the supplemental feeding of deer and tissues was determined after preparation stock solution by inductively coupled plasma spectrophotometry.

Results of concentrations for heavy metals and essential elements in soil samples shown considerable heterogeneity of the samples with a wide range of minimum and maximum values. The largest differences were determined in the concentrations of Pb and As in soil; ranged from 13.67 to 63.08 mg/kg for Pb and from 4.43 to 29.45 mg/kg for As. All of analyzed samples had concentrations within the permitted maximum concentration prescribed by the Regulations on pollution of soils (NN 39/2013).

The accumulation of heavy metals in plants depends on the plant species. How many plants acquire and accumulate metals depends on factors transfer of metals from the soil into the plant. The accumulation of heavy metals in plants increases the risk of transmission in to the ruminants, cattle and game. In the tree leaves samples were found higher average concentration of Pb in relation to the average concentration in samples of ground flora (0.54: 0.20 mg/kg) and the average value of Cd (0.28:0.10 mg/kg). The movement of the As concentration in samples of ground flora from 0.06 to 0.12 mg/kg was higher than in the samples of tree leaves. The highest concentration of heavy metals in the tree leaves composition is determined by the Pb (0.37 mg/kg) and Cd (0.38 mg/kg). Levels of Fe ranged from 118.60 to 168.10 mg/kg and Se from 0.03 to 0.06 mg/kg. The concentration of Pb and Cd in the composition of the ground flora was 0.31 mg/kg. Concentration of Se ranged from 0.02 to 0.03 mg/kg. Fodders for the supplemental feeding are also a potential source of heavy metals. Plants acquire them through the roots and leaves and represent a danger to the animals that feed with them. The mean Pb values in the compound feed was 0.10 mg/kg, Cd 0.07 mg/kg, As 0.01 mg/kg and Hg 0.02 mg/kg. The Fe concentration was ranged from 29.45 to 71.28 mg/kg, and the concentration of Se from 0.04 to 0.15 mg/kg.

In the second year of research, fallow deer are fed with a mixture enriched with the selenium. In the feed it was determined the highest concentration of Pb (0.18 mg/kg). The Hg concentration was below the detection limit. The concentration of essential element Fe ranged from 73.47 to 756.80 mg/kg, and the concentration of Se from 2.16 to 4.05 mg/kg.

Heavy metals are relatively easily absorbed from nutrition and they quickly accumulate in the tissues (muscles, liver and kidneys). The lack of essential elements in the body fluids and tissues of animals with excess heavy metal disrupts homeostasis and leads to a number of disorders.

During the first year of the experiment there was higher concentration of Pb in muscle (0.56 mg/kg in young and 0.37 mg/kg in adult) and Cd in kidney (0.41 in young and 3.82 mg/kg in adult). Concentration of Se in all tissues was low. After supplementary feeding with selenium, concentration of Pb was lower in all tissues (muscle = 0.07:0.44 mg/kg; kidney = 0.15:0.27 mg/kg; liver = 0.17:0.19 mg/kg; fat = 0.17:0.69 mg/kg; spleen = 0.06:2.14 mg/kg). Therefore, there was positive implication selenium with heavy metals in fallowdeer tissues.

Keywords: acid soil, heavy metals, fallow deer, selenium

Effect of liming on essential and detrimental trace elements transfer into food chain by grains and vegetables

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Abstract

Acid soils are widespread in the continental part of Croatia with acidity as limiting factor either for the field crops yield or their quality. On the other hand, liming caused a significant changes in soil properties modifying soil acidity, nutrients and trace elements availability. The solubility and therefore plant availability of most of the essential heavy metals and trace elements in soils is expected to be remarkably changed after liming. The consequences of changed solubility differ among elements, but also among various crops and vegetables, especially among different consumable parts of crops (grain) or vegetables (root, leaves, fruits). Considering crop grains and vegetables as a very important sources of minerals in food, liming may result in multiple positive results as increasing Mo content and decreasing content of Cd, Cr, Pb and Ni. Simultaneously, liming can decrease content of essential minerals as Fe, Mn, Zn and Cu. Therefore, the aim of this study was testing liming influence on essential and detrimental trace elements in crop grains and some vegetables.

The study is result of two separated experiments. First experiment was conducted in continental Croatia as four-year filed trials on very acid dystric luvisol (pH_{KCl} 4,02) limed with 0, 10 or 20 t ha^{-1} sugar factory waste lime on soil depth 30 cm. In second season, 14 months after liming, maize was grown, in third season winter wheat and in fourth season soybean. Second was pot experiment with very acid soil from orchard (pH_{KCl} 4,21) limed with 0 and 10 t ha^{-1} lime. In 3,5 L pots with 5 kg of soil, hot pepper and lettuce were grown. In both experiments edible plant parts (grains, fruits or leaves) were collected for analyses of Fe, Mn, Zn, Cu, Mo, Ni, Co, Cr and Cd concentrations after digestion of samples in microwave oven.

Liming didn’t cause consistently decreasing of iron (Fe) in grains and vegetables. Fe in wheat grain was decreased 6,6 % by lower liming dose and 9,5 % by higher dose, and in soybean by 1,9 and 4,8 %. On the other hand, liming didn’t affect Fe concentration in maize grain although maize was harvested 20, wheat 29 and soybean 44 months after liming. The liming experiment with vegetables resulted without effect on Fe in hot pepper but decreased Fe in lettuce leaves by 24 %.

Manganese (Mn) concentration was also decreased at least in maize grain (7,6 %) and the most in lettuce (77,6 %). Different liming doses decreased Mn in wheat 19,0 and 23,1 % and in soybean 11,3 and 24,5 %. Hot pepper also accumulated 56,5 % less Mn after liming than in acid soil. Very similar was influence on Zn concentrations since lower and higher doses decreased Zn in maize only 3,9 and 5,2 %, in wheat 4,1 and 9,4 % and in soybean 5,4 and 13,9 %. Decreasing Zn concentration was not significant in hot pepper (7,9 %), but was the highest in lettuce (32,3 %).

Copper (Cu) concentrations weren’t significantly affected by liming although concentrations were 6,3-7,8 % lower in soybean, 9,1 % in hot pepper and 17,4 % in lettuce.

Molybdenum (Mo) was the only trace element with increased concentrations as a result of liming. The lowest increasing was 37,2 % in lettuce and 39,7 % in hot pepper. Lowest liming dose increased Mo in maize 37,7 %, and in wheat and soybean 51,5 %. Highest liming dose increased Mo in maize grain also 51,5 %, but significantly more in wheat (67,1 %) and soybean (77,6 %).

Cobalt (Co) was in very low concentrations in all maize and wheat samples and therefore decreasing after liming was measured only in soybean grain (12,9 %). Co concentrations were also low in vegetables, but still significant decreasing as result of liming was measured in hot pepper (36,5 %) and lettuce (52,6 %).

Chromium (Cr) concentrations were also very low, especially in maize grain. Nevertheless, liming affected in further decreasing of Cr in wheat (24,3-32,3 %), soybean (20,5-21,3 %), hot pepper (19,4 %) and lettuce (18,2 %).

Nickel (Ni) concentrations were significantly decreased in all analyzed samples after liming, starting from maize (19-35 %), soybean (35-42 %), wheat (41-47 %) and ending whit highest decreasing in lettuce (50,2 %) and hot pepper (65,4 %).

Cadmium (Cd) concentrations were in maize grain below detection limit, in wheat grain was 0,105 mg kg^{-1} on very acid soil, but lower liming dose decreased Cd concentration 25,7 % and highest dose 37,1 %. Liming resulted in decreasing Cd in soybean by 43-54 %, in hot pepper 35,8 % and in lettuce 20,7 %.

Finally, liming had lowest influence on maize (Mn, Mo and Ni), stronger impact on wheat (no changes in Cu and Co, small changes in Zn and Fe concentrations), even highest on soybean (no changes only in Cu) and the highest changes were in trace elements concentrations in lettuce.

Liming caused the highest relative decreasing of Ni concentrations and lowest decreasing of Zn and Co. No decreasing were significant for Cu. The most consistent relative changes in concentrations caused by liming acid soils were increasing Mo concentrations.

Food quality: East Croatia consumers' perceptions and attitudes

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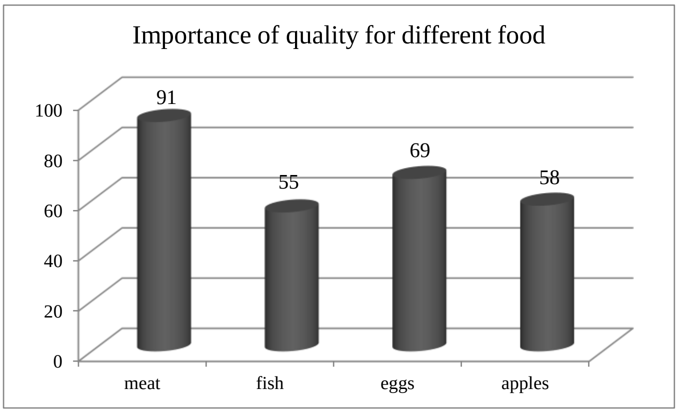
Abstract
Food quality is product of soil where it‘s produced. Acid soils can result significantly in desired higher nutrient content but simultaneously in higher harmful elements content. Food quality and safety have been highly topical for the past 20 years due to recent so called food crisis in Europe and wider. The aim of the paper is to define consumers' perceptions related to food quality via survey carried out in East Croatia. A survey of perception of consumers about food quality and safety was conducted during May 2010. Survey included 150 respondents older than 18 years from East Croatia who were asked about their concerns in terms of food quality, safety and health risks, importance of food quality for different food and whether their attitudes regarding food quality and safety is changed over time. The majority of respondents feel concern when buying food, they pay attention more when buying meat and eggs than apples and fish and consumers believe that food quality and safety is higher today than 15-20 years ago.

INTRODUCTION
Food quality is product of soil where it‘s produced. One of soil quality component is soil pH which affects the crop yield and quality components in food. Low pH indicates high content of essential mineral (iron, manganese, zinc, copper) and detrimental elements (cadmium, lead, chromium). At the other hand, food quality and safety have been highly topical for the past 20 years due to recent so called food crisis in Europe and wider. Before the crisis caused by mad cow disease (Burton and Young, 1996), dioxin crisis in Belgium (Verbeke, 2001) and daminozide (Alar-controversy) in the USA (Hermann et al., 1997; Auld, 1990) most consumers expect that every food on market has good quality and it is safe. Food quality is a complex concept that is frequently measured using objectives indices related to the nutritional, microbiological, or physicochemical characteristics of food or in terms of the opinions of designated experts. Food quality is a consumer-based perceptual/evaluative construct that is relative to person, place and time and that is subject to the same influences of context and expectations as are other perceptual/evaluative phenomena (Cardello, 1995). In order to regain consumer confidence in food quality, public institutions affect the food and feed industry by developing a total quality management system to enhance food safety, restructuring of the inspection system and to improve consumer information. Therefore, the majority of food producers in Europe, and in smaller numbers in Croatia, implemented systems to ensure food safety and quality: ISO 9001, ISO 22000: 2005. HACCP and others (Loncaric et al., 2011).

MATERIAL AND METHODS
A survey of perception of consumers about food quality and safety was conducted during May 2010. Survey included 150 respondents older than 18 years from East Croatia (Osijek, Đakovo and Našice).
The survey consisted of 13 questions that can be divided into several segments:
1. Respondents concerns regarding food quality, safety and health risks
2. Importance of food quality for different food
3. Consumers' attitudes regarding quality over time

RESULTS AND DISCUSSION
Respondents concerns regarding food quality, safety and health risks
The question regarding consumers concern when buying food is taken to initially determine their concern or indifference in terms of food quality, safety and related health risks (Table 1). A large number of respondents (79%) are worried when buying food: 25% of consumers are worried constantly, and 54% consumers sometimes. Furthermore, consumer perception is different regarding specific groups where they belong (gender, age, number of persons in the family etc.). The division by gender has not statistically significant differences in the responses, what is opposite to Wandel and Bugge results who find that women are more likely prioritize quality evaluation of food, and they are more likely to buy these products, than men. In general, a greater concern for food quality and safety feel younger respondents, respondents from smaller families and respondents without children. These respondents have statistically significant deviation compared to other respondents. Respondents who had small children (up to two years) at least feel concern when buying food.

Importance of food quality for different food
Assessing the importance of quality of certain products, the highest was graded meat. For 91% of respondents, quality component is the most important when buying meat, then eggs (69%), while purchasing apples (58%) and fish (55%) quality is less important. Consumers pay attention more to meat and eggs because those products are very perishable food what can cause health problems very easily.



Graph 1. Quality importance for meat, eggs, fish and apples

Quality components of different food are represented in table 1. Looking total scores by using Likert scale evaluation (where 1 is not important at all and 5 is extremely important), taste is the most important quality component (total score 4,72), followed by appearance, origin and price. Following results from graph 1, meat scored the highest rating in opposite to other food groups. Consumers rated eggs origin with highest score of all quality components. When consumers were asked about what food origin they preferred, the answers were as it follows: respondents prefer Croatia origin in all analyzed food (94% meat, 89% eggs, 74% apples and 60% fish). It is interesting that fish scored the lowest rating of all food. It can be explained with the fact that consumers in East Croatia (Croatian granary and the most important agricultural area in Croatia) rarely consume fish and they are pretty irrelevant on it.

Table 1. Purchasing criteria of certain foods

criteria	price	look	taste	place of purchase	producer	origin	quality label
Meat	4,45	4,85	<u>4,90</u>	4,12	4,46	4,68	4,48
Eggs	3,93	4,25	4,64	3,68	4,12	<u>4,44</u>	4,21
Apples	4,04	4,60	<u>4,69</u>	3,78	4,03	4,34	4,09
Fish	4,29	4,52	<u>4,65</u>	3,83	4,03	4,18	4,08
total	**4,18**	**4,56**	**4,72**	**3,85**	**4,16**	**4,41**	**4,22**

Consumers' attitudes regarding quality over time

Finally, we wanted to see whether respondents notice and respect the institutional efforts, activities in legislation as well as media promotion putting into food quality and safety in last 15-20 years. The results showed respondents respect the efforts, since 63% consider that food quality and safety is higher today compared to 20 years ago, 9% believe that there is no change, while 28% of respondents believe that today food quality and security is less than before.

CONCLUSION

Based on a questionnaire conducted in Eastern Croatian in the spring of 2010, it can be concluded that the majority of respondents feel concern when buying food. In general, a greater concern for food quality and safety feel younger respondents, respondents from smaller families and respondents without children. Consumers pay attention more to meat and eggs because those products are very perishable what can cause health problems very easily, so they are more careful when buying it. Quality components of different food show that taste is the most important quality component, followed by appearance, origin and price. However, consumers perceive the efforts that last 10-15 years was putted into improving food quality and security, because the majority of consumers believe that it is higher today than 15-20 years ago.

REFERENCES

Auld, E. 1990. Food risk communication: lessons from the Alar controversy, Health education research 5 (4). 535-543.

Burton, M., Young, T. 1996. Impact of BSE on the demand of beef and other meats in Great Britain. Applied Economics. Taylor and Francis Journals 28 (6). 687-693.

Cardello, A.V. 1995. Food quality: Relativity, context and consumer expectations. Food quality and preference 5 (3). 163-170.

Hermann, R. O., Warland, R. H., Sterngold, A. 1997. Who reacts to food safety scares. Agribusiness 13 (5): 511-520.

Loncaric, R., Gurdon, J., Zmaic, K., Sudaric, T. 2011. Percepcije potrošača o sigurnosti i rizicima povezanim s hranom. Proceedings. 46th Croatian and 6th International Symposium on Agriculture. Opatija. Croatia. 276-280.

Verbeke, W. 2001. Beliefs, attitudes and behaviour toward fresh meat revisited after the Belgian dioksin crisis. Food Quality and Preference 12 (8). 489-498.

Wandel, M. and Bugge, A. 1997. Environmental concern in consumer evaluation of food quality. Food quality and preference 8 (1). 19-26.

The influence of demographic and socioeconomic factors on youth attitudes about the impact of milk on human health

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Abstract
In the last couple of years, milk producers have been facing growing dissatisfaction of consumers with the safety of milk as a food product, caused by the short-term but unacceptable appearance of aflatoxin in milk. The results of the conducted survey have shown that young consumers generally find milk safe whereat female examinees, examinees coming from low-income households and those coming from farming households have expressed greater concern with respect to the food safety aspects of milk consumption.

INTRODUCTION
Milk is a product that can be said to contain all the substances necessary for optimal functioning of human body. The convenient proportion of proteins, carbohydrates, fat, vitamins and minerals makes milk a high-value product which should find its place in every day's human nutrition. Unsafe milk and various pathogenic microorganisms contained therein, which seem to affect even technologically developed producers or manufactures, apparently represent one of the main threats for expansion of milk consumption. Aflatoxin, a product of mould species called *Aspergillus*, has lately drawn great attention in this context. It was this mycotoxin that caused major problems to the milk producers in Slavonia and Baranya a couple of years ago. A combination of periods with a wide temperature and humidity range (Ćurin i Cetinić, 2007) and a low soil pH (Mejía-Teniente et al., 2011) sets fertile grounds for growth and development of these toxic food contaminants. The aforementioned reveals the main goal of this paper which is focused on research of the attitudes of young consumers towards the impact of milk as a product on human health through the demographic and socioeconomic characteristics of the examinees.

MATERIAL AND METHODS
The survey took advantage of the method of collection of primary data by means of a questionnaire as a research instrument and was conducted from March to June 2014. Due to the comprehensiveness of the research, the paper presents only the parts referring to the attitudes of consumers on the connection between milk and human health. The target group encompassed undergraduate and graduate students (18-25 years of age) and the sampling was purposive and comprised 1,157 examinees from eastern Croatia, out of which 1,060 declared themselves as milk consumers (91.6 %) and accordingly, only their answers were deemed as relevant. The data obtained in the survey were processed by a programme package named SPSS Statistics Desktop, V17.00, while the result processing referred to descriptive statistical analysis in order to describe samples (frequencies, percentages, arithmetic mean and standard deviation). What was also applied was the procedures of inferential statistics which serve to determine the probability of the validity of conclusions based on the data. In terms of parametric tests, the samples were subject to the independent samples t-test and one-way analysis of variance as to check the differences in particular attitudes between examinees.

RESULTS AND DISCUSSION
In order to find out what young consumers think about milk as a safe product, they were asked to evaluate four assertions by means of a Likert scale consisting of five levels (1 = strongly disagree, 5 = strongly agree). Their answers were used to calculate the arithmetic mean (M) and standard deviation (SD) for each assertion, as shown in Table 1.

Table 1 Examinees' attitudes on milk and its impact on health

Assertion	N	M	SD
Fresh (pasteurized) milk is safer than UHT milk (assertion 1)	1,157	3.81	1.096
Cow's milk is safe for humans (assertion 2)	1,157	4.00	0.997
Aflatoxins that might appear in milk do concern me (assertion 3)	1,157	2.92	1.269
I consume milk and dairy products exclusively for their impact on human health (assertion 4)	1,157	2.92	1.080

Key: N = total examinees; M = arithmetic mean; SD = standard deviation

The table discloses that young consumers find cow's milk, particularly if consumed fresh, safe for humans, though the reason for drinking it is not solely linked with human health and their considerations about potentially dangerous aflatoxins which might be found in milk are not so frequent. As to determine the differences in the examinees' opinions on the assertions stated in the table regarding their socioeconomic and demographic characteristics, the authors applied the independent samples t-test and one-way analysis of variance (ANOVA). Statistically significant differences in the examinees' attitudes occur to be related only to sex and the amount and source of income. Male examinees tend to agree with the assertion ''Fresh (pasteurized) milk is safer than UHT milk'' more than female examinees do ($t=2.473$, $p<0.05$), which corresponds to the research of Colić-Barić (2001) demonstrating that in comparison to females, males drink fresh milk more often. On the other hand, female examinees are more prone to the assertion ''Aflatoxins that might appear in milk do concern me'', which is understandable since women generally take more care of the food safety aspects (Krešić, 2010; Wolf, 2011). Comparing to the examinees with higher income, low-income examinees are characterized by a higher level of agreement with the assertions ''Aflatoxins that might appear in milk do concern me'' ($F=20.769$, $df=5$, $p=0.000$) and ''Fresh (pasteurized) milk is safer than UHT milk'' ($F=2.604$, $df=5$, $p=0.024$). The link between low-income examinees and higher consumption and appreciation of fresh milk has already been confirmed by Yayar (2012) whereas Buchler (2010) stressed that low-income consumers are more afraid of various risks of food contamination, regardless of its source (microorganisms, additives, pesticides or use of antibiotics). The examinees coming from farming households happen to approve all the assertions to a great extent: ''Fresh (pasteurized) milk is safer than UHT milk'' ($F=3.099$, $df=2$, $p=0.045$), ''Cow's milk is safe for humans'' ($F=4.153$, $df=2$, $p=0.016$), ''Aflatoxins that might appear in milk do concern me'' ($F=11.419$, $df=2$, $p=0.000$) and ''I consume milk and dairy products exclusively for their impact on human health'' ($F=12.501$, $df=2$, $p=0.000$). Their high level of agreement with the above assertions is logical since it can be assumed that farming households often drink milk produced on their own farms (Buzby, 2013) and as such represent a strong promo power in the development of milk consumption culture. What can be done for the purpose of diminishing the doubt about the safety of consumption of milk and dairy products pursuant to European and world examples (Wolf, 2011) is the issue of certificates, labels or declarations of safety (Miklavec, 2015) containing designations such as ''100% secure/safe'' or ''aflatoxin free'', which will certainly have positive effect on particular consumer segments when making decisions on consumption of milk and dairy products.

CONCLUSION

Milk is a high-value food product which should be frequently consumed by all age categories. If there is a slightest doubt of consumers about the safety of milk as a generic product, milk producers and manufacters ought to put efforts primarily into milk production which will continuously meet safety requirements and then into curtailing and eradicating various prejudices related to milk consumption. The results of the survey indicate that female examinees, examinees coming from low-income households and those coming from farming households raise greater concern about the food safety aspects of milk consumption, so they need to be approached by a different combination of the promotional mix. Labels or certificates issued by an independent and professional organization which can guarantee that a certain milk brand is a safe product without pathogenic microorganisms definitely represent a good choice in this view.

REFERENCES

Buchler, S., Smith, K., Lawrence, G. 2010. Food risks, old and new: Demographic characteristics and perceptions of food additives, regulation and contamination in Australia. Journal of Sociology. 46 (4): 353-374.

Buzby, J.C., Gould, L.H., Kendall, M.E., Jones, T.F., Robinson, T., Blayney, D.P. 2013. Characteristics of Consumers of Unpasteurized Milk in the United States. The Journal of Consumer Affairs. 47 (1): 153-166.

Colić Barić, I. 2001. A comparison of nutrition knowledge, attitudes and dairy consumption of school children according to age and gender. Mljekarstvo. 51 (1): 3-14.

Ćurin, K., Cetinić, E. 2007. Zdravstvena ispravnost i važnost mlijeka i mliječnih proizvoda. Medica Jadertina. 37 (1-2): 15-28.

Krešić, G., Herceg, Z., Lelas, V., Režek Jambrak, A. 2010. Consumers' behaviour and motives for selection of dairy beverages in Kvarner region: a pilot study. Mljekarstvo. 60 (1): 50-58.

Mejía-Teniente, L., Chapa-Oliver, A.M., Vazquez-Cruz, M.A., Torres-Pacheco, I., Guevara-González, R.G. 2011. Aflatoxins Biochemistry and Molecular Biology – Biotechnological Approaches for Control in Crops. In: Aflatoxins – Detection, Measurement and Control (Torres-Pacheco, I. , ed.). Available from: http://www.intechopen.com/books/ (10.06.2015)

Miklavec, K., Pravst, I., Gruner, K.G., Klopčić, M., Pohar, J. 2015. The influence of health claims and nutritional composition on consumers' yoghurt preferences. Food Quality and Preference. 43: 26-33.

Wolf, C.A., Tonsor, G.T., Olynk, N.J. 2011. Understanding U.S. Consumer Demand for Milk Production Attributes. Journal of Agricultural and Resource Economics 36 (2): 326-342.

Yayar, R. 2012. Consumer characteristics influencing milk consumption preference: The Turkey case. Theoretical and Applied Economics. 19 (7): 25-42.

The influence of pH and organic matter of soil on lead content in soil and fresh plums

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Abstract
The content of lead in fruits is matter of many research. Having in mind that the path for lead from soil to fruit product, in this case plum, is very long, and depends on many factors such as pH of soil, the content of organic matter, the kind of fruits etc. The purpose of this work is to present the differences in lead content in soil with different pH values, as well as organic matter content and their influences on lead content in fresh plums. The land which was used for production of fresh plums, cca 0, 1 ha, is placed near road which is very frequent. Taking above mentioned into consideration, it is very important to know if there is effect on lead content in fresh plums from different sources, including influence of road, contamination of soil with lead, the use of different pesticides, etc., the research performed in this work have showed, a significant differences of lead content in soil, but its concentration in fresh plums has been very low.

INTRODUCTION

There are micronutrients and minerals that concern us because of foods and food products consumption and/or occupational or residential exposure (Norom et al., 2007; Demirbas, 2010; Bakircioglu et al., 2011). However, fruit is important source of vitamins and microelements for animals and humans. Microelements present in them, can be divided into categories such as essential and other, where the first category can be toxic in very high concentrations, including Cu, Fe, Zn, Co, etc. The other category includes Cd, Ni, Pb which are very toxic for humans and plants in very small concentrations. Factors which affect the intake of microelements by plants, both fruits and vegetables depend on soil reaction, organic matter content, mineral colloids, soil humidity and microbiological activity. Organic matter, particularly humus compounds can form organometallic compounds with high mobility, which are then at reduced pH value, released into the soil solution and as such it is very available for plants (Kabata–Pendias and Pendias 2001). The lead is mineral which can be present in fruit, in this case in plums from different sources, including the use of pesticides, the residues of gasoline, contamination after flooding, etc. Taking this into consideration, there are different data on lead content in plums, 0,13 mg/kg of fresh products, but data from literature may vary, because the transportation path from soil to plums is very long and the considerations of heavy metal in tree fruit must include many factors (Bordean et al. 2011)., as above mentioned. The concentrations of lead in fruit, with exceptions of berries and small fruit, but in this case plum, the maximum level of lead is set to 0,1 mg/kg of wet weight (COMISSION REGULATION (EC) No 1881/2006, 2006; Regulation on maximum allowed concentrations of specific contaminants, Official Gazette of B&H, Regulation no. 68/2014).

The source of lead is from natural activities of soil, but also from the pollution. The acidity of soil is one of main factor influencing lead mobilization in soil and then uptake by plant, The correlation exist between soil reaction and level of lead accumulation in soil, expressed by very significant coefficients of correlation, which reveal the simultaneous increase of total lead concentration with decrease of the pH values. Researchers have also found the correlation between the humus content and the level of heavy metals accumulation in soil. Between these components, e.g. lead content in soil and its pH and organic matter, a direct relationship was found, because a large humus content leads to formation of stable lead compounds. Due to the degradation of components of the adsorptive complexwith time, the granulometric composition of soil, supports an increase of sand and silt fractions, in detriment of clay, which determines a weak lead retention, especially in acid soils.In such condtitions heavy metals can be easily absorbed by plants (Paulette, 2008)

The research performed by group of researchers, have also found that the increase of lead and cadmium intake by a plant is accompanied by the increase of soil acidity and decrease of organic matter, phosphorus, and calcium contents (Szymczak et al. 1993). Beside the influence of releasing of lead into soil solution and then into plums, in this case it is very important to emphasize the nearness of road which is very frequent. The nearness of road side to agricultural soil, the contamination with heavy metals may also results from vehicular emission and elevated heavy metals uptake by crops or fruit affects food quality and safety (Ho and Tai, 1988).

MATERIAL AND METHODS

Determination of lead, pH (H_2O), pH (KCl) and organic matter

The measurements were performed using PerkinElmer Optima 2100 DV ICP-OES instrument (PerkinElmer, Inc Shelton, Ct, USA) equipped with WinLab32 for ICP Version 4.0 software for simultaneous measurement of wavelengths of metals. In this work lead, at 220.353 nm, and calibration up to 10.02 mg/L. Determination of pH in water and KCl, as well as organic matter was estimated by using standard methods, pH using pH meter, and organic matter (humus), using bichromate method by Tjurin.

Sample preparation

The ground fruit sample (0.5 g) was accurately weighed into a high pressure PTFE vessel. Five-mL of concentrate nitric acid was added. The vessel was closed and placed inside the microwave oven unit. It was then heated following a one-stage digestion programmed at 900 W for 35 min. The acid digested solution was cooled and diluted to 25 mL final volume in volumetric flask with 10% (v/v) HCl.

RESULTS AND DISCUSSION

Very shortly in next figure, the dependence of the content of lead with pH value (determined in distilled water), is presented (Figure 1).

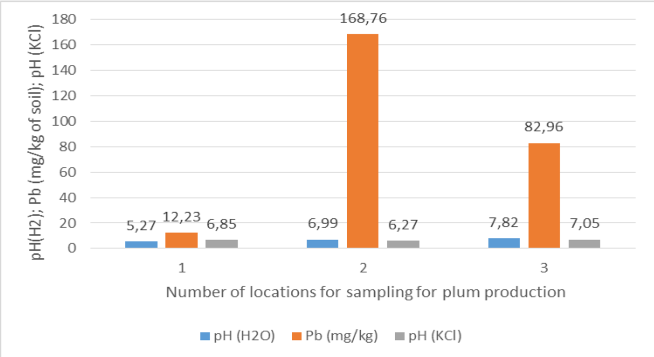

Figure 1. The lead content (dry matter) in soil with different locations with pH (H_2O), pH (KCl) values

Figure 1 shows an average concentration of lead in soil with pH values, which are determined in samples of soil taken from different locations of land used for plum production. The highest concentration of lead was determined for location no. 2, 168,76 mg/g, and the lowest was in location no. 1, 12,23 mg/kg. The pH values are also different, and the lowest vales has been estimated for samples for location no. 1, 5,27, and the biggest for location no. 3., 7,82. Considering the level of pH (KCl), the lowest value was recorded for samples from location no. 2., but the highest for samples from locations no. 3. 7,05.

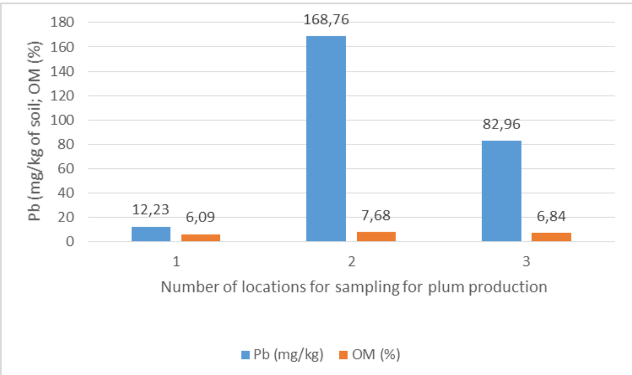

Figure 2. The lead content (dry matter) in soil with different location with organic matter

In Figure 2 the results of lead content and organic matter (%) have been presented for three locations, of plum production. As above mentioned, the location no. 2. had the highest lead content, 168,76 mg/kg, and lowest, location no. 1. 12,23 mg/kg of soil. The average content of organic matter was estimated as the highest value in samples of location no. 2, 7,68 %, but the lowest in samples of location no. 1.

The transfer coefficients are also important and they show possibility of intake and bioavailability of heavy metals to plant through root system. The transfer coefficient was calculated by dividing the concentration of heavy metals in plum by the total heavy metal concentration in the soil (Kachenko and Singh, 2006).

$$TF = C_{plant}/C_{soil}$$

where, C_{plant} = metal concentration in plant tissue (plum), mg kg^{-1} fresh weight and C_{soil} = metal concentration in soil, mg kg^{-1} dry weight. Results of transfer coefficients are respectively: location no. 1, 0,004, location no. 2, 0,0003 and location no. 3, 0,0006.

CONCLUSION

Based on results presented in this work the following is concluded;

- The concentration of lead in plums is under limits set by national and international regulations, an average concentration in fresh plums was 0,0495 mg/kg, which is significant less then it was set as maximum allowed by national and EU regulations for lead;
- The intake of lead in soil does not have large influence on its content in fresh plums, because the results of its content in soil have shown the large differences according to fresh products;
- When considering the influence of lead content in plums it is always important to provide more details on area where the production is organized because, of possible influence of environment as well as eventual pollution from industry;
- Although the level of lead in soil of location no. 2 was very high, 168,76 mg/kg, with confidence could be said, that plums are fruits which are not easy to be contaminated by lead, because of many factors, process of lead intake from soil solution to root system, transportation paths, resistance of plum tree against heavy metals, etc.
- The humus in soil for plum production is very high, scale to Gračanin (very humus soil, scale from 5 to 10, measuring less than 1, and more then 10 (Škorić, A. 1992.).
- Based on results of transfer coefficients and the total content of lead in plums, they are safe for consumption.

REFERENCES

Bakircioglu, D., Kurtulus, Y.B. and Ucar, G. 2011. Determination of some trace metal levels in cheese samples packaged in plastic and tin containers by ICP-OES after dry, wet and microwave digestion. Food and Chemical Toxicology 49: 202–207.

Bordean, D. M., Gergen, I., Gogoaşă, I., Oprea G., Pirvulescu L., Alda L., M., Alda S., Borozan A., B., Demirbas, A. 2010. Oil, micronutrient and heavy metal contents of tomatoes. Food Chemistry 118: 504–507.

COMISSION REGULATION (EC) No 1881/2006, 2006.: Setting maximum levels for certain contaminants in foodstuffs

Harmanescu M. 2011: Mathematical model evaluation of heavy metal contamination in vegetables and fruits, Journal of Food, Agriculture & Environment Vol.9 (1): 680-683.

Ho, I.B. and K.M. Tai, 1988. Elevated levels of lead and other. Metals in roadside soil and grass and their use to monitor aerial metal depositions in Hong Kong. Environ. Pollut., 49: 37-51.

Kabata–Pendias, A. and Pendias, H. 2001. Trace elements in soils and plants. 3rd edn. CRC Press, Boca Raton.

Nnorom, I.C., Osibanjo, O. and Ogugua, K. 2007. Trace heavy metal levels of some bouillon cubes, and food condiments readily consumed in Nigeria. Pakistan Journal of Nutrition 6 (2): 122-127.

Kachenko, A.G. and B. Singh, 2006. Heavy metals contamination in vegetables grown in urban and metal smelter contaminated sites in Australia. Water Air Soil Pollut., 169: 101-123.

Paulette L. 2008**.:** Lead acculumation in soil and the correlations between lead concentrations and soil properties, University of Agricultural and Veterinary Medicine, Cluj Napoca, Romania.

Regulation on maximum allowed concentrations of specific contaminants 2014:, Official Gazette of B&H, Regulation no. 68/2014

Szymczak J., Ilow R., Regulska-Ilow B. 1993: Cadmium and lead content in vegetables, fruit, cereals and soil from areas in the degree of industrial pollution and from greenhouses.. Roczn. PZH, 44, (4), 331.

Škoric A. 1992: Manual for pedological survey; University of Zagreb, Faculty of agriculture, Zagreb.

Biofortification of Fe and Zn in wheat grain on acid and calcareous soils

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Abstract
Wheat (Triticum spp.) is the most important bread grain and an important source of food and essential minerals and vitamins for humans, especially in developing countries where bread is the basic or the most common type of food. Low concentrations of Fe and Zn in wheat grain leads to many health problems and even deaths, mostly of young children and women. The lack of these micronutrients in human diet highlights the need to increase their levels in grain. The simplest and fastest way is biofortification of wheat, the application of Fe and Zn in the form of fertilizers in the soil and foliar or a combination thereof, but their content is determined by the influence of a large number of grain genotype, soil conditions, physical and soil chemical properties, types of fertilizers, and partly with their content and availability in the soil where they are grown. In acidic soils, heavy metals are more soluble and mobile and available to plants than in calcareous soils, therefore, the concentration of Fe and Zn in wheat grain are greater in more acidic than in alkaline soils.

The field trial was conducted during one season on two localities in eastern Croatia. Soil at locality Beravci was calcareous (3,7 % $CaCO_3$) and alkaline pH reaction (pH_{H2O} 7,56) with low level of available phosphorus (46 mg/kg) and moderate potassium availability (175 mg/kg). Soil at locality Novi Grad was acid (pH_{H2O} 6,04 and pH_{KCl} 4,88) with moderate level of available phosphorus (114 mg/kg) and potassium (184 mg/kg).

The total fertilization was carried out as standard on the basis of chemical analysis with 150: 125: 188 kg / ha of N: P_2O_5: K_2O. The Zn and Fe fertilization was carried out in 7 different treatments in the soil (sowing) and through the leaves (foliar application) in 3 replications: 1. Control 2. Application of Fe in the soil in the autumn (5 kg/ha Fe), 3. Zn (5 kg/ha Zn), 4. foliar fertilization Fe 0.5% solution in the tillering stage to spiking, 5. foliar Zn, 6. Fe + Zn in soil and 7. Fe + Zn foliar. At the same time, four winter wheat cultivars were planted: Srpanjka (Croatian standard for yield), Divana (Croatian standard for quality), Katarina and Zdenka. The aim of this study was to show the influence of cultivars and applications of Fe and Zn on their concentration in wheat grain.

Applied fertilization treatment with micronutrients Fe and Zn didn't significantly affect yield, although highest yield was obtained after foliar application of Fe and Zn, and lowest yield on control plots without any application of micronutrients. The highest yield on both localities was achieved by cultivars Zdenka and Katarina followed by significantly lower yields of Srpanjka and Divana. Also, higher yield was obtained on acid soil at locality Novi Grad and lower yield on calcareous soil at locality Beravci.

Biofortification with Fe resulted in significant average increase of Fe and Zn concentration in wheat grain. Foliar Fe or Zn application increased Fe 20,7 % (44,1 mg/kg) and Zn 19,1 % (42,7 mg/kg) comparing to control plots (36,6 and 35,8 mg / kg) and simultaneous foliar application of Fe and Zn resulted in 16,9 % Fe (42,8 mg/kg) and 17 % (41,9 mg/kg) Zn increase. Fe or Zn application in soil also increased Fe (13,3 %) and Zn (15,3 %) in grain, but increase was less significant.

Soil acidity and other soil properties significantly affected Fe and Zn concentrations in grain. Acid soil in Novi Grad resulted in 25 % higher Fe and 20 % higher Zn concentration in average of all treatments and cultivars comparing to calcareous soil in Beravci (44,6 and 35,7 mg/kg Fe, 43,2 and 35,1 mg/kg Zn). However, although Fe and Zn application resulted in similar increase on both localities, the increase wasn't significant on acid soil, regardless to fertilization treatment. Foliar application on calcareous soil increased Fe 23,8 % (39,4 comparing to 31,9 mg/kg) and Zn 27,2 % 39,7 mg/kg comparing to 31,2 mg/kg), and application in soil 16,9 % Fe (37,2 mg/kg) and 21,3 % Zn concentration (37,9 mg/kg). Therefore, all treatments significantly increased Fe and Zn concentration in grain on calcareous soil. On the other side, Fe and Zn application on acid soil increased Fe and Zn concentration, but none of these increase were significant.

Cultivars significantly affected Fe and Zn concentrations. The highest Fe and Zn concentration in average was in cv. Divana (54,2 and 54,7 mg/kg), significantly lower in cv. Srpanjka (38,0 and 37,8 mg/kg) and lowest in cv. Katarina (34,8 and 32,3 mg/kg) and Zdenka (33,5 Fe and 33,1 mg / kg Zn). The difference among cultivars were significant even on control plots without fortification: highest concentration were in cv. Divana (49,0 Fe and 48,5 mg/kg Zn) and lower in cultivars Srpanjka (36,0 and 35,1 mg/kg), Zdenka (30,8 and 30,9 mg/kg) and Katarina (30,5 and 28,8 mg / kg). Cultivar Divana had highest concentrations after foliar application (57,6 Fe and 60,5 mg/kg Zn) or after application in soil (56,2 and 50,9 mg/kg). Significantly lower concentrations were in cultivars Srpanjka (28-41 % lower), Katarina (29-39 %) and Zdenka (32-40 %) after all types of Fe or Zn applications.

Considering obtained results, it could be concluded that acid soil resulted in generally higher concentrations of Fe and Zn in wheat grain. Biofortification with Fe and Zn resulted in relative higher increment on calcareous soils, but absolute higher concentrations were by growing wheat on acid soil. Foliar application of micronutrient was more efficient than application in soil. Cultivars play very important role in Fe and Zn concentrations. The highest concentrations were determined by growing cultivar Divana after foliar application, what was almost double higher than by growing cultivar with lowest Fe and Zn grain accumulation.

Estimation of safety intake of nitrates from green leafy vegetables in Croatia

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Abstract
The main determining factors for presence of nitrates in green leafy vegetables are climate conditions and particular light. Climate conditions cannot be managed or changed by producer. All Member States shall monitor nitrate levels in vegetables which may contain significant levels, in particular green leafy vegetables, and communicate the result to European Food Safety Authority (EFSA) on a regular basis. The aim of this paper is to identify whether the exposure of consumers in Croatia is in safe limits with regard to determined amounts of nitrates in lettuce and spinach during market monitoring in 2013.

INTRODUCTION
Nitrates and nitrogen compounds naturally are present in the environment, and therefore are found in air, food (mainly vegetables and fruits) and water. Plants extracted nitrates from the soil by roots, distributed throughout and converted into high-energy protein compounds through photosynthesis. Nitrates by themselves are relatively safe. In vegetables containing nitrate, microbiological or enzymatic effects can cause conversion of nitrate into nitrite (BfR, 2013). Nitrate content in vegetables ranges widely (between 1 and 10,000 mg/kg) depending on the type and their source, as well as their cultivation or storage conditions (JECFA, 1995). Humans are mainly exposed to nitrates by diet and sources of nitrates are vegetables, canned meats and drinking water. Vegetables are providing between 80% and 85% of the daily amount consumed nitrate (Gangolli et al., 1994; van Velzen et al., 2008). However, daily consumption of nitrates depends on multiple factors, such as lifestyle, cultural considerations and geographic location (JECFA, 1995). Historically, the main concern for the public health is link between nitrates and stomach cancer. Although newly published prospective epidemiological cohort studies indicate, that there is no association between estimated intake of nitrite and nitrate in the diet and stomach cancer (Bryan et al., 2012). Maximum levels for nitrates in certain leafy vegetables are set in Regulation (EU) No 1258/2011. As well climatic factors (harvest in winter/summer) and horticultural factors (cultivation under cover or in the open air) are respected.

MATERIALS AND METHODS
The paper presents monitoring results of green leafy vegetables, lettuce and spinach, on Croatian market in 2013. All samples were taken from domestic breeding randomly, at markets, green markets and primary production. Vegetables are collected during the spring and fall on whole Croatian territory. Weight of the sampled specimen was at least 1 kg. In total, 40 samples were sampled and promptly delivered to the laboratory for analysis. Samples were homogenized within 24 hours of sampling. Analysis was performed at accredited laboratory of “Andrija Štampar Teaching Institute of Public Health” in Zagreb. For identification and quantification of nitrate a technique of high pressure liquid chromatography (HPLC) with UV detection was used. For result over MRL and for all median values, safe intake calculations were performed.

RESULTS AND DISCUSSION
Results of nitrates monitoring on Croatian market
In total, 40 samples of green leafy vegetables were analysed, 23 lettuces and 17spinachs. Seasonal distribution was equal which is significant for growth and harvest conditions and occurrence data on the presence of nitrates.
Lettuce is showing the highest values in spring time and spinach in autumn. This corroborates the way that sunlight influences nitrate content in spinach. Results show that all values are in the range set by regulation. Just one spinach sample from primary production, sampled in autumn, have increased value of 4957 mg/kg (MDK = 3500 mg NO_3/kg). In comparison with data from Spain (AESAN, 2011) about median nitrate content in spinach (1420 mg/kg in summer and 1904 mg/kg in winter), Croatia evidence a lower values (919 mg/kg for spinach samples in both seasons). EFSA (2008) reported median content of 1510 mg/kg on a Europe-wide level. Likewise, EFSA (2008) quote that lowest median nitrate concentration having Butterhead lettuce, just below 2000 mg/kg. Value of median nitrate concentration for different lettuce types in Croatia was 1218 mg/kg.

Results of nitrates exposure assessment for Croatian consumers
Due to large variation in the median concentrations of nitrate in different vegetables EFSA (2008) considered different scenarios for nitrate exposure estimations when assessing Acceptable Daily Intake (ADI) (Tamme et al., 2006). It has been estimated that vegetables constitute a major source of human exposure to nitrates contributing

approximately 80 to 92% of the average daily intake (Dich et al. 1996.). An ADI for nitrate of 3.7 mg/kg b.w./day, is equivalent to 222 mg nitrate per day for a 60 kg adult. It was established by the former Scientific Committee on Food (SCF) and was reconfirmed by the Joint FAO/WHO Expert Committee on Food Additives-JECFA (2002). Assuming that person eating 400g of mixed vegetables at typical median nitrate concentration levels would on average receive a dietary exposure to nitrate of 157 mg/day. According to EFSA (2008) this is within the ADI, even when the exposure to nitrate from other dietary sources is considered. With respect to given facts an exposure assessmen was performed for Croatian consumers regarding to monitoring results (Table 1.)

Table 1.The amount of nitrate in mg /150g of vegetables

Food type	Lettuce	Spinach
Mean value of nitrates (mg/kg)	1247	919
Mean value of nitrates in mg/157g	195,7	144,3
% ADI (222,0 mg/day)	88,2	65

It is evident that all results are within percentage of ADI. Largest seasonal intake is by consuming lettuce and the smallest by consuming spinach during spring time. Also, an intake calculation for grown up person was performed based to unpublished data of Croatian Food Agency about dietary habits in Croatia. Lettuce Portion amounts 38g and spinach 250g. Results had shown slightly increase value of ADI (103,49%) but it should be noted that different scientific studies shown reduction of nitrate levels between 16 to 79% when vegetables are cooked in water (Dejonckeere et al., 1994). Results of the exposure assessment of spinach sample with concertation of nitrates above MRL by intake of 157g lead to high exceedance of ADI (313,99%). That sample should be removed from the market and prevent the exposure of consumers.

CONCLUSION

The levels of nitrate and nitrite found in vegetables in this study were unlikely to pose any health risk to the general population. It could be considered that benefits of vegetable and fruit consumption outweigh any perceived risks from the consumption of nitrate in lettuce and spinach growth in Croatia. However, reducing nitrate contamination in vegetables can represent added value because vegetable are already rich in essential nutrients (Chunga et al., 2011). In order to maximise health benefits from eating vegetables, consumers should keep some good preparation practice. Farmers should comply with the guidelines of good agriculture practice.

REFERENCES

Bryan, N.S., Alexander, D.D., Coughlin, J.R., Milkowski, A.L., Boffetta, P. 2012. Ingested nitrate and nitrite and stomach cancer risk: an updated review. Food Chem Toxicol. 50(10):3646-65.

Commission Regulation (EU) No 1258/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for nitrates in foodstuffs. Official Journal of the European Union.

Chung, S., Trana, J., Tonga, K., Chenb, M., Xiaobc, Y.. Chand, H.C. 2011. Nitrate and nitrite levels in commonly consumed vegetables in Hong Kong. Food Additives & Contaminants. Volume 4, Issue 1: 34-41

Dejonckheere, W., Steurbaut, W., Drieghe, S., Verstraeten, R. Braeckman, H. 1994. Nitrate in food commodities of vegetable origin and the total diet in Belgium 1992-1993. Microbiologie-Aliments-Nutrition 12: 359-370.

Dich, J., Jarvinen, R., Knekt, P., Penttila, P.L. 1996. Dietary intakes of nitrate, nitrite and NDMA in the Finnish Mobile Clinic Health Examination Survey. Food Addit.Contam 13. 541-552.

European Food Safety Authority (EFSA). 2008. Opinion of the Scientific Panel on Contaminants in the Food chain on a request from the European Commission to perform a scientific risk assessment on nitrate in vegetables. The EFSA Journal number. 689. 1-79.

Federal Institute for Risk Assessment (BfR) 2013. Frequently Asked Questions on nitrate and nitrite in food. http://www.bfr.bund.de/en/frequently_asked_questions_on_nitrate_and_nitrite_in_food-187207.html. 14 July 2015.

Gangolli, S.D., Van den Brandt, P.A., Feron, V.J., Janzowsky, C., Koeman, J.H., Speijers, G.J. 1994. Nitrate, nitrite and N-nitroso compounds. European Journal of Pharmacology. 292: 1–38.

Joint FAO/WHO Expert Committee on Food Additives (JECFA). 1995. Nitrate and nitrite. Evaluation of Certain FoodAdditives. Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. Who Technical Report Series 859.

Joint FAO/WHO Expert Committee on Food Additives (JECFA) 2002. Evaluation of certain food additives and contaminants.Fifty-ninth report of the Joint FAO/WHO Experts Committee on Food Additives. WHO Technical Reports series 913. 20-32.

Spanish Agency for Food Safety and Nutrition (AESAN). 2011. Report from the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) in relation to the risk assessment of infants and young children's exposure to nitrates resulting from the consumption of chard in Spain. Reference number: AESAN-2011-004. http://aesan.msssi.gob.es/AESAN/web/home.shtml. 14 July 2015.

Tamme, T., Reinik, M., Roasto, M., Juhkam, K., Tenno, T., et al. 2006. Nitrates and nitrites in vegetables and vegetable-based products and their intakes by Estonian population. Food Additives and Contaminants. 23 (04): 355-361.

Van Velzen, A.G., Sips, A.J.A.M., Schothorst, R.C., Lambers, A.C. and Meulenbelt, J. 2008. The oral biovailability of nitrate rich vegetables in humans. Toxicological Letters. 181(3): 177-181.