Managing Soils for Food Security and Climate Change Adaptation and Mitigation

IAEA

Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

International Symposium on Managing Soils for Food Security and Climate Change Adaptation and Mitigation

Edited by L.K. Heng, K. Sakadevan, G. Dercon
and M.L. Nguyen

Soil and Water Management and Crop Nutrition Subprogramme
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture,
Department of Nuclear Sciences and Applications,
International Atomic Energy Agency, Vienna, Austria

Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

Food and Agriculture Organization of the United Nations
Rome, 2014

The designations employed and the presentation of material in this information product do not imply the expression of any opinion whatsoever on the part of the Food and Agriculture Organization of the United Nations (FAO) concerning the legal or development status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. The mention of specific companies or products of manufacturers, whether or not these have been patented, does not imply that these have been endorsed or recommended by FAO in preference to others of a similar nature that are not mentioned.

The views expressed in this information product are those of the author(s) and do not necessarily reflect the views or policies of FAO.

ISBN 978-92-5-108552-3 (print)
E-ISBN 978-92-5-108553-0 (PDF)

© FAO, 2014

FAO encourages the use, reproduction and dissemination of material in this information product. Except where otherwise indicated, material may be copied, downloaded and printed for private study, research and teaching purposes, or for use in non-commercial products or services, provided that appropriate acknowledgement of FAO as the source and copyright holder is given and that FAO's endorsement of users' views, products or services is not implied in any way.

All requests for translation and adaptation rights, and for resale and other commercial use rights should be made via www.fao.org/contact-us/licence-request or addressed to copyright@fao.org.

FAO information products are available on the FAO website (www.fao.org/publications) and can be purchased through publications-sales@fao.org.

Contents

PLENARY SESSION

SESSION 1
MANAGING SOILS FOR CROP PRODUCTION AND ECOSYSTEM SERVICES

SESSION 2
PRESERVING AND PROTECTING SOIL RESOURCES

SESSION 3
SOIL AND WATER CONSERVATION ZONES FOR POLLUTION CONTROL

SESSION 4
MANAGING SOILS FOR CLIMATE CHANGE ADAPTATION AND MITIGATION

SESSION 5
MANAGING AGRICULTURAL WATER FOR CLIMATE CHANGE ADAPTATION

SESSION 6
RECENT ADVANCES IN NUCLEAR TECHNIQUES AND INSTRUMENTATION

PREFACE

Global food production must increase by 70% to feed the projected growth in the world's population from about seven to nine billion people by 2050. This cannot be achieved without increasing land productivity and conserving soil and water resources in the face of the severe challenges posed, including climate change, soil erosion and salinization, and drought and flooding — all of which contribute to reducing the quantity and quality of soil and water resources. Global R&D efforts are accelerating to both develop and put into practice "win–win" systems of agricultural production, targeting a range of scales from field plots to farm and catchment levels, which are resilient against the negative consequences of these challenges, while at the same time enhance land productivity for sustainable food production and minimize the greenhouse gas (GHG) emissions which potentially contribute to climate change and variations.

This publication is a compilation of selected papers presented during both oral and poster sessions at the International Symposium on "Managing Soils for Food Security and Climate Change Adaptation and Mitigation", organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture on 23–27 July, 2012. The objective of this Symposium was to communicate the advances that have been made in using nuclear and conventional techniques to improve land management practices for: (i) enhancing productivity; (ii) increasing soil resilience to climate change; and (iii) reducing GHG emissions. The Symposium also sought to identify current gaps in our knowledge and to discuss ways in which soil (and water) resources can be better managed to meet the challenge of promoting food security through the dual approach of climate change adaptation and mitigation.

Approximately 400 delegates from 80 Member States and representatives of international organizations including the FAO attended. Overall 85 oral and 136 poster papers were presented during the 5-day Symposium. These covered a wide range of topics during the plenary and six thematic sessions, including (i) managing soils for crop production and on-farm and area-wide ecosystem service efficiency; (ii) preserving and protecting soil resources; (iii) establishing soil and water conservation zones for pollution control; (iv) managing soils for climate change adaptation and mitigation through increasing soil carbon stocks (C sequestration) and reducing greenhouse gas emissions; (v) managing agricultural water for climate change adaptation; and (vi) recent advances in nuclear techniques and applications in land management research.

It is hoped that the information presented in these Proceedings provides valuable guidance to scientists and land managers in both the public and private sectors, as well as to government and institutional policy- and decision-makers involved in addressing land management issues for climate smart agriculture and the conservation of natural resources for agricultural productivity and food security.

Qu Liang
Director
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture

ACKNOWLEDGEMENTS

The Symposium was organized by a Scientific Committee comprising: W.S. Aulakh (India), W.E.H. Blum (Austria), P.M. Chalk (IAEA), E.T. Craswell (Australia), T. Friedrich (FAO), R. Lal (USA), Q. Liang (FAO/IAEA), L. Montanarella (EC/JRC), M.L. Nguyen (FAO/IAEA), S. Nortcliff (UK), N. Sanginga (IITA), N. Senesi (Italy), J.C. Yang (China), P. Zdruli (Italy) and B. Zhang (China).

The Editors thank the following organizations for collaborating in the Symposium:

- East and Southeast Asia Federation of Soil Science Societies
- European Confederation of Soil Sciences Societies
- International Union of Soil Sciences
- nternational Centre for Advanced Mediterranean Agronomic Studies
- Soil Science Society of America
- Soil Science Society of China.

Thanks are also due to Mr. Jim Dargie, former Director of the Joint FAO/IAEA Division, for his meticulous work and valuable suggestions for ensuring consistency of style and editing the papers and to Mr. E. Izewski for type-setting.

Special thanks to Ms. Jordana Antal for her excellent administrative support and assistance, Mr. Phillip Chalk for the coordination, compilation and technical review of extended synopses of both oral and poster papers presented at the Symposium and to Mr. Liang Qu, Ms. Katherine Long, Ms. Lina Shakhashiro, Mr. Sereyvirak Dy, Ms. Rosario Leon De Mueller, Ms. Ksenija Ajvazi, Ms. Kyoko Makovicky and the SWMCN Laboratory team for their contributions.

Finally the assistance and support of Ms. Karen Morrison and her team (Ms. M. Khaelss and Ms. B. Keceli Meszaros) from the Conference Services Section of the Division of Conference and Document Services in organizing the Symposium and exhibitions are gratefully acknowledged.

The Editors
May 2014

PLENARY SESSION

Integrated Soil Fertility Management (ISFM) in sub-Saharan Africa: Concepts and Practice

B. Vanlauwe[1,*]

ABSTRACT

Agricultural intensification is a necessity in the densely populated areas of sub-Saharan Africa (SSA). The integrated soil fertility management (ISFM) paradigm has been accepted by the research and development community as a viable set of principles to foster agricultural intensification. Integrated soil fertility management involves a set of soil fertility management practices that include the use of fertilizer, organic inputs and improved germplasm combined with the knowledge on how to adapt these practices to local conditions, aiming at maximizing agronomic efficiency (AE) of the applied nutrients and improving crop productivity. All inputs need to be managed following sound agronomic principles. An alternative approach being promoted in SSA is conservation agriculture (CA). In this paper, the major concepts underlying ISFM are highlighted with a focus on nitrogen (N) fertilizer on maize across Africa through a meta-analytical study consisting of published papers with data on AE as affected by specific ISFM components. The summary data confirm that the components underlying ISFM do result in increased AE values for N fertilizer on maize. Application of ISFM principles within important farming systems is then considered with a focus on dual purpose grain legume–maize rotations. It is proposed that ISFM could be the initial trigger towards the production of sufficient amounts of crop residues to engage in CA. Due to the lack of organic resources within smallholder farms, when promoted in SSA, CA should be accompanied by a fourth principle: the appropriate use of inorganic fertilizer, applied in the right formulation at the right time, place and rate.

Key words: *agronomic efficiency, N fertilizer, conservation agriculture, maize–legume systems, organic resource quality, soil fertility gradients.*

INTRODUCTION

The need for intensification of agriculture in sub-Saharan Africa (SSA) has recently gained support, in part because of the growing recognition that enhanced farm productivity is a major entry point to break the vicious cycle underlying rural poverty. Recent events include the launching of the Alliance for a Green Revolution in Africa (AGRA), which aims at increasing fertilizer use from the current 8 kg to 50 kg fertilizer nutrients/ha (Abuja Fertilizer Summit, 2006), thereby acknowledging that sustainable intensification needs to rely on the sensible use of external nutrient sources. Since fertilizer is an expensive commodity and because the overuse of fertilizer can lead to undesirable environmental degradation, integrated soil fertility management (ISFM), aiming at maximizing the agronomic efficiency of nutrient inputs (Vanlauwe *et al.*, 2010), has been increasingly adopted by the research and development community as a framework for boosting crop productivity with minimal environmental impacts.

In recent years, conservation agriculture (CA) has also been promoted intensively as a paradigm towards sustainable intensification of smallholder farming systems in SSA, in some extreme cases infringing on the domain of religion (Baudron *et al.*, 2012). Conservation agriculture is commonly defined around a set of three principles: minimum tillage, soil surface cover, and crop rotations. One of the main justifications for 'pushing' CA is its impressive adoption under large-scale farming conditions in various parts of the world (Landers, 2001). Although some of the initially hypothesized benefits of CA, including soil C sequestration and higher yields are not unequivocally confirmed (Govaerts *et al.*, 2009), CA is usually observed to result in more stable and economically favourable yields, usually after a number of years after conversion from conventional agriculture to CA (Rusinamhodzi *et al.*, 2011). Also widely observed is the fact that minimal tillage without surface mulch usually results in depressed yields (Paul *et al.*, 2013), partly because mulch provides the necessary conditions for soil biota to thrive and ensure that the physical conditions of the topsoil are conducive for seed germination and initial crop growth. The CA revolution started in the 1970s with large-scale farmers in Brazil (Landers, 2001), and spread to countries in Latin America, Europe, and certain parts of South Asia (e.g. the Indo-Gangetic basin) (Rockstrom *et al.*, 2009). Another important benefit of minimum tillage was the energy saved by eliminating several ploughing operations. Fertilizer use was already a common practice in these systems where high yields were common. In a sense, reduced tillage automatically provided the required crop residues to provide sufficient soil cover due to the high crop productivity under these high input systems.

Any pathway to agricultural intensification needs to be adapted to smallholder farming conditions. Smallholder farming conditions in SSA are different from large-scale farming situations in many aspects. First of all, yields are low due to the limited use of agro-inputs and sub-optimal agricultural practices with the limited availability of crop residues as a consequence. Secondly, in many systems, several competing uses exist for available crop residues, e.g. for livestock feed (Giller *et al.*, 2009). Thirdly, under high population densities where fallow land is virtually absent, management-induced soil fertility gradients are created due to a concentration of scarcely available organic resources, either direct or processed, e.g. as manure or compost, in small areas, usually around the homestead (Tittonell *et al.*, 2005), further degrading more remote plots through nutrient mining and reduced organic matter recycling. Fourthly, farmer resources includ-

---

[1] International Institute of Tropical Agriculture (IITA), Nairobi, Kenya
[*] E-mail address of corresponding author: b.vanlauwe@cgiar.org

L.K. Heng, K. Sakadevan, G. Dercon and M.L. Nguyen (eds), Proceedings — International Symposium on Managing Soils for Food Security and Climate Change Adaptation and Mitigation. Food and Agriculture Organization of the United Nations, Rome, 2014: 3–9

ing land, cash or labour, are most often in limited supply thus limiting the opportunities farmers have to invest in agriculture.

Concepts underlying integrated soil fertility management

Definition

Integrated soil fertility management has been defined as "A set of soil fertility management practices that necessarily include the use of fertilizer, organic inputs and improved germplasm combined with the knowledge on how to adapt these practices to local conditions, aiming at maximizing agronomic use efficiency of the applied nutrients and improving crop productivity. In ISFM, all inputs need to be managed following sound agronomic principles (Vanlauwe *et al*., 2010). The goal of ISFM is optimized crop productivity through maximizing interactions that occur when fertilizers, organic inputs and improved germplasm and the required associated knowledge are integrated by farmers (Figure 1).

The definition focuses on maximizing the efficiency with which fertilizer and organic inputs are used since these are both scarce resources in the areas where agricultural intensification is needed. Agronomic efficiency (AE) is defined as incremental yield return to applied nutrient inputs:

$$AE \text{ (kg/kg)} = (Y_F - Y_C) / (F_{appl})$$

where Y_F and Y_C refer respectively to yields (kg/ha) in the treatment where nutrients have been applied and in the control, which did not receive nutrient, and F_{appl} is the amount of fertilizer and/or organic nutrients applied (kg/ha).

Note that maximal AE also leads to maximum value:cost ratios since both indicators are linearly related for specific input and output prices.

Important components

The ISFM definition has a number of concepts including the use of fertilizer and improved germplasm, combined application of fertilizer and organic inputs, adaptation to local conditions and rehabilitation of degraded soils (Figure 1). In terms of response to fertilizer, two types of soil are distinguished: (i) soils in which crop productivity responds to fertilizer — 'responsive soils' — (Path A, Figure 1), and (ii) soils in which crop productivity responds minimally or not at all to fertilizer — 'poor, less-responsive soils' — due to other constraints besides the nutrients contained in the fertilizer (Path B, Figure 1). Investment in overall soil fertility rehabilitation through, for example, organic resource management will be required before AE will increase on non-responsive soils (Path C, Figure 1). A third type of soil, 'rich, less-responsive soils', is not included in the graph since such soils are sufficiently fertile to supply most or all of the nutrients needed by a crop. Inclusion of such soils in Figure 1 would result in a line with N-AE close to 0 across all ISFM components.

The application of fertilizer to improved germplasm on responsive soils will boost crop yield and improve the AE relative to current farmer practice in SSA, which is characterized by traditional varieties receiving little nutrient inputs that are often inappropriately managed. Combining organic and inorganic inputs has been advocated as a sound management principle for smallholder farming in the tropics since neither of the two inputs is usually available in sufficient quantities and both are needed in the long-term to sustain soil fertility and crop production (Figure 1) (Vanlauwe *et al*., 2001). Inorganic inputs are often too expensive for smallholders to be applied at optimal rates and organic inputs applied at rates that are feasible for smallholder farmers seldom release sufficient nutrients for optimum crop yield (Vanlauwe *et al.,* 2001). Moreover, the combination of the two input types can provide added benefits if managed correctly.

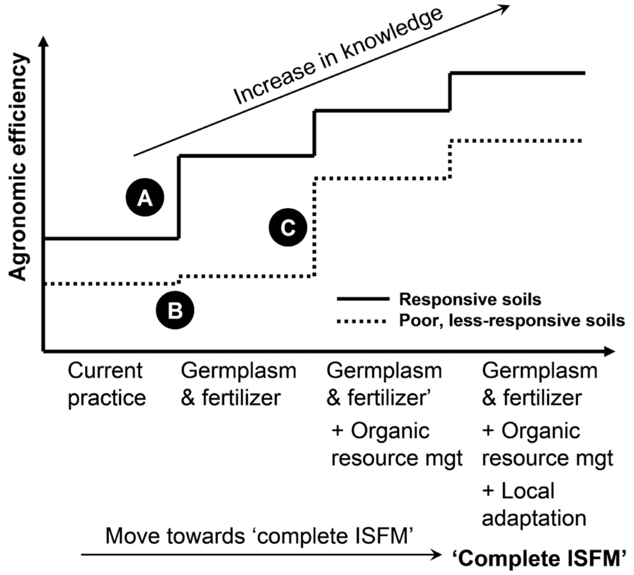


FIGURE 1. Conceptual relationship between the agronomic efficiency of fertilizers and organic resources and the implementation of various components of integrated soil fertility management (ISFM). "Current practice" assumes the use of the current average fertilizer application rate in sub-Saharan Africa of 8 kg per ha. Adapted from Vanlauwe *et al.* (2010).

Adjusting for site-specific soil conditions is a last requirement for maximizing AE because of the variability found in farming systems at different scales. Constraints to crop production can vary substantially between different fields within a single farm, creating what is often referred to as "soil fertility gradients" (Tittonell *et al*., 2005; Vanlauwe *et al*., 2006). Nutrient deficiencies related to soil type can occur at regional levels, but deficiencies related to cropping history and management can differ within short distances on a single farm. Such fertility gradients can have a substantial impact on fertilizer response and adjustment of inputs used along existing soil fertility gradients is one important aspect of local adaptation (Vanlauwe *et al*., 2006). Often, within-farm gradients of soil fertility are dissected by considering fields close to the homestead, referred to as "infields", separately from fields furthest away from the homestead, referred to as 'outfields' (Tittonell *et al.,* 2005; Zingore *et al*., 2007). Adaptation to local conditions also includes accompanying measures that are needed to address constraints that are unlikely to be resolved by fertilizer and/or organic inputs. These measures include the application of lime to acid soils, water harvesting techniques on soils susceptible to crusting, or soil erosion control in hillsides. Again, for poor, non-responsive soils, investment in overall soil fertility rehabilitation will be required before fertilizer AE will be enhanced (Path C, Figure 1). Zingore *et al.* (2007), for instance, demonstrated that responses to fertilizer on degraded outfields were only obtained after application of 17 $Mg \cdot ha^{-1} \cdot y^{-1}$ of farmyard manure during three consecutive years.

Towards complete ISFM

Complete ISFM comprises the use of improved germplasm, fertilizer, appropriate organic resource management, and local adaptation. Several intermediary phases have been identified that assist the practitioner's move towards complete ISFM, starting from the current average practice of applying eight kg/ha fertilizer nutrients to local

TABLE 1. Summary of selected properties related to the various integrated soil fertility management components considered in the meta-analysis, after removing data with low N fertilizer agronomic efficiency (N-AE) values due to relatively high N application rates or relatively high no-input control yields. The total number of data points retained is 721. Source: Vanlauwe *et al.* (2011).

Property	Farmer-lead fertilizer management	Researcher-lead fertilizer management	Improved varieties + researcher-lead fertilizer management	Organic inputs + researcher-lead fertilizer management	Researcher-lead fertilizer management on infields across soil fertility gradients
No. cases	24	324	73	272	28
N-AE mean	19	23	34	32	33
N-AE std. dev.	15	19	23	29	22
N-AE min.	–4	–23	–15	–26	6
N-AE max.	61	128	83	146	95
Upper quartile	20	30	52	46	39
Median	14	21	37	26	32
Lower quartile	11	9	13	12	20

varieties. Each step is expected to provide the management skills that result in an increase in yield and improvements in AE (Figure 1). Figure 1 is not intended to prioritize interventions but rather suggests a stepwise adoption of the elements of complete ISFM. It does, however, depict key components that lead to better soil fertility management. In areas, for instance, where farmyard manure is targeted towards specific fields within a farm, local adaptation is already taking place, even if no fertilizer is used.

Proof of the ISFM concept through meta-analysis of nitrogen (N) fertilizer response to maize in Africa

Data sources

A meta-analytical study was conducted using data obtained through specific searches within several agricultural databases (Vanlauwe *et al.*, 2011). Only peer-reviewed literature in journals and conference proceedings with information on control yields, yields after N fertilizer application and fertilizer N rates in maize-based cropping systems in SSA was included in the database. Considered papers included data from farm surveys, multi-locational on-farm trials and replicated on-station trials. A total of 90 peer-reviewed publications fulfilled all the criteria. Available data from different sites in Ethiopia, DR Congo, Botswana, Somalia, Rwanda and Tanzania recorded in the nutrient response database (Fertibase) of FAO (www.fao.org) were also included provided that they fulfilled the criteria.

Nitrogen agronomic efficiency (N-AE) under farmer managed conditions and researcher management as affected by improved maize germplasm

The average N-AE value for farmer-managed plots (24 cases) was 19 kg·grain·kg^{-1}·N (Table 1). The average N-AE value for researcher-managed plots (324 cases) was 23 kg·grain·kg^{-1}·N (Table 1). With improved hybrid maize varieties, an average N-AE value of 34 kg·grain·kg^{-1}·N (73 cases) was found (Table 1). Least square means calculations from studies that reported N-AE values for both local and improved germplasm showed that improved hybrid maize varieties increased N-AE significantly from 17 to 26 kg·grain·kg^{-1}·N, with no differences between local and improved, open-pollinated varieties (OPV) (Figure 2).

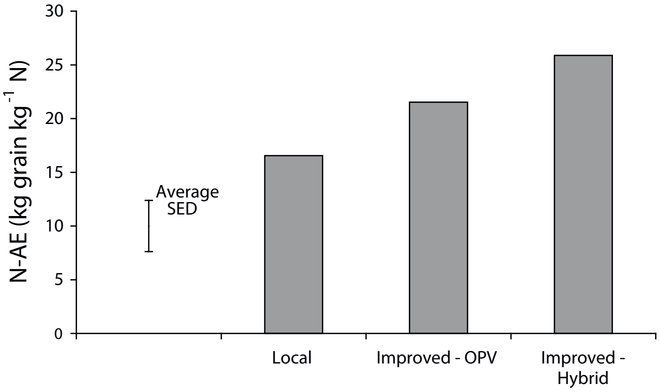


FIGURE 2. Agronomic efficiency of fertilizer N (N-AE) for local, improved open-pollinated (OPV), and improved hybrid maize varieties across the three regions. A total of 11 trial-years was included in the analysis (two from East/Central Africa, five from Southern Africa, and four from West Africa). SED refers to standard error of the difference. Source: Vanlauwe *et al.* (2011).

N-AE as affected by mixing fertilizer with organic inputs

Organic resources can be classified following their quality with consequences for their most appropriate use. Palm *et al*. (2001) proposed four classes of organic resources. Class I contains materials with high N (> 25 g/kg), low soluble polyphenol (< 40 g/kg), and low lignin (< 150 g/kg) content and are proposed to be applied directly to the crop. Class II organic resources have a high N (> 25 g/kg) and a high polyphenol (> 40 g/kg) or a high lignin content (> 150 g/kg), whereas class III organic resources have a low N (< 25 g/kg), a low polyphenol (< 40 g/kg), and a low lignin content (< 150 g/kg). Resources of Class II and III are proposed to be mixed with either fertilizer or class I organic resources to obtain optimal yields. Class IV organic resources have a low N (< 25 g/kg) and a high lignin content (> 150 g/kg) and are advised to be applied as surface mulch. Application of organic resources in combination with N fertilizer resulted in an average N-AE value of 32 kg·grain·kg^{-1}·N (272 cases) (Table 1). Following formal statistical testing including all retained data-points, N-AE values were significantly higher for the treatments where fertilizer was combined with manure or compost (38 kg·grain·kg^{-1}·N), but all other organic resources did not affect N-AE values significantly

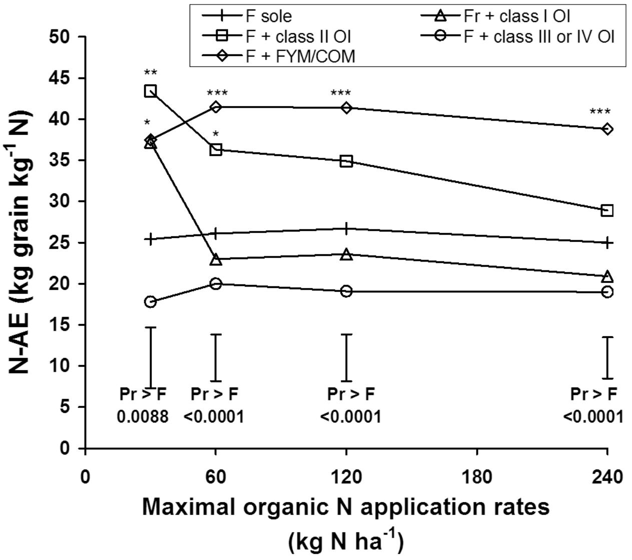


FIGURE 3. Agronomic efficiency of fertilizer N (N-AE) as affected by combination with different classes of organic inputs (Classes I, II, III + IV, and manure + compost) for organic N application rates ≤ 30 kg·N/ha (125 observations), ≤60 kg·N/ha (238 observations), ≤120 kg·N/ha (305 observations), or ≤240 kg·N/ha (352 observations). The classes are described in detail by Palm *et al.* (2001). Error bars are average standard errors of the difference. The symbols "*", "", and "***" indicate a significant difference with the sole fertilizer treatment at the 0.1, 1, and 5 percent level. In the legend, "F", "OI", "FYM", and "COM" refer respectively to fertilizer, organic inputs, manure and compost. Source: Vanlauwe *et al.* (2011).**

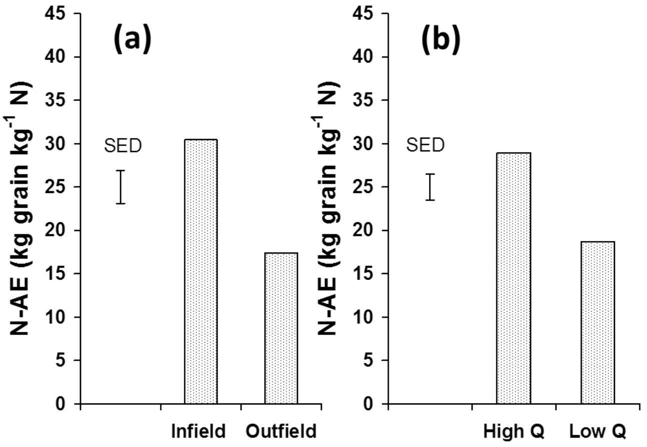


FIGURE 4. N fertilizer agronomic efficiency (N-AE) for paired comparisons between infields and outfields within single studies (a) and N-AE of fields with the maize control yields belonging to the lower (low Q) and upper quartile ranges (high Q) (b) across the three regions. SED refers to standard error of the difference. Source: Vanlauwe *et al.* (2011).

compared with the sole fertilizer treatment (25 kg·grain·kg^{-1}·N). However, when performing the statistical analysis on the data with maximum organic N application rates of 30 or 60 kg·N/ha, organic inputs belonging to Class II and manure/compost had significantly higher N-AE values than the sole fertilizer treatment or the Classes I and III/IV organic inputs (Figure 3). At higher organic N application rates, only the treatment with manure/compost gave significantly higher N-AE values than the sole fertilizer treatment (Figure 3).

N-AE as affected by targeting within-farm soil fertility gradients

The average N-AE for infields (28 cases) was 33 kg·grain·kg^{-1}·N (Table 1). When analysing studies where infields and outfields were included in the same study, average no-input control yields were 2 300 kg/ha for the infields and 1 400 kg/ha for the outfields and average N-AE values were 31 kg·grain·kg^{-1}·N for the infields and 17 kg·grain·kg^{-1}·N for the outfields (Figure 4a). When considering the lower and upper quartiles for the no-input control yields from all the sole fertilizer data, thereby assuming that the yield in the no-input control plots is a good indicator for soil fertility status, N-AE values were found to be consistently higher for the upper (29 kg·grain·kg^{-1}·N) than for the lower quartile (19 kg·grain·kg^{-1}·N) (Figure 4b).

Proof of concept

The above evidence from maize producing areas in Africa supports the main concepts underlying ISFM. Inclusion of improved maize germplasm, combining organic inputs of Classes II or manure/compost in combination with fertilizer and targeting fertilizer to responsive infields was shown to enhance N-AE substantially compared with N-AE values obtained under farmer management. Consequently, the basic principles underlying ISFM are well founded and good entry points for substantial improvements in N-AE. However, due to the high levels of variation inherent to meta-analysis and the lack of experimental designs that include all components embedded in ISFM, a consistent, multi-locational design is required, involving all ISFM components in an unbiased way, to obtain the optimal N-AE adapted to specific biophysical conditions across agricultural landscapes and to develop site-specific recommendations for fertilizer management.

Integrated soil fertility management in practice

Principles embedded within the definition of ISFM need to be applied within existing farming systems. Two examples clearly illustrated the integration of ISFM principles in existing cropping systems: (i) dual purpose grain legume–maize rotations with phosphorus (P) fertilizer targeted at the legume phase and N fertilizer targeted at the cereal phase in the moist savannah agro-ecozone (Sanginga *et al.*, 2003), and (ii) micro-dose fertilizer applications in legume–sorghum or legume–millet rotations with retention of crop residues and combined with water harvesting techniques in the semi-arid agro-ecozone (Bationo *et al.*, 1998; Tabo *et al.*, 2007).

As for the grain legume–maize rotations, application of appropriate amounts of mainly P to the legume phase ensures good grain and biomass production and biological N_2 fixation, the latter in turn benefiting a subsequent maize crop and thus reducing the need for external N fertilizer (Sanginga *et al.*, 2003). Choosing an appropriate legume germplasm with a low harvest index will favour accumulation of organic matter and N in the non-harvested plant parts and choosing adapted maize germplasm will favour a matching demand for nutrients by the maize. Application of a sufficient amount of legume crop residues can also improve other soil conditions, thus leading to enhanced fertilizer N use efficiency (Sanginga *et al.*, 2003). Selection of fertilizer application rates based on local knowledge of the initial soil fertility status within these systems would qualify the soil management practices as complete ISFM.

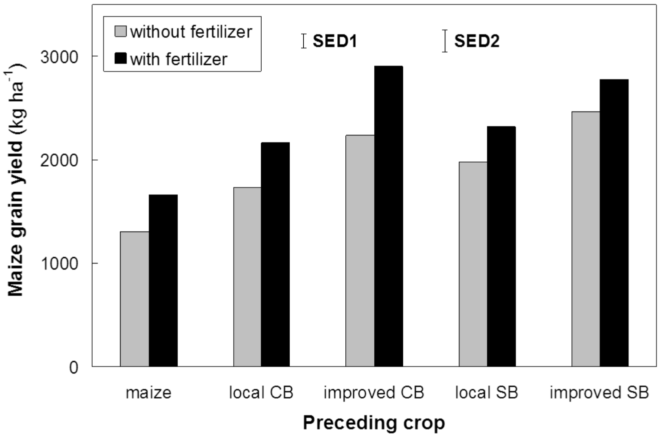


FIGURE 5. Maize grain yield as affected by application of compound fertilizer (NPK, 17:17:17) at 100 kg/ha and the crop grown in the preceding season (maize, climbing beans (CB) or soybean (SB) in South Kivu, DR Congo. SED — standard error of difference; * — significant at p < 0.05; ** — significant at p<0.01. There was no significant fertilizer × preceding crop interaction. Source: adapted from Pypers *et al.* (2012).

Pypers *et al*. (2012) observed a significant effect of previous cropping on maize yields both with and without fertilizer in field demonstrations in South Kivu, DR Congo (Figure 5). Yields of maize following soybean or climbing beans were 27–57 percent higher than those of maize following maize. Rotational benefits were also greater when improved, dual-purpose legume varieties with a low harvest index were grown. These legumes gave similar grain yields to local varieties (not shown), but grain yields of following maize crops were 20–34 percent higher than those of maize following local legume varieties. These yield improvements were related to greater biological N_2 fixation in the improved legumes, which derived a greater proportion of N from the atmosphere (due to their longer growing period relative to local varieties) and gave a higher biomass yield. Independently, application of compound fertilizer (N:P: potassium [K], 17:17:17) increased maize yields by between 22 and 39 percent. Combining crop rotation and fertilizer application resulted in yield increases up to 120 percent relative to the unfertilized maize–maize rotation, and a mean fertilizer value:cost ratio of 2.7.

As for the micro-dose technology, spot application of appropriate amounts of fertilizer to widely spaced crops such as sorghum or millet substantially enhances its use efficiency with further enhancements obtained when combined with physical soil management practices aiming at water harvesting. Recycling crop residues can reduce wind and water erosion (Bationo *et al*., 1998), and thus further benefit growth and nutrient demand of a following cereal. Rotating a legume such as cowpea, with sorghum or millet has been shown to further increase cereal yields (Aune and Bationo, 2008).

Integrated soil fertility management versus conservation agriculture: Different steps along an intensification gradient?

Adoption of conservation agriculture in Africa and the quest for organic inputs

Success with adoption of CA under smallholder farming conditions in SSA has been limited by a number of important constraints to widespread adoption, with the lack of organic resources to provide sufficient surface mulch consistently ranking amongst the top constraints, often related to low crop productivity and thus a low amount of crop residues and/or competing uses for organic resources (Giller *et al.*, 2009). The consequence of the above phenomena is that at planting of a subsequent crop, the area covered with crop residues from the previous crop is often below the 30 percent required, even if all crop residues are recycled. In central Kenya, for instance, Guto *et al*. (2012) showed that with maize yields below 2.5 t/ha, surface cover was less than 30 percent at the onset of the season. Aggregated maize yields in SSA are below 2 t/ha (www.fao.org), and assuming a harvest index of 45 percent, stover yields are thus expected to be usually below 2.5 t/ha.

Even before the current widespread promotion of CA, several attempts were made to enhance the availability of organic resources in smallholder farms, mainly driven by the search for low-input agricultural practices in the realm of "organic" or "green" agriculture and the widespread belief that fertilizer use was unrealistic for African smallholder farmers. Examples include alley cropping systems (e.g. Kang *et al.*, 1985), integration of herbaceous legumes (e.g. Carsky *et al.*, 2001), and biomass transfer systems (e.g. Gachengo *et al.*, 1999). Over time, adoption of the above practices has been disappointing, commonly due to the lack of immediate benefits to farmers who adopted them. With the recent interest in CA, some of these options are being reconsidered to provide the organic resources required to engage in CA, often placing CA amongst "green technologies". Obviously, earlier constraints to adoption of these practices are still valid when tested within the context of CA. Moreover, CA is not a green technology since fertilizer and herbicides are commonly used agro-inputs in areas were CA has taken off.

Fertilizer use as a fourth principle for conservation agriculture in Africa

Since the Abuja Fertilizer Summit in 2006, fertilizer use has regained emphasis in the context of agricultural intensification in Africa, but with a specific focus on maximizing the use efficiency and profitability of these agro-inputs. Consequently, one of the major arguments that led to the focus on low-input agriculture has lost relevance. Fertilizer, when applied appropriately, has been demonstrated to increase crop yields substantially in smallholder farming conditions. "Appropriate" refers to application of the right type of fertilizer at the right rate, time, and place (Zingore and Johnston, 2013), and accompanied by the appropriate agronomic practices (e.g. time of planting, crop spacing, weed control). "Appropriate" also refers to avoiding non-responsive soils or soils on which crops do not respond to the application of standard fertilizer due to other constraints limiting crop growth besides the nutrients contained in the fertilizer (Vanlauwe *et al*., 2010).

If the lack of organic resources is a major constraint to adoption of CA by smallholder farmers and if the appropriate use of fertilizer results in substantial increases in crop productivity and the availability of crop residues, then a logical consequence is to advocate the use of fertilizer in the context of CA activities. More crop residues will also allow alternative uses of these residues while retaining the minimally required soil cover. Since crop productivity in smallholder systems is generally low, adding the appropriate use of fertilizer as a fourth principle of CA will ensure that the appropriate approaches are taken when promoting CA and that valuable farmers' time — which is a limited resource in "hoe and cutlass" agriculture — is prevented from being spent on interventions with limited scalability. Obviously, the models for promoting CA would need to change if fertilizer is considered as a principle equal to the other three principles with input supply and market output value chains becoming necessary conditions for success. In this context, alternative paradigms for intensification such as ISFM, which aims at assimilating best practices to maximize the use efficiency of fertilizer, could serve as the initial steps to take towards the application of CA, thus moving the latter from a 'competitive' to a 'complementary' paradigm.

The use of isotopic and nuclear techniques in integrated soil fertility management and conservation agriculture

Due to the complexity of different management factors such as crop residues, soil organic matter status and tillage that may affect nutrient and water use efficiency and hence agronomic efficiency, isotopic and nuclear techniques are important tools in ISFM and CA to elucidate the roles of these factors and their interactions and to assess N use efficiency of fertilizer and organic inputs, quantify biological N_2 fixation and the effects of crop residues on soil evaporation–plant transpiration, soil organic carbon and nitrogen dynamics (Nguyen *et al.*, 2011).

CONCLUSIONS

Intensification of agricultural systems is a must in many areas in SSA. With fertilizer use back on the agenda, the ISFM paradigm, aiming at maximising the agronomic efficiency of fertilizer and organic inputs, is valid for intensifying agriculture. The principles of ISFM have been demonstrated to increase the AE of fertilizer, and these principles have been shown to be amenable to major cropping systems in SSA with AE values relatively easily exceeding 30 kg·grain per kg·N applied — far above commonly observed values under farmer management of 19 kg·grain per kg·N. This paper also argues that there is an urgent need to advocate a fourth principle which is the appropriate use of inorganic fertilizer for operationalizing CA under smallholder conditions to enhance crop productivity and thus produce the required crop residues to ensure sufficient soil cover.

REFERENCES

Abuja Fertilizer Summit. 2006. *Abuja declaration on fertilizer for African green revolution*. African Union Special Summit of the Heads of State and Government, Abuja, Nigeria.

Aune, J.B. & Bationo, A. 2008. Agricultural intensification in the Sahel – the ladder approach, *Agricultural Systems*, 98: 119–125.

Bationo, A., Lompo, F. & Koala, S. 1998. Research on nutrient flows and balances in West Africa: State-of-the-art. *Agric. Ecosys. Envir.,* 71: 19–35.

Baudron, F., Andersson, J.A., Corbeels, M. & Giller, K.E. 2012. Failing to yield? Ploughs, conservation agriculture and the problem of agricultural intensification: An example from the Zambezi Valley, Zimbabwe. *J. Dev. Studies*, 48: 393–41.

Carsky, R.J., Becke, M. & Hauser, S. 2001a. Mucuna cover crop fallow systems: Potential and limitations. *In* G Tian, F. Ishida & J.D.H Keating, eds. *Sustaining soil fertility in West Africa*. SSSA Special Publication No. 58, pp. 111–136. Madison, USA.

Gachengo, C.N., Palm, C.A., Jama, B. & Othieno, C. 1999. Tithonia and senna green manures and inorganic fertilizers as phosphorus sources for maize in western Kenya. *Agroforest. Syst.,* 44: 21–36.

Giller, K.E., Witter, E., Corbeels, M. & Tittonell, P. 2009. Conservation agriculture and smallholder farming in Africa: The heretics' view. *Field Crops Res.,* 114: 23–34.

Govaerts, B., Verhulst, N., Castellanos-Navarrete, A., Sayre, K.E., Dixon, J. & Dendooven, L. 2009. Conservation agriculture and soil carbon sequestration: Between myth and farmer reality. *Crit. Revs. Plant Sci.,* 28: 97–122.

Guto, S.N., Pypers, P., Vanlauwe, B., de Ridder, N. & Giller, K.E. 2012. Socio-ecological niches for minimum tillage and crop-residue retention in continuous maize cropping systems in smallholder farms of central Kenya. *Agron. J.,* 104: 188–198.

Kang, B.T., Grimme, H. & Lawson, T.L. 1985 Alley cropping sequentially cropped maize and cowpea with leucaena on a sandy soil in Southern Nigeria. *Plant Soil*, 85: 267–277.

Landers, J.N. 2001. How and why the Brazilian zero tillage explosion occurred. *In* D.E. Stott, R.H. Mohtar & G.C. Steinhardr, eds. *Sustaining the global farm,* pp. 29–39. Selected Papers from the 10th International Soil Conservation Organization Meeting, Purdue University, USA, 24–29 May 1999.

Nguyen, M.L., Zapata, F., Lal, R. & Dercon, G. 2011. Role of nuclear and isotopic techniques in sustainable land management: Achieving food security and mitigating impacts of climate change. *In* R. Lal & B.A. Stewart, eds. *World soil resources and food security,* pp. 345-418. Boca Ratan, FL, CRC Press.

Palm, C.A., Gachengo, C.N., Delve, R.J., Cadisch, G. & Giller, K.E. 2001. Organic inputs for soil fertility management in tropical agroecosystems: application of an organic resource database. *Agric. Ecosys. Environ*., 83: 27–42.

Paul, B.K., Vanlauwe, B., Ayuke, F., Gassner, A., Hoogmoed, M., Hurisso, T.T., Koala, S., Lelei, D., Ndabamenye, T., Six, J. & Pulleman, M.M. 2013, Medium-term impact of tillage and residue management on soil aggregate stability, soil carbon, and crop productivity. *Agric. Ecosys. Environ.*, 164: 14–22.

Pypers, P., Sanginga, J.M., Kasereka, B., Walangululu, J.M. & Vanlauwe, B. 2012. Rotational benefits of improved dual-purpose soybean and climbing bean varieties in the highlands of South Kivu, DR Congo. *Field Crops Res.,* (in press).

Rockstrom, J., Kaumbutho, P., Mwalley, J., Nzabi, A.W., Temesgen, M., Mawenya, L., Barron, J., Mutua, J. & Damgaard-Larsen, S. 2009. Conservation farming strategies in East and Southern Africa: Yields and rain water productivity from on-farm action research. *Soil Tillage Res.,* 103: 23–32.

Rusinamhodzi, L., Corbeels, M., van Wijk, M.T., Rufino, M.C., Nyamangara, J. & Giller, K.E. 2011. A meta-analysis of long-term effects of conservation agriculture on maize grain yield under rain-fed conditions. *Agron. Sustain. Dev.,* 31: 657–673.

Sanginga, N., Dashiell, K., Diels, J., Vanlauwe, B., Lyasse, O., Carsky, R.J., Tarawali, S., Asafo-Adjei, B., Menkir, A., Schulz, S., Singh, B.B., Chikoye, D., Keatinge, D. & Rodomiro, O. 2003. Sustainable resource management coupled to resilient germplasm to provide new intensive cereal–grain legume–livestock systems in the dry savannah. *Agric. Ecosys. Environ.,* 100: 305–314.

Tabo, R., Bationo, A., Gerard, B., Ndjeunga, J., Marchal, D., Amadou, B., Annou, G., Sogodogo, D., Taonda, J.B.S., Hassane, O., Diallo M.K. & Koala, S. 2007. Improving cereal productivity and farmers' income using a strategic application of fertilizers in West Africa, *In* A. Bationo, B. Waswa, J. Kihara & J. Kimetu, eds. *Advances in integrated soil fertility management in sub-Saharan Africa: Challenges and opportunities,* pp. 201–208. The Netherlands, Kluwer Publishers.

Tittonell, P., Vanlauwe, B., Leffelaar, P.A., Rowe, E. & Giller, K.E. 2005a. Exploring diversity in soil fertility management of smallholder farms in western Kenya. I. Heterogeneity at region and farm scale. *Agric. Ecosys. Environ.,* 110: 149–165.

Vanlauwe, B., Bationo, A., Chianu, J., Giller, K.E., Merckx, R., Mokwunye, U., Ohiokpehai, O., Pypers, P., Tabo, R., Shepherd, K., Smaling, E., Woomer, P.L. & Sanginga, N. 2010. Integrated soil fertility management: Operational definition and consequences for implementation and dissemination. *Outlook on Agriculture*, 39: 17–24.

Vanlauwe, B., Kihara, J., Chivenge, P., Pypers, P., Coe, R. & Six, J. 2011. Agronomic use efficiency of N fertilizer in maize-based systems in sub-Saharan Africa within the context of integrated soil fertility management. *Plant Soil,* 339: 35–50.

Vanlauwe, B., Tittonell, P. & Mukalama, J. 2006. Within-farm soil fertility gradients affect response of maize to fertilizer application in western Kenya. *Nutr. Cycl. Agroecosyst.,* 76: 171–182.

Vanlauwe, B., Wendt, J. & Diels, J. 2001. Combined application of organic matter and fertilizer. *In* G. Tian, F. Ishida, & J.D.H. Keatinge, eds. *Sustaining soil fertility in West Africa,* SSSA Special Publication No. 58, pp. 247–280. Madison, USA.

Zingore, S. & Johnston, A. 2013. The 4R nutrient stewardship in the context of smallholder agriculture in Africa. *In* B. Vanlauwe, P. Van Asten & G. Blomme, eds. *Agri-ecological intensification of agricultural systems in the African highlands*. Earthscan, UK. (in press).

Zingore, S., Murwira, H.K., Delve, R.J. & Giller, K.E. 2007. Soil type, management history and current resource allocation: Three dimensions regulating variability in crop productivity on African smallholder farms. *Field Crops Res.,* 101: 296–305.

Towards Sustainable Land Management for Enhancing Food Security While Mitigating Climate Change Impacts: The Role of Nuclear and Isotopic Techniques

M. L. Nguyen[1]

ABSTRACT

The continuing need to enhance food security and reduce the impacts of climate change demands an action plan that leads to sustainable soil and water management. Nuclear and isotopic techniques (NITs) are used as tracers to understand the processes occurring in soil-water-plant systems and their complex interactions, and to provide comprehensive soil-water management technologies tailored to specific agro-ecosystems. One of the missions of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture is to assist IAEA and FAO Member States to establish their capacities to use nuclear-based methods to develop land (soil and water) management technologies for sustainable food security and conservation of natural resources. This paper provides an overview of the strategies/approaches and relevant NITs used by national agricultural research and development institutes in projects supported by the Soil and Water Management & Crop Nutrition (SWMCN) Subprogramme of the Joint FAO/IAEA Division to increase natural (soil and water) and agricultural (e.g. applied fertilizers) resource use efficiency, enhance soil quality and its resilience against degradation (e.g. through salinization and erosion) and minimize the impacts of climate change on soil and agricultural water resources. These activities are grouped into: (i) managing soils for enhancing crop production; (ii) preserving and protecting soil resources; (iii) managing soils for climate change adaptation and mitigation; and (iv) agricultural water management under climate change. This overview highlights several advances in the development and use of NITs and the successful transfer of these to Member States for sustainable management of soil and water resources in agroecosystems. Although NITs offer comparative advantages of high specificity, accuracy and sensitivity over conventional techniques, neither should be used in isolation; rather they should be integrated to maximize their potential in unravelling processes that influence the complexity of soil-water-plant interactions at scales ranging from field plots to the catchment (area-wide) level.

Keywords: *land management, integrated soil-water management, climate change adaptation, climate change mitigation, isotopic techniques.*

INTRODUCTION

Background Situation Analysis

The world is facing the unprecedented dual challenge of enhancing food security while ensuring environmental sustainability, in particular, the conservation of soil and water as well as plant and animal genetic resources. The present world population of seven billion is projected to exceed nine billion by 2050. Worldwide, land degradation is currently estimated at 1.9 billion ha and it is increasing at a rate of five to seven million ha each year (Lal, 2006). This degradation reduces productivity and biodiversity, damages natural resources and ecosystems, resulting in long term socio-economic and environmental impacts and leading ultimately to human migration and socio-political unrest (Bruinsma, 2003; UNEP, 2010). Besides these urgent issues, several environmental problems that can impact on land productivity and sustainable agricultural development also need to be addressed. These include: (i) increasing risks and impacts of climate change (CC) and variability in crop yield; (ii) rising energy demands, in particular non-renewable energy sources; (iii) expanding urbanization and industrialization; and (iv) deteriorating water and air quality.

To enhance the vital function of soil productivity and ensure adequate provision of soil-water ecosystem services, the following main strategies are required: (i) agricultural intensification on the best arable lands which are already being farmed to enhance food security with minimal environmental degradation; (ii) rational utilization of marginal lands; and (iii) combating land degradation and restoring degraded soils (Lal, 2000). A key element across all land types and an integral part of sustainable agriculture is the need to enhance soil quality for the environmental sustainability of agro-ecosystems (Karlen, Andrews and Doran, 2001). In this context, there is a strong necessity for innovative research to develop specific technologies that address the most strategically important issues of sustainable soil/land and water management in agro-ecosystems.

Objectives

In 1964, two United Nations Organizations, the Food and Agriculture Organization (FAO) and the International Atomic Energy Agency (IAEA) established the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture at the IAEA Headquarters in Vienna, Austria. The aim of this strategic partnership was to help Member States solve practical agricultural problems with nuclear technology through international co-operation in research, capacity building, labora-

---

[1] Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy, PO Box 100, A-1400 Vienna, Austria
E-mail address of corresponding author: m.nguyen@iaea.or

L.K. Heng, K. Sakadevan, G. Dercon and M.L. Nguyen (eds), Proceedings — International Symposium on Managing Soils for Food Security and Climate Change Adaptation and Mitigation. Food and Agriculture Organization of the United Nations, Rome, 2014: 11–18

tory support and information dissemination (IAEA, 2014a; FAO/IAEA, 2014a and b). To achieve this, its activities span five separate but interrelated areas: Soil and Water Management and Crop Nutrition (SWMCN), Plant Breeding and Genetics, Animal Production and Health, Insect and Pest Control and Food and Environmental Protection. Activities in each area are planned and implemented through a Headquarters-based Section and a Laboratory Unit located in Seibersdorf near Vienna, Austria (FAO/IAEA, 2014a and b).

Through Co-ordinated Research Projects (CRPs) involving scientists from developing countries, international institutions (e.g. Consultative Group on International Agricultural Research, CGIAR) and advanced research organizations, agricultural issues of regional or global significance are studied. Technologies obtained from CRPs and from the Laboratory are then transferred to IAEA and FAO Member Countries through Technical Co-operation Projects (TCPs) (FAO/IAEA, 2014a).

This paper provides an overview of the SWMCN Subprogramme's activities involving the use of nuclear and isotopic techniques (NITs) to address challenges/issues related to: (i) managing soils for enhancing food production; (ii) preserving and protecting soil resources; (iii) managing soils for counteracting the adverse effects of climate change; and (iv) agricultural water management under climate change. In particular, it highlights the main findings and lessons learned from these activities and outlines future trends for the way forward. It is not an exhaustive review and therefore only key publications are included in the reference section. Further information can be found at http://www-naweb.iaea.org/nafa/swmn/index.html (FAO/IAEA, 2014b).

MANAGING SOILS FOR ENHANCING FOOD PRODUCTION

In the late 1990's, CRPs using NITs were initiated to enhance soil fertility and the productive capacity of selected cropping systems in the main agro-ecological zones (AEZs) of the world (Nguyen *et al.*, 2011). The underlying philosophy in the implementation of these CRPs was to adopt an integrated approach to soil, water and nutrient management in addressing issues of major concern and relevance for intensifying crop production under dryland and irrigated conditions. The development of an integrated nutrient management package (involving both manufactured fertilizers and natural sources of nutrients such as rock phosphates, biological nitrogen fixation [BNF], animal and green manures, etc., along with the recycling of crop residues), resulted in a greater demand for the use of nitrogen-15 (^{15}N), phosphorus-32 (^{32}P) and sulphur-35 (^{35}S) isotopes as tracers, to develop efficient agronomic practices tailored to local conditions and specific cropping systems that improve nutrient use efficiency and enhance soil fertility (Chalk, Zapata and Keerthisinghe, 2002; Nguyen and Zapata, 2006; Nguyen, Zapata and Dercon, 2010). Significant advances were also made during the past decade in developing and applying variations in the natural abundance (isotopic signatures) of stable isotopes (deuterium (^{2}H), carbon-13 (^{13}C), ^{15}N, oxygen-18 (^{18}O) and sulphur-34 (^{34}S) to assess the dynamics of nutrients and water in soil-plant systems. These developments were possible due to advances in automated online systems for stable isotope ratio measurements in soil, plant, water and gas samples (Chalk, Zapata and Keerthesinghe, 2002).

One lesson learnt was the need to move away from discipline-oriented research and adopt more holistic croppping systems approaches involving combinations of nutrient sources and nutrient-water interactions (Nguyen *et al.*, 2011). In this way, the Subprogramme significantly improved both the development and transfer of results to beneficiaries (scientific community) and end users (farmers). Another valuable lesson was the importance of targeting cropping systems in the main AEZs. This facilitated the identification of management practices designed to mitigate major soil-related constraints to crop production. A major outcome of this approach was the realization that use of the best adapted crop genotypes to local soil/climate conditions was a key requirement for improving the productivity and sustainability of the cropping systems in question. These studies using NITs, which focused initially on the search for crop genotypes with superior nutrient use efficiency, later demonstrated the great potential of such genotypes for identifying suitable germplasm with tolerance to particular abiotic stresses (drought, flooding, soil N or P deficiency, soil salinity, aluminum [Al] toxicity, etc.). In this area, two CRPs were successfully implemented: one on selecting wheat and rice genotypes with increased plant-water use efficiency under water stress (drought and salinity) using the carbon (C) isotope discrimination (^{13}C versus ^{12}C) technique (IAEA, 2012), and the second on identifying food crop (cereal and legume) genotypes tolerant to soils of low N and P fertility status using ^{15}N and ^{32}P isotopic techniques (IAEA, 2013).

PRESERVING AND PROTECTING SOIL RESOURCES

Soil erosion and the associated deposition-sedimentation are natural landscape-forming processes. However, they can be accelerated by human activities (land use change, farm mismanagement, deforestation and overgrazing), resulting in negative impacts on agricultural production and the environment (IAEA, 2002). Land degradation through soil erosion is associated with the irretrievable loss of basic soil resources and it is therefore a major threat to ecosystem services such as water and biogeochemical cycles, biodiversity and plant primary productivity. While soil erosion is the predominant land degradation process occurring worldwide, more than 75% of the total agricultural land area affected by erosion is situated in the developing world (Lal, 2000). Globally, the economic costs of soil loss by erosion have been estimated at US$ 400 billion per yr (Pimentel *et al.*, 1995). However, it is also important to recognize that effective soil conservation measures can successfully counter soil erosion losses and make a significant contribution to environmental sustainability. Measuring and identifying sources of soil erosion play the key role in designing effective soil conservation measures. Reliable data on the rates and patterns of soil redistribution (erosion/sedimentation) are required to provide a comprehensive assessment of the magnitude of the erosion problem and to underpin soil conservation measures, including the assessment of their economic and environmental impacts. Existing conventional techniques for monitoring soil erosion are capable of meeting some of these requirements, but they have a number of important limitations including lack of spatial soil distribution and time-consuming measurements (Nguyen *et al.*, 2011). The quest for alternative techniques to complement existing conventional methods for both assessing soil erosion and to meet new requirements has directed attention to the use of fallout radionuclides (FRNs) and in particular caesium-137 (^{137}Cs) as tracers for documenting rates and spatial patterns of soil redistribution within landscapes (Ritchie and Mc Henry, 1990; IAEA, 1995).

Initial CRPs using FRNs focused on the refinement and standardization of the ^{137}Cs technique for its worldwide application in agricultural landscapes under a range of environmental conditions (Zapata, 2002 and 2003; Nguyen *et al.*, 2011). Results from these CRPs paved the way to extend the use of both the ^{137}Cs technique and other FRNs as tracers for soil erosion/sedimentation investigations. In a follow-up CRP conducted between 2002 and 2009 (Nguyen *et al.*, 2011), FRN techniques involving beryllium-7 (^{7}Be), ^{137}Cs and lead-210 (^{210}Pb) as soil/sediment tracers were further developed to document short-term (<30 d), medium-term (~40 yr) and long-term (~100 yr) average soil redistribution rates and patterns in the landscape under different local conditions (climate, soil, topography and land uses). This combined application demonstrated that these are powerful tools to assess the relative impacts of soil conservation measures on soil erosion and land degradation (Zapata and Nguyen, 2010; Dercon *et al*., 2012).

The IAEA is assisting developing Member States to establish and strengthen their human and institutional capacities for using FRNs reliably to minimize land degradation and enhance sustainable agriculture through both national and regional TCPs which provide expert services and laboratory quality assurance support. Currently there are 37 Member States using FRN techniques to address issues relating to sustainable land management. For example, a major regional Asia-Pacific TC project involving 14 countries has recently been completed. Here, FRN methodologies were used successfully to assess soil erosion and evaluate soil conservation measures as well as to better understand the link between soil redistribution and soil quality (e.g. soil organic matter) in the landscape. Similarly, since 2009, the IAEA has supported a regional Latin America TC project using environmental radionuclides as indicators of land degradation to enhance soil conservation and environmental protection in different ecosystems in order to ensure sustainable agricultural production and reduce the impacts of land degradation (Dercon *et al*., 2012).

In order to target cost-effective soil conservation measures, it is important to determine not only the extent but also the source of soil erosion. In an ongoing CRP entitled “Integrated isotopic approaches for area-wide precision conservation to control the impacts of agricultural practices on land degradation and soil erosion”, isotopic and conventional approaches are being integrated to support the implementation of precision conservation at catchment scales (Nguyen *et al*., 2011). The FRN techniques are also being applied to develop sediment budgets on an area-wide basis (catchment) over different timescales. Furthermore, compound specific stable isotope (CSSI; e.g. ^{13}C and ^{15}N in amino acids) techniques (Gibbs, 2008) are being used to identify sources of soils in sediments (fingerprints) and apportion their relative contribution from different land uses (FAO/IAEA, 2008; Dercon *et al*., 2014). Such integrated applications will help identify critical areas (hot spots) of soil loss and assist extension workers, policy makers, land managers and farmers to target appropriate soil conservation measures, thus providing effective guidelines for area-wide sustainable management of land and water resources in agro-ecosystems (FAO/IAEA, 2008).

Other widespread soil degradation processes severely affecting the productivity and ecosystem services of agricultural lands are soil salinization and acidification. In these cases, a win/win option is needed to enhance the sustainable management of the natural resource base and mitigate climate change impacts on land degradation. This can be achieved through the development, pilot testing and adoption of integrated approaches to soil-water-plant/animal management. A strategic analysis of the components of the system and identification of major constraints are required to formulate appropriate interventions. A core element is the search for plant genotypes tolerant to the major soil constraint, e.g. salt-tolerant trees, shrubs, forage or crop cultivars in salt-affected lands, whereas P-efficient and Al-tolerant crop genotypes are required in tropical acid soils. Nuclear and isotopic techniques play a key role in developing specific and cost-effective management practices and for monitoring changes in nutrient, water and soil quality as well as overall productivity in the crop-livestock farming production systems under consideration. This integrated approach has been successfully applied in several CRPs and TCPs implemented by the Subprogramme (IAEA, 2006; Sakavedan and Nguyen, 2010).

MANAGING SOILS FOR CLIMATE CHANGE

Sustainable intensification of agricultural production systems demands a combined approach to improve crop productivity for food security and at the same time restore soil quality and enhance its resilience against degradation and risks associated with climate change (CC) impacts. Conservation Agriculture (CA), which seeks to meet such requirements, is based on the following principles: (i) minimum mechanical soil disturbance by tillage/cultivation; (ii) the retention of a permanent organic cover on the soil surface; and (iii) the use of crop rotations/plant associations, including cover/green manure crops (FAO, 2014a and 2014b). Such CA systems are currently used in about 100 million ha to enhance the food security of smallholders in the developing world (Derpsch and Friedrich, 2009).

In a CRP entitled “Integrated soil, water and nutrient management in conservation agriculture”, the influence of soil, water and crop management practices on soil organic matter (SOM) accumulation and its subsequent impacts on soil water, nutrient and C dynamics were investigated using NITs in various cropping systems worldwide. The results demonstrated that CA can bring benefits such as increased soil moisture retention, BNF, N retention and soil C sequestration. However, these effects were highly variable and site-specific, in some cases the benefits being negated by the influence of crop residues on plant diseases which could reduce crop yields and quality. One of the major lessons, with great implications for adoption strategies, is that CA can only be sustainable and successfully implemented if local constraints such as soil compaction, low soil fertility and lack of SOM are first removed (Dercon et al., 2010). Further research is therefore needed to develop and pilot test specific packages of integrated technologies and practices tailored to targeted agro-ecological zones and local agronomic management. Furthermore, this information is essential for a comprehensive assessment of socio-economic and environmental benefits and the development of appropriate policies to facilitate and encourage the adoption of CA by farmers. With this in mind, a new challenging CRP was initiated in 2011 (FAO/IAEA, 2014b) to investigate the potential of mulch-based cropping systems to enhance soil resilience against degradation and climate change risks and to increase soil fertility for sustainable food production in sub-Saharan Africa (SSA). Stable isotope techniques (15N and 13C) at enriched and/or natural abundance levels enabled an in-depth analysis and understanding of basic soil biological-physical processses, including soil C and nutrient cycling in mulch-based cropping systems. The selection and characterization of benchmark sites will provide a platform for extrapolation of results to other relevant agro-ecological zones in SSA (FAO/IAEA, 2014b).

AGRICULTURAL WATER MANAGEMENT UNDER CLIMATE CHANGE

Although the main focus of this Symposium was on soils and food security and climate change adaptation/mitigation, it is of utmost importance to also pay due attention to water because of soil-water interactions (Nguyen *et al.,* 2011) and their influence not only on food production but also on the provision of essential ecosystem services (UNEP, 2011). At present, agriculture uses around 70% of the total fresh water resources in the world. Rainfed agriculture accounts for 60% of world food production while the remaining 40% comes from irrigated agriculture. Managing agricultural water to enhance crop water productivity (more crop per drop) and water use efficiency in crop production systems is therefore of paramount importance. Studies conducted in CRPs and TCPs using NITs and related techniques have demonstrated that there is considerable scope to improve water use efficiency and crop productivity in rainfed agriculture. This can be achieved through the appropriate integration of soil-water-plant technological options such as water conservation practices (e.g. zero or reduced tillage, mulching, crop residue retention, crop rotation, inter-cropping), water harvesting techniques, use of crop varieties adapted to drought and saline conditions and improvement of soil fertility (IAEA, 1998). In a recently completed CRP entitled "Managing irrigation water to enhance crop productivity under water limiting conditions: A role for isotopic techniques", integrated soil-water-plant approaches and recent advances in isotopic techniques were used to better manage irrigation water for enhancing crop productivity under water limiting conditions. Stable isotopes of water (^{18}O and ^{2}H), soil moisture neutron probes and related conventional techniques (e.g. micro-lysimetry) were used to quantify soil evaporation (E) and plant transpiration (T) fluxes at different stages of crop development. Significant advances were made using NITs to partition crop evapo-transpiration (ET) into its E and T components for developing management practices that reduce E and improve the amount of biomass produced per unit of transpiration (Heng *et al*., 2014). Data generated in the project were also used to validate FAO's AquaCrop model for developing improved irrigation water, soil and crop agronomic practices to enhance crop water productivity and increase water use efficiency (Heng *et al*., 2009; Steduto *et al*., 2009; FAO/IAEA, 2014b). In addition, a CRP using ^{2}H, ^{15}N and ^{18}O and related techniques is being implemented to investigate the use of water conservation zones (wetlands, riparian buffer zones and farm ponds) to optimize water and nutrient storage, to reduce agricultural pollutants from runoff and deep drainage and to increase food/water security within agricultural catchments (FAO/IAEA, 2014b; Sakadevan, Heng & Nguyen, 2014).

THE WAY FORWARD: TOWARDS SUSTAINABLE LAND MANAGEMENT IN AGRO-ECOSYSTEMS

Recognizing that environmental degradation, poverty reduction and food security are strongly intertwined, one of the key elements in the strategic planning documents of UN organizations such as the FAO Strategic Framework (2010–2019) and IAEA's Mid-term Strategy (2012–2017) is to support environmental sustainability (FAO, 2014c; IAEA, 2014b). The overall aim is to develop and implement activities which will help countries and regions achieve sustainable management and utilization of natural resources and thereby contribute to achievement of the Millennium Development Goals (MDGs) for poverty reduction, food security and environmental sustainability (UN, 2012).

One strategic objective of the FAO (FAO, 2014c) is the sustainable management of land, water and genetic resources and improved responses to global environmental challenges affecting agriculture. The underlying philosophy in developing strategies for the SWMCN Subprogramme is, therefore, to use NITs to address global issues of major concern and relevance to sustainable land and water management in agro-ecosystems (FAO/IAEA, 2007 and 2008; Nguyen *et al*., 2011), taking holistic and ecosystem approaches (Table 1).

Conceptual Framework of the Ecosystem Approach

Soil is a thin layer covering the earth's surface, which acts as an interface between the biosphere, lithosphere, hydrosphere and atmosphere. The biogeochemical processes occurring in the soil are essential driving forces for the cycling of elements/nutrients and other chemicals, thus influencing key ecosystem functions such as plant primary productivity and water and air quality (UNESCO-SCOPE, 2006; Totsche *et al*., 2010). Interactive processes affecting coupled cycling of these elements (C, N, P and S) are strongly influenced by increasing global energy demand. However, present knowledge of global cycling of the elements, particularly the global C cycle remains limited by uncertainties in relation to quantitative aspects of soil C storage, loss and dynamics. In this regard, NITs offer considerable potential to both address process-level topics for a better understanding of biogeochemical cycles and to assess the relative value and effectiveness of novel management technologies designed to remove constraints/limitations to soil quality for productivity and/or sustainability in agro-

TABLE 1. Trends and outlook in developing and adopting holistic and ecosystem approaches to agricultural production systems by the SWMCN Subprogramme of the Joint FAO/IAEA Division

Description	Initial	Transitional	Future
Goals	Food security	Food security Preservation/protection Natural resource base Climate change (CC) response strategy	Food security Water security Poverty reduction Environmental sustainability
Objectives	Food production	Food and feed/fodder production CC adaptation/mitigation Soil, land and water, genetic resources conservation	Food and biomass production CC preparedness (risk assessment), response and rehabilitation Combating land and water degradation Promoting biodiversity
Activities	Agriculture (crops)	Agriculture/Livestock	Agriculture/Livestock Horticulture/Forestry/Aquaculture/Fisheries Linkage to other economic sectors
Soil-water functions	Agricultural production inputs	Production and a range of ecosystem services (resilience and sustainable use)	Production and a full range of ecosystem services
Main issues	Integrated soil fertility management	Soil quality/soil health. Agricultural water management	Sustainable land and water use and management
Scale	Farmer's fields/plots	Farm/landscape/area-wide approach	Agricultural watersheds/catchments
Systems	Crop production	Conservation agriculture Cropping/farming systems in targeted AEZ	Climate smart agro-ecosystems and neighboring natural ecosystems

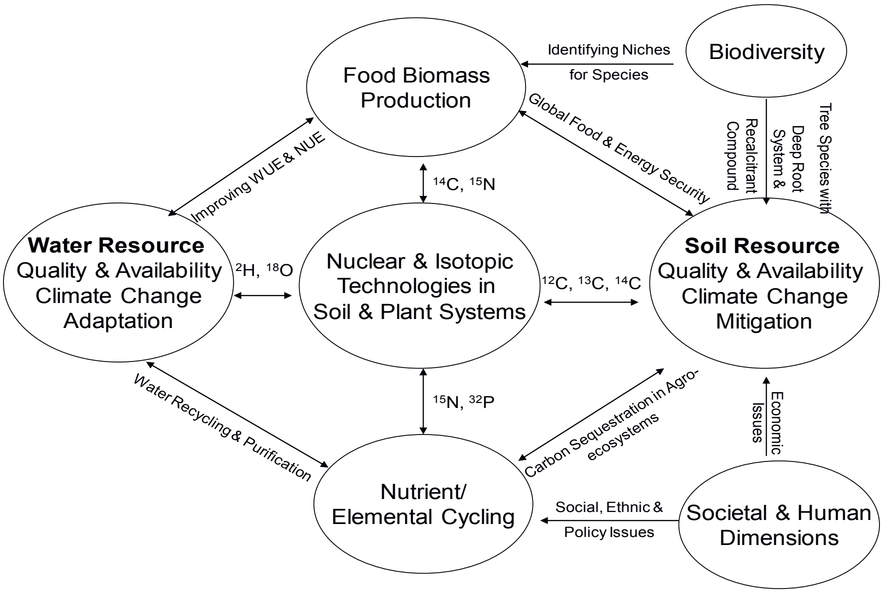


FIGURE 1. The role of nuclear and isotopic techniques in agro-ecosystems to address global issues of carbon sequestration to advance food security and mitigate climate change.

ecosystems (Nguyen *et al*., 2011). Moreover, this research is needed to provide technologies backed by scientific evidence so that land care practitioners/climate change policy makers can be informed of the merits of adopting such technological practices. Figure 1 shows the central role of NITs in addressing the important inter-related issues of food and biomass (feed, fodder, fibre and fuel) production, soil/land use and soil C sequestration and resilience of agro-ecosystems, nutrient/elemental cycling, and the declining availability and quality of water resources, as well as other relevant interactive factors (FAO/IAEA, 2007; Nguyen *et al*., 2011).

Ensuring food security and reducing the impacts from CC and variability necessitates a framework of actions for a multifunctional agriculture, addressing the urgent global issues of C sequestration (CC mitigation), greenhouse gas (GHG) emission minimization (CC mitigation), the agricultural system's resilience (CC adaptation) and food production. This approach represents a key requirement for the sustainable management of land and water resources (Lal, 2004). The primary focus of this multifunctional agriculture is to develop and adopt more holistic approaches, by integrating natural (soil and water) and genetic (plant and animal) resources to assess the impacts of food and biomass production (above-ground plant and below-ground root growth) on soil C sequestration and C budgets, while mitigating GHG emissions and enhancing resilience (adaptation) in agro-ecosystems.

Carbon Sequestration and Nutrient/Water Cycling Process-level Studies in Agro-ecosystems

Because of the need to stabilize the C stored in the soil, key mechanistic studies are required to obtain a better understanding of the processes and driving factors that control the dynamics (transformations/turnover) of specific compounds in soil organic carbon (SOC), as well as the functions of soil biota (biological component of soil quality). These studies demand the refined quantification of C, nutrient and water pools and fluxes in a given agro-ecosystem. Measurement of variations in the natural abundance of stable isotopes (^{2}H, ^{13}C, ^{15}N, ^{18}O, and ^{34}S) in components of the agro-ecosystem (soil organic matter, standing biomass, ground and surface water, atmospheric gases) can provide unique information on such pools and fluxes. This information is essential for the management of SOC sequestration in agricultural lands. For detailed information on the application of NITs in C sequestration studies, the reader is referred to selected publications (see Nguyen *et al*., 2011).

Recent developments also include novel isotopic techniques such as CSSI and advances in instrumental analytical chemistry and computational data acquisition/processing for isotope ratio measurements in soil, plant, water and gas samples (Crosson, 2008). Currently such techniques have been used to investigate the transformations and turnover of specific C compounds in soils and ecosystems (Glaser, 2005). Stable isotope probing (SIP) is a powerful technique in microbial ecological research. It allows the identification of *in situ* active microbial populations in the ecosystem that have been treated with ^{13}C or ^{15}N - labelled substrates, based on the incorporation of ^{13}C or ^{15}N into cellular biomarkers such as nucleic acids (DNA or RNA). The combined use of stable isotope techniques with biomarkers thus increases the potential to unravel the role of biodiversity in soil C cycling and improve understanding of the relationship between soil and vegetation in C modelling (Staddon, 2004; Amelung *et al*., 2008). However, this advanced technique needs to be further refined and the protocols harmonized for worldwide application in agricultural research. The advent of synchrotron facilities that accelerate electrons to almost the speed of light and the continuing development of synchrotron-based techniques (such as X-ray absorption, fluorescence and tomography) to improve spatial resolution and sensitivity offers exciting opportunities to unravel key processes and factors influencing soil-water-nutrient-plant-rhizosphere interactions (Lombi and Susini, 2009).

Applications for Sustainable Land Management in Agro-ecosystems

The design and implementation of successful soil and water management practices aimed at advancing food security and mitigating climate change requires integrated strategies (win/win options) to enhance the net rate of C sequestration in soils by increasing biomass production, enhancing soil quality and reducing GHG emissions from farm lands (Lal and Spruce, 1999). Conservation Agriculture has been proposed by FAO as an essential component of the action programme of the FAO-Global Soil Partnership to promote sustainable management of soil resources and improved governance for soil protection and sustainable productivity (FAO, 2014a and 2014b). Some of the benefits claimed for employing CA include increased SOM accumulation, enhanced soil fertility, improved soil water storage, reduced soil erosion and N leaching, better soil aggregation, enhanced resilience of farming systems to climate change and reduced GHG emissions (Lal, 2007; Govaerts et al., 2009). All of these interact to improve vital soil functions and ecosystem services, ensure agricultural productivity and promote systems sustainability, which consitute the basic principles of climate smart agriculture.

While initial CA investigations have focused on improving soil fertility and increasing agronomic crop yields, much research is still needed to assess CA benefits in terms of resource (water and fertilizer inputs and energy) use/conservation efficiency as well as its environmental impacts (SOC sequestration, GHG emissions and ecosystems services) to develop and promote novel climate-smart agricultural ecosystems in both rural and urban/peri-urban farming environments.

At present, area-wide (watershed scale) agricultural water management in both rainfed and irrigated agriculture and water quality are the most challenging issues for agricultural production systems worldwide. Integrating land and water resources management in agro-ecosystems is an essential requirement to tackle these issues (FAO/IAEA, 2007). The development and adoption of integrated water resources management in climate smart agro-ecosystems includes the design and implementation of a series of watershed studies on: (i) soil-water-plant interactions across the landscape; (ii) area-wide assessment of water use efficiency; and (iii) enhancement of water ecosystem services such as water capture-storage and reuse, wetlands construction/management, drought/flooding risk assessment and rehabilitation and non-point source (diffuse) pollution control. This approach involves a suite of NITs and conventional techniques and requires the formation of multi-disciplinary and often inter-institutional teams, thus demanding more effort in terms of networking, co-ordination and information technology.

Techniques that offer great potential include variations in the natural abundance of water stable isotopes, CSSIs, cavity ring-down laser absorption spectroscopy (CRD-LAS) and the cosmic ray soil moisture observing system (COSMOS). Area-wide agricultural water management also calls for the use of databases to integrate large and complex sets of data obtained under a range of agro-ecological conditions. Such data can also be used to validate and refine existing models, as well as to develop decision support systems to make better decisions about the technologies suitable for specific farming and catchment systems. Because of the nature of sustainability issues, long-term experiments are needed to develop and pilot test appropriate technologies over time. In view of the large spatial variability over short distances across the landscape, innovative area-wide (catchment/watershed) upscaling approaches and related geo-information systems and techniques (GIS and geo-statistical tools) are required to assess land and water connectivity issues and also the interactions between neighbouring natural ecosystems and agro-ecosystems (Nguyen *et al*., 2011).

In all cases it is envisaged that a core element of climate smart agro-ecosystems will be the development of soil-water-plant approaches for the identification/selection of best adapted crop genotypes to climate change impacts. In this context, there is great scope for interdisciplinary research between plant breeders and soil/water management specialists in the area of crop resilience to climatic change and resulting abiotic stresses (drought, flooding, salinity, variable nutrient/water availability, etc.). Research is needed to predict the evolutionary response of different species to climate change, i.e. extinction versus adaptation, and to develop best-fit genotypes for particular environments. Soil type matching plus appropriate "external" nutrient and water inputs could be the key to climate change adaptation. Isotopic techniques can be used in crop improvement programmes to examine the physiological response/adaptation of plant genotypes to climate variations and extreme events.

CONCLUSIONS

This overview describes relevant strategies/approaches and NITs used in the main project areas of the SWMCN Subprogramme to address soil and water management issues in crop production systems for food security and climate change adaptation/mitigation. These are outlined in terms of managing soils for enhancing crop production, preserving and protecting soils and managing soils and agricultural water under climate change. A conceptual framework for adopting an ecosystem approach, the potential strategies and the role of NITs as cutting edge tools for sustainable land and water management in agro-ecosystems are outlined. In view of the complexity of the systems and scale (area-wide) of the studies, a suite of approaches/strategies and NITs and related conventional techniques, databases and modeling will be needed. In addition, the adoption of more holistic approaches calls for more inter-disciplinary research, networking and co-ordination.

Like all other techniques, NITs have advantages and limitations. They offer the comparative advantages of high specificity, accuracy and sensitivity over conventional non-nuclear techniques. Because of these advantages, they generate quantitative data, providing direct answers to the questions posed and thus saving time and effort. However, certain preconditions have to be met to take advantage of the NITs chosen — in particular the availability of skilled and trained human resources and access to adequate instrumental analytical facilities. These techniques cannot be used in isolation, but rather must be integrated with others in national and/or regional research activities to maximize their potential in terms of achieving successful outcomes and people-centred benefits.

Human and institutional capacity-building, networking, co-ordination, information exchange and communication technologies are important tools in the development and application of NITs for sustainable land and water management in agro-ecosystems. Partnerships and innovative collaboration modalities such as involvement in the FAO Global Soil Partnership play important roles in fostering technology dissemination and enhancing developmental efforts. In this context, greater advocacy is needed for the key role of sustainable land management in achieving the MDGs of food security/poverty alleviation and environmental sustainability and their overall contribution to sustainable development.

ACKNOWLEDGEMENTS

To Mr. F. Zapata, a former staff member, for his excellent inputs to this manuscript and to Jim Dargie, Sharon Nguyen, Lee Heng and

Karuppan Sakadevan for editorial assistance. Also, my appreciation for the support from both current and former colleagues in the Soil and Water Management & Crop Nutrition Subprogramme who have contributed to the activities of the Subprogramme.

REFERENCES

Amelung, W.S., Brodowsky, S., Sandhage-Hofmann, A. & Bol, R. 2008. Combining biomarker with stable isotope analyses for assessing the transformation and turnover of soil organic matter. *Adv. Agron*., 100: 155–250.

Bruinsma, J. 2003. *World Agriculture: Towards 2015/2030: An FAO Perspective*. London, Earthscan Publications. 432pp.

Chalk, P.M., Zapata, F. & Keerthisinghe, G. 2002. Towards integrated soil, water and nutrient management in cropping systems: The role of nuclear techniques. *In Transactions 17th World Congr. Soil Science*, August 2002, CD-ROM, pp. 2164/1–2164/11, Bangkok, Thailand.

Crosson, E.R. 2008. WS-CRDS: Precision trace gas analysis and simplified stable isotope measurements. *Am. Lab*., Nov.-Dec. Issue: 37–41.

Dercon, G., Nguyen, M.L., Aulakh, M., Boddey, R., Dalal, R., Govaerts, B., Halitligil, M., Ibragimov, N., Ismaili, M., Lopez, S., Mohammad, W., Pulleman, M., Vanlauwe, B. & Zagal, E. 2010. Soil, water and nutrient management under conservation agriculture across agroecosystems worldwide. *In Proceedings 11th Eur. Soc. Agron. (ESA) Congr.*, Agro-2010, Montpellier. pp.437–438.

Dercon G., Mabit, L., Hancock, G., Nguyen, M.L., Dornhofer, P., Bacchi, O.O.S.,Benmansour, M., Bernard, C., Froehlich, W., Golosov, V.N., Haciyakupoglu, S., Hai,P.S., Klik, A., Li, Y., Lobb, D.A., Onda, Y., Popa, N., Rafiq, M., Ritchie, J.C., Schuller, P., Shakashiro, A., Wallbrink, P., Walling, D.E., Zapata, F. & X. Zhang. 2012. Fallout radionuclide-based techniques for assessing the impact of soil conservation measures on erosion control and soil quality: An overview of the main lessons learnt under a FAO/IAEA Coordinated Research Project. *J. Environ. Radioact.,*107: 78–85.

Dercon, G., Gibbs, M., Rasche, F., Brandt, C., Cadisch, G., Boeckx, P., Bodé, S., Hancock, G., Blake, W., Walling, D.E., Mabit, L., Benmansour, M., Lam, N., Lobb, D., Schuller, P., Golosov, V., Zhang, X., Li, Y., Froehlich, W., Asfary, A. & Nguyen, M.L 2014. Compound-specific stable isotope techniques for improving soil conservation strategies: An overview of the lessons learned from an FAO/IAEA coordinated research project. These Proceedings.

Derpsch, R. & Friedrich, T. 2009. Global overview of conservation agriculture adoption. *In* Lead Papers, *4th World Congr. Conservation Agriculture*, 4-7 February 2009, pp. 429–438. New Delhi.

FAO/IAEA. 2007. *Integrated soil-water-plant solutions for biomass production and environmental performance as influenced by climate change,* by M.L. Nguyen. Report of an FAO/ IAEA Consultants Meeting, Vienna, 12–14 November 2007. Unpublished.

FAO/IAEA. 2008. *Integrated approaches for the assessment of land use impacts on soil loss/sediment production and related environmental problems,* by M.L. Nguyen. Report of an FAO/IAEA Consultants Meeting, Vienna, 5–7 November 2007. Unpublished.

FAO. 2014a. *Conservation agriculture* (available at http://www.fao.org/ag/ca/).

FAO. 2014b. *Global soil partnership* (available at http://www.fao.org/nr/water/ landandwater_gsp.html).

FAO. 2014c. *Strategic framework 2010–2019* (available at http://www.fao.org/uploads/media/PC102-2-FC-128-Strategic Framework(English).pdf).

FAO/IAEA. 2014a. *Joint FAO/IAEA Food and Agriculture Programme* (available at http://www-naweb.iaea.org/nafa/index.html).

FAO/IAEA. 2014b. *Subprogramme Soil and Water Management and Crop Nutrition* (available at http://www-naweb.iaea.org/nafa/swmn/index.html).

Glaser, B. 005. Compound-specific stable isotope (δ^{13}C) analysis in soil science. *J. Plant Nutr. Soil Sci*., 168: 633–648.

Gibbs, M. 2008. Identifying source soils in contemporary estuarine sediments: A new compound specific isotope method. *Estuaries Coasts,* 31: 344–359.

Govaerts, B., Verhulst, N., Castellanos-Navarrete, A., Sayre, K.D., Dixon, J. & Dendooven, L. 2009. Conservation agriculture and soil carbon sequestration: Between myth and farmer reality. *Crit. Rev. Plant Sc*i., 28: 97–122.

Heng, L.K., Hsiao, T., Evett, S., Howell, T. & Steduto, P. 2009. Validating the FAO AquaCrop model for irrigated and water deficient field maize. *Agron. J.*, 101: 488–498.

Heng, L.K., Hsiao, T. C., Williams, D., Fereres, E., Cepuder, P., Denmead, T., Amenzou, N., Dhan, N., Duong, H., Kale, S., Li, B.G., Mahmood, K., Mei, X.R., Phiri, E., Zingore, S. & Nguyen, M.L. 2014. Managing irrigation water to enhance crop productivity under water-limiting conditions: A role for isotopic techniques. These Proceedings.

IAEA. 1995. *The use of fallout radionuclide measurements in soil erosion measurements*, by D.E:Walling & T. Quine. Proceedings Internat. Symp. Nuclear Techniques in Soil-Plant Studies for Sustainable Agriculture and Environmental Preservation, pp. 597–619. IAEA STI/PUB/947. Vienna.

IAEA. 1998. *Management of nutrients and water in rainfed arid and semi-arid areas.* IAEA-TECDOC-1026. Vienna. 66 pp.

IAEA. 2002. *Recent advances in the use of environmental radionuclides in soil erosion investigations*, by D.E. Walling. *In* Proceedings Internat. Symp. Nuclear Techniques in Integrated Plant Nutrient, Water and Soil Management, pp. 279–301. IAEA-CSP-11P. Vienna.

IAEA. 2006. *Management practices for improving sustainable crop production in tropical acid soils.* IAEA Proceedings Series, STI/PUB/1285. Vienna. 351 pp.

IAEA. 2012. *Greater agronomic water use efficiency in wheat and rice using carbon isotope discrimination*. IAEA-TECDOC-1671. Vienna. 280 pp.

IAEA. 2013. *Optimizing productivity of food crop genotypes in low nutrient soils*. IAEA-TECDOC-1721. Vienna. 327 pp.

IAEA. 2014a. *Our work* (available at http://www.iaea.org/OurWork).

IAEA. 2014b. *Medium term strategy for 2012–2017* (available at http://www.iaea.org/About/mts.html).

Karlen, D.L., Andrews, S.S. & Doran, J.W. 2001. Soil quality: Current concepts and applications. *Adv. Agron*., 74: 1–40.

Lal, R. 2000. Soil management in the developing countries. *Soil Sci.,*165: 57–72.

Lal, R. 2004. Soil carbon sequestration impacts on global climate change and food security. *Science* 304: 1623–1627.

Lal, R. 2006. *Encyclopaedia of Soil Science*. Second Edition, Boca Rattan, Fl., CRC Press. 1959 pp.

Lal, R. 2007. Farming carbon. *Soil Till. Res.,* 96: 1–5.

Lal, R. & Spruce, J.P. 1999. The potential of world cropland soils to sequester C and mitigate the greenhouse effect. *Environ. Sci. Policy,* 2: 177–185.

Lombi, E. & Susini, J. 2009. Synchrotron-based techniques for plant and soil science: Opportunities, challenges and future perspectives. *Plant Soil,* 320: 1–35.

Nguyen, M.L. & Zapata F. 2006. Use of nuclear techniques in addressing soil-water-nutrient issues for sustainable agricultural production. *In Proceedings 18th World Congr. Soil Sci.*, 9-15 July 2006, CD-ROM Abstracts — Workshop Pre-Congr. Session No.1–1, Philadelphia, USA.

Nguyen, M.L., Zapata, F. & Dercon, G. 2010. Zero tolerance on land degradation for sustainable intensification of crop production. *In* P. Zdruli, M. Pagliai, S. Kapur & F. Faz Cano, eds. *Land degradation and desertification: Assessment, mitigation and remediation*, pp. 37–47. Dordrecht, Heidelberg, New York, London, Springer.

Nguyen, M.L., Zapata, F., Lal, R. & Dercon, G. 2011. Role of isotopic and nuclear techniques in sustainable land management: Achieving food security and mitigating impacts of climate change. *In* R. Lal, & B.A. Stewart, eds. *World Soil Resources and Food Security. Adv. Soil Sci.*, 18: 345–418. Boca Raton, Fl., CRC Press.

Pimentel, D., Harvey, C., Resosudarmo, P., Sinclair, K., Kurtz, D., McNair, M., Cris, S., Shpritz, L., Fitton, L., Saffouri, R. & Blair, R. 1995. Environmental and economic cost of soil erosion and conservation benefit. *Science,* 267: 1117–1123.

Ritchie, J.C. & Mc Henry, J.R. 1990. Application of radioactive fallout cesium-137 for measuring soil erosion and sediment accumulation rates and patterns: A review. *J. Environ. Qual.,* 19: 215–233.

Sakavedan, K. & Nguyen, M.L. 2010. Global response to soil and water salinization in agricultural landscapes. *In* R.P. Thomas, ed. *Proceedings Global Forum on Salinization and Climate Change,* CD-ROM Paper, pp. 119–125. Valencia.

Sakadevan, K., Heng, L. & Nguyen, L. 2014. Water conservation zones in agricultural catchments for biomass production, food security and environmental protection. These Proceedings.

Staddon, P.L. 2004. Carbon isotopes in functional soil ecology. *Trends Ecol. Evol*., 19: 148–154.

Steduto, P., Hsiao, T.C., Raes, D. & Fereres, E. 2009. AquaCrop - The FAO crop model to simulate yield response to water: I. Concepts and underlying principles. *Agron. J.* 101: 426–437.

Totsche, K.U., Rennert, T., Gerzabek, M.H., Kogel-Knabner, I., Smalla, K., Spiteller, M. & Vogel, H.J. 2010. Biogeochemical interfaces in soil: The interdisciplinary challenge for soil science. *J. Plant Nutr. Soil Sci.,* 173: 88–99.

United Nations. 2012. *UN millennium development goals* (available at http://www.un.org/ millenniumgoals/).

UNEP. 2010. *The United Nations System-wide Earthwatch* (available at http://earthwatch.unep.ch/ emergingissues/desertification/landdegradation.php).

UNEP. 2011. *An ecosystem services approach to water and food security*. Nairobi, UNEP and Colombo, IWMI. (available at http://www.unepdhi.org/~/media/Microsite_UNEPDHI/Publications/ documents/unep).

UNESCO-SCOPE. 2006. *The global carbon cycle*. UNESCO-SCOPE Policy Briefs, October 2006, No. 2. Paris. (available at http://unesdoc.unesco.org/images/0015/001500/150010e.pdf).

Zapata, F., ed. 2002. *Handbook for the assessment of soil erosion and sedimentation using environmental radionuclides*. Dordrecht, the Netherlands, Kluwer Academic Publishers, 221 pp.

Zapata, F., ed. 2003. Field application of the Cs-137 technique in soil erosion and sedimentation studies. Special issue. *Soil Till. Res.,* 69: 1–162.

Zapata, F. & Nguyen, M.L. 2010. Soil erosion and sedimentation studies using environmental radionuclides. *In* K. Froehlich, ed. *Environmental radionuclides - tracers and timers of terrestrial processes*, pp. 295–322, Elsevier B.V.

SESSION 1

MANAGING SOILS FOR CROP PRODUCTION AND ECOSYSTEM SERVICES

Contributions of Fertilizer Nitrogen in Global Cereal Production, Soil Organic Matter Status and Nitrogen Balance

J.K. Ladha[1,*]

ABSTRACT

Presently, 50 percent of the human population relies on synthetic nitrogen (N) fertilizer for food production. In the subsistence agriculture of the pre-chemical era, biological N_2 fixation (BNF) was the primary source of reactive N but, in recent years, chemical N fixation (synthetic N) has become more important in global agriculture. Today, the Haber-Bosch and cultivation-induced BNF processes of converting N_2 from the atmosphere to ammonia introduce reactive N of over 100 Tg N/year into the global environment to increase food production (Galloway *et al*., 2004). Although this has sustained the large human population of Earth in meeting dietary needs, a large population in the world still lacks available N to sustain crop production. This together with increasing population obviously means that the future global demand for reactive N is bound to grow substantially (Cassman *et al.,* 2003; Wood, Henao and Rosegrant, 2004). However, since a substantial amount of N created for food production is lost to the environment, this has also greatly increased the contribution of reactive N to a wide variety of environmental problems (Galloway *et al.,* 2004; Vitousek *et al.,* 2010). Unlike nonreactive gaseous N_2, reactive N has magnified the adverse effects because the same atom of N can cause multiple effects in the atmosphere, in terrestrial ecosystems, in freshwater and marine systems, and on human health. This paper (i) analyses the global consumption and demand for fertilizer N in relation to cereal production, (ii) evaluates the nitrogen-15 (^{15}N) and N difference methods to determine synthetic N recovery efficiency in current and succeeding crops grown across agro-climatic regions, (iii) examines long-term use of N on the sustenance of soil organic matter (SOM), (iv) constructs global N balances, and (v) analyses various strategies available to improve the overall use efficiency of N.

Key words: *cereals, N fertilizer, recovery efficiency, biological N_2 fixation, use efficiency, soil organic matter.*

GLOBAL N CONSUMPTION AND DEMAND FOR MAJOR CEREALS

During and after the Green Revolution, synthetic nitrogen (N) fertilizer has played a crucial role in increasing crop productivity to alleviate the ever-increasing food insecurity caused by a worldwide increase in population. Since the 1960s, the application of synthetic N fertilizer to fertilizer-responsive and lodging-resistant short-stature cultivars of cereals boosted food production by about 260 percent or an average growth of about 6.4 percent per year. Today, fertilizer N supplies approximately 45 percent of the total N input for global food production and world use is around 100 million metric tons (Mt) (FAO, 2010). Currently, about 52 percent of global N fertilizer consumed worldwide is used for the world's three most important cereal (rice, wheat and maize) production (Ladha, 2010). The N application rate (kg/ha) showed a curvilinear time trend with averages of 80.4, 65.7 and 52.4 in maize, rice and wheat, respectively (Figure 1). In 2009, maize and rice approached similar N application rates (109.4 vs 98.3 kg/ha) followed by wheat (83.5 kg/ha). It is projected that to meet the global cereal demand of three billion tons (t) by 2050 and with projected increase of 7 percent in harvested area, fertilizer application rates to the three cereals will have to increase on average by about 54 percent assuming no change in N use efficiency. In terms of total global synthetic N use, it would increase from 51.8 Tg in 2009 to 85.6 Tg in 2050 (Ladha *et al.,* unpublished).

FERTILIZER N RECOVERY EFFICIENCY BY MAJOR CEREALS

Nitrogen fertilizers are expensive inputs, costing agriculture more than US$50 billion per year. Most agricultural crops use fertilizer N inefficiently. Trials with major cereals (maize, rice and wheat) show recovery efficiencies of fertilizer N varying from 44 to 55 percent (Table 1). Our review of published data showed that the average

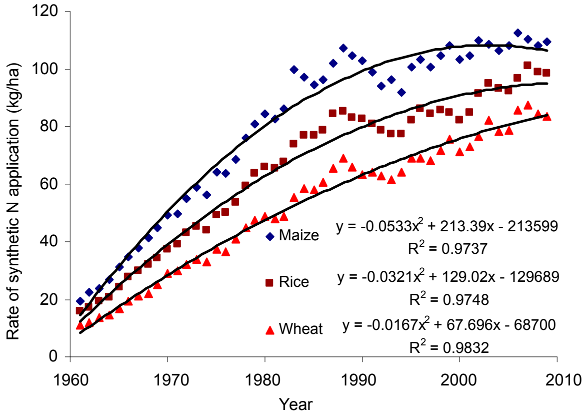


FIGURE 1. Trends in global averages of fertilizer N application rates to maize, rice and wheat.

---

1 International Rice Research Institute, DPS Marg, Pusa, New Delhi - 110 012, India

* E-mail address of corresponding author: j.k.ladha@irri.org

L.K. Heng, K. Sakadevan, G. Dercon and M.L. Nguyen (eds), Proceedings — International Symposium on Managing Soils for Food Security and Climate Change Adaptation and Mitigation. Food and Agriculture Organization of the United Nations, Rome, 2014: 21–24

Table 1. Range of N use efficiency for cereals in various regions

Region / Crop	RE[*] Mean	RE^{15}N[**] Mean
Africa	0.63	0.37
Australia	0.46	0.41
Europe	0.68	0.61
America	0.52	0.36
Asia	0.50	0.44
Average / total	0.55	0.44
Maize	0.65	0.40
Rice	0.46	0.44
Wheat	0.57	0.45
Average / total	0.55	0.44

[*] RE = recovery efficiency of fertilizer N based on total plant N (kg N taken kg^{-1} N applied)

[**] RE ^{15}N = recovery of ^{15}N-labelled fertilizer N based on total plant N (kg N taken kg^{-1} N applied)

recovery efficiencies across all regions and crops was seven percent lower when estimated by the nitrogen-15 (^{15}N) dilution method than by the N-difference method.

The amount of fertilizer N that remains available for subsequent crops can only be quantified with the use of labelled ^{15}N fertilizer. In a limited number of studies, the uptake of residual fertilizer was monitored for several growing seasons. The IAEA (2003) reported that the average accumulated recovery of ^{15}N fertilizer by subsequent crops during five growing seasons amounted to 6.5 percent, which is equal to 16 percent of the total fertilizer N recovered during the first growing season. With an average ^{15}N fertilizer recovery (RE^{15}N) of 44 percent in the first growing season (Ladha *et al*., 2005), the total recovery of ^{15}N fertilizer, including the recovery by the five subsequent crops, is approximately 50 percent.

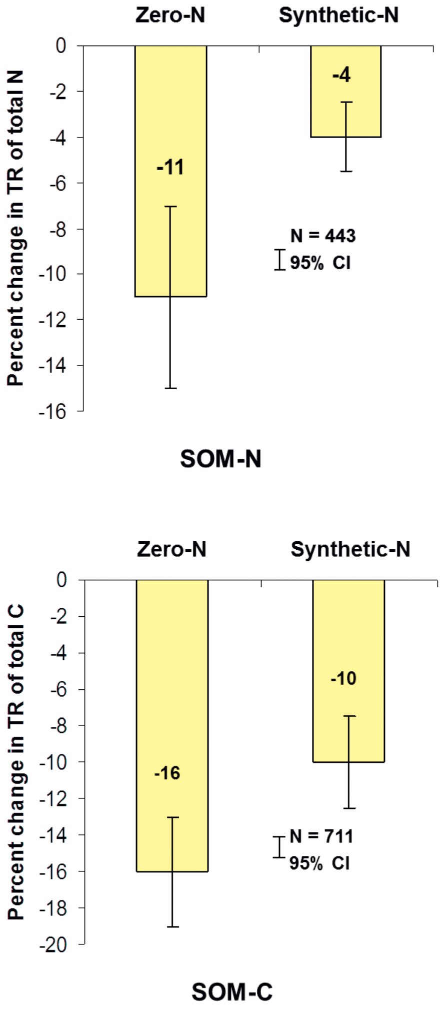


FIGURE 2. Meta analysis of change in time response ratio of SOM-N and SOM-C: A global analysis of 114 long-term experiments.

ROLE OF N FERTILIZER IN SUSTAINING SOIL ORGANIC MATTER IN CEREAL CULTIVATED SOILS

Soil organic matter (SOM) is essential for sustaining the food production and maintaining ecosystem services and is a vital resource base for storing N. The impact of long-term use of synthetic-N fertilizer on SOM, however, has been questioned recently. We tested the hypothesis that long-term use of synthetic fertilizer-N results in a decrease in SOM. We analysed peer-reviewed data from 100 long-term field experiments with controlled N fertilizer treatments representing a wide range of climatic zones, soil types, crops, and management practices. Results demonstrate dramatic world-wide declines of 7–16 percent of SOC and 7–11 percent of SOM with no N amendments. In soils receiving synthetic fertilizer N, the rate of SOM loss decreased (Ladha *et al*., 2011). There was an average increase of respectively 8 percent and 12 percent for SOC and SOM following the application of synthetic fertilizer N treatments compared with no N fertilizer applied (Figure 2). On the other hand, long-term application of an organic source either alone or in combination with synthetic fertilizer N consistently increased both soil carbon (C) and N ranging from 9–34 percent over control (Ladha *et al*., 2011). Among the land types, flooded dryland (i.e. rice–wheat rotation) responded better to organic amendment than dryland–dryland (i.e. maize–wheat) or flooded–flooded (i.e. rice–rice). Although synthetic N fertilizers showed a lower potential to improve the long-term N-supplying capacity than organic matter, both amendments are vital to increase the intrinsic capacity of agricultural soils to sustain crop productivity. The flooded soils showed higher SOM content than the soils going through flooding and dry conditions. Increases in SOC and SOM content in flooded soils is attributed to increased C input (Tanji *et al*., 2003; Kogel-Knabner *et al*., 2010) in combination with flooded conditions, leading to a reduction in the rate of decomposition of plant material caused by a lack of oxygen (Neue *et al*., 1997). Increased non-symbiotic BNF in these flooded rice systems also leads to higher SOC and SOM (Ladha *et al*., 2000; Pampolino *et al*., 2008). When rice is grown in rotation with wheat (lowland–upland system), soil is exposed to an increase in aeration, leading to a higher loss of SOM, which can ultimately lead to a reduction in the cropping systems' sustainability (George *et al*., 1992; Ladha *et al*., 2011). As these cropping systems developed inherently low SOM content, they also became more responsive to organic amendments.

GLOBAL N BALANCE IN MAIZE, RICE AND WHEAT CROPPING SYSTEMS

Since there is a continual loss of reactive N in an agro ecosystem, an important question arises as to whether the system is reaching

N disequilibrium. An agro ecosystem would be in N equilibrium if the sum of N inputs equaled the sum of N outputs. Among various inputs and outputs, inputs from BNF and synthetic N sources and outputs through crop harvest and losses are the most important. Therefore, constructing N balance sheets is the key to both increasing our understanding of N transformation and N transfers, and to quantifying the size of various N reservoirs that ultimately are needed to conserve N in various transformations and biological processes of the system (Legg and Meisinger, 1982). Many efforts have been made to construct N balances but they were limited to small scale greenhouse, lysimeter, and field studies. Our ability to integrate various loss and gain processes and to construct N balances at a higher level such as a food production system on a global scale continue to be an obvious gap in our knowledge. Greenland and Watanabe (1982) identified three difficulties associated with the origin of the enigma: the difficulty in measuring the change in total N content of a given mass of soil, the difficulty in measuring the amount of N added to the soil–plant system by BNF and the difficulty in measuring losses of N from a soil–plant system. However, since then, major progress has been made in all three areas in terms of developing methodologies and generating relatively accurate numbers of various components of N gains and N losses required to construct N balances in different agricultural systems. Using a wealth of knowledge accumulated over the years, global N balance sheets covering 49 years (1961–2009) were constructed for rice, wheat, and maize production systems (Ladha *et al.*, unpublished). Results show that a significant amount of crop N demand by the world's major cereals is met from sources other than synthetic fertilizer N and inherent soil N reserves. It appears that the majority of this third source of N input is from non-symbiotic N fixation and recycling of N. The results highlight the need to consider all sources of N (synthetic, soil organic reserve and non-symbiotic N fixation) when designing strategies to improve N use efficiency.

STRATEGIES TO IMPROVE THE N FERTILIZER USE EFFICIENCY

Reducing N losses through achieving synchrony between crop demand and supply from soil and other sources is most crucial to achieving high levels of N use efficiency. There is an inverse relationship between loss and synchrony of N (Figure 3). Compared with plant genetic improvement, the resource management approach is likely to have more losses of N and less synchrony between supply and demand. If N losses are to be eliminated completely from a soil–plant system, a regulated N supply through a plant fixing its own N is an ideal approach. If BNF could be assembled in cereals, it would amplify the potential for N supply because fixed N would be available to the plant directly, with little or no loss. However, management of fertilizer N which has played and will continue to play important role in meeting the N demand of food crops would be of primary importance.

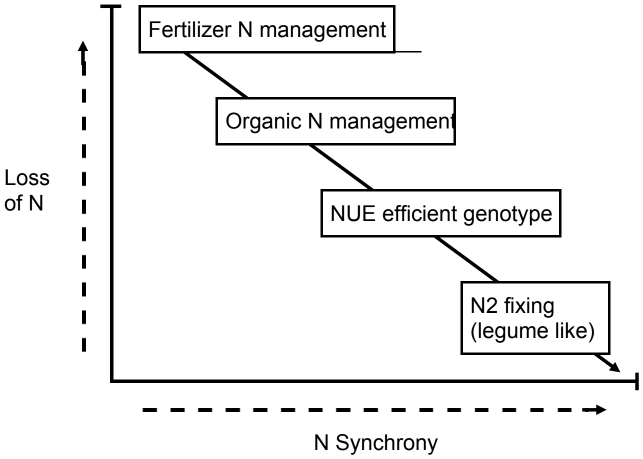


FIGURE 3. N loss – N synchrony relationship for various strategies.

TABLE 2. Comparative evaluation of tools/tactics of enhancing fertilizer N use efficiency

Tools/tactics	Benefit:cost	Limitations
Site-specific N management	High	Has to be developed for every site, infrastructure required
Chlorophyll meter	High	Initial high cost
Leaf colour chart	Very high	None
Plant analysis	High	Facilities need to be developed
Controlled-release fertilizer	Low	Low profitability and lack of interest by industry
Nitrification inhibitors	Low	Low profitability and lack of interest by industry
Fertilizer placement	High	Lack of equipment, labor-intensive
Foliar N application	High	Lack of equipment, risk involved
Breeding strategy	Very high	Varieties yet to be developed
N-fixation in non legumes	High	Technology yet to be developed for field scale
Models and decision support systems	Medium	Tools are not available
Remote-sensing tools	Low	Technology needs to be fine-tuned
Geographic information systems	Low	Technology needs to be fine-tuned
Precision farming technology	High	Technology needs to be fine-tuned
Resource-conserving technologies	High	Technology needs to be evaluated for long-term impacts
Integrated crop management	High	Technology needs to be evaluated for long-term impacts

Source: modified from Ladha *et al.* (2005)

Removing plant growth-limiting factors would increase crop demand for N, leading to a greater use of available N and consequently higher efficiency (Ladha *et al.,* 2005). This is possible by adopting fertilizer, soil, water, and crop management practices that will maximize crop N uptake, minimize N losses and optimize indigenous soil N supply including non-symbiotic N fixation. Management decisions that increase fertilizer N use by crops can focus on two approaches: increase fertilizer N use during the growing season when the fertilizer is applied, and decrease fertilizer N losses, thereby increasing the potential recovery of residual fertilizer N by the subsequent crops. Approaches suggested for increasing NUE include optimal time, rate, and methods of application for matching N supply with crop demand; the use of specially formulated forms of fertilizer, including those with urease and nitrification inhibitors;

the integrated use of fertilizer, manures, and/or crop residues; and optimizing irrigation management. In addition, some modern tools such as precision farming technologies, simulation modeling, decision support systems, and resource-conserving technologies also help to improve NUE. A full account of these strategies can be found elsewhere (Ladha *et al.,* 2005). In large-scale agriculture practised in industrialized countries, precision farming studies have demonstrated that variable-rate N fertilizer application has the potential to reduce significantly the amounts of N required to achieve yields similar to those obtained with standard uniform management practices. In agriculture with small to medium farm sizes in developing countries, the use of a simple and inexpensive leaf colour chart assists farmers in applying N when the plant needs it. The use of this simple tool has been shown to reduce misuse of fertilizer N. An analysis of various strategies to improve NUE together with their cost/benefit assessment and limitations is provided in Table 2.

CONCLUSIONS

The primary function of synthetic fertilizer N is to provide the crop with an immediately available source of available N, often the most limiting nutrient for plant growth. The secondary function is to reduce the decline in SOM content, a function which has long-term consequences on the sustainability of the systems as SOM plays multiple roles in maintaining soil quality and ecosystem services. Recent N balance studies also show that although about half of the synthetic N leaks out resulting in adverse impacts on the environment, a positive augmentative role of synthetic N in the overall economy of cereal production is to be recognized. The results therefore highlight the need to consider all N sources (synthetic, soil organic reserve and non-symbiotic N fixation) when designing strategies to improve N use efficiency.

REFERENCES

Cassman, K.G., Dobermann, A., Walters, D.T. & Yang, H. 2003. Meeting cereals demand while protecting natural resources and improving environmental quality. *Ann. Rev. Environ. Resour.,* 28: 315–358.

FAO. 2010. *FAO statistical databases*. (http:/apps.fao.org/).

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R. & Vorosmarty, C.J. 2004. Nitrogen cycles: Past, present, and future. *Biogeochemistry,* 70: 153–226.

George, T., Ladha, J.K., Buresh, R.J. & Garrity, D.P. 1992. Managing native and legume fixed N in lowland rice-based cropping systems. *Plant Soil,* 141: 69–91.

Greenland, D.J. & Watanabe, I. 1982. The continuing nitrogen enigma. *Transactions 12th International Congress Soil Science 5,* pp. 123–137, New Delhi, India.

IAEA. 2003. *Management of crop residues for sustainable crop production.* IAEA-TECDOC-1354, Vienna.

Kogel-Knabner, I., Amelung, W., Cao, Z., Fiedler, S., Frenzel, P., Jahn, R., Kalbitz, K., Kolbl, A. & Schloter, M. 2010. Biogeochemistry of paddy soils. *Geoderma,* 157: 1–14.

Ladha, J.K. 2010. Managing nitrogen for crop productivity and environmental quality. *Proceedings Fertilizer Association of India Annual Seminar*, New Delhi.

Ladha, J.K. Dawe, D., Ventura, T.S., Singh, U., Venture, W. & Watanabe, I. 2000. Long-term effects of urea and green manure on rice yields and nitrogen balance. *Soil Sci. Soc. Am. J.,* 64: 1933–2001.

Ladha, J.K., Pathak, H.P., Krupnik, T.J., Six, J. & Kessel, Chris V. 2005. Efficiency of fertilizer nitrogen in cereal production: Retrospect and prospects, *Adv. Agron.,* 87: 85–156.

Ladha, J.K., Reddy, C.K., Padre, A.T. & Kessel, Chris V. 2011. Role of nitrogen fertilization in sustaining organic matter in cultivated soils. *J. Environ. Qlty.,* 40: 1756–1766.

Legg, J.O. & Meisinger, J.J. 1982. Soil nitrogen budgets. *In* E.J. Stevenson, ed. *Nitrogen in agricultural soils – Agronomy.* Agronomy Monograph 22, pp. 503–566. Madison, USA, American Society of Agronomy, Crop Science Society of America, Soil Science Society of America.

Neue, H.U., Grant, J.L., Wang, Z.P., Heidmann, Becker P. & Quijano, C. 1997. Carbon in tropical wetlands. *Geoderma,* 79: 163–185.

Pampolino, M.F., Laureles, E.V., Gines, H.C. & Buresh, R.J. 2008. Soil carbon and nitrogen changes in long-term continuous lowland rice cropping. *Soil Sci. Soc. Am. J.,* 72: 798–807.

Tanji, K.K., Gao, S., Scardaci, S.C. & Chow, A.T. 2003. Characterizing redox status of paddy soils with incorporated rice straw. *Geoderma,* 114: 333–353.

Vitousek, P.M., Naylor, R., Crews, T., David, M.B., Drinkwater, L.E., Holland, E., Johnes, P.J., Katzenberger, J., Martinelli, L.A., Matson, P.A., Nziguheba, G., Ojima, D., Palm, C.A., Robertson, G.P., Sanchez, P.A., Townsend, A.R. & Zhang, F.S. 2010. Nutrient imbalances in agricultural development. *Science,* 324: 1519–1520.

Wood, S., Henao, J. & Rosegrant, M. 2004. The role of nitrogen in sustaining food production and estimating future nitrogen fertilizer needs to meet food demand. *In* **A. Mosier, J.K. Syers & J.R. Freney,** eds. *Agriculture and the nitrogen cycle – assessing the impacts of fertilizer use on food production and the environment*, pp. 245–260. Scope Series, Washington, Island Press.

Ammonia Volatilization Losses from Urea Fertilizer Applied on Wheat

M.M. Terrada[1,*], L. Benavides[1] and S.C. López[1]

ABSTRACT

Ammonia (NH_3) emissions represent a major loss of nitrogen (N) in agricultural production. A field experiment was performed in the Pampean region to evaluate the effect of different urea rates applied to wheat (*Triticum aestivum* L.) on NH_3 losses through volatilization using a direct method (^{15}N as tracer) and an indirect method (N-difference). Volatilization was quantified using static semi-open N-NH_3 collectors during eight d after fertilization with urea N at rates of 0, 50 and 100 kg/ha (W0, W50 and W100) in 2008, and W0 and W100 during 52 d in 2009. In both years, NH_3 volatilization correlated with soil accumulated temperature during the first eight d after fertilization and the percent of N derived from fertilizer (%Ndff) increased markedly between 24–48 h after fertilization, especially in W100, where %Ndff reached 100 percent. The total NH_3-N losses during the whole wheat growing season were 6.3, 7.4 and 9.6 kg/ha for W0, W50 and W100 in 2008, and 7.3 and 13.4 kg/ha for W0 and W100 in 2009, being significantly higher in W100. The N-NH_3 losses, in proportion to the fertilizer applied, were 2.2 and 3.3 percent for W50 and W100 using the indirect method, and 0.9 percent and 3.3 percent for W50 and W100 by the direct method, and 6.2 percent for W100 by both methods in 2009. There were no significant differences between methods. Volatilization under wheat increased with rate of urea application but the losses were less than 6.5 percent of the applied fertilizer.

Key words: *nitrogen-15, ammonia volatilization, urea, %Ndff, wheat, Pampean region.*

INTRODUCTION

Nitrogen (N) is a dynamic and mobile element which can undergo substantial losses in agricultural systems. Nitrogen is lost by leaching of nitrate (NO_3^-), surface runoff, volatilization of ammonia (NH_3), emissions of nitrous oxide (N_2O), nitric oxide (NO) and nitrogen dioxide (NO_2) and dinitrogen (N_2) gas (Delgado, 2002). Ammonia emissions also represent a major loss of nutrient N (Rochette *et al.*, 2009) and also result in degradation of air and water quality (Galloway *et al*., 2003).

Wheat is one of the most important crops in Argentina, but efficient use of fertilizer and conservation tillage are required to increase or maintain productivity without damaging the soil and the environment (Melaj *et al*., 2003). The most popular fertilizer used for wheat production is urea, which is transformed to NH_3 in the soil and can be volatilized to the atmosphere. After surface application, urea is quickly hydrolyzed within 1–2 d by urease to ammonium (NH_4^+), hydroxyl (OH^-) and carbonate (CO_3^{-2}) ions, leading to a high pH and very high concentrations of NH_4^+ around the urea granule; consequently, it increases the gaseous NH_3 losses to the atmosphere (Zaman *et al.*, 2008). The extent of NH_3 losses depends mainly on soil factors and the season of year (Videla *et al.*, 1994).

The aims of this study were to evaluate the effect of different urea rates applied to wheat on the losses of NH_3 through volatilization using two different methods, namely, a direct method (using ^{15}N as tracer) and an indirect method (N-difference) and to evaluate the relation between NH_3 volatilization and soil temperature.

MATERIALS AND METHODS

A field experiment was performed during the 2008 and 2009 spring wheat (*Triticum aestivum* L.) growing seasons in an experimental field at the Centro Atómico Ezeiza, Comisión Nacional de Energía Atómica, Buenos Aires, Argentina (34º49´S, 58º34´W). The soil was classified as a typical Argiudoll with 13.7 percent clay, 47.5 percent silt and 38.8 percent sand. Within the 0–10 cm layer the soil bulk density was 1.07 g/cm^3, pH was 6.8, organic matter was 4.5 percent and extractable P was 46 µg·P·g^{-1}. The experimental site was located in the Pampean region.

The experimental design was fully randomized blocks with four replications. Different N fertilization rates were applied to wheat grown under conventional tillage on each block and receiving: 0, 50 and 100 kg N-urea per ha (W0, W50 and W100) during 2008; and 0 and 100 kg N-urea (W0 and W100) during 2009. The wheat (Baguette 13, Nidera) was sown in August 2008 and in July 2009 with a row spacing of 15 cm and a sowing density of 6 × 10^6 seeds/ha, and was harvested in December 2008 and 2009. The N fertilizer was applied at tillering in September of both years.

Ammonia volatilization was measured over eight d in 2008 and over 52 d in 2009. To quantify the N volatilized derived from fertilizer (Ndff) and from soil (Ndfs), a micro-plot of 5 × 5 m was established on each fertilized plot and received nitrogen-15 (^{15}N) enriched urea (5% ^{15}N atom excess) as a tracer.

Volatilization of NH_3 was quantified using static semi-open NH_3-N collectors, adapted from Nömmik (1973), Videla *et al.* (1994) and Lara Cabezas *et al*. (1999). The collectors consisted of PVC cylindrical structures 0.5 m high and 0.15 m in diameter. They were inserted into the soil at a depth of 0.10 m and had two absorber foams soaked with 35 ml of 0.25M sulphuric acid (H_2SO_4) solution with 3% (v/v) glycerine. The upper foam absorbed atmospheric NH_3 coming from outside the collector and protected the lower foam, which retained the NH_3 volatilized from the soil surface inside the collector.

In each plot, an NH_3 collector was inserted into the soil immediately after fertilization and periodically the lower foam from each

---

[1] División Agronómica, Centro Atómico Ezeiza, Comisión Nacional de Energía Atómica, Presbítero González y Aragón 15, CP B1802AYA, Ezeiza, Provincia de Buenos Aires, Argentina

[*] E-mail address of corresponding author: maltert@cae.cnea.gov.ar

L.K. Heng, K. Sakadevan, G. Dercon and M.L. Nguyen (eds), Proceedings — International Symposium on Managing Soils for Food Security and Climate Change Adaptation and Mitigation. Food and Agriculture Organization of the United Nations, Rome, 2014: 25–28

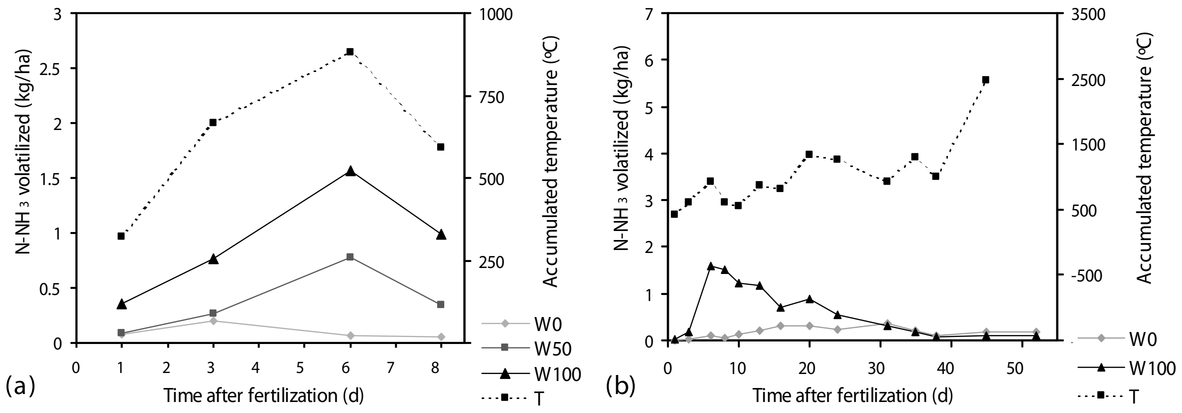


FIGURE 1. Ammonia volatilized and soil accumulated temperature (T) between sampling after fertilization in W0, W50 and W100 in 2008 (a) and in W0 and W100 in 2009 (b).

collector was removed to measure NH_3 retained; it was replaced immediately by new foam. In fertilized plots, the NH_3 collector was inserted inside the ^{15}N labelled micro-plots. The same methodology was used to recover NH_3 in unfertilized plots or $^{15}NH_3$ in fertilized plots. The NH_3 or $^{15}NH_3$ retained was removed by two washings with 200 ml of deionized water using a shaker and shake for 30 min. The extract was acidified with 1 ml of 0.1 M H_2SO_4 and concentrated at 50ºC to a volume of 50 ml. The NH_3 in the extract was determined by Kjeldahl distillation and titration. The ^{15}N-NH_3 was analysed for N isotope ratios with an optical emission spectrometer NOI 6PC (Fisher Analysen Instrumente GmbH, Leipzig, Germany).

The percent N derived from fertilizer (% Ndff) was calculated as follows (IAEA, 2001):

$$\%\text{Ndff} = \frac{\text{atom }\%^{15}\text{N excess (foam)}}{\text{atom }\%^{15}\text{N excess (urea)}} \times 100$$

The soil temperature of each plot was measured with a soil temperature sensor and data logger (Logger16, Cavadevices, Argentina), to evaluate the relation between NH_3 volatilization and the soil accumulated temperature between sampling dates.

RESULTS AND DISCUSSION

In both growing seasons, NH_3 volatilization in wheat fertilized plots showed an increasing trend during the first six d and then began to diminish (Figures 1a and 1b).

During 2008, NH_3 volatilization correlated ($r^2 = 0.92$) with changes in soil accumulated temperature between sampling dates, as has been reported by other authors (Barbieri *et al.,* 2010). Total N-NH_3 volatilized during the first eight d after fertilization were 0.4, 1.5 and 3.7 kg·N-NH_3·ha^{-1} for W0, W50 and W100, respectively, with significant differences between W0 and W100 ($p < 0.01$) and between W50 and W100 ($p < 0.05$). Cumulative NH_3 losses were calculated using these values. In order to estimate volatilization during the whole wheat growing season the baseline volatilization measured in W0 was considered to be occurring in all treatments before and after the measurement period.

The total N-NH_3 losses were 6.3, 7.4 and 9.6 kg/ha for W0, W50 and W100, respectively, being significantly higher in W100 plots ($p < 0.01$).

The %Ndff increased markedly between 24 and 48 h after fertilization, especially in W100, with all volatilized N derived from applied fertilizer reaching values of 100 percent 72 h after fertilization (Figure 2). Sainz Rozas *et al.* (1997) described a similar situation in a soil with an initial pH of 5.8, because the highest rate of volatilization would occur when the increase in soil pH due to urea hydrolysis reaches its maximum value, two or three d after fertilization. On average, the weighted percentages of N losses by volatilization derived directly from fertilizer during the eight d were 33 percent and 90 percent for W50 and W100, respectively, with significant differences due to fertilization rates ($p < 0.05$). These values were the equivalent of 0.4 and 3.3 kg·N-NH_3·ha^{-1} as Ndff during the eight d after fertilization.

In the 2009 season, the NH_3-N losses by volatilization during the first eight d were 3 kg/ha by either the indirect or direct method, without a significant difference between these two methodologies and between different wheat seasons. However, volatilization of NH_3 continued to be higher ($p < 0.05$) in fertilized plots than in non-fertilized plots until 16 d after fertilization (Figure 1b). As NH_3 volatilization in wheat fertilized plots decreased with time, and soil temperature began to increase in relation to external climatic conditions (beginning of spring), accumulated volatilization correlated with changes in soil accumulated temperature between sampling dates only during the first eight d after fertilization ($r^2 = 0.73$), as in the previous season.

The amounts of N-NH_3 volatilized during the 52 d after fertilization were 2.4 and 8.6 kg/ha for W0 and W100, being significantly higher in the fertilized plots ($p < 0.01$). The total N-NH_3 losses during the wheat season were 7.3 and 13.4 kg/ha for W0 and W100, respectively.

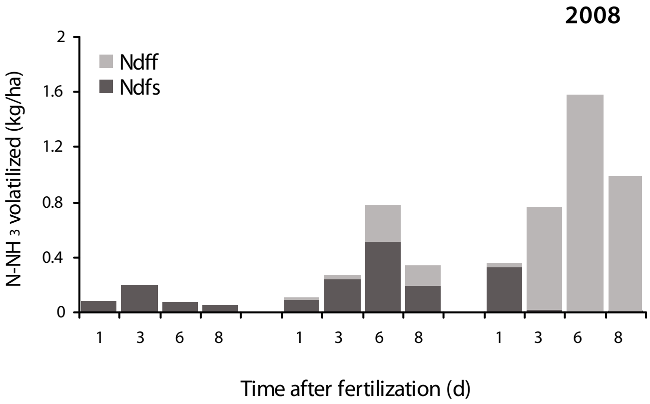


FIGURE 2. Ammonia volatilized derived from fertilizer (Ndff) and from soil (Ndfs) in the 2008 season.

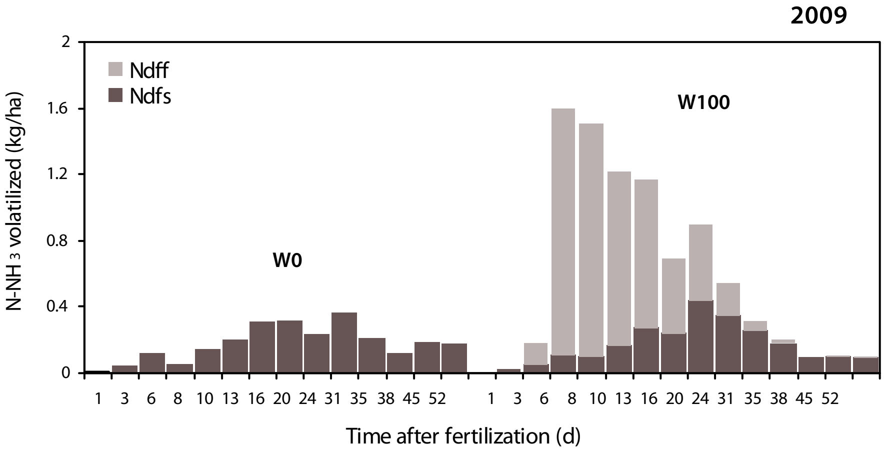


FIGURE 3. Ammonia volatilized derived from fertilizer (Ndff) and from soil (Ndfs) in the 2009 season.

TABLE 1. The N-NH_3 losses by volatilization during eight d (2008) and 52 d (2009) after fertilization in proportion to the fertilizer applied (%), by the ^{15}N method and the indirect method without ^{15}N

2008	^{15}N method	Indirect method
W50	0.9 ± 0.9 aA	2.2 ± 1.9 aA
W100	3.3 ± 1.5 aB	3.3 ± 1.5 aA
2009	**^{15}N method**	**Indirect method**
W100	6.2 ± 2.0 a	6.2 ± 2.0 a

The same lower case letter in a line indicates no significant differences ($p < 0.05$).
The same upper case letter in a column in 2008 indicates no significant differences ($p < 0.05$)

As in the previous season, the %Ndff increased markedly between 24 and 48 h after fertilization and the volatilized N derived from applied fertilizer reached values of almost 100 percent (Figure 3). On average, the weighted percentage of N losses from volatilization derived directly from fertilizer during the 52 d was 71 percent for W100. The Ndff during the 52 d after fertilization was 6.2 kg/ha, almost twice the amount registered during eight d in 2008.

A comparison between the percentage fertilizer lost by volatilization using the indirect method and the ^{15}N method was made (Table 1). The indirect method, also called the N-difference method, evaluated the apparent volatilization in fertilized plots by the difference between NH_3-N volatilized from fertilized and non-fertilized plots. Differences between methods were not significant in either year. During 2008, estimates of NH_3-N losses as a proportion of fertilizer applied using the indirect method were respectively 2.2 and 3.3 percent for W50 and W100. When ^{15}N was used as tracer, the NH_3-N losses as a proportion of fertilizer applied were 0.9 percent and 3.3 percent for W50 and W100, respectively (Table 1).

During 2009, estimates by both methods produced the same value, i.e. 6.2 percent of applied fertilizer was lost by volatilization over 52 d (Table 1) although the ^{15}N dilution method may lead to different results than the indirect or N-difference method when evaluating N use efficiency (Ladha *et al.*, 2005).

Although relatively expensive, ^{15}N techniques usually provide results that have lower variability and are of higher sensitivity, resulting in more precise information in a shorter period of time (IAEA, 2008). In this case, variability was the same with both methods although higher with the lower rate of fertilization. On the other hand, the use of ^{15}N would increase the cost of trials but measurements only in fertilized plots would be enough to assess cumulative NH_3-N volatilization, thereby reducing costs in conventional laboratory analysis.

A longer period of measurement ensured a better estimation of cumulative NH_3-N volatilization. Little NH_3-N volatilization is expected in cold weather (Ladha *et al.*, 2005) as occurs during the sowing of winter wheat. When fertilization is delayed and it is applied at tillering as in this case, it is expected that N uptake by the crop can be improved (Melaj *et al.*, 2003) and use efficiency increased. However, at the same time it is possible that NH_3-N volatilization increases because of rising external temperature. Nevertheless, a correlation between NH_3-N volatilization and temperature was obtained only during the first eight d after fertilization because of the trend of decreasing volatilization with growth period related to N-fertilizer incorporation into crops and N dynamics in soil including physicochemical and biological processes.

In the south east Pampa region, Sainz Rozas *et al.* (1997) observed N losses through volatilization of 9 percent in a maize crop receiving a lower rate of urea fertilization (70 kg/ha). There is a lower risk of volatilization in winter wheat compared with summer-grown crops, but warm conditions in early spring would increase volatilization losses.

CONCLUSIONS

In the literature, NH_3 volatilization is generally recorded during the first eight d after fertilization, but this study showed that volatilization may continue to be important during a longer period of time. Baseline volatilization was reached 25–30 d after the addition of urea-N at a rate of 100 kg/ha with significant differences between fertilized and unfertilized plots being found until 16 d after fertilization.

Losses of N by volatilization in wheat increased with the rate of urea application but the losses were lower than 6.5 percent of the applied fertilizer. In general, ammonia volatilization in winter wheat is lower than in summer-grown crops. However, its importance in the N budget would not be negligible and must be considered.

REFERENCES

Barbieri, P.A., Echeverría, H.E., Saínz Rozas, H.R. & Maringolo, M. 2010. Fertilización de maíz con urea de liberación lenta: pérdida por volatilización y eficiencia de uso de nitrógeno. *Ci. Suelo,* 28: 57–66.

Delgado, J.A. 2002. Quantifying the loss mechanisms of nitrogen. *J. Soil Water Conserv.,* 57: 389–398.

Galloway, J.N., Aber, J., Erisman, J., Seitzinger, S., Howarth, R., Cowling, E. & Cosby, B. 2003. The nitrogen cascade. *Bioscience*, 53: 341–356.

IAEA. 2001. *Use of isotope and radiation methods in soil and water management and crop nutrition*. IAEA Training Course Series No. 14. Vienna.

IAEA. 2008. *Guidelines on nitrogen management in agricultural systems*. IAEA Training Course Series No. 29. Vienna.

Ladha, J.K., Pathak, H., Krupnik, T.J., Six, J. & van Kessel, C. 2005. Efficiency of fertilizer nitrogen in cereal production: Retrospects and prospects. *Adv. Agron*., 87: 85–156.

Lara Cabezas, A.R., Trivelin, P.C.O., Bendassolli, J.A., de Santana, D.G. & Gascho, G.J. 1999. Calibration of a semi-open static collector for determination of ammonia volatilization from nitrogen fertilizers. *Commun. Soil Sci. Plant Anal.,* 30: 389–406.

Melaj, M., Echeverria, H., López, S., Studdert, G., Andrade, F. & Bárbaro, N. 2003. Timing of nitrogen fertilization in wheat under conventional and no tillage system. *Agron. J.*, 95: 1525–1531.

Nömmik, H. 1973. Assessment of volatilization loss of ammonia from surface-applied urea on forest soil by N^{15} recovery. *Plant Soil*, 38: 589–603.

Rochette, P., Angers, D.A., Chantigny, M.H., Macdonald, J.D., Gasser, M.O. & Bertrand, N. 2009. Reducing ammonia volatilization in a no till soil by incorporating urea and pig slurry in shallow bands. *Nutr. Cycl. Agroecosyst*., 84: 71–80.

Saínz Rozas, H., Echeverría, H.E., Studdert, G.A. & Andrade, F.H. 1997. Volatilización de amoníaco desde urea aplicada al cultivo de maíz bajo siembra directa. *Ci. Suelo*, 15: 12–16.

Videla, C., Navarro, C., González, N. & Miñon, D. 1994. Volatilización de amonio a partir de orina de vacunos aplicada a suelos de la pampa deprimida. *Ci. Suelo*, 12: 1–6.

Zaman, M., Nguyen, M.L., Blennerhassett, J.D. & Quin, B.F. 2008. Reducing NH_3, N_2O and NO_3^- –N losses from a pasture soil with urease or nitrification inhibitors and elemental S-amended nitrogenous fertilizers. *Biol. Fertil. Soils*, 44: 693–705.

Influence of Fine Particle Suspension of Urea and Urease Inhibitor on Nitrogen and Water Use Efficiency in Grassland using Nuclear Techniques

M. Zaman[1,*], M.M. Barbour[2], M.H. Turnbull[3] and L.V. Kurepin[4]

ABSTRACT

Controlled environment experiments were conducted to assess the effects of urea applied as fine particle suspension (FPA) together with urease inhibitor, (N-(n-butyl) thiophosphoric triamide (nBTPT), trade-name Agrotain® to ryegrass swards on nitrogen use efficiency (NUE) and water use efficiency (WUE). Perennial ryegrass was sown 7–10 seeds per plastic pot. After eight weeks of sowing, the ryegrass sward pots (36 pots) were transferred to a growth cabinet which was maintained at 20°C, 70% relative humidity (RH), 700 $mmol \cdot m^{-2} \cdot s^{-1}$ photosynthetic available radiation (PAR) during the 16-h light period, and 15°C, 70% RH during the 8-h dark period. After two weeks in the growth cabinet, ryegrass from each pot was trimmed to 6 cm height to achieve uniformity followed by applying six treatments that include: (1) control (no N), (2) control + leaf spray irrigation (equivalent of 10 mm of rain to wash applied urea) after d-1, (3) urea applied as fine particle suspension (FPA) on d 0, (4) urea applied on d 0 + leaf irrigation one d after of urea application, (5) Agrotain treated urea in FPA form, and (6) Agrotain treated urea + leaf irrigation after d-1. Each treatment had three replicates. To determine herbage ^{15}N uptake, chosen swards of ryegrass were treated with ^{15}N labelled urea (10% atom excess) with or without Agrotain at a rate equivalent to 25 $kg \cdot N \cdot ha^{-1}$. Twenty-eight d after initial treatment application, ryegrass plants were harvested, separated into new (newly grown) and old (tissue grown after uniformity cut before application of treatments) plant tissue, weighed and analysed for ^{15}N content. For intrinsic water use efficiency (WUE_i) measurements, 18 additional pots of ryegrass receiving the above six treatments were chosen. Pre- and post-treatment measurements of leaf-level gas exchange and on-line photosynthetic carbon and oxygen isotope discrimination were carried out to calculate WUEi. Urea applied with Agrotain in FPA form significantly increased herbage dry matter yield and WUE_i compared to urea alone. The herbage ^{15}N data showed that Agrotain improved NUE. Ryegrass receiving the urea+Agrotain+irrigation treatment took up 49% of N from applied urea, compared to 38% by the urea+irrigation treatment. Irrigating the leaves one d after applying urea + Agrotain further increased ryegrass growth and also resulted in the highest WUEi compared to no leaf irrigation.

Key words: *Urea, Agrotain, Fine Particle Suspension, Pasture, N uptake, N and water use efficiency, stable isotopes.*

INTRODUCTION

Increasing demand for dairy and meat products in fast-growing economies like China and India is encouraging countries, such as New Zealand, to intensify dairy production. In New Zealand, the major farming system is animal grazing, with mixed ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) as the pasture for animals. Nitrogen (N) is an important plant nutrient for increasing pasture productivity. According to the International Fertilizer Industry Association (IFA), world N fertilizer consumption is estimated to grow at an average annual rate of 2.5% and is projected to reach 112.4 Mt·N in 2015 (IFA, 2011). However, the increase in N use in pastoral systems will have a high environmental cost.

Urea [$(Co(NH_2)_2$] is the predominant form of N fertilizer worldwide (>50%) (Watson 2000; IFA 2011) and also in New Zealand (ca. 80%), mainly because of its lower cost per unit N and higher N content (46% N), in comparison to other N based fertilizers. Furthermore, urea is easy to transport, store, blend and spread. However, urea has been reported to have lower N use efficiency (NUE) than ammonium- and nitrate-based fertilizers (Black *et al.,* 1985; Zaman *et al.,* 2008). Here, NUE is defined as kg of additional dry matter produced per kg of applied N. This reduced NUE is partly due to N losses (10% to 30% of the applied N) from urea as ammonia (NH_3) (Zaman *et al.,* 2013a). Some of these N losses like gaseous emission of NH_3, nitric oxide (NO) and nitrous oxide (N_2O) will of course be unavoidable. That's because gaseous emissions of NH_3, N_2O and NO are part of the natural N cycle and can even occur after application of any ammonium-based fertilizer. Hence, controlling N losses, especially gaseous N losses, is critical for improving the NUE of all N-based fertilizers to minimize negative effects on the environment and decrease costs.

Granular urea efficiency can be considerably improved by coating it with a urease inhibitor (UI), such as Agrotain® (N-(n-butyl) thiophosphoric triamide (nBTPT, Watson *et al.,* 2008; Zaman *et al.,* 2008, 2013b). After application to soil, nBTPT is quickly converted to its oxygen analog N-(n-butyl) phosphoric triamide (nBTPTO) (Byrnes and Freney, 1995), which temporarily blocks the action of the urease enzyme (Christianson *et al.,* 1990), thereby slowing the process of urea hydrolysis (Zaman *et al.,* 2008). This delayed urea hydrolysis by nBTPTO reduces the rate of increase of soil pH (around the fertilizer

---

1 Ballance Agri-Nutrients Limited. Private Bag 12503, Tauranga Mail Centre, Tauranga 3143, New Zealand
2 Faculty of Agriculture and Environment, the University of Sydney, Australia.
3 School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand.
4 Department of Biology, Western University, London, Ontario, Canada, N6A 5B7.
* E-mail address of corresponding author: zamanm_99@yahoo.com

L.K. Heng, K. Sakadevan, G. Dercon and M.L. Nguyen (eds), Proceedings — International Symposium on Managing Soils for Food Security and Climate Change Adaptation and Mitigation. Food and Agriculture Organization of the United Nations, Rome, 2014: 29–32

granule) as well as decrease the amount of ammonium (NH_4^+) in the soil, all of which reduces the potential for NH_3 volatilization and other N losses. Also, the NUE of applied urea is reported to be improved further if urea is applied in fine particle application form (Dawar *et al.,* 2012). Fine particle application of urea results in a more even distribution of the urea on a per plant basis, thus minimising localized hot spots for N losses. However, there is limited information on fine particle suspension applications of urea applied together with Agrotain in grazed pastures.

Plant WUE, calculated as the amount of plant biomass produced per unit of water used depends on irrigation techniques, soil fertility, crop variety, and soil and water conservation practices. Leaf intrinsic water use efficiency (WUEi) defined as the ratio of instantaneous photosynthetic and transpiration rates, has been shown to be positively related to the stable ^{13}C isotope composition of plant (Brugnoli and Farquhar 2000). We anticipated that plants with increased N supply (through the combined effects of urea and Agrotain) will increase leaf photosynthetic rate leading to increased WUE, increased $\delta^{13}C_{p,}$ combined with more modest changes in stomatal conductance and $\delta^{18}O_p$. We further anticipated that spray irrigation after urea applied with Agrotain in FPA form will increase both WUE and NUE. With both fertilizer and irrigation representing a substantial cost to the farmer, a major aim of our study was to assess the effect of urea with UI "Agrotain" on ryegrass water- and N-use efficiency, using stable isotopic techniques ^{15}N and ^{13}C.

MATERIALS AND METHODS

We carried out two controlled environment experiments in a growth cabinet. The soil used for the study was collected (0–7 cm depth) from a dairy pasture site near Lincoln, Canterbury, New Zealand. It was Paparua silt loam, Typic Haplustepts (Soil Survey Staff, 1998), had a silt loam texture with a pH of 5.65, total N of 0.38%, organic matter of 7%, Olsen P of 20 mg/L, CEC of 14 $cmol_c$ /kg. The soil was sieved (4 mm) to remove plant litter and roots. Sieved soil was adjusted to 80% of field capacity and 1.5 kg of moist soil was transferred to plastic pots (140 mm in diameter) to a depth of 150 mm. Each pot also received a basal dose of phosphorus and sulphur at an equivalent rate of 40 kg/ha. Perennial ryegrass cv. "Nui" was sown in each pot at 7-10 seeds per pot. Eight weeks after sowing, the ryegrass sward in each pot was cut to 6 cm height to achieve uniformity and then transferred to a growth cabinet. The growth cabinet was maintained at 20°C, 70% relative humidity (RH), 700 $mmol \cdot m^{-2} \cdot s^{-1}$ photosynthetic available radiation (PAR) during the 16-h light period, and 15°C, 70% RH during the 8-h dark period. After two weeks in the growth cabinet, ryegrass from each pot was trimmed again to 6 cm height followed by applying the six treatments (1) control (no N), (2) control + leaf spray irrigation (equivalent of 10 mm of rain to wash applied urea) after one d, (3) urea applied as fine particle suspension (FPA) on d 0, (4) urea applied on d 0 + leaf irrigation one d after of urea application, (5) Agrotain treated urea in FPA form, and (6) Agrotain treated urea + leaf irrigation after one d. Each treatment had three replicates. To determine herbage ^{15}N uptake, chosen swards of ryegrass were treated with ^{15}N labelled urea (10% atom excess) with or without Agrotain at a rate equivalent to 25 $kg \cdot N \cdot ha^{-1}$. Twenty-eight d after treatment application, ryegrass plants were harvested separated into new and old (previously cut before application of treatments) plant tissue, weighed fresh, washed with deionized water, and then dried at 60°C for 7 d. Dried herbage weight was recorded to calculate herbage dry matter yield. The dried herbage samples were then ground.

Total N and ^{15}N in herbage were measured using a Dumas elemental analyser (Europa Scientific ANCA-SL) interfaced to an isotope mass spectrometer (Europa Scientific 20-20 Stable Isotope Analyser; Europa Scientific Ltd, Crewe, U.K.). Total N uptake in each treatment was determined using the product of herbage N content and herbage dry matter. Calculations of ^{15}N recovery in plant were carried out as described by the International Atomic Energy Agency (IAEA 1976).

$$\textit{Percentage N derived from fertilizer (\%Ndff)} =$$

$$\left(\frac{\%^{15}\mathrm{N}\ \textit{excess in sample}}{\%^{15}\mathrm{N}\ \textit{excess in fertilizer}}\right)\times 100$$

$$\textit{Percentage uptake of applied N} = \left(\frac{\%N\,dff \times \textit{Yield of } N}{\textit{Rate of N application}}\right)$$

For intrinsic water use efficiency (WUE_i) measurements, 18 additional pots of ryegrass receiving the above six treatments were chosen. Pre- (one d before treatment application) and post-treatment (one d before harvest) measurements of leaf-level gas exchange and on-line photosynthetic carbon-13 and oxygen-18 isotope discrimination (Barbour & Farquhar, 2000; Barbour *et al.,* 2000) were carried out. After post-treatment measurements of leaf-level gas exchange and on-line photosynthetic carbon and oxygen isotope discrimination, ryegrass from each pot was harvested to determine ^{13}C in plant tissue.

RESULTS AND DISCUSSION

Herbage Dry Matter Yield and N Uptake

Urea applied with Agrotain-in FPA form produced significantly ($P<0.05$) higher herbage dry matter yield than did urea alone (Figure 1). Applying irrigation to ryegrass leaves after one d of applying treatments further increased growth in all cases. This resulted in the highest growth for the urea with Agrotain + irrigation treatment. Similarly, the new plant tissues (new tissue emerged after treatment application) contributed more toward plant dry matter production than the old plant tissue (the leaves which were previously trimmed). Considering both new and old plant tissues, urea applied with Agro-

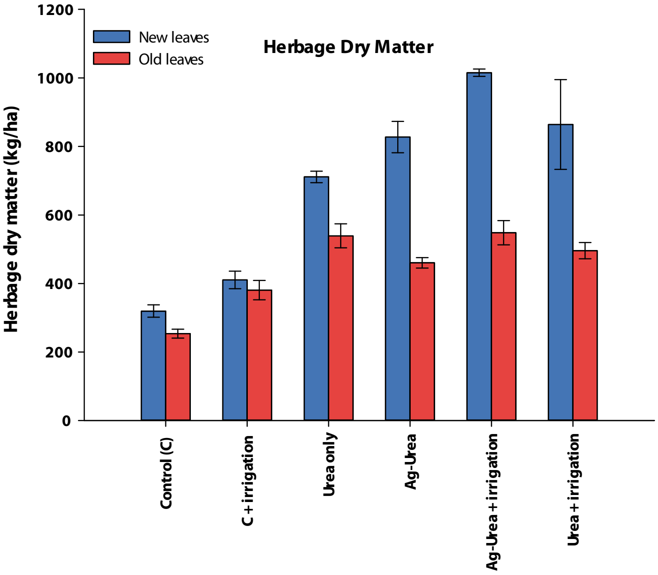


FIGURE 1. Herbage dry matter yield of new and old plant tissues of ryegrass as influenced by application of urea with the urease inhibitor (Agrotain) by the fine particle suspension method. Values are means (n = 3). Vertical bars represent one standard error of the mean.

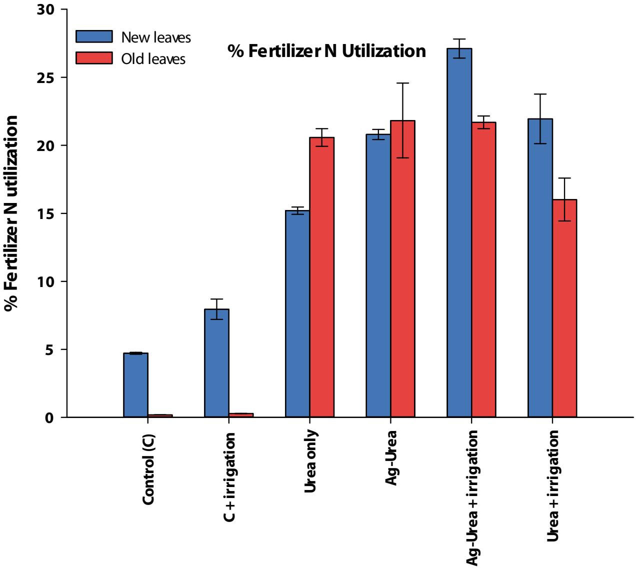


FIGURE 2. Percentage recovery of applied ^{15}N by new and old plant tissues of ryegrass as influenced by urea applied together with the urease inhibitor (Agrotain), using the fine particle suspension method. Values are means (n = 3). Vertical bars represent one standard error of the mean.

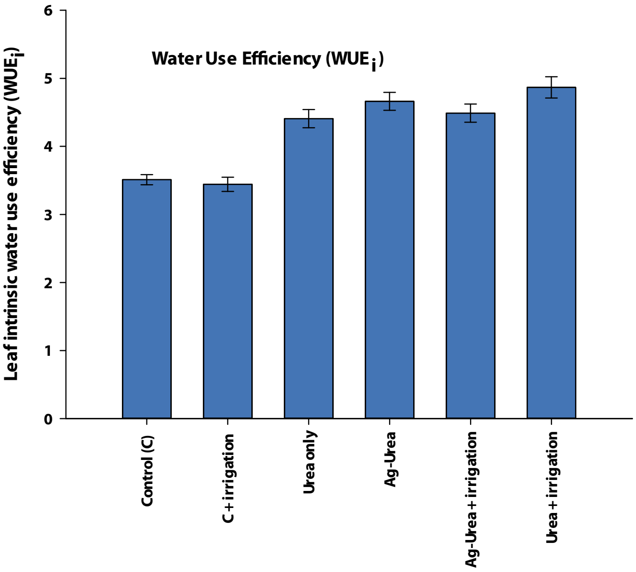


FIGURE 3. Leaf intrinsic water use efficiency (WUEi) of ryegrass as influenced by application of urea with the urease inhibitor (Agrotain) applied via the fine particle suspension method. Values are means (n=3) of a dimensionless ratio between assimilation rate and transpiration rate. Vertical bars represent one standard error of the mean.

tain in FPA form increased herbage dry matter by 14% over urea alone. Such increases in herbage dry matter yield can be attributed to the positive effects of Agrotain on soil microbial, chemical, and physical processes and plant physiological processes. Among the soil microbial process, Agrotain delays soil urease activity which minimizes the rate of NH_3 losses (Zaman *et al.,* 2008, 2013a; Soares *et al.,* 2012). Urea is an uncharged particle and can thus move both laterally and downward in soil. Since Agrotain retains urea in the soil for 7 to 10 d, this facilitates urea dispersion in sub-surface soil layers where it is likely to make good contact with the plant under moist condition (Dawar *et al.,* 2011). Urea applied with Agrotain is also reported to reduce nitrification (Sanz-Cobena *et al.,* 2012), which conserves N against denitrification and nitrate leaching. Since urea with Agrotain was applied to plant leaves, which is likely to delay plant urease activity and facilitate direct uptake of applied urea N through plant leaves. In our earlier field trial we observed that urea applied to ryegrass with Agrotain by the fine particle application method, followed by light irrigation (5 mm), significantly improved NUE, compared to granular urea (Zaman and Blennerhassett., 2009). Light irrigation after one d also washed urea from plant leaves and the surface soil, thus potentially minimizing the risk of leaf burning and NH_3 volatilization (Zaman *et al.,* 2013a).

There were significant treatment differences in the proportion of plant N derived from the labelled urea (Figure 2). Urea applied with Agrotain exhibited significantly ($p < 0.05$) higher N uptake than did urea alone; while irrigating the leaves after one d further increased the proportion of N derived from labelled urea. The higher N uptake in the treatment receiving both irrigation and Agrotain treated urea in FPA form suggests that the inhibitor needs a "dewfall" in order to gain the maximum benefits from the applied urea. Over all, plants receiving the urea+Agrotain+irrigation treatment took up 49% of N from applied urea, compared to 38% by the urea+irrigation treatment. Similarly, the two treatments of control (control only and control + irrigation) also showed that plants took up a small amount (between 5 and 8%) of plant N derived from the labelled urea. This was probably due to the dry deposition of $^{15}N\text{-}NH_3$ onto the ryegrass sward leaves in the growth cabinet. Also, the control plants that were spray irrigated (artificial dewfall) after one d, had taken up more labelled N than the control plants without irrigation, suggesting that wet leaves are better sinks for atmospheric NH_3.

Intrinsic leaf water use efficiency

Pre-treatment measurements (one d before treatment application) showed that ryegrass height, photosynthesis parameters and isotopic values were not significantly different between pots (data not shown). As shown above, urea applied with Agrotain to the pasture leaves significantly increased growth of the sward as well as increasing N uptake (Figures 1 and 2); which was strongly related to an increase in photosynthetic rate (data not shown), probably due to higher N availability for Rubisco. The higher photosynthetic rate in response to applied urea, combined with a smaller increase in stomatal conductance and thus transpiration, resulted in higher leaf intrinsic WUE (photosynthetic rate divided by transpiration rate, as measured in the leaf chamber) (Figure 3). Again, leaf irrigation one d after urea treatment resulted in higher WUE, and Agrotain presence gave a small (non-significant) increase. On-line (Barbour *et al.,* 2000) and tissue carbon isotope discrimination analysis was strongly correlated to WUE$_i$ suggesting that discrimination may provide a good record of changes in WUE$_i$.

CONCLUSIONS

Applying urea together with Agrotain as fine particle suspension method showed appreciably improved NUE and WUE$_i$ compared with urea alone. This is likely due to increase in N assimilation rate. Applying light leaf irrigation (10 mm) one d after applying N treatments further improved both NUE and WUE$_i$. The ^{15}N data showed that ryegrass plants receiving the urea + Agrotain + irrigation treatment took up 49% of N from applied urea, compared to 38% by the urea+irrigation treatment. Our results suggest that applying urea with Agrotain followed by light irrigation is a useful management tool for enhancing both NUE and WUE_i and has a good potential for improving profitability.

REFERENCES

Barbour, M.M., McDowell, N.G., Tcherkez, G., Bickford, C.P. & Hanson, D.T. 2007. A new measurement technique reveals rapid post-illumination changes in the carbon isotope composition of leaf-respired CO_2. *Plant, Cell & Envir*., 30: 469–482.

Barbour, M.M. & Farquhar, G.D. 2000. Relative humidity- and ABA-induced variation in carbon and oxygen isotope ratios of cotton leaves. *Plant Cell & Envir*., 23: 473–485.

Barbour, M.M., Fischer, R.A., Sayre, K.D. & Farquhar, G.D. 2000. Oxygen isotope ratio of leaf and grain material correlates with stomatal conductance and grain yield in irrigated wheat. *Aust. J. Plant Phys*., 27: 625–637.

Black, A.S., Sherlock, R.R., Smith, N.P., Cameron, K.C. & Goh, K.M. 1985. Effects of form of nitrogen, season, and urea application rate on ammonia volatilisation from pastures. *NZ. J. Agr. Res***.,** 28: 469–474.

Byrnes, B.H. & Freney, J.R. 1995. Recent developments on the use of urease inhibitors in the tropics. *Fert. Res.*, 42: 251–259.

Christianson, C.B., Byrnes, B.H. & Carmona, G. 1990. A comparison of the sulfur and oxygen analogs of phosphoric triamide urease inhibitors in reducing urea hydrolysis and ammonia volatilization. *Fert. Res*., 26: 21–27.

Dawar, K., Zaman, M., Rowarth, J.S., Blennerhassett, J.D. &Turnbull, M.H. 2011. Urea hydrolysis and lateral and vertical movement in the soil: effects of urease inhibitor and irrigation. *Biol. Fertil. Soils.* 47: 139–146.

Dawar, K., Zaman, M., Rowarth, J.S., Blennerhassett, J.D. &Turnbull, M.H. 2011. Urea hydrolysis and lateral and vertical movement in the soil: effects of urease inhibitor and irrigation. *Biol. Fertil. Soils.* 47: 139-146.

Dongmann, G., Nurnberg, H.W., Förstel, H. & Wagener, K. 1974. On the enrichment of $H_2{}^{18}O$ in the leaves of transpiring plants. *Radiat. & Envir. Biophys.,* 11: 41–52.

IFA. 2011. International Fertiliser Association statistics, Nitrogen Fertilizer Consumption by Region, In: Wilson, J.R. (Ed.), Advances in Nitrogen Cycling in Agricultural Ecosystems.

International Atomic Energy Agency. 1976. Tracer Manual on Crops and Soils. Guidelines on Nitrogen Management in Agricultural Systems. (F. Zapata, ed). Technical Reports Series No. 171: 119–148. Vienna.

Sanz-Cobena, A., Sánchez-Martín, L., García-Torres, L. & Vallejo, A. 2012. Gaseous emissions of N_2O and NO and NO_3^- leaching from urea applied with urease and nitrification inhibitors to a maize *(Zea mays)* crop. *Agric. Ecosyst. Environ***.,** 149: 64–73.

Soares, J.R., Cantarella, H. & Menegale, M.L.C. 2012. Ammonia volatilization losses of surface –applied urea with urease and nitrification inhibitors. *Soil Biol. Biochem*., 52: 82–89.

Soil Survey Staff. 1998. Keys to soil taxonomy. 8th Edn. Washington, DC, United States Department of Agriculture.

Watson, C.J., Akhonzada, N.A., Hamilton, J.T.G. & Matthews, D.I. 2008. Rate and mode of application of the urease inhibitor N-(n-butyl) thiophosphoric triamide on ammonia volatilization from surface-applied urea. *Soil Use Manag.*, 24: 246–253.

Zaman, M., Nguyen, M.L., Blennerhassett, J.D. & Quin, B.F. 2008. Reducing NH_3, N_2O and NO_3^--N losses from a pasture soil with urease or nitrification inhibitors and elemental S-amended nitrogenous fertilizers. *Biol. Fert. Soils*. 44: 693–705.

Zaman, M. & Blennerhassett, J.D. 2009. Can fine particle application of fertilisers improve N use efficiency in grazed pastures? *In* L.D. Currie and C.L. Lindsay, eds. *Proceedings of a Workshop on Nutrient Management in a Rapidly Changing World,* pp. 257–264. Fertilizer and Lime Research Centre, Massey University, Palmerston North..

Zaman, M., Saggar, S. & Stafford A.D. 2013a. Mitigation of ammonia losses from urea applied to a pastoral system: The effect of nBTPT and timing and amount of irrigation. *NZ. Grass. Assoc.,* 75: 121–126.

Zaman, M., Zaman, S., Adhinarayanan, C., Nguyen, M.L. & Nawaz S. 2013b. Effects of urease and nitrification inhibitors on the efficient use of urea for pastoral systems. *Soil Sci. & Plant Nut.* 1–11. DOI: 10.1080/00380768.2013.812940.

Stabilized Nitrogen Fertilizers to Reduce Greenhouse Gas Emissions and Improve Nitrogen Use Efficiency in Australian Agriculture

H.C. Suter[1,*], D. Chen[1] and D. Turner[1]

ABSTRACT

Loss of nitrogen (N) from applied fertilizer is a major cause of inefficiency in N fertilizer utilization. This loss of N can occur through many pathways including ammonia (NH_3) volatilization, nitrate (NO_3^-) leaching and emissions of gases such as nitrous oxide (N_2O). One way of addressing these losses is to amend N fertilizers with compounds that slow the production of the forms of N that can be lost. Two such compounds are urease inhibitors, designed to reduce NH_3 loss, and nitrification inhibitors, designed to reduce NO_3^- leaching and gaseous emissions. However, the impact of these compounds on N loss is variable across soil type, region, cropping system and temperature. An examination of where these compounds may be beneficial requires detailed laboratory investigation plus field validation. This paper reports on the results of experiments performed under both laboratory and field conditions and draws conclusions regarding the suitability of these compounds for improving N use efficiency in Australian agriculture.

INTRODUCTION

Nitrogen (N) use efficiency from applied fertilizer in a variety of Australian agricultural systems ranges from 8 to 88 percent with an average of around only 50 percent of the N recovered in the plant (Chen *et al.*, 2008). Losses from applied N fertilizers occur mostly as gaseous emissions (ammonia [NH_3], nitrous oxide [N_2O] and other gases [N_2, NO_x]) or nitrate (NO_3^-) leaching. Some fertilizer N is retained by the soil where it is either immobilised or remains available for later crop use. Losses of up to 30 percent of applied N as NH_3 have been reported from pasture systems in Australia (Eckard *et al.,* 2003; Prasertsak *et al.,* 2001). Denitrification, can also be a major pathway of N lost as N_2O, N_2 and NO_3^- during periods of irrigation or waterlogging, with losses of up to 80 percent of applied N recorded in irrigated cotton (Chen *et al.,* 1994). In Australia, N_2O emissions from agriculture account for 71 percent of the national total. Fertilizer usage is a major contributor, with current fertilizer emission factors for N_2O ranging from 0.05 to 2.8 percent (DCCEE, 2011).

Use of stablized N fertilizers, those treated with urease and/or nitrification inhibitors, is one approach to reduce N losses from fertilizers and to mitigate both direct (N_2O) and indirect (NH_3) nitrogenous greenhouse gas emissions from agriculture. The urease inhibitors work by slowing the pathway of N transformations through inhibition of the activity of the urease enzyme which is involved in hydrolyzing urea. The nitrification inhibitors suppress the activity of NH_3 oxidizing bacteria and hence slow the conversion of NH_3 to nitrite and subsequently NO_3^- . Research has found that the impact of these inhibitors in reducing N loss and improving crop yield is highly variable with temperature and soil type (Carmona *et al.*, 1990; Barth 2006).

Australia has a wide range of climatic conditions and soil types and hence the few studies examining the use of stabilized fertilizers are not sufficient to assess comprehensively their potential use across Australian agricultural systems.

This research presents findings from laboratory and field experiments investigating the impact of stabilized fertilizers on N transformations and losses on a range of soil types from different regions in Australia, covering pasture, cropping and sugarcane production systems. The stabilized fertilizers used were urea treated with the urease inhibitor *N*-(n-butyl) thiophosphoric triamide (NBPT), and the nitrification inhibitors dicyandiamide (DCD), 3,4-dimethylpyrazole phosphate (DMPP) and nitrapyrin (N-serve).

MATERIALS AND METHODS

Laboratory incubation experiments

Air-dried and sieved (< 2mm) top soil (0–20 cm depth from sugarcane sites and 0–10 cm from all others) was placed into an incubation vessel (250–500 mL capacity) and pre-wetted to 60 percent water filled pore space for all experiments.

Soils of varying physical and chemical properties were collected from both pasture (dairy) and cropping sites in Victoria, Australia (Table 1) for assessing the impact of the urease inhibitor NBPT on urea hydrolysis rates. The methodology followed that described in Suter *et al.* (2011) with slight modifications for soil mass (40 to 150 g of oven-dried equivalent used), N application rate, temperature of incubation (5, 15, 25 and 35 ^{o}C) and extraction soil:solution ratio (1:5 for all except those reported in Suter *et al.,* 2011). Urease activity was measured following the method of Douglas and Bremner (1971). The minor variations in methodology as described above were not considered to have a major influence on the results achieved because the trends observed for different soil types were consistent, and so it is possible to compare results from all experiments.

For experiments on the impact of nitrification inhibitors (DCD, DMPP and N-serve) on nitrification rates and N_2O emissions, urea with or without the nitrification inhibitors was applied either as a buried granule or as a liquid. Five different soils, three from Victoria and two from Queensland were examined. The selected properties

---

1 Department of Agriculture and Food Systems, Melbourne School of Land and Environment, The University of Melbourne 3010, Victoria, Australia
* E-mail address of corresponding author: helencs@unimelb.edu.au

L.K. Heng, K. Sakadevan, G. Dercon and M.L. Nguyen (eds), Proceedings — International Symposium on Managing Soils for Food Security and Climate Change Adaptation and Mitigation. Food and Agriculture Organization of the United Nations, Rome, 2014: 33–36

TABLE 1. Selected properties of soils used for the urease inhibitor incubation experiments

Soil	Soil texture	Industry	pH_w	Clay (%)	Silt (%)	Sand (%)	Organic C (%)	Urease activity (mg urea-N/g soil/h)
1	Clay loam	Dairy	5.5	20	25	55	3.9	134
2	Fine sandy clay loam	Dairy	5.4	21	28	51	10*	186
3	Silty loam	Dairy	5.5	22	38	40	2.4	97
4	Clayey sand	Cropping	7.8	9	2	89	1.3	54
5	Medium clay	Cropping	8.1	37	24	39	1.0	78
6	Medium clay	Cropping	8.1	39	21	40	1.3	90

*Note: this soil included the pasture thatch layer and hence a large organic component

TABLE 2. Selected properties of the soils used for nitrification inhibitor incubation experiments

Soil no.	Soil texture	Region	Industry	pH_w	Clay (%)	Organic C (%)
7	Clay loam	Queensland	sugarcane	5.3	39	1.9
8	Loam	Queensland	sugarcane	4.8	13	1.5
9	Fine sandy loam	Victoria	dairy	5.4	21	10
10	Medium clay	Victoria	dairy	5.5	33	2.4
11	Clay loam	Victoria	grains cropping	7.8	30	1.3

*Note: this soil included the pasture thatch layer and hence a large organic component

of these soils are provided in Table 2. The methodology followed for the cropping soil from Victoria is provided in Chen *et al.* (2010) and the studies on the pasture and sugarcane soils followed a similar method with the modification of soil mass used (40 and 80 g of oven dried equivalent for the sugarcane and pasture soils, respectively) and soil:solution ratio of the extraction (1:5).

Field experiments

A field experiment was conducted on a ryegrass seed crop in Victoria, Australia where NH_3 loss, N_2O emissions, soil mineral N transformation and biomass production were measured regularly over an eight-month period where regular fertilization occurred. Details of the soil properties at the field site and the methodology used for the NH_3 volatilization study are reported in Suter *et al.* (2013). Another NH_3 volatilization field experiment was conducted on cropping soils from southern Australia and details of the methodology are reported in Turner *et al.* (2010).

The N_2O emissions from surface applied granular urea with and without the addition of the nitrification inhibitor DMPP were investigated using manual chambers (23 cm diameter, 25 cm height) in a small (2 x 1 m) plot trial in the ryegrass seed crop experiment. Fertilizer was applied every 1 to 2 months while the pasture was cut to five cm height above ground level to simulate grazing in the same period. A nitrogen-15 (^{15}N) micro-plot (25 cm internal diameter × 20 cm depth) study using ^{15}N granular urea to determine the fate of applied fertilizer N was also conducted at the ryegrass seed crop site and is described in Suter *et al.* (2013).

RESULTS AND DISCUSSION

Urease inhibitors

In the laboratory incubation experiments, use of NBPT reduced urea hydolysis across all soil types examined, with the level of reduction dependent upon soil properties including soil urease activity and temperature. Typical responses from pasture and cropping soils are shown in the data provided for two of the soils (Soils 1 and 4) in Figure 1. In the cropping soils (Soils 4, 5 and 6), NBPT was found to reduce urea hydrolysis rates markedly under cooler conditions, but increasing temperature reduced the level of inhibition. In the more organic pasture soils (Soils 1, 2 and 3), the trend was for rapid urea hydrolysis for both urea and urea with the added NBPT. The differences in urea hydrolysis and in the impact of NBPT between the soils are attributed mainly to the urease activity (54–90 mg urea-N g·soil/h measured in cropping soil compared with 134–186 mg urea-N g·soil/h in pasture soils).

In field experiments on cropping soils, NBPT reduced NH_3 loss from urea by 89 percent compared with no NBPT (from 10 to 1 percent of applied N lost) (Turner *et al.,* 2010), and on pasture soils by between 42 and 67 percent depending upon the season (reducing losses from 30 to 9 percent of applied N in autumn and from 2 to 1 percent of applied N in spring) (Suter *et al.*, 2013). These results show that the urease inhibitor NBPT can reduce N losses in high urease activity systems. Despite the large N saving with the use of the urease inhibitor, biomass production was not altered in the pasture site. This is likely due to the presence of sufficient N in the soil to support the pasture growth, as indic ated by the results obtained from the ^{15}N micro-plot study which showed that of the total N taken up by the plant (52 kg·N/ha in the biomass) the fertilizer supplied only around 30 percent (17.7 kg·N/ha) of the required N to the plant (Suter *et al.,* 2013). Of the applied ^{15}N, 27 percent was unaccounted for and considered to be lost as NH_3 based on measured loss of NH_3 on the site of 30 percent of applied N, 42 percent was taken up by the plant and the remainder (31 percent) was found in the soil.

Nitrification inhibitors

Nitrification inhibitors (DCD, DMPP and N-serve) were found to reduce N_2O emissions across a range of soils, temperatures and moisture contents by between 15 and 98 percent in laboratory experiments, whilst their impact on NO_3^- production was variable (Table 3). The reason for the variability between soil types could not be clearly identified from the dataset developed to date. It is hypothesized that

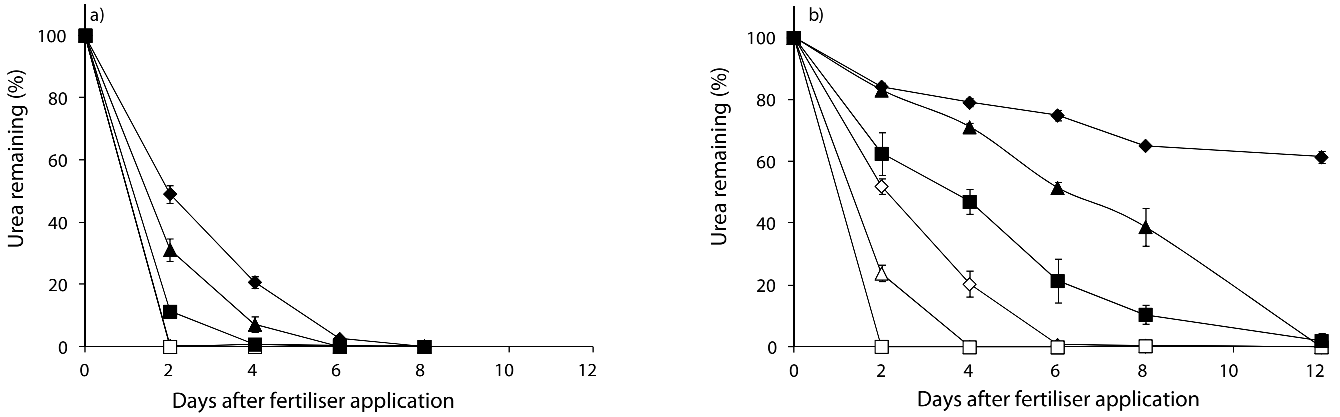


FIGURE 1. Urea remaining in soil during incubation for: (a) clay loam dairy pasture soil (Soil 1), and (b) clayey sand wheat cropping soil (Soil 4), with application of granular urea with and without NBPT. Urea at 15°C (◇), 25°C (△)and 35°C (□); Urea + NBPT at 15°C (◆), 25°C (▲) and 35°C (■)

TABLE 3. Summary of incubation experiments with Queensland and Victorian soils showing the effect of nitrification inhibitors on NO_3^- formation and cumulative N_2O emissions over the indicated range of temperatures. Note the values presented are percent reduction relative to the control.

Soil texture	Region	Temp. (°C)	NO_3^- reduction (%)			N_2O reduction (%)		
			DMPP	DCD	N-serve	DMPP	DCD	N-serve
Clay loam	Queensland	25–35	> 85	—	—	> 94	—	—
Loam	Queensland	25–35	0	—	—	19–64	—	—
Fine sandy loam	Victoria	5–25	0	0	—	36–68	15–83	—
Medium clay	Victoria	5–25	0–51	0–81	—	31–76	44–88	—
Clay loam	Victoria	5–25	4–70	—	83–98	> 95	—	> 95

the soil microbial community may have an important role in determining whether the inhibitors work as they are only considered to target the autotrophic NH_3 oxidizing bacteria whilst other microbes such as heterotrophic bacteria or archaea can perform the same function and are not affected by the inhibitors (Amberger 1993; Di *et al.,* 2010). Differences in soil properties such as texture, carbon, pH and nutrient status, will impact on the microbes found in the soils and consequently the response to the use of the inhibitors (Nicol *et al.,* 2008).

In the field experiment on the ryegrass seed crop, the use of the nitrification inhibitor DMPP reduced the fertilizer induced N_2O emissions over an eight-month period by 64 percent. The greatest impact was seen during spring when soils that were saturated over winter were drying and soil temperatures were warming. The saved N from reduced N_2O emissions did not translate into increased biomass as the amount lost was small relative to the amount applied. No other nutrient limitation to plant growth (such as phosphorus, sulphur and potassium deficiency) was observed on-site. At this site, NO_3^- leaching was considered to be minimal due to the nature of the soils (texture contrast) and the site topography (flat to slightly undulating). This helps to explain the lack of observed difference in biomass production with the use of the nitrification inhibitor.

CONCLUSIONS

Stabilized fertilizers, those amended with urease or nitrification inhibitors, show promise for reducing N losses from applied fertilizers and mitigating emissions of both N_2O and NH_3 gases in targeted Australian agricultural industries. Further research is required into the productivity effects of the use of urease inhibitors, the variable response seen with the nitrification inhibitors and the role of the soil microbial community in nitrification inhibition.

REFERENCES

Amberger, A. 1993. Efficient management of nitrogen fertilization in modern cropping systems. *In* M.A.C. Fragoso & M.L. van Beusichem, eds. *Optimization of plant nutrition. Developments in Plant and Soil Sci.,* 53: 619–622.

Barth, G. 2006. *Influence of soil properties on the effect of 3,4 - dimethylpyrazole — phosphate as nitrification inhibitor*. Munich, Technischen Universität München.

Carmona, G., Christianson, C.B. & Byrnes, B.H. 1990. Temperature and low concentration effects of the urease inhibitor *N*-(n-butyl) thiophosphoric triamide (nBTPT) on ammonia volatilization from urea. *Soil Biol. Biochem.,* 22: 933–937.

Chen, D., Suter, H.C., Islam, A. & Edis, R. 2010. Influence of nitrification inhibitors on nitrification and nitrous oxide (N_2O) emission from a clay loam soil fertilized with urea. *Soil Biol. Biochem.,* 42: 660–664.

Chen, D., Suter, H.C., Islam, A., Edis, R., Freney, J.R. & Walker, C.N. 2008. Prospects of improving efficiency of fertilizer nitrogen in Australian agriculture: A review of enhanced efficiency fertilizers. *Aust. J. Soil Res.,* 46: 289–301.

DCCEE. 2011. *Australian National Greenhouse Accounts: National Inventory Report 2009.* Canberra, Australian National Greenhouse Accounts Volume 1.

Di, H.J., Cameron, K.C., Shen, J-P., Winefield, C.S., O'Callaghan, M., Bowatte, S. & He, J-Z. 2010. Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. *FEMS Microbiol. Ecol.,* 72: 386–394.

Douglas, L.A. & Bremner, J.M. 1971. A rapid method of evaluating different compounds as inhibitors of urease activity in soils. *Soil Biol. Biochem*., 3: 309–315.

Eckard, R.J., Chen, D., White, R.E. & Chapman, D.F. 2003. Gaseous nitrogen loss from temperate perennial grass and clover dairy pastures in south-eastern Australia. *Aust. J. Agric. Res.,* 54: 561–570.

Nicol, G.W., Leininger, S., Schleper, C. & Prosser, J.I. 2008. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. *Environ. Microbiol.,* 10: 2966–2978.

Prasertsak, P., Freney, J.R., Denmead, O.T., Saffigna, P.G. & Prove, B.G. 2001. Significance of gaseous nitrogen loss from a tropical dairy pasture fertilized with urea. *Aust. J. Exp. Agric*., 41: 625–632.

Suter, H.C., Pengthamkeerati, P., Walker, C. & Chen, D. 2011. Influence of temperature and soil type on inhibition of urea hydrolysis by N-(n-butyl) thiophosphoric triamide in wheat and pasture soils in Southeastern Australia. *Soil Res.,* 49: 315–319.

Suter, H.C., Sultana, H., Turner, D.A., Davies, R., Walker, C. & Chen, D. 2013. Influence of urea fertiliser formulation, urease inhibitor and season on ammonia loss from ryegrass *Nutr. Cycl. Agroecosys.,* doi:10.1007/s10705-013-9556-y.

Turner, D.A., Edis, R.B., Chen, D., Freney, J.R., Denmead, O.T. & Christie, R. 2010. Determination and mitigation of ammonia loss from urea applied to winter wheat with N-(n-butyl) thiophosphorictriamide. *Agric. Ecosys. Environ.,* 137: 261–266.

Selection and Evaluation of Maize Genotypes Tolerant to Low Phosphorus Soil

J.C. Yang[1,*], H.M. Jiang[1], J.F. Zhang[1], L.L. Li[1] and G.H. Li[1]

ABSTRACT

Genotype selection for tolerance to low phosphorus (P) stress is an important strategy for the development of maize cultivars growing on soils low in available P. In this study, 15 maize genotypes were selected from 116 inbred lines during a three-year field experiment based on their 100-grain weight at maturity. Some morphological and physiological changes associated with low P stress were investigated at the seedling stage. In order to understand the mechanisms used by genotypes tolerant to low P soil to utilize P from the sparing soluble P forms, five typical maize genotypes selected from the inbred lines, were used in a phosphorus-32 (^{32}P) isotope tracer experiment. The results showed that root growth of the low P tolerant genotypes was accelerated under low P conditions, leading to wider exploration of soil space for the uptake of nutrients. Low P tolerant genotypes had higher P uptake efficiencies and could capture more available soil P under P deficiency than low P sensitive ones. A higher rate of transformation for water-soluble P to slowly available P in P deficient soil than in soil with sufficient P. L-values showed that different cultivars had different soil P use efficiencies and low P tolerance mechanisms. The low P tolerant cultivar DSY-32 regulated soil P use efficiency and plant P content according to the L-value under P fertilizer application, but another low P tolerant cultivar, DSY-2, utilized soil P more efficiently regardless of the L-value.

Key words: *phosphorus-32, L-value, maize, phosphorus use efficiency, phosphorus fractions, root traits.*

INTRODUCTION

Phosphorus (P) deficiency limits plant growth and crop productivity in many regions of the world, especially in developing countries (Tomscha, *et al.,* 2004). To increase crop productivity, farmers often apply substantial amounts of phosphate fertilizers. However, applying large amounts of fertilizer not only reduces limited P resources, but also leads to environmental pollution. Maize is an important grain and forage crop in the world after wheat and rice. It is both sensitive to P and grown widely in areas where P, although abundant, is largely unavailable to plants. Consequently, an approach that has received increasing attention is to adapt crops to unfavourable soil conditions by selecting and evaluating genotypes with enhanced nutrient use efficiency in soils with a low nutrient status, and/or requiring moderately low external inputs to induce expression of their genetic potential for adequate production. This strategy is now considered a promising, energy-efficient, eco-friendly and socio-economically feasible approach (Guo *et al.*, 2002).

Plants differ greatly in their ability to grow on low P soil because they have developed specific physical, chemical and biological mechanisms to utilize P compounds under such conditions (Hiradate *et al.*, 2007). Naruzzaman *et al.* (2006) showed that the evaluation and identification of crop plants for genotype variation in their ability to access and utilize sparingly soluble forms of soil P (Ca–P, Al–P, Fe–P) is a possible means to overcome P deficiency in soils and optimize P fertilizer use in cropping systems where P is poorly available.

In a pre-screening experiment, 116 maize inbred lines with various genetic backgrounds were employed in a field experiment in 2007 which resulted in the selection of 15 maize inbred lines for developing an index system for assessing low P tolerant maize genotypes in a two-year field experiment in 2008 and 2009. To confirm P uptake from sparingly soluble P soils by different maize genotypes, a ^{32}P tracer technique was used as an additional criterion to evaluate maize tolerance in five lines selected from the 15 maize inbred lines.

MATERIALS AND METHODS

Field experiment

Plant materials

A total of 116 maize inbred lines with various genetic backgrounds were collected from several agricultural universities and institutes in China and used in a field experiment (experiment 1) in 2007. Of these, 15 maize cultivars were selected based on differences in 100-grain weight at the mature stage when grown in a P deficient soil and P sufficient soil. Eleven low P tolerant genotypes (DSY-30, DSY-2, DSY-31, DSY-20, DSY-21, DSY-39, DSY-101, DSY-33, DSY-32, DSY-23 and DSY-93), and four low P sensitive genotypes (DSY-113, DSY-79, DSY-129 and DSY-48) were selected for experiments 2 and 3, respectively in 2008 and 2009.

Experimental site

The study was carried out in 2007, 2008 and 2009 at Langfang experimental station, located in Hebei Province (E116°35′41, N39°36′). The general soil characteristics were pH 8.49, available P 4.9 mg/kg, available nitrogen (N) 55.8 mg/kg, available potassium (K) 92.9 mg/kg and organic matter 14.4 g/kg. These parameters were measured as described by Lu (1999). The soil was characterized as a typical P deficient soil.

Experimental design

The experiment involved two treatments, namely, P0 (P deficient) receiving no P fertilizer and P1 (P sufficient) receiving P at

---

1 Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, P. R. China
E-mail address of corresponding author: jcyang@caas.ac.cn; yangjuncheng@caas.cn

L.K. Heng, K. Sakadevan, G. Dercon and M.L. Nguyen (eds), Proceedings — International Symposium on Managing Soils for Food Security and Climate Change Adaptation and Mitigation. Food and Agriculture Organization of the United Nations, Rome, 2014: 37–42

52 kg·P ha^{-1}. Urea, superphosphate, and potassium chloride were applied with N (225 kg·N·ha^{-1} in total, half of which was applied basally and the remainder as a top-dressing during the bell-mouthed or pre-anthesis stage), P (applied basally) and K (87 kg·K·ha^{-1} applied basally). To prevent cross pollination, maize cover package pollination was utilized.

Measurements and statistical analyses

In 2007, ten representative plants were sampled at maturity and the 100-grain weights of the inbred lines were determined. During 2008 and 2009, the plants were harvested at both the seedling and maturity stages. Plants collected during the seedling stage were separated into roots and shoots to assess morphological traits, dried at 65°C in an air-forced oven for 48 h and weighed to determine root and shoot dry weights. The roots were placed in a transparent water-filled tray (15 cm × 25 cm) on a scanner (Epson Perfection 4990 Photo; Model J1318) to facilitate root spreading. The system was used to scan all fine root fragments and the program calculated the traits of all fine roots such as length, volume and surface area. The data obtained were then analysed using the digital image analysis system, WinRHIZO Pro 2007a. After harvesting, the 100-grain weight was determined. Phosphorus uptake efficiency was measured as the amount of P in the plant materials including the roots and shoots at the seedling stage (mg P per plant) (Morris and Garrity, 1993). The P content of the plant materials was determined using the Mo-Sb-Vc colorimetric method (Barry and Miller, 1989) after digestion in sulphuric-perchloric acids.

The inhibition (percent) (due to P limitation) was defined as:

$$\text{Inhibition (percent)} = (P1–P0) / P1 \times 100 \text{ percent}$$

where P1 is plant P uptake in the P1 (P sufficient) treatment (52 kg·P·ha^{-1}), and P0 is plant P uptake in the P0 (P deficient) treatment (0 kg·P·ha^{-1}).

^{32}P isotopic tracer experiment

Plant materials

Based on the results of a previous three-year field experimental selection, five typical cultivars were used: genotypes DSY-30, DSY-2 and DSY-32 (low P tolerant genotypes) and DSY-48 and DSY-79 (low P sensitive genotypes).

Preparation of ^{32}P carrier-free solution

Carrier-free ^{32}P solution was prepared (100 mL) with an activity concentration of 1.85 MBq/mL. Four ml (7.4 MBq) of the solution was diluted in 500 mL deionized water, added to the soil of each pot, and the soil with solution homogenized carefully using a stainless steel rod to avoid spilling. The pots were covered with aluminum foil to avoid light during equilibration and analysis of P fractions.

Treatments

To evaluate the P pool of the low P soil for the different maize genotypes, the pots were separated into two groups, one group with plants and the other group without. Each group included treatment 1 (the P0 treatment: no P application but 200 mg·N·kg^{-1} and 166 mg·K·kg^{-1} soil), and treatment 2 (the P1 treatment: included 66 mg P·kg^{-1} soil, 200 mg·N·kg^{-1} and 166 mg·K·kg^{-1} soil). Each treatment was replicated three times. One week after pot preparation, the experiment was conducted in a greenhouse. Soil samples were taken on days 0, 3, 7, 14 and 25 while plant samples were taken only on day 25.

Measurements

Sequential P fractionations of Ca_2–P, Ca_8–P, Al–P, Fe–P, O–P (occluded P) and Ca_{10}–P were performed by methods described by Gu and Jiang (1990). Measurements of ^{32}P radioactivity in the fractions were made using a liquid scintillation counter (LSC, LS-6500, Beckman, USA). Radioactivity in 5 mL extracts was determined by Cherenkov counting, with counting efficiency being determined using the standard ^{32}P solution provided by the HTA Co. Ltd.

The L-value (µg·P·g^{-1} soil) was calculated as follows:

$$\text{L-value} = [^{32}\text{P Bq·g}^{-1}\text{ soil}] / [^{32}\text{P Bq·µg}^{-1}\text{ P in plant}]$$

Statistical analysis

All data are presented as the means of three replicates with standard errors. Differences between treatment means were compared by least significant difference (LSD) test at $p < 0.05$

RESULTS

Field experiment

Screening of maize genotypes tolerant of low P soil

Figure 1 shows the reductions in 100-grain weights of the different maize genotypes. Overall, the results were normally distributed (KS test, Z value = 0.928, p value = 0.356). Maize genotypes tolerant to low P soils were defined as those showing a change in inhibition lower than -5 percent under low P conditions, while low P sensitive genotypes were selected when the inhibition was higher than 10 percent.

Four quadrant analyses for the selected typical maize genotypes (Figure 2) showed that maize genotypes tolerant to low P were distributed primarily in the first (responsive and efficient) and fourth quadrants (non-responsive and efficient). However, low P sensitive genotypes were distributed mainly in the second (responsive and inefficient) and third quadrants (non-responsive and inefficient). Based on the depression in 100-grain weight and the four quadrant analysis, the following typical 15 inbred lines were selected for further analysis: DSY-30, DSY-2, DSY-31, DSY-20, DSY-21, DSY-39,

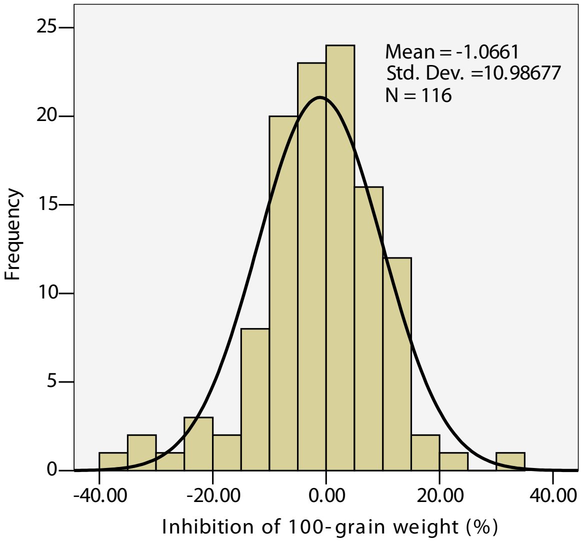


FIGURE 1. Reductions in 100-grain weights of 116 maize inbred lines in field experiment 1.

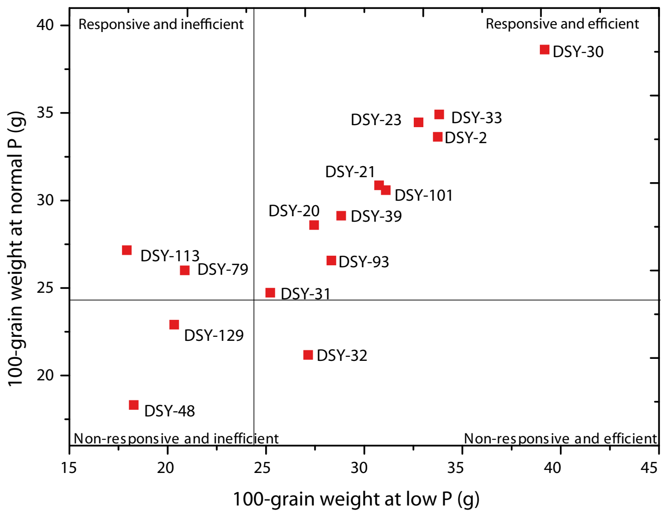


FIGURE 2. Selected P-tolerant and P-sensitive genotypes out of 116 lines. DSY-30 and DSY-2 (responsive and efficient, i.e. tolerant); DSY-32 (non-responsive and efficient, i.e. tolerant); DSY-79 (responsive and inefficient, i.e. sensitive); and DSY-48 (non-responsive and inefficient, i.e. sensitive).

DSY-101, DSY-33, DSY-93, DSY-23 and DSY-32 (genotypes tolerant to low P), and DSY-113, DSY-79, DSY-129 and DSY-48 (genotypes sensitive to low P).

Root architectural traits at seedling stage

Root morphological parameters are shown in Table 1. In 2008, enhancements of root lengths were observed for maize genotypes grown under low P conditions. Specifically, 64 percent of the genotypes that were tolerant to low P showed enhanced root lengths, while only 25 percent of the genotypes that were sensitive to low P displayed this feature under P deficiency. The total root surface areas and root volumes of low P tolerant genotypes were higher than those of low P sensitive genotypes under low P conditions, except for DSY-113.

The data also show that inhibition of root length ranged from around 44 percent to 48 percent in 2009. The total root lengths of low P tolerant genotypes (especially DSY-30, DSY-2, DSY-31, DSY-21, DSY-39, DSY-33 and DSY-32) increased dramatically under P deficiency. Among the low P sensitive genotypes, the inhibition of root length in DSY-21 was significantly lower than those of other genotypes, indicating that low P tolerant genotypes developed longer roots. By contrast, low P sensitive genotypes developed smaller roots under P deficiency compared with P sufficiency. These results indicate that the longer root lengths of low P tolerant genotypes were essential for their higher ability to acquire soil P. Comparing the inhibition of total root surface area with that of total root volume between low P tolerant and sensitive genotypes, the inhibition of root surface area and total root volume ranged from around 45.5–47.0 percent and from 50.0–58.0 percent respectively. Inhibitions of root surface area and total root volume were lower in low P tolerant than in low P sensitive genotypes.

P nutrient characteristics of genotypes at seedling stage in field experiments 2 and 3

There was a close relationship between plant P uptake and the level of soil P supply. The efficiency of P uptake of the different genotypes was lower under P deficiency than under P sufficiency in both field experiments (Figure 3). Under P deficiency, P uptake efficiency in DSY-21 and DSY-23 (both are low P tolerant genotypes) was significantly higher than that of DSY-113 (low P sensitive genotype) in experiment 2 (Figure 3A). Compared with the low P sensitive genotypes, those that were low P tolerant had higher P contents under P deficiency, indicating that the ability of low P tolerant genotypes to take up P was relatively stronger in field experiment 2 (Figure 3B). For example, P uptake efficiencies in DSY-30, DSY-2, DSY-39, DSY-101 and DSY-93 were significantly higher than those of DSY-113, DSY-79, DSY-129 and DSY-48. The level of inhibition of P uptake by low P tolerant genotypes was much lower than that of most low P sensitive genotypes

TABLE 1. Changes in root morphological characteristics of seedlings in field experiments 2 and 3

Genotype	No.	Inhibition of root length (%)		Inhibition of root surface area (%)		Inhibition of root volume (%)	
		2008	2009	2008	2009	2008	2009
P tolerant	DSY-30	–6.9	–6.7	–16.1	–12.2	–25.6	–20.0
	DSY-2	–56.7	–41.6	–10.1	–45.5	22.1	–49.6
	DSY-31	11.0	–29.0	18.1	–28.5	25.0	–27.8
	DSY-21	–17.3	–44.0	5.5	17.3	23.8	52.5
	DSY-39	–7.4	–19.2	8.3	–14.6	21.2	–10.0
	DSY-101	–1.1	29.7	–5.4	27.9	–9.9	26.0
	DSY-33	50.3	–13.0	34.4	–10.7	13.0	–8.3
	DSY-32	34.1	1.4	40.0	–8.0	45.0	–18.0
	DSY-23	11.8	47.7	5.3	50.4	–1.0	53.0
	DSY-93	–127.4	22.0	–53.2	46.5	–3.0	54.3
	DSY-20	–45.5	14.9	–47.6	–1.1	–49.7	–20.1
P sensitive	DSY-113	–68.9	34.8	–56.4	41.0	–45.6	46.7
	DSY-79	17.6	32.9	26.7	32.2	34.5	31.5
	DSY-129	28.2	24.5	22.7	29.1	16.7	33.4
	DSY-48	2.3	32.9	3.3	47.1	4.4	58.3

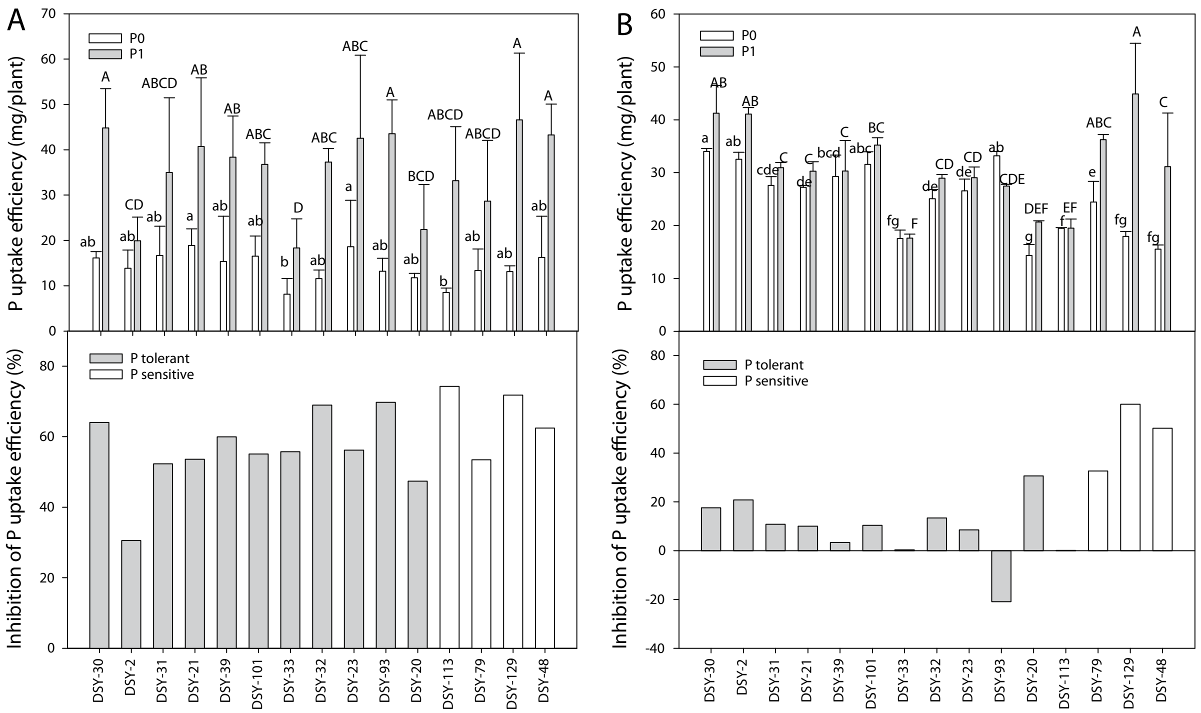


FIGURE 3. P uptake efficiency and inhibition of typical maize inbred lines. A: field experiment 2 (2008), B: field experiment 3 (2009). Different uppercase and lowercase letters mean significant at 5% level.

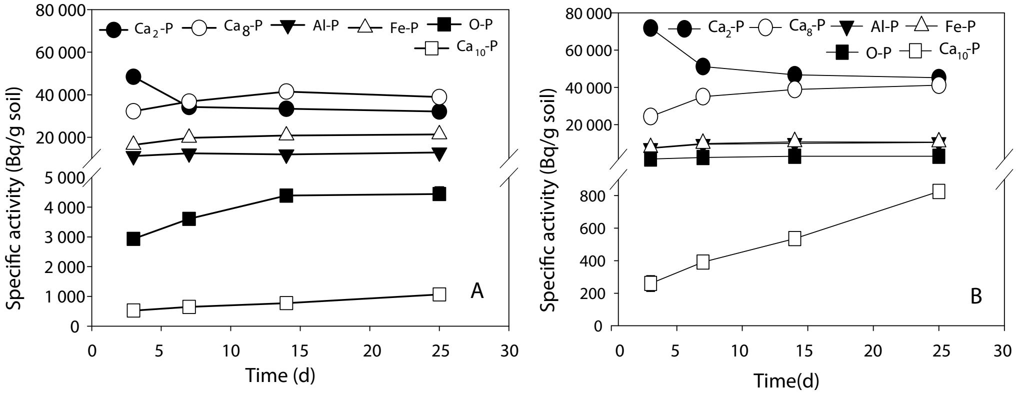


FIGURE 4. Changes over time in ^{32}P specific activities of inorganic P fractions with (A) and without (B) P fertilizer.

in field experiments 2 and 3, indicating that the ability of low P tolerant lines to take up P was relatively strong under low P conditions.

^{32}P isotope tracer experiment

Dynamic variations of P fractions

Without P fertilizer, the ^{32}P activity in Ca_2–P decreased rapidly during the early period. However, the reverse was observed in Ca_8–P, i.e. a rapid increase during the early period was followed by a slow increase. The ^{32}P activities in Fe–P and Al–P increased steadily at a moderate rate and reached a steady state, while those in O–P and Ca_{10}–P increased slowly and steadily during the entire test period of 25 d (Figure 4A). When P fertilizer was added to the soil, the transformation dynamics of ^{32}P in all P soil fractions were consistent with the transformation dynamics without P fertilizer (Figure 4B). Nevertheless, the ^{32}P activities of different fractions showed different characteristics. First, in Ca_2–P the activities were significantly higher than those without P fertilizer in the soil. Second, the ^{32}P activities in both slowly available P (Ca_8–P, Al–P and Fe–P) and unavailable P (O–P and Ca_{10}–P) were significantly lower than without P fertilizer in the soil. These results suggest that the rate at which water-soluble P was transformed to slowly available and unavailable P in the soil deficient in P was higher than in the soil with sufficient P.

Inorganic P pools and L values

As shown in Table 2, the DSY-30 and DSY-2 genotypes had higher L-values and intermediate P uptakes irrespective of whether or not external P fertilizer was applied, indicating slight dependency on insoluble and soluble P and that the two genotypes were likely low P tolerant. The L-value of DSY-32 was the highest under low P limitation, suggesting that this genotype activated and utilized different Pi forms in soil and reduced the dependence on soil soluble P. After P

TABLE 2. L values of different maize genotypes with or without external P fertilizer

	Without external P fertilizer			With external P fertilizer		
Variety No.	P specific activity of plant (Bq/μg)	Specific activity of soil (Bq/g soil)	L value ($ug{\cdot}P{\cdot}g^{-1}$ soil)	P specific activity of plant (Bq/μg)	Specific activity of soil (Bq/g soil)	L value ($ug{\cdot}P{\cdot}g^{-1}$ soil)
DSY-30	22.4±2.5	4 216.0±144.4	190.0±27.6	16.8 ± 0.9	4 015.3±339.1	239.4±32.3
DSY-2	20.0±0.1	4 483.9±153.6	224.7±6.8	14.0 ± 1.0	3 517.3±120.5	252.2±25.9
DSY-32	7.8±1.0	3 964.6±135.8	513.3±45.4	20.4 ± 1.7	3 792.5±129.9	250.8±5.1
DSY-79	11.4±0.8	3 572.4±122.4	314.7±32.4	8.3 ± 0.4	3 820.9±130.9	459.8±17.1
DSY-48	64.9±2.9	3 622.5±124. 1	55.8±0.6	28. 8±2.7	5 089.3±312.2	133.6±36.2

fertilizer was applied, the L-value of DSY-32 decreased rapidly, indicating that more P was utilized from the fertilizer. Therefore, DSY-32 is a typical low P tolerant genotype. Although the L-value of DSY-79 was higher than DSY-30 and DSY-2, the radioactivity of its shoot was also higher (data not shown), indicating that DSY-79 required a much higher P content in the plant to maintain normal crop growth. Therefore, DSY-79 is a typical low P sensitive genotype. The L-value of DSY-48 was significantly lower than that of the remaining four maize genotypes with or without external P fertilizer. One probable explanation is that this genotype had a high dependency on, and was sensitive to the supply of soil available P.

DISCUSSION

The primary objective of most maize breeding programmes is to develop high yielding and well adapted cultivars. The data reported here support these findings since the 100-grain weights of low P tolerant genotypes were higher than those of the low P sensitive genotypes under low P conditions. This suggests that 100-grain weight could be an indicator for screening maize genotypes tolerance to low P. A similar "specific mechanism(s)" has been described by Gourley, Allan and Russelle (1993).

A larger root system provides greater root-soil contact, which is particularly important for uptake of P. Mobile nutrients such as nitrate can be depleted at low rooting density, while for less mobile ions like P, uptake is often closely related to root length. There are a number of reports concerning root length under P deficient conditions. For example, root elongation associated with P deficiency was observed in *Arabidopsis* (Ma *et al.*, 2003), barley (Steingrobe, Schmidt and Claasen, 2001), and rice (Kirk and Du, 1997). The screening of maize germplasm in the present study also revealed root elongation induced by P deficiency since low P tolerant germplasm groups were found to elongate their roots specifically under P deficiency. This could result in roots exploring more soil space for uptake of nutrients (Table 1). This finding is consistent with previous reports of maize root system growth and development being influenced by P deficiency (Mollier and Pellerin, 1999).

Zhang *et al.* (2005) reported that during the seedling stage, P uptake efficiency was the main contributor to P tolerance because the high P uptake and P accumulation features at the seedling stage of P tolerant genotypes were the nutritional foundation for producing more biomass. In this study, low P tolerant genotypes were able to maintain higher uptake efficiencies and absorb more P from the soil to satisfy their growth requirements under low P conditions while low P sensitive genotypes were less able to do so.

Elucidating the transformation dynamics of P fractions is important for better understanding the availability of different P fractions. Jiang and Gu (1989) reported that Ca_2–P was the most rapidly available P source to plants, with Ca_8–P, Fe–P and Al–P being slowly available sources, and P in Ca_{10}–P and O–P being unavailable. Previous studies have shown that after applying P fertilizer to a calcareous soil, the initial P form was Ca_2–P (Dai *et al.*, 2006). The data reported here indicate that the ^{32}P activity in Ca_2–P reached a maximum within three days and then decreased rapidly between 3 to 7 days after ^{32}P-labelled fertilizer P was applied to the Langfang low P soil. It is therefore proposed that Ca_2–P in Langfang low P soil was a fast transformation process in the soil and is the main P fraction available to the plant. A continuously increasing trend in ^{32}P activity was observed with Ca_8–P, Fe–P and Al–P during the entire 25-d test period, and this increasing rate was moderate. Therefore, Ca_8–P, Fe–P and Al–P can be considered as a moderate transformation process. Among these three P fractions, the ^{32}P activity and its increasing rate in Ca_2–P and Ca_8–P reached maximum values, possibly in part because the concentration of Ca is greater than that of Fe and Al in calcareous soils. Therefore, Ca_8–P might be another important fraction of available P in Langfang low P soil. Nevertheless, the ^{32}P activity in Ca_{10}–P and O–P increased very slowly, suggesting that these forms represented a slow transforming phase (Figure 4). Additionally, after fertilizer P application the ^{32}P activity and its increasing rate in O–P decreased radically, indicating that a larger proportion of water-soluble P in P deficient soils without external P fertilizer would be transformed to the plant-unavailable O–P fraction, i.e. the plant would have more difficulty in acquiring P from a P deficient soil. The results presented here are further evidence that external P fertilizer was especially essential for optimum crop growth in the Langfang low P soil.

In low P soils, the P efficiency of plants differs between genotypes within a given plant species (Ozturk *et al.*, 2005). The L-value has considerable theoretical advantages as a measurement of plant available P from the soil P. In this study, the L-values were determined to evaluate the P efficiency of different maize genotypes using a ^{32}P tracer technique after the maize plants were grown in the Langfang low P soil for 25 d. The results showed that the P efficiency of five maize genotypes differed significantly. DSY-32, which is a typical low P tolerant genotype, actively regulated the P use ratio between the soil and exogenous fertilizer P. When exogenous P was supplied, DSY-32 absorbed exogenous P preferentially; otherwise, it would try to exploit soil P when no exogenous P was supplied. However, another low P tolerant genotype, DSY-2, exhibited a different low P tolerant pathway, utilizing soil P efficiently regardless of exogenous P application. This result suggests that different maize genotypes have very different low P tolerance pathways and therefore that it would be useful to fully exploit the limited P resources in low P soils by screening and planting low P tolerant crop varieties. Additionally, the results confirmed the L-value as a very useful parameter for evaluating plant P efficiency.

CONCLUSIONS

This field study clearly demonstrated that maize germplasm differ in their ability to take up P from a low P soil and that the differences were attributed to plant morphological and physiological features. Based on these results, an effective method for enhancing P efficiency is to develop P tolerant cultivars for achieving high yields under conditions of P deficiency. The results indicated that soil P availability during maize seedling development is critical for the early growth and grain yield. Inhibition of root length, surface area, root volume and P uptake efficiency under P deficiency were preliminarily defined as screening indexes for rapid selection of low P tolerant genotypes during the seedling stage. In addition, the 100-grain weight was defined as the screening index for low P tolerant genotype during the mature stage.

The ^{32}P tracer technique was a powerful adjunct for better understanding soil P availability and sources of P pools in a low P soil–plant system. Results indicated that the rate at which water-soluble P was transformed into slowly available and unavailable P in the P deficient soil was higher than in the soil with sufficient P. Ca_2–P was a fast transformation process, while Ca_8–P might be another important available P fraction and Ca_{10}–P and O–P were transformed slowly. The L-values showed that low P tolerant cultivars regulated soil P use efficiency and plant P content.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (grant no. 21107139), the Ministry of Agriculture Public Benefit Research Foundation of China (grant no. 201103007), an FAO/IAEA research contract (no. 13783), the Special Fund of Research Institute Technology Development (grant no. 2012EG134235) and the National Basic Research Program of China (2013CB127406).

REFERENCES

Barry, D.A.J. & Miller, M.H. 1989. Phosphorus nutritional requirement of maize seedlings for maximum yield. *Agron. J.,* 81: 95–99.

Dai, K.J., He, F., Guan, H.L., Shen, Y.X. & Zhang, G.M. 2006. Research advances in plant and its low-phosphorus environment – inducement, adaptation and counter measures. *Chin. J. Ecol.,* 25: 1580–1585.

Gourley, C.J.P., Allan, D.L. & Russelle, M.P. 1993. Defining phosphorus efficiency in plants. *Plant Soil,* 155/156: 289–292.

Gu, Y.C. & Jiang, B.F. 1990. Assay method of inorganic phosphorus fractionation in calcareous soils. *Soils,* 22: 101–102.

Guo, Y.C., Lin, W.X., Shi, Q.M., Liang, Y.Y., Chen, F.Y., He, H.Q. & Liang, K.J. 2002. Screening methodology for rice (*Oryza sativa*) genotypes with high phosphorus use efficiency at their seedling stage. *Chin. J. Appl. Ecol*., 13: 158–159.

Hiradate, S., Ma, J.F., Matsumoto, H. & Donald, L.S. 2007. Strategies of plants to adapt to mineral stress in problem soils. *Adv. Agron.,* 96: 66–133.

Hou, Y.N., Yang J.C., Jiang, H.M., Zhang J.F., Wu, Q.Y. & Li, J. 2009. P content and grain characters of different maize genotypes under low-phosphorus soil environment. *Acta Agric. Nucl. Sin.,* 23: 327–333.

Jiang, B.F. & Gu, Y.C. 1989. A suggested fractionation scheme of inorganic phosphorus in calcareous soils. *Nutr. Cycl. Agroecosys.*, 20: 156–165.

Kirk, G.J.D. & Du, L.V. 1997. Changes in rice root architecture, porosity, and oxygen and proton release under phosphorus deficiency. *New Phytol*., 135: 191–200.

Lu, R.K. 1999. *Soil agro-chemical analysis.* Beijing, China Agricultural Science and Technology Press.

Ma, Z., Baskin, T.I., Brown, K.M. & Lynch, J.P. 2003. Regulation of root elongation under phosphorus stress involves changes in ethylene responsiveness. *Plant Physiol.,* 131: 1381–1390.

Mollier, A. & Pellerin, S. 1999. Maize root system growth and development as influenced by phosphorus deficiency. *J. Exp. Bot.*, 55: 487–489.

Morris, R.A. & Garrity, D.P. 1993. Resource capture and utilization in intercropping: non-nitrogen nutrients. *Field Crops Res.,* 34: 319–334.

Naruzzaman, M., Lambers, H., Michael, D.A. & Veneklaas, E.L. 2006. Distribution of carboxylates and acid phosphatase and depletion of different phosphorus fractions in the rhizosphere of a cereal and three grain legumes. *Plant Soil,* 281: 109–120.

Ozturk, L., Eker, S., Torun, B. & Cakmak, L. 2005. Variation in phosphorus efficiency among 73 bread and durum wheat genotypes grown in a phosphorus-deficient calcareous soil. *Plant Soil,* 269: 69–80.

Steingrobe, B., Schmid, H. & Claassen, N. 2001. Root production and root mortality of winter barley and its implication with regard to phosphate acquisition. *Plant Soil,* 237: 239–248.

Tang, J.H., Yan, J.B., Ma, X.Q., Teng, W.T., Meng, Y.J., Dai, J.R. & Li, J.S. 2007. Genetic dissection for grain yield and its components using an "immortalized F2 population" in maize. *Acta Agron. Sin.*, 33: 1–7.

Tomscha, J.L., Trull, M.C., Jill, D. Lynch, J.P. & Guiltinan, M.J. 2004. Phosphatase under-producer mutants have altered phosphorus relations. *Plant Physiol.,* 135: 334–345.

Zhang, L.M., He, L.Y., Li, J.S. & Xu, S.Z. 2005. Phosphorus nutrient characteristics of different maize (*Zea mays* L.) inbreds for tolerance to low-P stress. *Agric. Sci., China,* 4: 281–287.

Overview of FAO/IAEA Coordinated Research Project on Crop Genotypes Tolerant to Low N and P Soils

J.J. Adu-Gyamfi[1,*] and G. Dercon[1]

ABSTRACT

A five-year coordinated research project (CRP) entitled "Selection and Evaluation of Food (Cereal and Legume) Crop Genotypes Tolerant to Low Nitrogen and Phosphorus Soils through the Use of Isotopic and Nuclear Related Techniques" that supports Member States in their efforts to optimize crop yields and soil productivity in low nitrogen (N) and phosphorus (P) environments was initiated in 10 developing countries in Africa, Asia and Latin America. The overall objective of the CRP was to develop integrated crop, soil and nutrient management practices to increase crop production in marginal lands by identifying and promoting the development of food crop genotypes with enhanced N and P use efficiency and greater productivity. The studies conducted within this CRP concerned two major food security cereal crops namely upland rice (*Oryza sativa* L.) and maize (*Zea maize* L.), and three legumes, namely common bean (*Phaseolus vulgaris* L.), soybean (*Glycine max* L.) and cowpea (*Vigna unguiculata* L.). Protocols for characterization of root traits contributing to enhanced N and P acquisition from low fertility soils with emphasis on rapid root phenotyping methods were developed and used to evaluate and select crop genotypes with superior N and P acquisition and/or utilization. Results showed that out of 150–200 genotypes from the five crops that were tested, 3–5 cultivars identified as N and P efficient in low N and P soils, had better root architecture and produced 15–20 percent higher yields than those with poor root architecture. Branching angle interval was identified as a suitable root selection parameter for soil N use efficiency, while adventitious rooting and root hair formation were identified as suitable plant parameters for selecting P use efficiency. The genotypes of rice, common bean, maize, soybean and cowpea identified in a number of cases provide valuable resources for plant breeding programmes aimed at enhancing P and N use efficiency in crops. The CRP created a database on how cereals and legumes can acquire N and P in low nutrient soils, and this database could be further expanded and interpreted using multivariate analysis on how cereal and legume crops can acquire N and P in low nutrient soils.

Key words: *genotypes, low N and P soils, N use efficiency, P use efficiency, root architecture, root traits.*

INTRODUCTION

Global climate change is likely to exacerbate plant abiotic stresses in the coming decades by increasing water stress and by accelerating soil fertility degradation (St Clair and Lynch, 2010; Lynch and Brown, 2013). To respond to this set of challenges, there is a need to develop agricultural systems with significantly greater productivity and resilience while at the same time use limited natural resources more efficiently. Low phosphorus (P) and nitrogen (N) availability are primary limitations to productivity in low-input agriculture and fertilizers are primary resource inputs in intensive agriculture. A critical feature of future agricultural systems will be new crop varieties with improved conversion of soil resources to yields. These new cultivars would have improved productivity in low-input systems and decrease input requirements in high-input systems.

There are two distinct avenues to improve crop resource use efficiency: (i) improve resource acquisition, and (ii) improve physiological utilization of acquired resources. While both avenues merit exploration, improving resource acquisition represents the greater opportunity for crop breeding (Lynch, 2011). For example, over half of N fertilizer applied in intensive agriculture is not taken up by the crop; crops typically acquire only 5–8 percent of applied fertilizer during the growth season. Improved N and P recoveries by crops would therefore translate into significant economic and environmental benefits along with reduced production of greenhouse gases from denitrification and fertilizer production, and reduced water pollution from leaching and runoff (Cordell, Drangert and White, 2009). Scientists are currently turning their attention to roots — the hidden half of the plant — as central to their efforts to develop crops with greater yields without causing environmental damage. Root biology and the mechanisms of taking up nutrients from the soil are therefore central components of efforts to develop crops with improved soil resources acquisition. Roots are most efficient when their architecture is tailored to their environment. For example, deep, shallow and fine roots can exploit soils in which limiting nutrients are trapped in the soil. Several root traits are known to be associated with P and N acquisition efficiency in low P and N soils. Several of these have the potential to be deployed in crop breeding programmes including: rhizosphere modification to mobilize nutrients (Ryan, Delhaize and Jones, 2001), enhanced symbioses with N_2-fixing bacteria and longer, denser root hairs to enhance the acquisition of N and P and other immobile resources (Lynch and Brown, 2013). Possible root system architecture (RSA) and morphological traits include root hair length, root length, root branching angle and root density and basal root whorl number. Nitrate is highly mobile in soil and is readily lost to leaching and therefore a crop root system with lateral root development and branching may be useful for capturing N as it

---

[1] Soil and Water Management & Crop Nutrition Laboratory, International Atomic Energy Agency, Seibersdorf, Austria

[*] E-mail address of corresponding author: J.Adu-gyamfi@iaea.org

L.K. Heng, K. Sakadevan, G. Dercon and M.L. Nguyen (eds), Proceedings — International Symposium on Managing Soils for Food Security and Climate Change Adaptation and Mitigation. Food and Agriculture Organization of the United Nations, Rome, 2014: 43–48

FIGURE 1. (a) A growth chamber cigar roll method, (b) field sampling and (c) transparent glass tube for evaluating genotypic variations in roots (root architecture and morphology) and plant growth traits associated with N and P acquisition efficiencies in maize at the vegetative growth stage.

moves deeper through the soil. Crop species differ in their ability to take up P and N from the soil, and these differences are attributed to the root system architecture, morphology and physiology of the crops relative to their germplasm base. The identification of root traits for enhanced P and N acquisition is enabling crop breeders to develop new genotypes with better yield in low fertility soils of Africa, Asia, and Latin America.

However, in order to use a trait as a selection criterion for crop improvement through either direct phenotypic selection or marker-assisted selection, it is necessary to develop protocols to measure accurately root traits that enhance N and P acquisition in the glasshouse and in the field.

THE COORDINATED RESEARCH PROJECT

This five-year coordinated research project (CRP) entitled “Selection and Evaluation of Food (Cereal and Legume) Crop Genotypes Tolerant to Low Nitrogen (N) and Phosphorus (P) Soils through the Use of Isotopic and Nuclear-Related Techniques” established a research network and supported the efforts of teams of scientists in sixteen Member States (Australia, Benin, Burkina Faso, Brazil, Cameroon, China, Cuba, France, Germany, Ghana, Kenya Malaysia, Mexico, Mozambique, Nigeria and the United States). The aim of the CRP was the development of integrated crop, soil and nutrient management practices to increase crop production in marginal lands by identifying and promoting the development of food crop genotypes (cereal and legume) with enhanced N and P use efficiency and greater productivity. n Studies were conducted along four main areas of investigation to (i) develop and validate screening protocols for plant traits that enhance N and P acquisition and utilization in major food cereal and legume crops grown in low fertility soils, (ii) employ validated screening protocols to identify genotypes with superior N and P acquisition and/or utilization, (iii) identify mechanisms for adaptation and high productivity of selected legumes and cereals to low N and P soils using isotopic techniques (stable ^{15}N and radioactive phosphorus-32 [^{32}P] and phosphorus-33 [^{33}P], and (iv) assess the selected genotypes with different enhanced root architecture and morphology that capture and/or utilize nutrients from different soil depths under field conditions from the standpoints of yield and productivity.

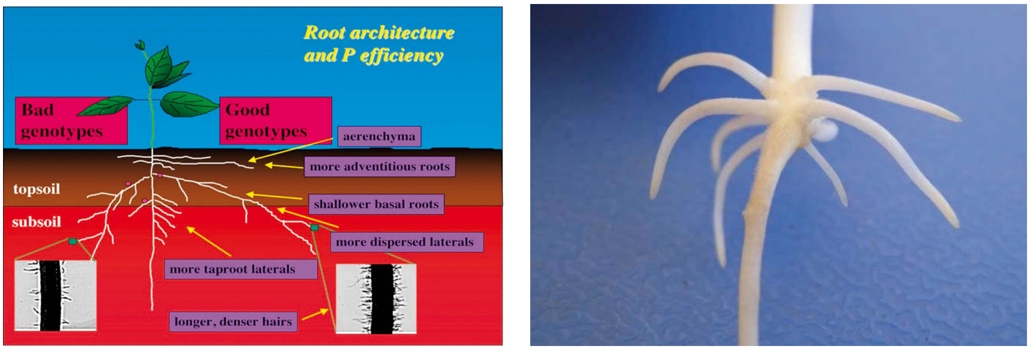
The studies concerned two major food security cereal crops, namely, upland rice (*Oryza sativa* L.) and maize (*Zea maize* L.), and three legumes, namely common bean (*Phaseolus vulgaris* L.), soybean (*Glycine max* L.) and cowpea (*Vigna unguiculata* L.). They were conducted across a wide geographical area in both the northern and southern hemispheres under a wide range of environmental and edaphic conditions. Experiments were conducted in the laboratory or in glasshouses for rapid screening at the early seeding stage using the paper-rolled cigar method, and under field conditions for final evaluation and selection of the genotypes. The main results and recommendations arising from the CRP are summarized below according to the following four main outputs.

Protocols for evaluating root traits (architecture and morphology) contributing to enhanced P acquisition from low-P soils.

Root architectural phenotype influences P and water acquisition from the soil. Crop genotypes with shallow roots, many basal root whorls, adventitious roots and basal roots have advantages in acquiring P from low P soils, while genotypes with deeper basal roots and longer primary roots will acquire water from deeper soil horizons. Developing protocols which can provide robust and rapid evaluation of RSA traits that enhance N and P acquisition by different crops in the glasshouse and in targeted production environments is vital. A simple visual method to evaluate crop root phenotypes at the early seedling stage using the paper-rolled cigar method and at the late growth stage in the field using shovelomics were developed and validated. This method is available in three different languages at http://roots.psu.edu or http://www.naweb.iaea.org/nafa/swmn/news-swmcn.hmtl. Field phenotyping using shovelomics should be useful for evaluating food crop genotypes for low P and drought tolerance in developing countries. During the CRP, a new version of the SIMROOT model was created for simulating large diversity of root systems. In addition, protocols for fractionation of soil P using ^{32}P to elucidate the mechanisms of P acquisition from different soil P pools were developed and fine-tuned at the Seibersdorf Laboratories to support the CRP.

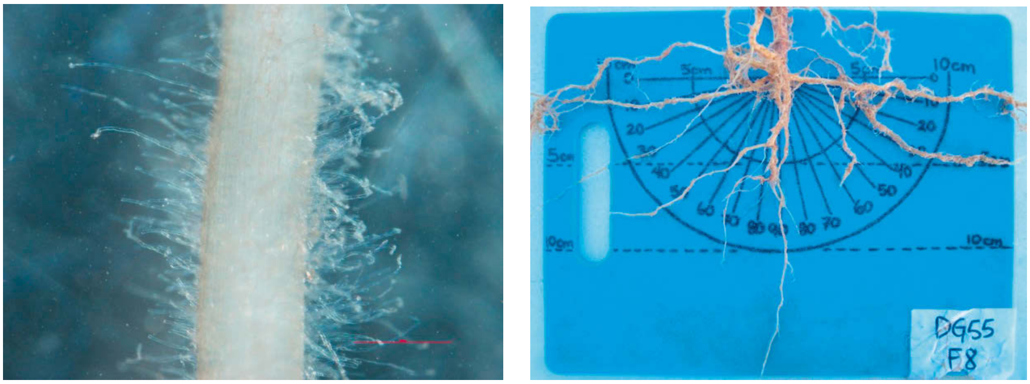
Validation of screening protocols to select genotypes with superior N and P acquisition and/or utilization

Germplasm of maize, upland rice, common bean, cowpea and soybean were exchanged among the participants or were acquired from four centres belonging to the Consultative Group on International Agricultural Research (CGIAR): soybean and cowpea lines from the International Institute of Tropical Agriculture (IITA), maize from the Centro Internacional de Mejoramiento de Maiz y Trigo (CYMMT), common bean from the Centro Internacional de Agricultura Tropical (CIAT) and upland rice from The African Rice Center [formerly West



Root Architecture

Basal Root Whorls Number (BRWN)



Root Hairs

Basal Root Growth Angle (BRGA)

FIGURE 2. Root phenotypes associated with genotypic differences in adaptation to low nitrogen and phosphorus.

Africa Rice Development Association (WARDA)]) and other advanced research institutes. One hundred and fifty to two hundred genotypes were screened at the early seedling stage for enhanced N and P acquisition using the paper-rolled cigar method using PVC tubes of length 15.0 cm and 3.4 cm inner diameter (Figure 1). Twenty five genotypes with different abilities to grow in low P and N conditions selected from the different crops at the seedling stages were further evaluated under field conditions (Figure 1) at two or more sites under diverse agro-ecological environments. Data on environmental variables (latitude, longitude, altitude, rainfall and temperature), soil classification and soil physico-chemical characteristics and systems studied were recorded.

Root characteristics evaluated included: basal root whorls number (BRWN), root hair length density (RHLD), basal root growth angle (BRGA), root length (RL), root length density (RLD), root angle (RA), root branching (RB), adventitious root length (ARL), adventitious root number (ARN), adventitious root branching (ARB), basal root length (BRL) basal root number (BRN), basal root branching (BRB), basal root depth (BRD), primary root depth (PRD), primary root branching (PRB), seminal root length (SRL), lateral root length (LRL), lateral root number (LRN), seminal root elongation (SRE), root AM colonization (RAMC) and root biomass (RB). In addition, data were obtained on: shoot biomass (SHB), grain yield (GY), stem diameter (STDIA), leaf area index (LAI), plant height (PLHT), leaf chlorophyll (LCHL) and nodule weight (NODWT) for legumes (Figure 2)

The results from the 16 countries for the five crops showed that:

- branching angle interval and seminal root length were suitable root selection parameters for soil N use efficiency, while adventitious rooting and root hair formation were identified as suitable parameters for selecting P use efficiency.
- P efficiency correlated strongly with genotypic differences in root hair length, root hair plasticity and lateral root length.
- genotypes with more root cortical aerenchyma (RCA) had deeper roots and produced twice the amount of shoot biomass in low N conditions than genotypes with less (RCA). For beans, RHLD, BRGA, BRWN, ARL and BRN were identified as the most suitable traits (Figure 3 and Table 1).

It is concluded that (i) seedling screening tools demonstrated significant genotypic variation for root traits. These included root length, angle, number of axial roots and branching as well as root hair parameters (length and density), (ii) cultivars identified with some of these traits proved superior for uptake of P and N under conditions of nutrient stress, and (iii) cultivars with superior growth, nutrient acquisition and efficiency obtained good yields of grain under conditions of nutrient stress (Figure 3).

Effects of selected genotypes on cropping system performance

The assessment and selection of crop genotypes with different enhanced root characteristics that explore nutrients from different soil depths under field conditions is relevant for promoting food security and long-term sustainability of soil fertility. Five to ten genotypes were further assessed for their productivity in low-input systems. Five rice and maize genotypes were selected that had the highest N use efficiency (66–80 percent) and the highest P use efficiency (6–8 percent), and which also had 15–30 percent greater yields than

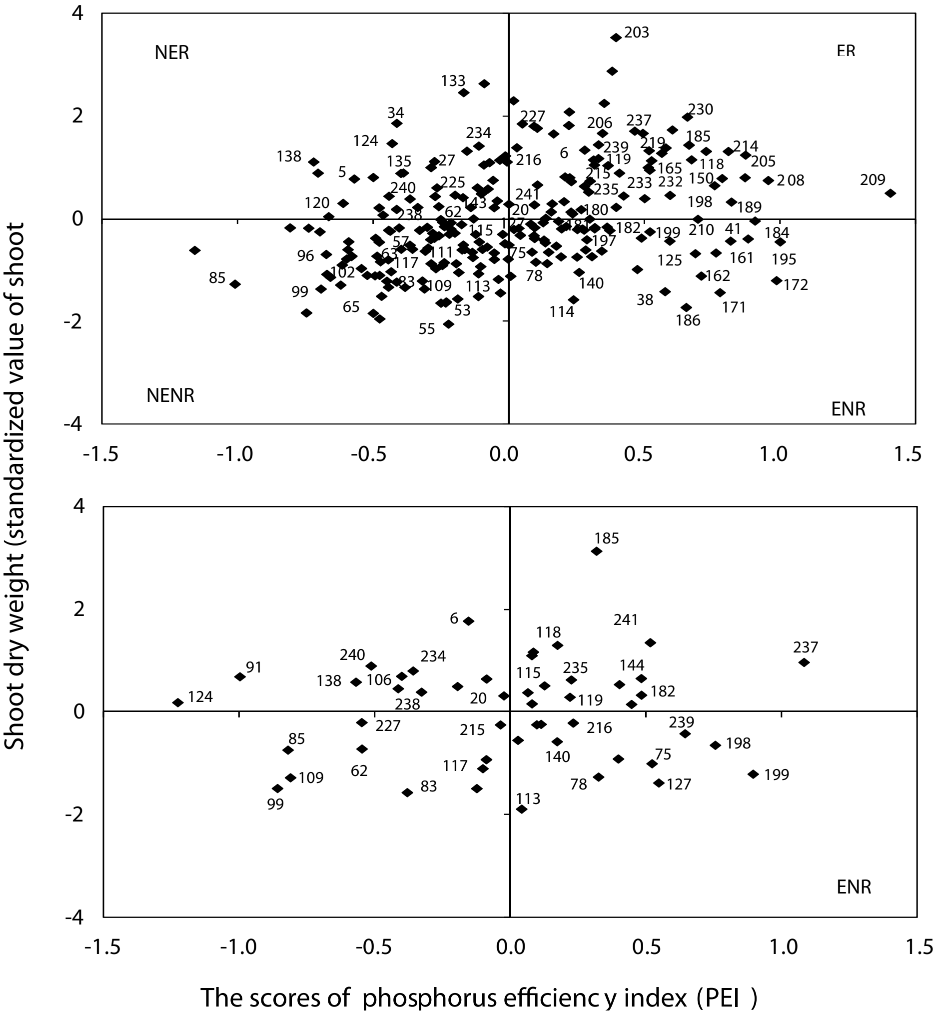


FIGURE 3. Division of charts of 242 and 50 common accessions according to P efficiency and standardized value of shoot dry weight under high P conditions. Phosphorus efficiency is expressed as PEI, which is an assessment index calculated from principal component analysis. Standardized values of shoot dry weight were estimated as the following function: $Xs = (X - \bar{X})/SD$. Categories represented by efficient and responsive (ER), non-efficient and responsive (NER), non-efficient and non-responsive (NENR), and efficient and non-responsive (ENR). Accession numbers are indicated (Bayuelo-Jiménez *et al.*, 2011).

the others. For common bean, soybean and cowpea genotypes were identified with deep root systems produced 20–40 percent better yields, 45 percent increases in biological N_2 fixation (BNF), and 40 percent less soil erosion in low P soils. Phosphorus efficient legumes contributed to soil fertility by enhanced biological BNF, which is quite sensitive to P supply. Economically, the greater productivity of P- and N- efficient genotypes would give third world farmers greater flexibility in soil management options, purchasing fertility inputs etc., in addition to greater household incomes and food security.

Mechanisms for adaptation and high productivity to low soil N and P using isotopic techniques

Nuclear, isotopic and related conventional techniques were employed to obtain quantitative estimates on optimization of plant nutrient (N and P) uptake and utilization from fertilizers and soils. For instance, stable N-15 and radioactive P-32/P-33 techniques were employed to obtain quantitative estimates for optimization of plant nutrient (N and P) uptake or utilization by N and P efficient crop genotypes in low N and P environments. In order to understand the mechanisms of the genotypic tolerance to low P soil to utilize P from the sparing soluble P forms, five maize genotypes selected from 116 maize inbred lines were used in a ^{32}P isotope tracer experiment to follow the recovery of ^{32}P in soil P fractions. The L-value and P availability of soil was also assessed.

The ^{32}P tracer results showed that after the addition of ^{32}P-Pi to the soil with no P fertilizer applied for 25 d, 29.0 percent of ^{32}P was quickly transformed into Ca_2–P (rapidly available P), and 66.1 percent was transformed into Al–P, Fe–P and Ca_8–P (slowly available P). Only 5.0 percent of ^{32}P was transformed into O–P and Ca_{10}–P (plant-unavailable P). Moreover, in the soil with P fertilizer applied, ^{32}P transformation into Ca_2–P increased, and the transformation into Ca_8–P, Fe–P, Al–P, O–P and Ca_{10}–P decreased significantly compared with the soil with no P fertilizer applied ($p < 0.05$). This result suggested a higher rate for water-soluble P transformation to slowly available and plant-unavailable P in P deficient soil than in soil with sufficient P. Low P tolerant cultivar DSY-32 regulated soil P use efficiency and plant P content according to exogenous P fertilizer application. How-

TABLE 1. Phenotypic correlations among nodal root traits and shoot biomass: number of nodal roots (Nodal_No.), nodal root length (Nodal_RL), nodal branching (Nodal_Br), nodal root angle (Nodal_Ra), shoot dry weight (ShDW), and grain yield (Gy)

HP/LP [a]	Nodal_No.	Nodal_RL	Nodal_Br	Nodal_Ra	ShDW	Gy
Experiment 1 (n = 242)						
Nodal_No	0.49***	0.25***	0.39***	0.37***	0.38***	0.10
Nodal_RL	0.42***	0.39***	0.50***	0.48***	–0.09	0.24**
Nodal_Br	0.55***	0.51***	0.49***	0.51***	0.10	0.21**
Nodal_Ra	0.33***	0.31***	0.46***	0.32***	–0.02	0.07
ShDW	0.49***	–0.03	0.21**	–0.02	0.50***	0.09
Gy	0.18*	0.07	0.28**	0.10	0.16	0.68***
Experiments 1 + 2 (n = 50)						
Nodal_No	0.32**	0.36**	0.32**	0.13	0.41**	0.09
Nodal_RL	0.19	0.39***	0.52***	0.31**	0.00	0.21*
Nodal_Br	0.25*	0.54***	0.26*	0.24	–0.03	0.18
Nodal_Ra	0.05	0.21	0.45**	0.32**	0.04	0.25*
ShDW	0.48***	–0.01	–0.01	–0.24*	0.36**	0.05
Gy	0.01	0.38***	0.35**	–0.06	0.02	0.54***

[a] For each environment, values below the diagonal represent correlations within the low P treatment; values above the diagonal represent correlations within the high P treatment; values on the diagonal (italic) correspond to across-phosphorus treatment correlations.

*, **, *** Significant respectively at $p < 0.05$, $p < 0.01$, $p < 0.001$

TABLE 2. L-values of maize genotypes without external P fertilizer

Variety no.	Specific activity Plant (Bq/µg)	Soil (Bq/g · soil)	L-value (µg P/g · soil)
DSY-30	22.4±2.5†	4 216 ± 144	190 ± 28
DSY-2	20.0 ± 0.1	4 484 ± 154	225 ± 7
DSY-32	7.8 ± 1.0	3 965 ± 136	513 ± 45
DSY-79	11.4 ± 0.8	3 572 ± 122	315 ± 32
DSY-48	64.9 ± 2.9	3 623 ± 124	56 ± 1

† Values following means are ± standard errors

TABLE 3. ^{15}N enrichment, amount of N derived from fertilizer and percentage nitrogen use efficiency of upland rice

Variety	^{15}N (atom % excess)		N derived from fertilizer (kg/ha)			% N use efficiency
	Plant	Grain	Plant	Grain	Total	
Nabawan	0.671 a	0.553 a	40.2 de	23.0 bc	63.2 bc	42.1 bc
Tenom	0.721 a	0.614 a	31.3 e	29.7 abc	61.0 c	40.7 c
WRDA 20	0.629 a	0.684 a	27.7 e	41.5 ab	69.2 bc	46.2 bc
WRDA 99	0.731 a	0.696 a	48.3 cde	51.8 a	100.1 abc	66.7 abc
Sintok	0.711 a	0.654 a	64.0 bcd	38.8 ab	102.7 ab	68.5 ab
Pulut Petai	0.770 a	0.758 a	70.6 bc	43.0 ab	113.6 a	75.7 a
Merah	0.729 a	0.714 a	109.3 a	11.7 c	121.0 a	80.7 a
Kuku Belang	0.753 a	0.685 a	78.6 b	21.1 bc	99.7 abc	66.4 abc

Data within a column followed by the same lower case letter are not significantly different ($p < 0.05$)

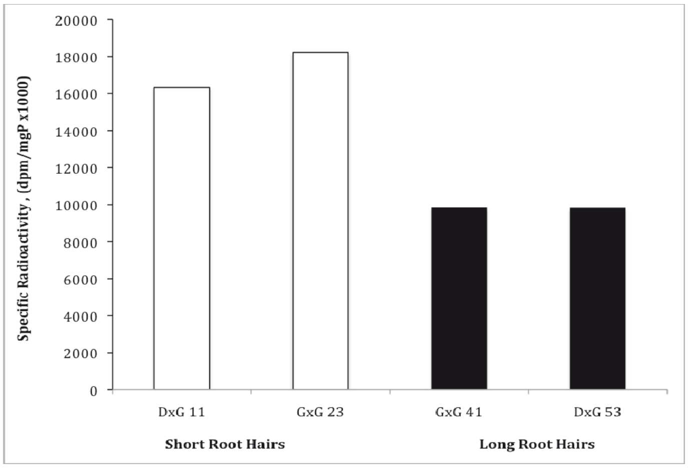


FIGURE 4. Specific radioactivity in plant tissue of four genotypes with contrasting root hairs: Genotypes with short root hairs (open bars) and genotypes with long root hairs (full bars). Genotypes G x G 41 and D x G 53 have long root hairs, while genotypes G x G 23 and D x G 11 have short root hairs.

ever, another low P tolerant cultivar, DSY-2, used soil P more efficiently, regardless of the application of exogenous P (Yang *et al.,* 2012). It was therefore concluded that the ^{32}P tracer technique was a valuable tool for understanding the physiology behind superior genotype performance (Table2).Similar results were reported by Adu-Gyamfi, Aigner and Gludovacz (2009) who showed that in a low-P soil, maize was more efficient than soybean in taking up soil P. The available P (Bray II) and the Ca–P were the fractions most depleted by plants followed by the Fe–P fractions. For common bean, the results showed that efficient genotypes with long root hairs had lower specific activity values compared with inefficient genotypes, since these were able to take up P from two different pools with a greater total P accumulation (Figure 4). For upland rice, ^{15}N and ^{32}P were employed to obtain quantitative estimates for optimization of plant N and P by N and P efficient crop genotypes in low N and P environments. The results presented here showed that (i) the ^{15}N enrichment in plants ranged between 0.629 and 0.753 atom % excess, while the ^{15}N enrichment in upland rice seeds ranged from 0.553 to 0.757 atom % excess. Variety Merah showed the highest N use efficiency in upland rice (80.7 percent) while the lowest N use efficiency was obtained from variety Tenom (40.7 percent). Nitrogen use efficiency by these upland rice genotypes was high (40–80 percent of applied N), with good grain yield (Table 3), while P use efficiency was similar to the other crops (2.4–8 percent) (Data not shown).

CONCLUSIONS

The main conclusions from the CRP are summarized as: (i) seedling screening tools demonstrated significant genotypic variation for root traits; these included root length, angle, number of axial roots and branching as well as root hair parameters (length and density); (ii) cultivars identified with some of these traits proved superior for uptake of P and N under conditions of nutrient stress; (iii) cultivars with superior growth, nutrient acquisition and efficiency had good yields of grain under conditions of nutrient stress; (iv) in some cases, positive agro-ecological outcomes were identified that were related to the performance of cultivars selected for favourable root traits; (vi) nuclear tools, specifically the use of ^{15}N and ^{32}P as tracers, proved valuable in studies that sought physiological explanations for superior genotype performance; and (v) some genotypes of rice, common bean, maize, soybean and cowpea were identified that provide valuable resources for plant breeding programmes aimed at enhancing P and N use efficiency.

REFERENCES

Adu-Gyamfi, J.J., Aigner, M. & Gludovacz, D. 2009. *Variations in phosphorus acquisition from sparingly soluble forms by maize and soybean in low-P and medium-P soil using ^{32}P.* Proceedings XVI International Plant Nutrition Colloquium, Sacramento, USA. 10 pp. (available at: http://repositories.cdlib.org/ipnc/xvi/1317).

Bayuelo-Jiménez, J.S., Pérez-Decelis, V.A., Magdaleno-Armas, M.L., Gallardo-Valdéz, M., Ochoa, I. & Lynch, J.P. 2011. Genetic variation for root traits of maize (*Zea mays* L.) from P'urhépecha Plateau, under contrasting phosphorus availability. *Field Crops Res.*, 121: 350–362.

Cordell, D., Drangert, J.-O. & White, S. 2009. The story of phosphorus: Global food security and food for thought. *Global Environ. Change,* 19: 292–305.

FAO. 2010. *Current world fertilizer trends and outlook to 2014*. Rome.

Lynch, J.P. 2011. Root phenes for enhanced soil exploration and phosphorus acquisition: tools for future crops. *Plant Physiol*., 156: 1041–1049.

Lynch, J.P. & Brown, K.M. 2013. New roots for agriculture: Exploiting the root phenome. *Phil. Trans. R. Soc. B.,* 367: 1598–1604.

Ryan, P.R., Delhaize, E. & Jones, D.L. 2001. Function and mechanism of organic anion exudation from plant roots. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., 52: 527–560.

St Clair, S.B. & Lynch, J.P. 2010. The opening of Pandora's Box: Climate change impacts on soil fertility and crop nutrition in developing countries. *Plant Soil,* 335: 101–115.

Yang, J.C., Wang, Z.G., Jiang, H.M., Zhang, J.F., Pan, P., Han, Z., Lu,C., Li, L.L. & Ge, C.L. 2012. Inorganic phosphorus fraction and its translocation dynamics in a low-P soil. *J. Environ. Radioactiv.,* 112: 64–69.

Using Boron Isotope (^{10}B) to Study Boron Uptake and Translocation by Peach and Plum Trees Grown in Sandy and Calcareous Soils Under Different Levels of Calcium

R.A. El-Motaium[1,*], S.H. Badawy[2] and P.H. Brown[3]

ABSTRACT

The objective of this study was to investigate boron (B) uptake and translocation using boron-10 (^{10}B), in response to calcium (Ca) supplementation of the substrate in an attempt to understand B function in plant metabolism. A pot experiment was designed using two-year old Marianna plum and Lovell peach trees. They were grown in 20 litre (L) pots filled with sandy or calcareous soil. Treatments included two concentrations of B (2.5 and 5.0 mg/kg) and three concentrations of Ca (80, 200 and 400 mg/kg). Boron was applied as H_3BO_3 enriched in ^{10}B and Ca as $(CH_3COO)_2\ Ca.H_2O$. The pots were arranged in a completely randomized design. Leaf, stem, fine root, root tip and root tip cell wall samples were collected at the end of the growing season for determination of total B, ^{10}B and Ca. Plant samples were extracted using 0.20 N HNO_3 and analysed using an inductively coupled plasma mass spectrometer (ICP/MS). Cell wall extraction was performed on 0.5–1.0 g fresh weight of the root tips. Results indicated that in sandy soil, there was an inverse relationship between B concentration in the nutrient solution and plant dry weight. However, as Ca concentration in the nutrient solution increased the dry weight increased. Analysis of variance showed that both B and Ca in the irrigation solution had significant effects on B partitioning in the three plant organs. Calcium application at 200 and 400 mg/kg reduced the B concentration significantly in the leaf and stem but linearly increased B concentration in the fine root. Under calcareous soil, less B was recorded in the leaf and stem but more in the fine root compared with its concentrations in the plants grown in sandy soil. The root tips maintained the highest B concentration. In both peach and plum the ^{10}B:^{11}B ratio increased as B concentration in the nutrient solution increased. However, Ca application decreased the ^{10}B:^{11}B ratio in the leaf indicating less B was translocated to the leaf. There were significant differences between B concentrations in Lovell peach and Marianna plum trees. Marianna accumulated less B than Lovell in the leaf and stem but more B in the root tips. Under calcareous soil, less B was translocated to the shoot than under sandy soil. The lower B translocation to the shoot system could be explained by the high fixation capacity (precipitation and adsorption) of calcium carbonate to B under calcareous soil. Boron and Ca levels in the root tip cell wall showed that Ca followed the same pattern as B. As Ca concentration in the irrigation solution increased (400 mg/kg), significant increases occurred in the B concentration in root tip cell walls. The percent increase of B in the cell wall reached about five- and seven-fold of its concentration at low Ca level (80 mg/kg) in sandy and calcareous soil, respectively.

Key words: *stable isotope, boron-10, ICP/MS, cell wall, boron partitioning, root tip.*

INTRODUCTION

Egypt is one of the countries most affected by climate change. It is located in an arid/semi-arid zone, north of the Equator, characterized by low erratic rainfall, high temperature and periodic drought. As a consequence, levels of boron (B) are often high in soils and groundwater and can reach toxic levels in plants causing crop yield reduction. Reclamation of high B soils requires about three times as much water as reclamation of saline soils (Keren and Bingham, 1985).

Since 1923, B was established as an essential element (Warington, 1923) and significant advances have been made in understanding its functions in plant metabolism (Brown *et al*., 2002). These include synthesis of plant cell walls, involvement in reproductive growth and development (flowering and fruit set), in phenol metabolism, influence on photosynthesis, role in membrane structure and function, involvement in membrane electron transport (Brown *et al*., 2002) and role in cross-linking of pectin substances in the cell wall (Kobayashi, Matoh and Azuma, 1996; O'Neill *et al.,* 2004).

The functions of calcium (Ca) in plant metabolism are: maintenance of the integrity of cell membranes including permeability, stability and selective ion uptake (Mengel and Kirkby, 1982). Calcium is also required for cell elongation, cell division (Burstrom, 1968), pollen germination and growth of pollen tubes. Calcium increases the rigidity of the cell wall by binding with pectin (Burstrom, 1968).

Functional similarities between Ca and B have also been established, the main one being their absence from the phloem sap and their extracellular apoplastic function (Marschner, 1995). Boron and Ca move along the transpiration stream and they share the same deficiency symptoms (Crisp *et al.,* 1976). They both exist in the same location in the plant cell wall (Marschner, 1995) and play similar roles in lignification. Both are involved in the basipetal transport of the plant hormone indole acetic acid (IAA; Tang and Dela Fuente, 1986).

There have been difficulties in studying B uptake and mobilization in higher plants due mainly to the lack of radioactive isotope for

---

1 Plant Research Department, Nuclear Research Center, Atomic Energy Authority, Cairo, Egypt
2 Soil Sciences Department, Faculty of Agriculture, Cairo University, Egypt
3 Department of Plant Sciences, The University of California, Davis, USA
* E-mail address of corresponding author: elmotaiumr@yahoo.com

L.K. Heng, K. Sakadevan, G. Dercon and M.L. Nguyen (eds), Proceedings — International Symposium on Managing Soils for Food Security and Climate Change Adaptation and Mitigation. Food and Agriculture Organization of the United Nations, Rome, 2014: 49–54

B. Several researchers have shown the possibility of using boron-10 (^{10}B) as a tracer to study B uptake and transport in plants (Martini and Thellier, 1980). Also, the development of inductively coupled plasma mass spectrometry (ICP-MS) and the use of the enriched ^{10}B isotope have made it possible to study B uptake and movement within the plant.

Boron adsorbing surfaces in soils include: aluminum and iron oxides, clay minerals, calcium carbonate and organic matter (Goldberg, 1997). Calcium carbonate acts as an important B adsorbing surface in calcareous soils (Goldberg and Forster, 1991). Retention of B on calcium carbonate results from an adsorption mechanism (Ichikuni and Kikuchi, 1972) which could involve exchange with carbonate groups.

A limited amount of work has been carried out on the internal distribution of B in plants although it has been shown to be present in the free space, cytoplasm, vacuole and cell wall (Thellier, Duval and Demarty, 1979).

The objectives of this research were to study: (i) the uptake, translocation and partitioning of B in plants at the organ and cellular levels (compartmentation of B) using ^{10}B, (ii) the interaction between B and Ca in plants, and (iii) the use of Ca supplementation approach as a substitute for leaching to reclaim soils high in B. It was anticipated that the results might shed light on the functions of B in plant metabolism.

MATERIALS AND METHODS

Two-year-old Mariana plum (local variety) and Lovell peach (imported variety) plants were grown in 20 L pots filled with either sandy or calcareous soil. Lovell peach and Marianna plum were chosen because they have proven to be the most sensitive rootstocks to high B concentrations in the substrate (El-Motaium *et al.,* 1994). The general properties of the soils used are given in Table 1. The original concentrations of hot water extractable B and available Ca were determined in the two soils. These were respectively 1.3 and 1.4 mg/kg, and 0.25 and 20 mM for the sandy and calcareous soils.

Treatments included two levels of B (2.5 and 5.0 mg/kg) and three levels of Ca (80, 200 and 400 mg/kg). Boron was applied as H_3BO_3 with enriched ^{10}B and Ca as $(CH_3COO)_2$ Ca.H_2O. Modified half-strength Hoagland (Johnson *et al.,* 1957) solution was applied as the irrigation solution.

The pots were arranged in a completely randomized design, a treatment being assigned randomly to each pot and each treatment was replicated eight times. Plants were grown for six months (March–August). Plants were separated into leaf, stem, fine roots and root tips. Leaves of Marianna plum were separated from the stem in three different categories: young leaves, at the top portions of the stems, mature leaves, at the middle portions of the stems, and old leaves, at the bottom portions of the stems.

Determination of total B, ^{10}B and Ca of the three organs was performed after oven drying at 65°C for 24 h. Root tips were collected from the fine roots for determination of total B, ^{10}B and Ca. Plant samples were extracted using 0.2 N HNO_3 and analysed using ICP/MS. To test for the accuracy of the method, a standard reference material was used. Cell wall extraction was performed on 0.5–1.0 g fresh weight of the root tips according to Selvendran and O'Neill (1987). The experiment was repeated for two years using new trees each year.

RESULTS AND DISCUSSION

Plant growth

Plant growth was measured as leaf, stem and root dry weight (Table 2). The data showed an inverse relationship between B concentration in the nutrient solution and plant dry weight. As the B concentra-

Table 1. Chemical and physical characteristics of sandy and calcareous soils

Soil	pH	EC (dS/m)	OM (%)	$CaCO_3$ (%)	CEC (meq 100g)	WHC (%)	BD (g/cm)	Clay (%)	HWE (mM)	Ca Avail. (mM)	Texture
Sandy	7.5	0.40	0.45	1.40	1.45	12.5	1.40	1.53	0.13	0.25	Sand
Calcareous	8.4	0.75	0.60	45.0	5.32	26.6	1.30	5.60	0.14	20.0	Loamy sand

SE — saturation extract; EC — electrical conductivity; OM — organic matter (%); CEC — cation exchange capacity; WHC — water holding capacity; BD — bulk density; and HWE — hot water extract

Table 2. Dry weight (g) of plum and peach organs grown in sandy and calcareous soils as affected by B and Ca in the substrate

		Sandy						Calcareous					
		Leaf		Stem		Fine root		Leaf		Stem		Fine root	
	Ca (mg/kg)	B (mg/kg)						B (mg/kg)					
		2.5	5	2.5	5	2.5	5	2.5	5	2.5	5	2.5	5
Plum	80	20	15	55	50	90	75	30	20	60	56	105	90
	200	25	20	65	57	120	100	40	28	70	61	140	120
	400	30	25	70	65	140	125	50	38	80	72	170	150
LSD(0.05)	B	1.88		2.01		2.87		2.01		2.48		2.01	
	Ca	2.30		2.46		3.52		2.46		3.04		2.46	
Peach	80	40	30	50	40	150	130	50	36	56	45	160	140
	200	45	33	60	50	200	160	55	44	65	55	230	180
	400	52	37	70	60	250	200	62	50	75	66	270	220
LSD(0.05)	B	2.01		2.22		5.67		2.01		3.19		6.80	
	Ca	2.46		2.72		6.95		2.46		3.91		8.33	

tion in the nutrient solution increased the dry weight decreased significantly in the three organs. However, as the Ca concentration in the media increased the dry weight increased significantly. The same trends were noted for plants grown in sandy and in calcareous soils.

In both plum and peach trees, B and Ca levels in the substrate had significant effects on the dry weight of leaf, stem and root. Boron had a greater effect on the dry weight of plant organs than Ca. In fine roots, B levels in the substrate increased as root dry weight decreased but Ca increased fine root dry weight significantly at both B levels. The effect of Ca on dry weight was more pronounced in the fine roots. This was not only related to the Ca effect but also to the high pH (8.4) particularly in the calcareous soil. The data indicated some kind of binding between B and Ca that could result in the formation of non-toxic compounds. Trees showed a stunted appearance under high B but vigorous growth when higher amounts of Ca were added to the substrate.

Boron partitioning in the different plant organs

Analysis of variance showed that both B and Ca in the irrigation solution had significant effects on B partitioning in the three plant organs (leaf, stem and fine root). Boron concentrations in leaf, stem and fine root increased as the B concentration in the irrigation solution increased. However, at the same B concentration (2.5 or 5.0 mg/kg), B concentrations in the three organs were considerably modified by levels of Ca in the irrigation solution. Boron was concentrated mainly in the fine root and less was translocated to the shoot under high Ca application rate.

Calcium application at 200 and 400 mg/kg reduced significantly B concentrations in the leaf and stem but linearly increased B concentration in the root (Table 3). Calcium application at 200 and 400 mg/kg reduced significantly the B concentrations in leaf and stem compared with the 80 mg/kg concentration. By contrast, B accumulation in the fine root increased significantly with increasing Ca supplementation. Similarly, as the Ca concentration in the irrigation solution increased its concentration in the fine root increased (data not shown). Calcium in the irrigation solution increased significantly the concentration of B in the fine root. This increase was linearly related to the increase in Ca in the substrate. These results applied to both species.

In the calcareous soil, the concentration of B was lower in the leaf and stem but higher in the root compared with the corresponding values in sandy soil. The root tips maintained the highest B concentration. The lower B content of the shoot system could be explained by the high B fixing capacity of calcium carbonate (precipitation and adsorption) in the calcareous soil. The higher B concentration in the root was presumably due to the higher pH and higher background Ca in the soil.

The correlation coefficients confirmed our previous finding. Highly significant correlation coefficients were found between B concentrations in the plant leaf and fine root (p = 0.01) and their dry matter yield (r = –0.977 and +0.959, respectively) (Figure 1). The cor-

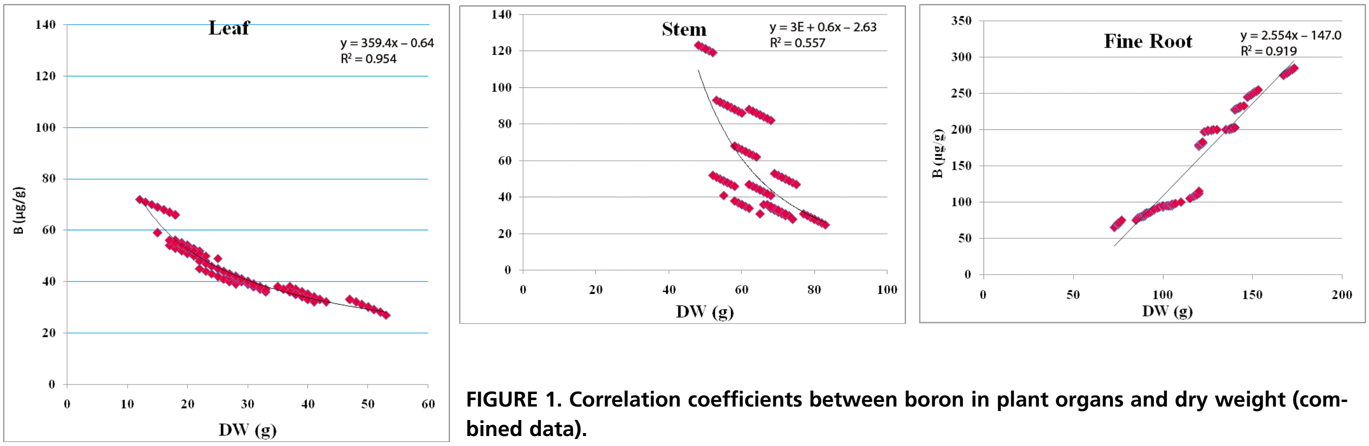


FIGURE 1. Correlation coefficients between boron in plant organs and dry weight (combined data).

Table 3. Total B content (mg/kg) of plum and peach organs grown in sandy and calcareous soils as affected by B and Ca in the substrate

		Sandy						Calcareous					
		Leaf		Stem		Fine root		Leaf		Stem		Fine root	
	Ca (mg/kg)	B (mg/kg)						B (mg/kg)					
Plum		2.5	5	2.5	5	2.5	5	2.5	5.0	2.5	5	2.5	5
	80	54	69	49	121	80	95	40	53	36	90	73	95
	200	42	51	44	89	110	250	35	42	33	65	100	200
	400	39	45	32	85	200	280	30	35	28	50	180	250
LSD(0.05)	B	2.26		2.01		5.75		2.01		2.78		6.86	
	Ca	2.77		2.46		7.04		2.46		3.41		8.41	
Peach	80	80	100	70	130	80	100	53	75	55	100	70	85
	200	60	80	65	100	110	220	45	60	45	75	95	200
	400	50	65	55	80	180	270	38	50	35	60	160	230
LSD 0.05)	B	2.48		3.22		7.89		2.22		1.88		3.75	
	Ca	3.04		3.94		9.66		2.72		2.30		4.59	

Table 4. 10B uptake and long distance transport of plum and peach organs grown in sandy and calcareous soils as affected by B and Ca in the substrate

		Sandy						Calcareous					
		Leaf		Stem		Fine root		Leaf		Stem		Fine root	
	Ca (mg/kg)	B (mg/kg)						B (mg/kg)					
		2.5	5	2.5	5	2.5	5	2.5	5	2.5	5	2.5	5
Plum	80	26	34	25	65	55	58	20	28	18	45	42	50
	200	21	25	22	50	109	143	17	21	16	32	90	100
	400	18	22	16	42	120	150	15	17	14	25	100	125
LSD(0.05)	B	1.62		2.37		3.02		2.18		1.84		4.02	
	Ca	1.99		2.91		3.70		2.67		2.25		4.93	
Peach	80	40	50	35	65	47	55	26	37	27	50	21	25
	200	30	40	31	50	91	130	22	30	22	37	45	50
	400	25	31	26	40	110	150	19	25	17	30	60	80
LSD(0.05)	B	2.22		2.33		3.22		2.65		2.10		2.41	
	Ca	2.72		2.86		3.94		3.25		2.57		2.95	

Table 5. B content (mg/kg) in the root tip cell wall of plum and peach organs grown in sandy and calcareous soils as affected by B and Ca in the substrate

	B:Ca (mg/kg)	Sandy			Calcareous		
		Root tip	Cell wall	% increase	Root tip	Cell wall	% increase
Plum	2.5:80	111	19		120	30	
	2.5:200	218	38	200	230	60	200
	2.5:400	242	100	526	280	150	500
	5:80	130	48		160	50	
	5:200	286	145	322	295	180	360
	5:400	310	270	555	380	350	700
LSD(0.05)		9.27	9.50	27.75	15.41	9.22	39.10
Peach	2.5:80	95	16		100	20	
	2.5:200	190	33	206	200	60	300
	2.5:400	220	80	500	240	105	525
	5:80	110	36		120	40	
	5:200	240	100	278	260	120	300
	5: 400	300	200	555	360	280	700
LSD(0.05)		28.59	6.08	30.14	9.64	6.08	49.14

relation was negative in leaf but positive in root, whereas the stem B concentration correlated significantly with the dry matter yield. The correlation coefficient matched well with B toxicity symptoms which were concentrated mainly in the leaf (leaf margins and midrib) followed by less marked symptoms in the stem. The positive correlation between B content and root dry weight indicates the possibility of the formation of B–Ca complex in the cell wall that is non-toxic.

^{10}B uptake and long distance transport

Boron (^{10}B) uptake and long distance transport was affected significantly by the B and Ca concentrations in the substrate. The uptake and translocation of ^{10}B was shown to be a function of the total B level.

As boron concentration in the substrate increased, high ^{10}B activity was recorded in the three organs, indicating that large amounts of ^{10}B were taken up and translocated. However, as the Ca concentration in the substrate increased, reduced ^{10}B levels were recorded in the leaf and stem indicating that less ^{10}B was translocated to the two organs (Table 4). The lower the Ca concentration in the substrate the higher was the ^{10}B activity, but as Ca supplementation of the substrate increased, ^{10}B levels decreased.

Plum trees accumulated less B than peach trees in leaf and stem but higher levels in the fine root. Significant differences were recorded for boron uptake and translocation between Ca concentrations (200 and 4000 mg/kg) in the irrigation solution. In the leaf, the effect of Ca on B translocation was mainly linear.

In the calcareous soil, less B was translocated to the leaf and stem but more was taken up by the root compared with the corresponding values on sandy soil. The root tips maintained the highest B concentration (Table 5). The lower B content of the shoot system could be explained by the high B fixation capacity (precipitation and adsorption) of calcium carbonate in the calcareous soil.

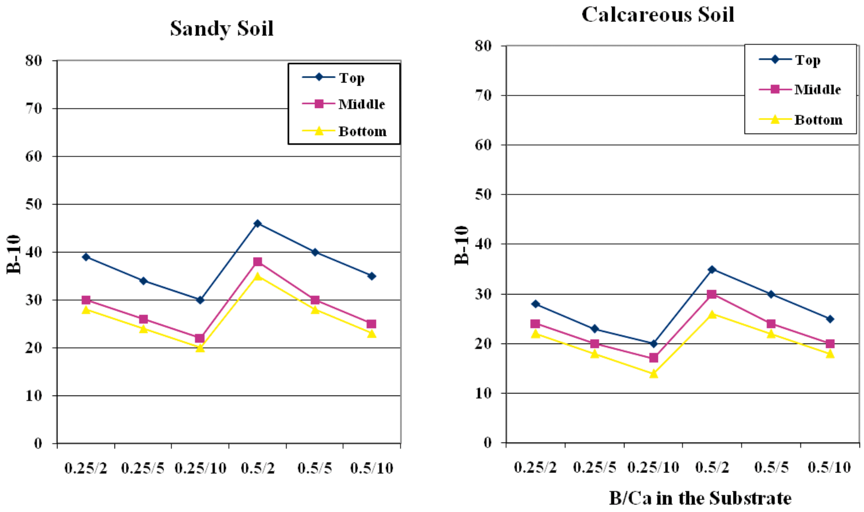


FIGURE 2. Effect of leaf age on B-10 translocation to plum leaf grown in sandy and calcareous soils.

Data for ^{10}B showed that the younger the leaf the more B was translocated. Concentrations of ^{10}B showed the following descending order: top > middle > bottom (Figure 2). ^{10}B activities were higher in the top (young leaf) followed by the middle (mature leaf) then the bottom (old leaf). These date indicate greater B translocation to the young leaf than to the older ones. However, as Ca levels in the substrate increased, the translocation of B was reduced.

These results provide evidence of passive B transportation via the transpiration stream. Young leaves have higher transpiration rates than older leaves due to less lignification, and as a result ^{10}B levels were higher in the young top leaf.

Boron and calcium accumulation in the cell walls of root tips

Boron accumulation in the root tip increased dramatically with Ca supplementation, most noticeably at levels of 200 and 400 mg/kg Ca in the substrate (Table 5).

Concerning B concentration in the root tip and its cell wall, the data indicated that as B and Ca levels in the substrate increased, B in the root tip increased significantly. Calcium in the irrigation solution increased B concentration significantly in the root tip as well as in its cell walls. Calcium in the root tip and cell walls followed the same pattern as B, with levels in the tissue being a function of levels in the substrate. At both B levels, there was a significant increase in B concentration in the cell wall at 400 mg/kg Ca compared with the values at 80 mg/kg Ca. The maximum increases in B and Ca in the root tip and in its cell walls were with the highest Ca application rate.

As the Ca concentration in the irrigation solution increased (400 mg/kg) there was a significant increase in B concentration in the cell wall (Table 5). Calcium in the cell wall of the root tips followed the same pattern as B, the percentage increase of B in the cell wall (at high Ca application rate, 400 mg/kg) reaching respectively about five and seven times its concentration at low Ca application rate (80 mg/kg) in sandy and calcareous soils.

CONCLUSIONS

Boron-10 and ICP-MS are good tools to study B uptake and translocation in plants. Boron concentrations in leaf, stem and root were modified significantly by levels of Ca in the substrate. Under high Ca and B applications, both elements accumulated in the cell walls of the root tip. This confirms that B and Ca have an extracellular function in the cell wall. Both elements could form a complex in the cell wall that prevents B transport to the shoot system and thereby reduces its toxicity

In a calcareous soil, less B was taken up and translocated to the shoot system. This indicates that high levels of B would have less harmful effects on plants grown on such soils. In arid regions, where soil and/or groundwater B levels are high, Ca supplementation could be used to reclaim such soils to reduce the adverse effect of B on plant growth.

REFERENCES

Brown, P.H., Bellaloui, N., Wimmer, M.A., Bassil, E.S., Ruiz, J., Hu, H., Pfeffer, H., Dannel, F. & Romheld V. 2002. Boron in plant biology. *Plant Biol*., 4: 205–223.

Burstrom, H.G. 1968. Calcium and plant growth. *Biol. Rev*., 43: 287–316.

El-Motaium, R., Hu, H. & Brown, P.H. 1994. The relative tolerance of six *Prunus* rootstocks to boron and salinity, *J. Am. Soc. Hort. Sci*., 119: 1169–1175.

Goldberg, S. 1997. Reactions of boron with soils. *Plant Soil*, 193: 35–48.

Goldberg, S. & Forster, H.S. 1991. Boron sorption on calcareous soils and reference calcites. *Soil Sci.,* 152: 304–310.

Ichikuni, M. & Kikuchi, K. 1972. Retention of B by travertines. *Chem. Geol*., 9: 13–21.

Johnson C.M., Stout P.R., Broyer T.C. & Carlton A.B. 1957. Comparative chlorine requirements of different plant species. *Plant Soil*, 8: 337–353.

Keren, R. & Bingham, F.T. 1985. Boron in water, soils and plants. *Adv. Soil Sci*., 1: 229–276.

Kobayashi, M., Matoh, T. & Azuma, J.I. 1996. Two chains of rhamnogalacturonan II are cross-linked by borate-diol ester bonds in higher plant cell walls. *Plant Physiol*., 110: 1017–1020.

Martini, F. & Thellier, M. 1980. Use of an (n,a) nuclear reaction to study the long-distance transport of boron in *Trifolium repens* after foliar application. *Planta*, 150: 197–205.

Marschner, H. ed. 1995. *Mineral nutrition of higher plants.* 2nd Edition, California, Academic Press, 889 pp.

Mengel, K. & Kirkby, E.A. 1982. *Principles of plant nutrition*. 3rd. Edition. Worblanfen-Bern/Switzerland, International Potash Institute.

O'Neill, M.A., Ishii, T., Albersheim, P. & Darvill, A.G. 2004. Rhamnogalacturonan II: Structure and function of a borate cross-linked cell wall pectic polysaccharide. *Ann. Rev. Plant Biol.*, 55: 109–139.

Selvendran, R.R. & O'Neill, M.A. 1987 Isolation and analysis of cell walls from plant material. *In* D. Glick, ed. *Methods of biochemical analysis*, 32, 25–153. New York, John Wiley Interscience.

Tang P.M. & Dela Fuente R.K. 1986. The transport of indole-3-acetic acid in boron and calcium deficient sunflower hypocotyl segments. *Plant Physiol.*, 81: 646–650.

Thellier, M., Duval, Y. & Demarty, M. 1979. Borate exchange of *Lemna minor L.* as studied with the help of enriched stable isotopes and of a (n,α) nuclear reaction. *Plant Physiol.*, 63: 283–288.

Warington, K. 1923. The effect of boric acid and borax on the broad bean and certain other plants. *Ann. Bot.*, 37: 629–672.

The Use of Isotopic Techniques to Quantify the Potential Contributions of Legumes to the Mitigation of Climate Change and Future Food Security

M.B. Peoples[1,*], D.F. Khan[2] and I.R.P. Fillery[3]

ABSTRACT

The large number of diverse legume species used to produce food for humans and livestock hold considerable promise as a means of adaptation to climate change. Legume systems have also been implicated as effective means of mitigating climate change through reductions in emissions of greenhouse gases such as carbon dioxide (CO_2) from fossil fuels and nitrous oxide (N_2O) from soil, and by accelerating soil carbon (C) sequestration. However, predicting how elevated concentrations of carbon dioxide (e[CO_2]) and future climates will affect soil nitrogen (N) cycling, soil C accretion and productivity in legume based farming systems remains a challenge. This paper identifies a number of opportunities where the isotopes of N and C could be applied to provide information on the key interaction between e[CO_2] and climate variables on the capacity for legumes to fix atmospheric N_2, influence the above and below ground allocation of C and N, and change the C and N dynamics of soils.

Key words: *legume agroecosystems, climate change, nitrogen and carbon isotopes, N fixation, N_2O emissions, C sequestration.*

INTRODUCTION

Legumes contribute directly to food security by providing protein-rich food for humans and livestock, and indirectly by enhancing the grain yield of following cereal crops (e.g. Espinoza *et al.*, 2012; Seymour *et al.*, 2012). Legumes also provide a renewable source of organic nitrogen (N) input for soils via their ability to fix atmospheric nitrogen (N_2) in symbiosis with rhizobia (Fillery 2001; Peoples *et al.*, 2009a). Annual global inputs of biologically fixed N by legumes in agricultural systems represent between 33–46 million tonnes (t) of N (Herridge, Peoples and Boddey, 2008), and it is widely recognised that the inclusion of N_2 fixing legumes in cropping sequences helps mitigate climate change by decreasing the fertilizer N requirements of subsequent food crops and thereby reducing the net emissions of fossil fuel derived carbon dioxide (CO_2; Jensen *et al.*, 2012). Some circumstantial data also indicate that legume-based systems may be responsible for lowering the emissions of the potent greenhouse gas nitrous oxide (N_2O), and contributing to soil carbon (C) sequestration (Jensen *et al.*, 2012). However, additional research is required to fully define the extent to which legumes can assist in mitigating climate change. There are many uncertainties about how legumes and soil management might need to be adapted to ensure adequate food production in the future when atmospheric concentrations of CO_2 and ambient temperatures are expected to be higher, and rainfall to be more variable, than experienced today (Tuiello *et al.*, 2007; Vadez *et al.*, 2012). The aim of this paper is to review the existing scientific literature to identify gaps in knowledge and to propose research opportunities where the isotopes of N and/or C could be employed to improve our understanding of the potential future role for legume systems.

The role of legumes in food security and adaptation to future climates

There are 20 000 species of legumes, several hundred of which have been used as traditional foods for humans or livestock in different geographic regions of the world. However, much of the 300 million hectares (ha) of crop and forage legumes grown globally each year is dominated by just 20–30 species (Herridge *et al.*, 2008). It has long been recognised that beyond these major species there are many underexploited legumes that hold considerable promise of providing valuable sources of grain, vegetables, fruits, root crops, forage or green manure with further domestication and selection (e.g. ~200 species listed in NAS 1975 and NAS 1979). Many of these underexploited species are already adapted across a wide range of environments, including dry climates and impoverished soils, and represent an untapped pool of genetic material to respond to future climate challenges. In principle there should be enough diversity within the large number of legume species with either the right phenology to minimize exposure to climatic stresses, or with suitable tolerances to temperature extremes, or the incurions of new diseases and pests in regions where climate change threatens the reliability of food supply (Vadez *et al.*, 2012). The indeterminate growth patterns of many legumes and their multi-purpose nature (i.e. grain, forage or green manure) could also assist in adapting to climate change where future farming systems will need to continue to produce food, generate income and protect the soil resource in the face of increased

---

1 CSIRO Sustainable Agriculture Flagship, CSIRO Plant Industry GPO Box 1600 Canberra, ACT 2601, Australia
2 Agricultural Research Station Bannu, Model Farm Service Centre Bannu, Bannu Township, Khyber Pakhtunkhwa (formerly North West Frontier Province), Pakistan
3 CSIRO Sustainable Agriculture Flagship, CSIRO Plant Industry Private Bag 5 Wembley, WA 6014, Australia
* E-mail address of corresponding author: mark.peoples@csiro.au

L.K. Heng, K. Sakadevan, G. Dercon and M.L. Nguyen (eds), Proceedings — International Symposium on Managing Soils for Food Security and Climate Change Adaptation and Mitigation. Food and Agriculture Organization of the United Nations, Rome, 2014: 55–60

climatic variability (Vadez *et al.,* 2012). Furthermore, it has been demonstrated that legumes tend not to exhibit the marked decline in foliage N content and seed protein that is observed in grasses and cereals growing under elevated concentrations of atmospheric CO_2 (e[CO_2]; Rogers, Ainsworth and Leakey, 2009; Lam *et al.,* 2012b), so in respect to nutritional security, legumes could help offset the anticipated reduction in protein intake in cereal-based human diets and forage provided to livestock.

Since many of the benefits derived from including legumes in farming systems arise from their ability to fixed atmospheric N_2 and to contribute to soil N fertility (Fillery, 2001; Peoples *et al.,* 2009a) it will be necessary to confirm that these benefits will continue to accrue under changing environmental conditions. The following sections consider how nitrogen-15 (^{15}N) techniques might be used to provide new information on the impact of future climates, particularly e[CO_2], on biological N_2 fixation and N cycling in legume-based farming systems. Since the amounts of N_2 fixed by legumes are generally closely related to biomass production (Unkovich *et al.,* 2010), discussion will also be included on whether carbon-13 (^{13}C) discrimination can be utilized as a means of comparing the efficiency of water use by different legume germplasm.

Legume inputs of fixed N

There are two main stable isotopes of N, nitrogen-14 (^{14}N) and ^{15}N. Of these, the lighter isotope, ^{14}N, is naturally more abundant than ^{15}N. If the ^{15}N concentration of the two main sources of N used by legumes for growth — namely atmospheric N_2 and soil N differ sufficiently then it is possible to calculate the proportion of the legume N accumulated during growth that was derived from N_2 fixation on the basis of the comparison of the ^{15}N composition of the legume with that of a non-N_2 fixing reference plant which is assumed to provide a measure of the ^{15}N signature of the plant-available soil N (Peoples *et al.,* 2009b). With appropriate analytical procedures and a suitably precise mass spectrometers it is possible to utilise the slight elevation in ^{15}N abundance of plant-available soil N (0.3673–0.3733 atom% ^{15}N) relative to atmospheric N_2 (0.3663 atom% ^{15}N) that occurs naturally in many soils. It is also possible to obtain or generate different sources of N that are artificially enriched in ^{15}N (5–99 atom% ^{15}N) that can be added to the soil to expand the difference in ^{15}N compositions of soil N and atmospheric N_2 to facilitate measures of N_2 fixation (Peoples *et al.,* 2009b). Both ^{15}N approaches have been applied to quantify N_2 fixation in many legume studies comparing legumes grown with ambient or e[CO_2]. In the majority of cases e[CO_2] was found to result in a stimulation of N_2 fixation as the result of the increased legume demand for N associated with enhanced plant growth (Roger *et al.,* 2009). Meta-analysis of the results of 127 studies suggests that N_2 fixation could be increased by 35–40 percent on average under e[CO_2] equivalent to twice current levels (Lam *et al.,* 2012b).

However, much of the data reviewed by Roger *et al.* (2009) and Lam *et al.* (2012b) came from short-term CO_2 enrichment studies, generally with only one legume variety and often with only one of the dominate legume species currently used in agriculture. Generally, little is known about the degree of genetic variation in the potential magnitude of response either within, or between, legume species, and certainly almost nothing is known about the symbiotic capacity of undomesticated species which may need to be exploited in future. The only data we are aware of which examine genetic differences in legume responses to e[CO_2] come from recent comparisons of:

(i) Five field pea varieties at an Australian FACE (Free Air CO_2 Enrichment) field facility which indicated considerable differences in productivity gains between varieties (from < 10 to > 40 percent) under e[CO_2] (550 ppm) compared with the ambient conditions (380 ppm; Fitzgerald, Brand and Mollah, 2012);

(ii) Two soybean cultivars at a FACE system in northern China which showed no significant effect of e[CO_2] on N_2 fixation by one cultivar (~80 kg fixed N/ha under both treatments), but a 66 percent stimulation (from 166 to 275 kg fixed N/ha) by the other (Lam *et al.,* 2012c);

(iii) The impact of soil type and levels of nutrition on the performance of the crop legumes field pea and chickpea, and the pasture legume barrel medic (*Medicago truncatula*). This glasshouse study showed a large influence of soil type on the relative responses by the three species with e[CO_2] with N_2 fixation increasing by 20–86 percent for chickpea, 44–51 percent for field pea, and 114–250 percent for barrel medic (Lam *et al.,* 2012a).

To complicate matters the climate changes projected for future decades could well modify, and may often limit, the direct CO_2 effects on crop and pasture productivity and N_2 fixation (Tubiello, Soussana and Howden, 2007). For example, there is evidence that higher N_2 fixation experienced under e[CO_2] can be lost in the presence of higher temperatures (Lilley *et al.,* 2001), or if plant growth is resticted by low soil phosphorus availability (Edwards, McCaffery and Evans, 2006; Lam *et al.,* 2012a). Several Free Air CO_2 Enrichment studies have also now observed "progressive N limitation" and longer-term "acclimation responses" to e[CO_2] which result in a decline in plant response to CO_2 and the amount of N harvested in biomass over time so that yield responses are much smaller than previously expected (Leakey *et al.,* 2009; Newton *et al.,* 2010). However, the effects on soil N availability may not necessarily occur if e[CO_2] is accompanied by a 2°C warming (Hovenden *et al.,* 2008).

Isotopic methodologies need to be employed to fully evaluate adaptation strategies. Especially to: (a) explore genetic variation in N_2 fixation response to e[CO_2] across a much wider range of legume species, (b) study the interdependence of C and N metabolism in legumes under different combinations of nutrition, temperature and water regimes, (c) quantify the impact of the timing of environmental stresses on N_2 fixation, and (d) determine the role of indeterminate growth patterns may play in the resilience of legume productivity and capacity to fixed N in the face of climate change.

Identifying more efficient use of variable rainfall

Most assessments of anticipated climate change impacts on agriculture predict more variable rainfall and an increased frequency of climatic extremes (Tubiello *et al.,* 2007). The major function of stomata is to maximize the rate at which CO_2 can diffuse into the leaf for photosynthesis while minimizing the simultaneous loss of water vapour. There is overwhelming evidence that e[CO_2] decreases both stomatal conductance and plant transpiration, and consequently is expected to improve plant water use efficiency (WUE; i.e. kg plant dry matter accumulated per mm rainfall; Leakey *et al.,* 2009). Nonetheless, it will still be necessary for researchers to be able to evaluate what effect management and environmental variables might have on the WUE of different legume germplasm when developing the most appropriate strategies to adapt legumes to climate change (Vadez *et al.,* 2012).

It has been demonstrated that plant species with the C_3 photosynthetic pathway discriminate against carbon-13 ($^{13}C)O_2$ during photosynthesis, and that there is less discrimination delta carbon-13 ($\Delta^{13}C$) in plant species or varieties which have a greater transpiration efficiency (Farquhar and Richards, 1984). The improved WUE results from lower stomatal conductance, greater photosynthetic capacity, or a combination of the two effects. The close inverse relationship between $\Delta^{13}C$ and transpiration efficiency has been successfully utilized in cereals to identify genetic material with intrinsically high WUE

TABLE 1. Examples of estimates of the below-ground partitioning of legume N derived from ^{15}N shoot-labelling techniques (derived from Khan et al., 2002; Unkovich, Baldock and Peoples, 2010)

Species	Common names	% Total plant N below-ground
Cajanus cajan	Pigeonpea	32–47
Cicer arietinum	Chickpea	48–68
Glycine max	Soybean	38
Lupinus angustifolius	Narrow leaf lupin	28–35
Ornithopus compressus	Yellow serradella	30–46
Pisum sativum	Field pea	32
Trifolium subterraneum	Subterranean clover/subclover	42
Vicia faba	Faba bean	24–40
Vigna radiata	Mung bean	20–39

TABLE 2. The use of ^{15}N methodologies to quantify the fate of faba bean N remaining in shoot residues or nodulated roots after grain harvest over a subsequent cropping cycle in an Australian rainfed system where wheat was grown following the faba bean crop (unpublished data, Khan, 2000)

Crop component	Faba bean N remaining after harvest (kg N/ha)	Faba bean N recovered by following wheat[1] (kg N/ha)	Faba bean N recovered in the soil (kg N/ha)	Faba bean N assumed to be lost after 1 year (kg N/ha)
Shoot residues	44	2 (4%)[2]	22 (50%)[2]	20 (45%)[2]
Below ground	51	4 (8%)[2]	43 (84%)[2]	4 (8%)[2]

[1] Total wheat N content at maturity represented 97 kg N/ha.

[2] Values in brackets represent the percentage of the faba bean N originally present.

(e.g. Condon *et al.,* 2004; IAEA, 2012). The $\Delta^{13}C$ approach has also been used as: (a) supporting data for better WUE by tall conventional field pea to semi-dwarf types (Armstrong, Pate and Tennant, 1994), (b) a means to compare WUE by five cowpea (*Vigna unguiculata*) genotypes grown in different densities on mixed culture with sorghum (*Sorghum bicolor*; Makoi, Chimphango and Dakora, 2010), and (c) a tool to select cowpea (Hall, Thiaw and Krieg, 1994) and peanut (groundnut, *Arachis hypogoea*; Hubick, Farquhar and Shorter, 1986; Wright, Rao and Farquhar, 1994) germplasm with more drought tolerance. Unfortunately, the use of leaf ^{13}C discrimination to screen for variations in plant WUE does not appear to be applicable to chickpea, lentil, or narrow leaf lupin (Turner *et al.,* 2007).

Before genetic variabilty in WUE can be explored further using the $\Delta^{13}C$ technique it will be necessary to explain the apparent inconsistencies observed between legume species. At very least it would be instructive to compare species where $\Delta^{13}C$ seems to be a useful guide to WUE with those where it apparently does not under the same conditions. This could help define the experimental and sampling protocols where the technique is likely to be most reliable.

The partitioning of legume N and the subsequent fate of legume N

Various ^{15}N-enrichment approaches have been used for many decades to investigate the fate of legume shoot N following a legume phase in a cropping sequence and to quantify the subsequent uptake of legume N by following cereal crops (e.g. Thompson and Fillery, 1997; Russell and Fillery 1999; Peoples *et al.,* 2009a). It has only been in recent years that different *in situ* ^{15}N shoot labelling techniques have been developed to quantify the above- and below-ground partitioning of legume N in the glasshouse (McNeill, Zhu and Fillery, 1998; Khan *et al.,* 2002) and field (Russell and Fillery 1996; Khan *et al.,* 2003; McNeill and Fillery, 2008). Collectively, these studies indicate that between 24 and 68 percent of the total N accumulated by crop or pasture legumes over a growing season may be associated with, or derived from, the nodulated roots (Table 1). This pool of below-ground N has often been ignored in previous research. Although such ^{15}N shoot labelling methodologies could also be utilized to follow the fate of above- and below-ground legume N separately, there have been relatively few attempts to do so. Data available from rainfed cropping systems in Australia using ^{15}N shoot labelling methods suggest that between 8 and 15 percent of the below-ground N from crop legumes may be recovered by a subsequent wheat crop (*Triticum aestivum*) which represented a 2–5 fold greater uptake than the N contributed from above-ground residues in the same trials (Table 2; McNeill and Fillery, 2008; Peoples *et al.,* 2009a). There is also some evidence that the below-ground pool of N could be less susceptible to loss processes than shoot residues (Table 2). These observations contrast with data collected from agroforestry systems which, while reporting comparable levels of partitioning of below-ground N for a range of woody perennial legume species (34–51 percent of total plant N; IAEA, 2008) as determined for crop and pasture legumes (Table 2), found lower recoveries of below-ground legume N by maize (*Zea mays*) than from legume shoot prunings (Seiter and Horwarth, 1999; IAEA, 2008). It was concluded that in agroforestry systems below-ground legume N played a more important role in contributing to soil structural stability than nutrient supply (IAEA, 2008).

Few studies have ascertained what effect e[CO_2] may have on the below-ground partitioning of legume N. Increased root nodulation has often been observed under e[CO_2] (Roger *et al.,* 2009; Lam *et al.,* 2012b), although circumstantial evidence based on comparisons of soil ^{15}N natural abundance following soybean grown under ambient and e[CO_2] suggests that e[CO_2] could result in a net decrease in below-ground allocation of biologically fixed N (Decock *et al.,* 2012), and no significant effect of atmospheric CO_2 concentration was observed in the proportion of total plant N partitioned below-ground in a recent pot study with field pea (Lam *et al.,* 2013).

It is also not clear what influence high CO_2 environments might have on the availability of legume N from either shoot or root residues for the benefit of other crops. It has been concluded from a number of (predominantly) non-legume studies that the gross N mineralization rates in soil tend to be unaffected by e[CO_2], but N immobilization by the microbial biomass can be 30 percent greater than under current ambient conditions (van Groenigen *et al.,* 2006). This is consistent with observations of progressive N limitations in long-term e[CO_2] treatments (Newton *et al.,* 2010). We are aware of only one study that has specifically investigated the effect of e[CO_2] on the recovery of legume N, and in this case wheat derived much less of its N supply from a previous field pea crop under e[CO_2] than ambient conditions (11 percent *cf* 20 percent, respectively; Lam *et al.*, 2013).

Clearly there are opportunities to apply *in situ* ^{15}N shoot labelling techniques to a wider range of legumes and systems to assess the above- and below-ground partitioning of legume N, and to quantify the contribution of both sources to the N assimilated by following crops under both ambient and e[CO_2] conditions.

The role of legumes in mitigation of climate change

Contributions of legume N to N_2O emissions

The soil emissions of N_2O from legume systems collated from the published literature (1.29 kg N_2O-N/ha on average) tend to be lower than measurements from N fertlized crops and pastures (4.49 kg N_2O-N/ha on average; Table 3). Jensen *et al.* (2012) concluded from such data that legume systems emit less N_2O than where fertilizer N is used, and that emissions under legumes may be comparable with those derived from native soil organic N (1.20 kg N_2O-N/ha; Table 3). Yet only a few of these data came from direct comparisons of legume or fertilizer sources undertaken within the same study, and many experiments did not include appropriate controls so it was not always possible to identify conclusively the actual source of the N_2O (i.e. endogenous soil N, legume or fertilizer N). Neither is there much information on N_2O emissions from legume residues or unutilized N fertilizer beyond the first subsequent growing season (Jensen *et al.,* 2012). Legume residues enriched in ^{15}N could be used to address this shortcoming and to allow the specific losses of legume N as N_2O to be quantified.

There are also very few measures of the impact of e[CO_2] on N_2O emissions from legume systems. A number of e[CO_2] trials supplied with N fertilizer indicate higher N_2O emissions (Lam *et al.,* 2012b). However, in the absence of additions of fertilizer N there are several potentially counteracting factors that can influence the extent of N_2O losses. Emission could be higher with e[CO_2] because of an association between microbial mediated N_2O losses and enhanced availability of soil C substrate (Lam *et al.,* 2012b), and the improved WUE by plants growing with e[CO_2] resulting in higher soil water contents and possibly resulting in an increased risk of denitrification (Leakey *et al.,* 2009). But N_2O emissions due to denitrification could also be lower due to e[CO_2] enhanced microbial immobilization of available forms of soil N reducing the likelihood of nitrate accumulation (van Groenigen *et al.,* 2006). A recent report from the SoyFACE facility in Illinois in the USA indicated no major effects of e[CO_2] on field measures of N_2O emissions from soybean growing in a a soybean-maize rotation (Decock *et al.,* 2012). The question remains whether this result is widely representative of other legume systems.

Contributions to soil C sequestration

Several long-term studies have demonstrated significant increases in soil C stocks where legumes have been included in farming systems (Jensen *et al.,* 2012). However, it is not clear whether the additional C had come directly from the legume component, or whether the soil organic C was present in labile or stable forms. Whether or not the soil organic C is vulnerable to short-term losses has important repercussions for land sustainability and the success of climate change mitigation strategies (Tuiello *et al.,* 2007). The well described differences in discrimination of ^{13}C during photosynthesis in C_4 plants ($\delta^{13}C \sim -13‰$) compared with C_3 plants ($\delta^{13}C \sim -27‰$) can be used to determine the proportion of soil C derived from C_4 or C_3 species. The advantage of this approach is that the effect of C_3 plants on C sequestration in mixed or rotation systems can be assessed using existing land use systems. For example, a study comparing continuous maize with a maize–lucerne (alfalfa, *Medicago sativa*) system showed that maize residues contribute < 15 percent and lucerne > 50 percent of the soil C (Gregorich, Drury and Baldock, 2001). Nevertheless, it should be noted that isotopic fractionation of ^{13}C during decomposition of different plant residues could confound the interpretation of data derived from analyses of the ^{13}C natural abundance of soil organic matter (Schweizer, Farr and Cadisch, 1999).

Pulse-labelling legumes with C isotopes is another approach that can be used to quantify the contribution of legume biomass to soil C sequestration. Although it is simpler to use ^{13}C, the comparatively high natural abundance of this stable isotope typically requires use of expensive highly enriched ^{13}C compounds to improve the precision of measurements of changes in ^{13}C content in soil C pools. Because of its very low natural abundance it is often more convenient to use ^{14}C to quantify the above- and below-ground allocation of C, describe the effect of the presence or absence of legumes on soil C sequestration and estimate flow of C through different soil OM pools (e.g. Bhupinderpal-Singh, Hedley and Sagger, 2005; Sanaullah *et al.,* 2012).

TABLE 3. Summary of field measurements of N_2O emissions from legume and N fertilized systems (derived from data presented by Jensen *et al.,* 2012)

Land use	Number of site-years of data collated	kg N_2O-N/ha per growing season or year	
		Range	Mean
Legume-based pasture	25	0.10–4.57	1.38
N fertilized grass pasture	19	0.30–18.16	4.49
Crop legume	46	0.03–7.09	1.02
N fettilized crop	48	0.09–12.67	2.71
No legume or added N	33	0.03–4.80	1.20

The effect of e[CO_2] on soil C has proved to be quite variable across different studies, but it appears that the rate of N supply is a key factor influencing changes in total soil C (van Groenigen *et al.,* 2006). No significant difference in soil C content between ambient and e[CO_2] treatments tend to occur with annual N inputs < 30 kg N/ha, but an 8 percent increase in soil C can result under e[CO_2] where > 150 kg N/ha was applied (van Groenigen *et al.,* 2006). Therefore, inputs of fixed N and high N legume residues should encourage C accretion (Jensen *et al.,* 2012). However, inputs of nutrients such as phosphorus, and sulphur in addition to N could be crucial in determining the rate of change in soil C stocks (Kirkby *et al.,* 2011). In many non-legume systems much of the increase in soil C with e[CO_2] seems to be in labile pools of C rather than stable forms (van Groenigen *et al.,* 2006). When determining the role legumes can play in mitigating climate change it will be important to resolve how vulnerable the accumulated legume organic C in soil may be to short-term losses, and this is a significant gap in current knowledge.

Isotopic methods could be applied to a range of legume crops or pasture legumes under different management scenarios to: (a) quantify the below-ground partitioning of C and the change of distribution of that C in nodulated roots, soil microbial biomass and different soil organic C fractions over time, (b) identify genotype x management combinations to accelerate the rate of soil C sequestration, (c) determine how management influences whether legume C is predominantly accumulated in labile (e.g. particulate organic matter) or stable (humus) soil C pools, and (d) investigate the impact of the C-rich legumes residues generated under e[CO_2] on soil microbial activity, and the ability of soils to store C.

CONCLUSIONS

The use of N and C isotopes could greatly assist in resolving some of the uncertainties concerning how e[CO2] and future climates could affect soil N cycling in legume agroecosystems and enable a better informed assessment of the potential for legumes to contribute to global food security.

ACKNOWLEDGEMENTS

The financial support of the Grains Research and Development Corporation to investigate the system effects of including legumes in cropping sequences is gratefully acknowledged. We are also indebted to Jason Brand and Roger Armstrong from the Victorian Department of Primary Industries in Horsham for the provision of data on the impact of e[CO2] on legumes and soil N dynamics.

REFERENCES

Armstrong, E.L., Pate, J.S. & Tennant, D. 1994. The field pea crop in south Western Australia – Patterns of water use and root growth in genotypes of contrasting morphology and growth habit. *Aust. J. Plant Physiol.,* 21: 517–532.

Bhupinderpal-Singh, Hedley, M.J. & Sagger, S. 2005. Characterization of recently ^{14}C pulse-labelled carbon from roots by fractionation of soil organic matter. *Eur. J. Soil Sci.,* 56: 329–341.

Condon, A.G., Richards, R.A., Rebetzke, G.J. & Farquhar, G.D. 2004. Breeding for high water use efficiency. *J. Exp. Bot.,* 55: 2447–2460.

Decock, C., Chung, H., Venterea, R., Gray, S.B. & Leakey, A.D.B. 2012. Elevated CO_2 and O_3 modify N turnover rates, but not N_2O emissions in a soybean agroecosystem. *Soil Biol. Biochem.,* 51: 104–114.

Edwards, E.J., McCaffery, S. & Evans, J.R. 2006. Phosphorus availability and elevated CO_2 affect biological nitrogen fixation and nutrient fluxes in a clover-dominated sward. *New Phytol.,* 169: 157–167.

Espinoza, S., Ovalle, C., Zagal, E., Matus, I., Tay, J., Peoples, M.B. & del Pozo, A. 2012. Contribution of legumes to wheat productivity in Mediterranean environments of central Chile. *Field Crops Res.,* 133: 150–159.

Farquhar, G.D. & Richards, R.A. 1984. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Aust. J. Plant Physiol.,* 11: 539–552.

Fillery, I.R.P. 2001. The fate of biologically fixed nitrogen in legume-based dryland farming systems: A review. *Aust. J. Expl. Agric.,* 41: 361–381.

Fitzgerald, G., Brand, J. & Mollah, M. 2012. *Field pea response to elevated CO_2.* Proceedings 16th Australian Society Agronomy Conference, Armidale, Australia, October 2012.

Gregorich, E.G., Drury, C.F. & Baldock, J.A. 2001. Changes in soil carbon under long-term maize in monoculture and legume-based rotation. *Can. J. Soil Sci.,* 81: 21–31.

Hall, A.E., Thiaw, S. & Krieg, D.R. 1994. Consistency of genotypic ranking for carbon isotope discrimination by cowpea grown in tropical and subtropical zones. *Field Crops Res.,* 36: 125–131.

Herridge, D.F., Peoples, M.B. & Boddey, R.M. 2008. Global inputs of biological nitrogen fixation in agricultural systems. *Plant Soil,* 311: 1–18.

Hovenden, M.J., Newton, P.C.D., Carran, R.A., Theobald, P., Wills, K.E., Vander Schoor, J.K., Williams, A.L. & Osanai, Y. 2008. Warming prevents the elevated CO_2-induced reduction in available soil nitrogen in a temperate, perennial grassland. *Global Change Biol.,* 14: 1018–1024.

Hubick, K.T., Farquhar, G.D. & Shorter, R. 1986. Correlation between water use efficiency and carbon isotope discrimination in diverse peanut (*Arachis*) germplasm. *Aust. J. Plant Physiol.,* 13: 803–816.

IAEA. 2008. Quantifying the contribution of above- and below-ground N in legumes to soil N pools and subsequent maize in an improved fallow system using *in situ* ^{15}N labeling techniques in western Kenya, by J.K. Nduf, A. Albrecht, G. Keerthisinghe & G. Cadisch. *In* IAEA-TECDOC-1606, *Management of agroforestry systems for enhancing resource use efficiency and crop productivity*, pp. 143–155. Vienna.

IAEA 2012. *Greater agronomic water use efficiency in wheat and rice.* IAEA-TECDOC-1671, Vienna. 275pp.

Jensen, E.S., Peoples, M.B., Boddey, R.M., Gresshoff, P.M., Hauggaard-Nielsen, H., & Alves, B.J.R. 2012. Legumes for mitigation of climate change and the provision of feedstock for biofuels and biorefineries. A review. *Agron. Sustain. Dev.,* 32: 329–364.

Khan, D.F. 2000. *Reconciling effects of legume N with reference to the role of the nodulated root*. Melbourne University, Melbourne. (PhD. thesis).

Khan, D.F., Peoples, M.B., Chalk, P.M. & Herridge, D.F. 2002. Quantifying below-ground nitrogen of legumes. 2. A comparison of ^{15}N and non-isotopic methods. *Plant Soil,* 239: 277–289.

Khan, D.F., Peoples, M.B., Schwenke, G.D., Felton, W.L., Chen, D. & Herridge, D.F. 2003. Effects of below-ground nitrogen on N balances of field-grown fababean, chickpea and barley. *Aust. J. Agric. Res.,* 54: 333–340.

Kirkby, C.A., Kirkegaard, J.A., Richardson, A.E., Wade, L.J., Blanchard, C. & Batten, G. 2011. Stable soil organic matter: a comparison of C:N:P:S ratios in Australian and international soils. *Geoderma* 163: 197-208.

Lam, S.K., Chen, D., Norton, R. & Armstrong, R. 2012a. Does phosphorus stimulate the effect of elevated [CO_2] on growth and symbiotic nitrogen fixation of grain and pasture legumes? *Crop & Pasture Sci.,* 63: 53–62.

Lam, S.K., Chen, D., Norton, R. & Armstrong, R. 2013. The effect of elevated atmospheric carbon dioxide concentration on the contribution of residual legume and fertilizer nitrogen to a subsequent wheat crop. *Plant Soil,* 364: 81–91.

Lam, S.K., Chen, D., Norton, R., Armstrong, R. & Mosier, A.R. 2012b Nitrogen dynamics in grain crop and legume pasture systems under elevated atmospheric carbon dioxide concentration: A meta-analysis. *Global Change Biol.,* 18: 2853–2859.

Lam, S.K., Hao, X., Lin, E., Han, X., Norton, R., Mosier, A.R., Seneweera, S. & Chen, D. 2012c. Effect of elevated carbon dioxide on growth and nitrogen fixation of two soybean cultivars in northern China. *Biol. Fert. Soils,* 48: 603–606.

Leakey, A.D.B., Ainsworth, E.A., Bernacchi, C.J., Rogers, A., Long, S.P. & Ort, D.R. 2009. Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. *J. Exp. Bot.,* 60: 2859–2876.

Lilley, J.M., Bolger, T.P., Peoples, M.B. & Gifford, R.M. 2001. Nutritive value and the nitrogen dynamics of *Trifolium subterraneum* and *Phalaris aquatica* under warmer, high CO_2 conditions. *New Phytol.,* 150: 385–395.

Makoi, J.H.J.R., Chimphango, S.B.M. & Dakora, F.D. 2010. Photosynthesis, water-use efficiency and delta C-13 of five cowpea genotypes grown in mixed culture and at different densities with sorghum. *Photosynthetica,* 48: 143–155.

McNeill, A.M. & Fillery, I.R.P. 2008. Field measurement of lupin belowground nitrogen accumulation and recovery in the subsequent cereal-soil system in a semi-arid Mediterranean-type climate. *Plant Soil,* 302: 297–316.

McNeill, A.M., Zhu, C. & Fillery, I.R.P. 1998. A new approach to quantifying the N benefit from pasture legumes to succeeding wheat. *Aust. J. Agric. Res.,* 49: 427–436.

National Academy of Sciences. 1975. *Underexploited tropical plants with promising economic value*. Washington, DC, National Academy of Sciences. 188 pp.

National Academy of Sciences. 1979. *Tropical legumes: Resources for the future*. Washington, DC, National Academy of Sciences. 331 pp.

Newton P.C.D., Lieffering, M., Bowatte, W.M.S.D., Brock, S.C., Hunt, C.L., Theobald, P.W. & Ross, D.J. 2010. The rate of progression and stability of progressive nitrogen limitation at elevated atmospheric CO_2 in a grazed grassland over 11 years of free air CO_2 enrichment. *Plant Soil,* 336: 433–441.

Peoples, M.B., Brockwell, J., Herridge, D.F., Rochester, I.J., Alves, B.J.R., Urquiaga, S., Boddey, R.M., Dakora, F.D., Bhattarai, S., Maskey, S.L., Sampet, C., Rerkasem, B., Khan, D.F., Hauggaard-Nielsen, H., Jensen, E.S. 2009a. The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. *Symbiosis,* 48: 1–17.

Peoples, M.B., Unkovich, M.J. & Herridge, D.F. 2009b. Measuring symbiotic nitrogen fixation by legumes. *In* D.W. Emerich & H.B. Krishnan, eds. *Nitrogen fixation in crop production,* Agronomy Monograph 52, pp. 125–170. Madison, USA, American Society of Agronomy, Crop Science Society of America, Soil Science Society of America.

Rogers, A., Ainsworth, E.A. & Leakey, A.D.B. 2009. Will elevated carbon dioxide concentration amplify the benefits of nitrogen fixation in legumes? *Plant Physiol.,* 151: 1009–1016.

Russell, C.A. & Fillery, I.R.P. 1996. Estimates of lupin below-ground biomass N, dry matter and N turnover to wheat. *Aust. J. Agric. Res.,* 47: 1047–1059.

Russell, C.A. & Fillery, I.R.P. 1999. Turnover of nitrogen from components of lupin stubble to wheat in sandy soil. *Aust. J. Soil Res.,* 37: 575–592.

Sanaullah, M., Chabbi, A., Rumpel, C. & Kuzyakov, Y. 2012. Carbon allocation in grassland communities under drought stress followed by ^{14}C pulse labeling. *Soil Biol. Biochem.,* 55: 132–139.

Schweizer, M., Fear, J. & Cadisch, G. 1999. Isotopic (^{13}C) fractionation during plant residue decomposition and its implications for soil organic matter studies. *Rapid Comm. Mass Spec.,* 13: 1284–1290.

Seiter, S. & Horwarth, W.R. 1999. The fate of tree root and pruning nitrogen in a temperate climate alley cropping system determined by tree injected N-15. *Biol. Fert. Soils,* 30: 61–68.

Seymour, M., Kirkegaard, J.A., Peoples, M.B., White, P.F. & French, R.J. 2012. Break-crop benefits to wheat in Western Australia – insights from over three decades of research. *Crop & Pasture Sci.,* 63: 1–16.

Thompson, R.B. & Fillery, I.R.P. 1997. Transformation in soil and turnover to wheat of N from components of grazed pasture in south Western Australia. *Aust. J. Agric. Res.,* 48: 1033–1047.

Tubiello, F.N., Soussana, J-F. & Howden, S.M. 2007. Crop and pasture response to climate change. *Proc. Nat. Acad. Sci., USA*, 104: 19686–19690.

Turner, N.C., Palta, J.A., Shrestha, R., Ludwig, C., Siddique, K.H.M. & Turner, D.W. 2007. Carbon isotope discrimination is not correlated with transpiration efficiency in three cool-season grain legumes (pulses). *J. Integ. Plant Biol.,* 49: 1478–1483.

Unkovich, M.J., Baldock, J. & Peoples, M.B. 2010. Prospects and problems of simple linear models for estimating symbiotic N_2 fixation by crop and pasture legumes. *Plant Soil,* 329: 75–89.

van Groenigen, K.J., de Graaff, M.A., Six, J., Harris, D., Kuikman, P. & van Kessel, C. 2006. The impact of elevated atmospheric [CO_2] on soil C and N dynamics: A meta-analysis. *In* J. Nosberger, S.P. Long, R.J. Norby, M. Stitt, G.R. Hendrey & H. Blum, eds. *Managed ecosystems and CO_2 case studies, processes, and perspectives* Ecological Studies Vol. 187, pp. 373–391. Berlin, Springer-Verlag.

Vadez, V., Berger, J.D., Warkentin, T., Asseng, S., Ratnakumar, P., Rao, K.P.C., Gaur, P.M., Munier-Jolain, N., Larmure, A., Voisin A.S., Sharma, H.C., Pande, S., Sharma, M., Krishnamurthy, L. & Zaman, M.A. 2012. Adaptation of grain legumes to climate change: A review. *Agron. Sustain. Dev.,* 32: 31–44.

Wright, G.C., Rao, R.C.N. & Farquhar, G.D. 1994. Water-use efficiency and carbon-isotope discrimination in peanut under water-deficit conditions. *Crop Sci.,* 34: 92–97.

Contribution of Nitrogen from Biofertilizer Inoculum to Young Oil Palm under Field Conditions

F.A.A. Zakry[1,2,*], K.A. Rahim[3], Z.H. Shamsuddin[1], Z.Z. Zakaria[4] and A.A. Rahim[1]

ABSTRACT

Continuous and excessive use of chemical fertilizers leads to deterioration of soil and environmental health especially under tropical climates which could accelerate losses of nutrients in the soil through leaching and other processes. The use of plant growth–promoting rhizobacteria (PGPR) as a biofertilizer inoculant could potentially mitigate the problem. However, the effectiveness of microbial inoculants in the field is still questionable. The present study was conducted on young oil palms to compare the contribution of nitrogen (N) through N_2 fixation from a PGPR biofertilizer with that from chemical fertilizer using the nitrogen-15 (^{15}N) isotopic dilution method. The results indicated that inoculated young oil palm accumulated 63.4 percent N through the activities of biofertilizer microorganisms, similar to uninoculated young oil palms which were fertilized totally by chemical fertilizer and accumulated 58.8 percent N. Therefore, biofertilizer inoculant can be potentially exploited for improving food crop productivity and environmental health.

Key words: *biofertilizer, biological nitrogen fixation, Elaeis guineensis Jacq., fertilizer, inoculation, ^{15}N isotope dilution, PGPR.*

INTRODUCTION

Improper and excessive use of synthetic fertilizers have damaged the environment, bringing about water pollution from algal blooms which are detrimental to aquaculture (Shumway, 1990; Tanga *et al.*, 2003), increased greenhouse gas emissions (Zou *et al.*, 2005; Kim and Dale, 2008), groundwater pollution which negatively affects water quality for human consumption (Johnson, Adams and Perry, 1991) and soil acidification which makes soil less fertile for agricultural production (Campbell *et al.*, 1995; Barak *et al.*, 1997). These impacts are cascading, difficult to manage, and cost billions of dollars to rehabilitate. Oil palm is highly traded on international markets and generates large incomes for countries like Malaysia and Indonesia which are major oil palm producers. An estimated 74 percent of global palm oil usage is for food products and 24 percent is for industrial purposes (USDA, 2010). Since the 1990s, the area occupied by oil palm cultivation has expanded by around 43 percent worldwide driven mainly by demand from India, China and the European Union (RSPO, 2011). However, the cultivation of oil palm contributes to ecosystem imbalances through activities such as manuring since oil palm is a nutrient-demanding crop.

Application of biofertilizer containing plant growth-promoting rhizobacteria for agricultural production is seen as one of the potential solutions to mitigate the harmful effects of synthetic fertilizers (Mohamed and Babiker, 2012; Khan *et al.,* 2012) since biofertilizers could reduce dependency for synthetic fertilizer and may even be an alternative. Plant growth-promoting rhizobacteria (PGPR) belong to several genera namely *Azospirillum*, *Bacillus*, *Pseudomonas* and several others (Esquivel-Cote *et al*., 2010; Mia *et al*., 2010; Prasanna *et al*., 2011; Aziz *et al*., 2012). Plant growth promotion by rhizobacteria involves several direct and indirect mechanisms such as biological nitrogen (N_2) fixation, phosphate solubilization, phytohormone production and antagonism against plant diseases (Mia *et al*., 2010; Aziz *et al*., 2012; Sayyed *et al.,* 2012; Zakry *et al.,* 2012). However, the value of biofertilizers for commercial agricultural production especially in oil palm is still in doubt. Questions have been raised, for example, about their cost effectiveness in delivering nutrients to the crop as compared with synthetic or inorganic fertilizer applications and also due to inconsistent results obtained from field experiments (El-Sirafya *et al*., 2006).

The present study was conducted using the nitrogen-15 (^{15}N) isotope dilution method to estimate N accumulation by field grown young oil palms from a biofertilizer containing diazotrophic plant growth promoting rhizobacteria and to compare this with amounts accumulated from a conventional nitrogenous fertilizer in delivering N nutrition

MATERIALS AND METHODS

The experiment was conducted in a field plot at Tangkah Estate, Sime Darby Plantation Berhad (formerly Golden Hope Plantation Berhad), Tangkak, Johor, in southern Peninsular Malaysia (2° 21′ N, 102° 40′ E). Soil chemical data are presented in Table 1. Fourteen-month-old GH500 cloned oil palms were established for five months after transplantation in the field. The upkeep and maintenance of the trial plots included a normal estate fertilizer application schedule of inorganic fertilizers, comprising N as ammonium sulphate, phosphorus (P) as Christmas Island rock phosphate, potassium (K) as muriate of

---

1 Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
2 Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus, 97008 Bintulu, Sarawak, Malaysia
3 Agrotechnology and Biosciences Division, Malaysian Nuclear Agency, Bangi, 43000 Kajang, Selangor, Malaysia
4 Biology Division, Malaysian Palm Oil Board, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia
* E-mail address of corresponding author: zakryfitri@upm.edu.my

L.K. Heng, K. Sakadevan, G. Dercon and M.L. Nguyen (eds), Proceedings — International Symposium on Managing Soils for Food Security and Climate Change Adaptation and Mitigation. Food and Agriculture Organization of the United Nations, Rome, 2014: 61–65

TABLE 1. Chemical properties of the soil[2] (Ultisol) from the oil palm experimental field

pH (KCl)	Available (mg/kg)				
(1:2.5)	Total N	P[1]	K	Ca	Mg
4.7	16.0	22.3	19.0	55.0	15.0

[1] Extracted with an aqueous solution of 0.05 M HCl and 0.0125 M H_2SO_4

[2] Bungor sandy clay loam soil with 1.2 percent total carbon content

potash, magnesium (Mg) as kieserite and boron (B) as borate (Goh and Härdter, 2003).

Bacillus sphaericus UPMB-10, isolated in Malaysia from oil palm roots (Amir *et al.,* 2003), was sub-cultured on tryptic soy agar (TSA) (Merck KGaA) to produce a pure mother culture for inoculum production as described by Zakry *et al*. (2012). The minimum population of strain UPMB-10 was ≥ 10^9 colony-forming unit (cfu)/g during field inoculation.

In the field, the plants were laid down in a randomized complete block design with four treatments and four replicates (Figure 1).

The four treatments include (1) the un-inoculated + 0 percent N + ^{15}N, (2) un-inoculated + 100 percent N + ^{15}N, (3) inoculated + 67 percent N + ^{15}N, and (4) un-inoculated + 67 percent N + ^{15}N. Treatments (1) and (2) served as negative and positive controls and also as benchmarks for deficient N (negative control) and optimum N (positive control). The treatment (3) involved inoculation with *B. sphaericus* strain UPMB-10 inoculum. Treatment (4) had a similar N rate (67 percent) to the inoculated treatment. All un-inoculated treatments were provided with killed inoculum (gamma-irradiated at 50 kGy) per palm. The "100 percent N" and "67 percent N" refer respectively to the full and 67 percent recommended inorganic N fertilizer application rates as described by Zakry *et al*. (2012).

Recordings were made from 16 palms for each of the 16 plots. Palms in the two outermost rows served as a buffer. The ^{15}N-labelled fertilizer used was $(^{15}NH_4)_2SO_4$ (ammonium sulphate) with 10.13 atom percent ^{15}N excess serving as a tracer. The field experiment was initiated by the application of ^{15}N-labelled fertilizer five months after transplanting. Within the 16 recording palms, two palms (micro-plot) received labelled ^{15}N fertilizer, with 10.13 at. percent ^{15}N ammonium sulphate at a rate of 1 g·N/m^2. The ^{15}N-labelled fertilizer was applied uniformly in liquid form using 2 L distilled water per isotopic plot of 1 m^2 area. The plots were then covered evenly with black polythene sheets to reduce ^{15}N-labelled fertilizer loss. A week later, the black polythene sheets at the ^{15}N isotopic micro-plots were removed after the inner surface of each sheet was rinsed with water prior to inoculum application. The black polythene sheets were used once only for all inoculated and un-inoculated ^{15}N isotopic micro-plots. Inoculum for the first inoculation was then applied followed by the second inoculation four months later. The inoculated +67 percent N + ^{15}N treatment was carried out at a rate of 2 kg inoculum (containing more than 10^9 cfu/g *B. sphaericus* UPMB-10) by raking the surface of the soil to a depth of approximately 5 cm within an area of 1 m^2, and at a rate equivalent to 296 kg/ha.

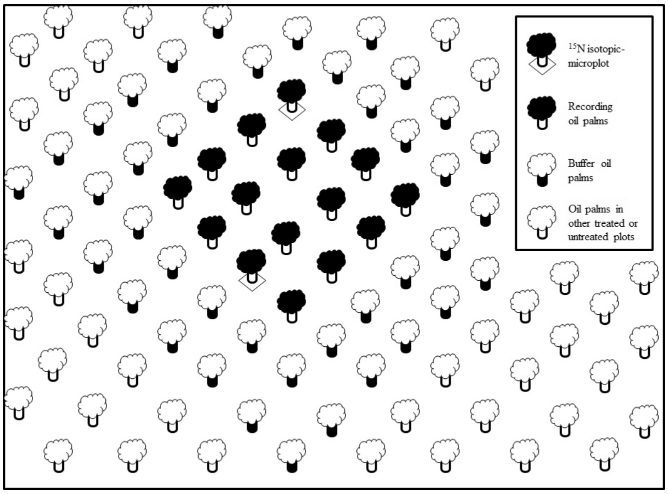


FIGURE 1. A plot with 16 recording oil palms (including two randomly selected palms receiving ^{15}N-labelled fertilizer) and two outermost rows serving as a buffer. The buffer oil palms help to prevent cross contamination between plots. They were treated the same as the recording palms in the ^{15}N isotopic-micro-plot. Recording palms were also used to conduct vegetative growth measurements (Zakry *et al.,* 2012).

Harvesting was carried out 240 days (8 months) after application of the ^{15}N-labelled fertilizer. Four palms from each treatment were harvested destructively, and separated into leaflets, rachis, stems and roots. The major roots were extracted with a backhoe tractor, and the remaining roots were excavated by shovelling and sieving the soil within the area occupied by the harvested palm. Fresh biomass and oven-dried (70°C for 72 h) sub-samples were weighed and recorded. Samples were ground to pass through 0.5 mm sieves and analysed for total N by the semi-micro Kjeldahl method (Bremner, 1996) and ^{15}N excess using an NOI-6PC emission spectrometer at the Malaysian Nuclear Agency, Bangi. The ^{15}N abundance found in palm tissue was corrected for the atom percent^{15}N excess present in the atmosphere (0.3663 at. percent^{15}Ne) (Warembourg, 1993).

Nitrogen fixation in the whole palm was calculated from weighted atom excess (WAE) in the inoculated palm (inoculated + 67 percent N + ^{15}N) and un-inoculated palm (un-inoculated + 67 percent N + ^{15}N), using the following formula (Zakry *et al.,* 2012):

$$WAE = \frac{AE(Lf) \times TN(Lf) + AE(Rc) \times TN(Rc) + AE(St) \times TN(St) + AE(Rt) \times TN(Rt)}{TN\ (Lf + Rc + St + Rt)} \times 100$$

where AE, TN, Lf, Rc, St and Rt refer respectively to atom percent ^{15}N excess in total N, leaflets, rachis, stems and roots.

To determine the proportions of N from the unlabelled fertilizer (percent Ndff [normal fertilizer]), labelled fertilizer (percent Ndff [^{15}N fertilizer]) and from the soil (percent Ndfs), the following formula was used:

$$\%Ndff\ (^{15}N\ fertilizer) = \frac{atom\ \%^{15}N\ excess\ in\ plant\ tissue}{atom\ \%^{15}N\ excess\ in\ labelled\ fertilizer} \times 100$$

$$\%\ Ndfs = 100 - \%Ndff\ (^{15}N\ fertilizer)$$

$$\%\ Ndff\ (normal\ fertilizer) = 100 - \%Ndff\ (^{15}N\ fertilizer) - \%Ndfs$$

RESULTS AND DISCUSSION

After 240 days of plant growth, the inoculated young oil palm had accumulated 0.23 percent N from the ^{15}N-labelled fertilizer (%Ndff) and 36.40 percent N from the soil (%Ndfs), besides the 63.37 percent from N_2 in the atmosphere (% Ndfa) (Figure 2). The un-inoculated young oil palm fertilized at the usual rate accumulated 0.20 percent Ndff (^{15}N fertilizer), 40.97 percent Ndfs and 58.82 percent Ndff (normal fertilizer). On average, the inoculated young oil palms had accumulated slightly higher amounts of N from the atmosphere than the fully fertilized young oil palms that received fertilizer-N at 58.82 percent (Figure 2). These results indicate that young oil palms that received biofertilizer containing *B. sphaericus*

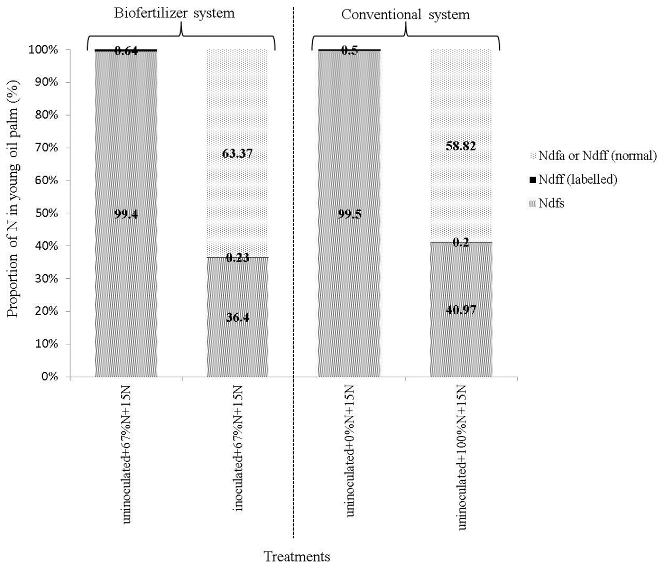


FIGURE 2. Percentages of N derived from atmosphere (%Ndfa [normal]), N derived from normal fertilizer (%Ndff), N derived from labelled fertilizer (%Ndff [labelled]) and N derived from soil (%Ndfs) after 240 d inoculation. Comparison of two systems: biofertilizer system (biofertilizer containing diazotroph *B. sphaericus* strain UPMB-10) and conventional system (the recommended and commonly practiced fertilizer programme at the oil palm estate).

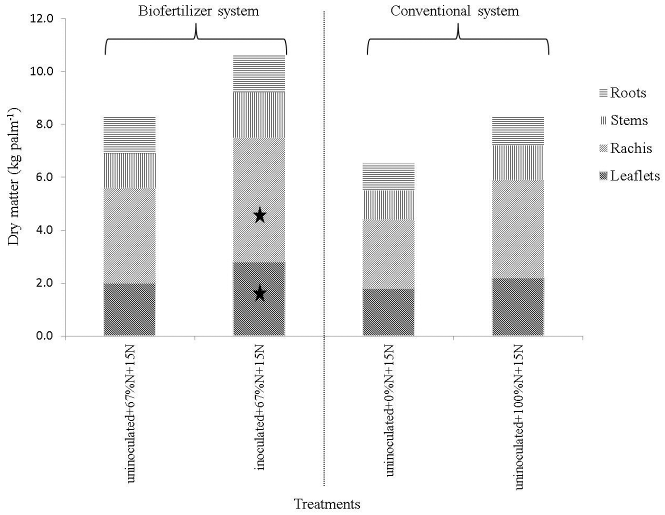


FIGURE 3. Dry matter yield and its distribution in immature young oil palm. Black star refers to $p < 0.05$ versus un-inoculated+67%N + ^{15}N (control) using one-way ANOVA and Dunnett's *post-hoc* test. Rachis and leaflet dry matter increased significantly in inoculated young oil palm compared with un-inoculated young oil palm. Leaflets, rachis, stems and root dry matter levels of inoculated young oil palm were similar to fully fertilized young oil palm and may be even better. Adapted from Zakry *et al.* (2012).

UPMB-10 had accumulated around the same percentage of N from the atmosphere as trees given normal fertilizer.

Values for the former may be an underestimate since the biofertilizer containing *B. sphaericus* UPMB-10 also improved dry matter and N yields and its distribution in immature young oil palm (Figures 3 and 4).

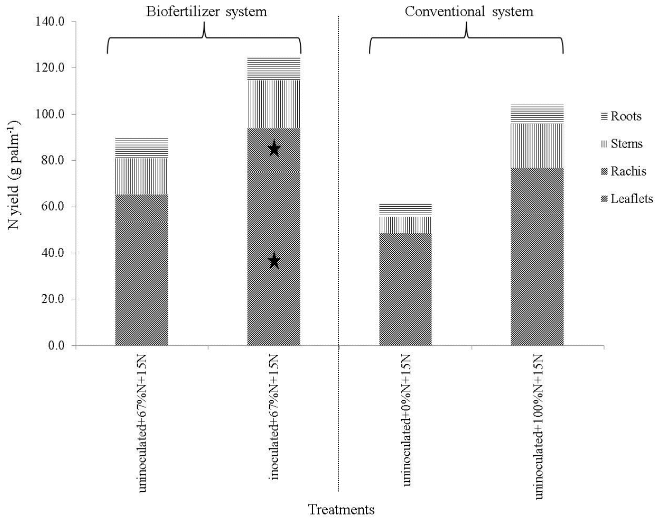


FIGURE 4. Total N yield and its distribution in immature young oil palm. Black star refers to $p < 0.05$ versus un-inoculated+67%N + ^{15}N (control) using one-way ANOVA and Dunnett's *post-hoc* test. Rachis and leaflet N yields increased significantly in inoculated compared with un-inoculated young oil palm young oil palm. Leaflet, rachis, stem and root N yield of inoculated young oil palm were similar to fully fertilized young oil palm and may be even better. Adapted from Zakry *et al.* (2012).

For example, rachis and leaflet dry matter levels increased significantly in inoculated compared with un-inoculated young oil palm, while stem and root dry matter accumulation of inoculated and un-inoculated young oil palm were similar. Increments in dry matter accumulation were paralleled by similar and consistent increases in total N yields of young immature oil palm. Rachis and leaflet N uptake were significantly higher in inoculated than in un-inoculated plants. These results indicate that increased N uptake by oil palms especially to rachis and leaflets which are the most nutrient demanding parts of oil palm and where photosynthetic activity is most active. Photosynthetic rate correlated positively with total leaf N content and subsequently contributed to vegetative growth (Cassman, Peng and Kropff, 1995). In addition, Shaobing, Daniel and Fekade (1991) reported that leaf photosynthetic rate in sorghum correlated significantly with biomass and grain production.

These results suggest that biofertilizers hold great promise for commercial application and this is supported by other studies which demonstrate their effectiveness in promoting nutrient uptake and plant growth. For example, Ahmad *et al.* (2008) conducted two-year pot and field trials which revealed that the organic fertilizer supplemented with 88 kg N/ha was as effective as the full dose of N fertilizer (175 kg/ha) in increasing root weight, fresh biomass, and ear and grain yields of maize. Interestingly, biofertilizer supplemented either with 88 or 132 kg N/ha significantly increased the growth and yield of maize over the full recommended rate of N fertilizer and was superior to organic fertilizer. Adesemoye, Torbert and Kloepper (2009) reported that application of a mixture of PGPR strains of *Bacillus amyloliquefaciens* IN937a and *Bacillus pumilus* T4 (a formulated PGPR product), and the arbuscular mycorrhiza fungus (AMF) *Glomus intraradices* and supplemented with 75 percent of the recommended fertilizer rate produced plant growth rates, yields, and N and phosphorus (P) uptakes that were equivalent to the full recommended fertilizer rate without inoculants.

The advantage of biologically fixed N_2 over fertilizer N may be related to the characteristics of N supply by the two modes of application. *Bacillus sphaericus* UPMB-10 fixes N_2 directly from the atmosphere within the plant itself, so "uptake" is almost complete (100 percent) with no losses to the environment. However, much of the fertilizer N applied may be lost (volatilized, denitrified and leached) (Bijay-Singh, Yadvinder-Singh and Sekhon, 1995) or simply remain in the soil unabsorbed. For example, applying urea will generally only lead to a recovery of less than 50 percent by the plants (Halvorson *et al*., 2002). Moreover, biofertilizer application has been shown to be more cost effective in terms of fertilizer management than conventional inorganic fertilizer application. The present study indicated that the cost of fertilizer N is potentially reduced by approximately 63 percent of total plant N requirement through biological N_2 fixation (Figure 2). Biofertilizer is an organic input containing living microorganisms that act with "flexibility" and differ from chemical fertilizers which deliver nutrients to the plant in a direct and inflexible manner. In effect, biofertilizers act according to plant need and insufficient nutrient in the soil will be rectified or rebalanced through biochemical–microbial action, i.e. biological N_2 fixation, biodegradation of organic material etc. This is different from the chemical fertilizer mechanism which can be ineffective and even harmful to the plant ecosystem. For example, inappropriate application of chemical fertilizers, especially excessive applications, will acidify the plant–soil ecosystem.

Therefore, the use of biofertilizers in agricultural production can be exploited for promoting environmentally friendly and sustainable agricultural crop production and environmental health. Although the present study demonstrated that biofertilizer containing PGPR *Bacillus sphaericus* strain UPMB-10 can deliver nutrients efficiently, its effect on crop productivity is still unclear. Thus, future field trials involving mature palms need to be conducted to evaluate the effect of biofertilizer inoculation on growth, yield and oil productivity in oil palm.

CONCLUSIONS

Comparison of N uptake from N_2 fixation using PGPR biofertilizer and chemical fertilizer showed that inoculated young oil palm accumulated 63.4 percent N through the activities of biofertilizer microorganisms compared to 58.8 percent N in un-inoculated and applied with chemical fertilizer. Therefore, biofertilizer inoculant can be potentially exploited for improving food crop productivity and environmental health. Further field studies are required to assess and quantify the role of biofertilizers on N uptake by mature oil palm leaves.

REFERENCES

Adesemoye, A.O., Torbert H.A. & Kloepper, J.W. 2009. Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. *Microb. Ecol*., 58: 921–929.

Ahmad, R., Arshad, M., Khalid, A. & Zahir, Z.A. 2008. Effectiveness of organic-/bio-fertilizer supplemented with chemical fertilizers for improving soil water retention, aggregate stability, growth and nutrient uptake of maize (*Zea mays* L.). *J. Sust. Agric*., 31: 57–77.

Aziz, Z.F.A., Saud, H.M., Rahim, K.A. & Ahmed, O.H. 2012. Variable responses on early development of shallot (*Allium ascalonicum*) and mustard (*Brassica juncea*) plants to *Bacillus cereus* inoculation. *Malaysian J. Microbiol*., 8: 47–50.

Barak, P., Jobe, B.O., Krueger, A.R., Peterson, L.A. & Laird, D.A. 1997. Effects of long-term soil acidification due to nitrogen fertilizer inputs in Wisconsin. *Plant Soil*, 197: 61–69.

Bijay-Singh, Yadvinder-Singh & Sekhon, G.S. 1995. Fertilizer-N use efficiency and nitrate pollution of groundwater in developing countries. *J. Contam. Hydrol*., 20: 167–184.

Bremner, J.M. 1996. Nitrogen-total. *In* D.L. Spark, ed. *Methods of soil analysis, Part 3. Chemical Methods,* pp. 1085–1121. American Society of Agronomy, Inc., Madison, Wisconsin.

Campbell, C.A., Bouman, O.T., Ukrainetz, H., Curtin, D. & Biederbeck, V.O. 1995. Soil acidification from long-term use of anhydrous ammonia and urea. *Soil Sci. Soc. Am. J.,* 59: 1488–1494.

Cassman, K.G., Peng, S. & Kropff, M.J. 1995. Relationship between leaf photosynthesis and nitrogen content of field-grown rice in tropics. *Crop Sci*., 35: 1627–1630.

El-Sirafya, Z.M., Woodard, H.J. & El-Norjarc. E.M. 2006. Contribution of biofertilizers and fertilizer nitrogen to nutrient uptake and yield of Egyptian winter wheat. *J. Plant Nutr*., 29: 587–599.

Esquivel-Cote, R., Ramírez-Gama, R.M., Tsuzuki-Reyes, G., Orozco-Segovia, A. & Huante, P. 2010. *Azospirillum lipoferum* strain AZm5 containing 1-aminocyclopropane-1-carboxylic acid deaminase improves early growth of tomato seedlings under nitrogen deficiency. *Plant Soil*, 337: 65–75.

Goh, K.J. & Härdter, R. 2003. General oil palm nutrition. *In* T.H. Fairhurst & R. Härdter R., eds. Oil palm: Management for large and sustainable yields, pp. 191–230. Switzerland, Potash and Phosphate Institute.

Halvorson, A.D., Follett, R.F., Bartolo, M.E. & Schweissing. F.C. 2002. Nitrogen fertilizer use efficiency of furrow-irrigated onion and corn. *Agron. J*., 94: 442–449.

Johnson, S.L., Adams, R.M. & Perry, G.M. 1991. The on-farm costs of reducing groundwater pollution. *Am. J. Agric. Econ*., 73: 1063–1073.

Khan, Z., Tiyagi, S.A., Mahmood, I. & Rizvi, R. 2012. Effects of N fertilisation, organic matter, and biofertilisers on the growth and yield of chilli in relation to management of plant-parasitic nematodes. *Turkish J. Bot*., 36: 73–81.

Kim, S. & Dale, B.E. 2008. Effects of nitrogen fertilizer application on greenhouse gas emissions and economics of corn production. *Environ. Sci. Technol*., 42: 6028–6033.

Mia, M.A.B., Shamsuddin, Z.H., Wahab, Z. & Marziah, M. 2010. Rhizobacteria as bioenhancer and biofertilizer for growth and yield of banana (*Musa* spp. cv. 'Berangan'). *Scientia Horticulturae*, 126: 80–87.

Mohamed, S.S.E. & Babiker, H.M. 2012. Effects of Rhizobium inoculation and urea fertilization on faba bean (*Vicia faba* L.) production in a semi-desert zone. *Adv. Environ. Biol*., 6: 824–830.

Prasanna, A., Deepa, V., Balakrishna Murthy, P., Deecaraman, M., Sridhar, R. & Dhandapani, P. 2011. Insoluble phosphate solubilization by bacterial strains isolated from rice rhizosphere soils from Southern India. *Int. J. Soil Sci*., 6: 134–141.

RSPO. 2011. *Promoting the growth and use of sustainable palm oil*. Roundtable on Sustainable Palm Oil (RSPO), Zurich.

Sayyed, R.Z., Reddy, M.S., Kumar, K.V., Yellareddygari, S.K.R., Deshmukh, A.M., Patel, P.R. & Gangurde, N.S. 2012. Potential of plant growth-promoting rhizobacteria for sustainable agriculture. *In* D.K. Maheshwari, ed. *Bacteria in agrobiology*: *Plant probiotics*, pp. 287–313. New York, Springer.

Shaobing, P., Daniel, K. & Fekade, G. 1991. Leaf photosynthetic rate is correlated with biomass and grain production in grain sorghum lines. *Photosynthesis Res*., 28: 1–7.

Shumway, S.E. 1990. A review of the effects of algal blooms on shellfish and aquaculture. *J. World Aquacult. Soc*., 21: 65–104.

Tanga, D., Kesterc, D.R., Ni, I., Qi, Y. & Kawamura, H. 2003. *In situ* and satellite observations of a harmful algalbloom and water condition at the Pearl River estuary in late autumn 1998. *Harmful Algae*, 2: 89–99.

USDA. 2010. *Indonesia: Rising global demand fuels palm oil expansion*. Washington, DC, United States Department of Agriculture, Foreign Agriculture Service.

Warembourg, F.R. 1993. Nitrogen fixation in soil and plant system. *In* R. Knowles & T.H. Blackburn, eds. *Nitrogen isotope technique,* pp. 127–155. Academic Press, London.

Zakry, F.A.A., Shamsuddin, Z.H., Abdul Rahim, K., Zakaria, Z.Z. & Abdul Rahim, A. 2012. Inoculation of *Bacillus sphaericus* UPMB-10 to young oil palm and measurement of its uptake of fixed nitrogen using the ^{15}N isotope dilution technique. *Microbes Environ*., 27: 257–262.

Zou, J., Huang, Y., Jiang, J., Zheng, X. & Sass. R.L. 2005. A 3-year field measurement of methane and nitrous oxide emissions from rice paddies in China: Effects of water regime, crop residue, and fertilizer application. *Global Biogeochem. Cycles*, 19: GB2021.

Legume Nitrogen Derived from Different Sources as Affected by Rhizobial Inoculant in a Bangladesh Soil

M.E. Haque[1,*] and M.A. Sattar[1]

ABSTRACT

Field experiments were conducted to study the effect of rhizobial inoculant on the quantity of legume nitrogen (N) derived from different sources using nitrogen-15 (^{15}N) tracer. Three different legumes (lentil, mungbean and soybean) were tested with and without rhizobial inoculant in ^{15}N isotope treated micro-plots laid out in a split-plot arrangement and a randomized complete block design with three replications and a non-nodulated wheat crop included as reference in a separate plot. Results showed that soybean produced the maximum number of nodules (33.4 nodules per plant) and the highest nodule dry weight (190.5 mg/plant) under inoculated conditions. The maximum total N recorded in inoculated soybean seed and stover were 112.6 and 77.0 kg N/ha, respectively, while the minimum values of 23.0 and 18.1 kg N/ha were obtained from un-inoculated mungbean. N derived from the atmosphere (Ndfa) ranged from 25.9 kg N/ha in un-inoculated mungbean to 159.6 kg N/ha in inoculated soybean. Due to inoculation, Ndfa increased by 123.87, 118.52 and 212.18 percent in lentil, mungbean and soybean, respectively. The maximum N derived from the soil (Ndfs) was 34.3 kg N/ha in un-inoculated soybean, while the inoculated mungbean derived the lowest N (10.4 kg N/ha) from the soil. The study revealed that all legumes received more N from fertilizer sources under un-inoculated than inoculated conditions. However, under inoculated conditions most (80.7–84.1 percent) of total plant N came from the atmosphere. With inoculation, comparatively less N was taken by the legume plants from soil (12.2–14.8 percent) and fertilizer (3.6–4.3 percent) sources.

Key words: *legume, nitrogen sources, rhizobial inoculant.*

INTRODUCTION

Managing nitrogen (N) inputs in crop production systems to achieve economic and environmental sustainability is a major challenge facing agriculture. Relying less on commercial fertilizer N and more on biological N_2 fixation by legumes has been suggested as a way to meet this challenge (Keeney and Nelson, 1982). The N benefits of legume-rhizobium symbiosis include N_2 fixation and mineralization, sparing of soil inorganic N and reduced immobilization of soil inorganic N (Fedorova *et al.*, 2005). Symbiotic N_2 fixation enhances soil fertility and productivity and increases carbon sequestration and nutrient conservation (Morgan, 1997). Efficient utilization of symbiotic N_2 fixation in agricultural practice is one of the important strategies for establishing sustainable agriculture in the 21st century. Using rhizobia inoculants can be a key part of accelerating rehabilitation of degraded land and ecosystem functions, enhancing survival and growth of plants and reducing costs in establishment and maintenance.

Declining soil fertility, particularly N, is recognized as a major threat to continued rice/cereal cropping in Bangladeshi soils, especially on the Tista Meander floodplain. It is widely believed that legumes improve soil fertility because of their N_2 fixing ability. Legumes vary in the amount of N_2 fixed and in the proportion of plant N derived from different sources – atmosphere, fertilizer and soil. We need therefore to identify legumes and genotypes that yield more and derive a large part of their N requirements from fixation (Wani, Rupela and Lee, 1995). This study was therefore conducted to quantify the amount of N_2 fixed by three important legumes (lentil, mungbean and soybean) with and without inoculation and to estimate the amount of N they derived from different sources.

MATERIALS AND METHODS

Field experiments were conducted during 2008–09 and 2009–10 at the sub-station farm of the Bangladesh Institute of Nuclear Agriculture (BINA), Rangpur, located at 25°43′ N latitude and 89°16′ E longitude in the north-west part of Bangladesh. The soil of the experimental site was silt loam (19 percent clay, 51 percent silt and 30 percent sand), having pH 7.2 (in water) with carbon (C) 0.73 percent, total N 0.074 percent, Olsen's phosphorous (P) 5.03 ppm and exchangeable potassium (K) 0.12 cmol/kg. Three different legumes – lentil (*Lens culinaris* Medik; cv. Binamusur 1), mungbean (*Vigna sinensis*; cv. Binamoog 4) and soybean (*Glycine max* L.; cv. Sohag) were tested with and without rhizobial inoculant by following a randomized complete block design with a split-plot arrangement having three replications.

For quantification of biological N_2 fixation by the different legumes, isotope labelled ammonium sulphate ($[^{15}NH_4]_2SO_4$) with 10.48 percent atom excess (a.e.) @ 20 kg N/ha was sprayed uniformly onto the 1 m^2 area of the legume plots. The reference crop wheat (*Triticum aestivum*) received 100 kg N/ha in the isotopic sub-plot. Unlabelled urea was applied to the remaining part of each plot to keep the N doses uniform for whole of the plot and as per treatment plan. The P, K and S doses for wheat were respectively 20 kg P/ha, 50 kg K/ha and 5 kg S/ha. The size of each main plot was 6 m x 5 m and each plot contained one ^{15}N micro-plot (1m x 1m), which was assigned for the isotopic study. Crops were harvested at physiological maturity. For total N and ^{15}N estimation, the aerial parts of the plants were dried in an oven at 65°C and the percent N determined using a Micro-

---

1 Soil Science Division, Bangladesh Institute of Nuclear Agriculture (BINA)
2 Bangladesh Agricultural University Campus, Mymensingh-2202, Bangladesh
* E-mail address of corresponding author: ekrambina@yahoo.com

L.K. Heng, K. Sakadevan, G. Dercon and M.L. Nguyen (eds), Proceedings — International Symposium on Managing Soils for Food Security and Climate Change Adaptation and Mitigation. Food and Agriculture Organization of the United Nations, Rome, 2014: 67–71

Kjeldahl digestion apparatus. Nitrogen-15:Nitrogen-14 ($^{15}N/^{14}N$) ratios were determined using an NOI-7 emission spectrophotometer (IAEA, 2002) and ^{15}N related calculations were performed using the equations of IAEA (1990) and Toomsan *et al.* (1995). Analysis of variance was performed and means were classified following Duncan's new multiple range test ($p < 0.05$).

RESULTS

Effect of rhizobial inoculant on nodulation and legume yield

The rhizobial inoculant had significant effects on the number of nodules per plant. Most nodules (25–41 per plant) were found in the plots where soybean seeds were inoculated with *Bradyrhizobium* and the least (2–3 per plant) in un-inoculated soybean (Table 1). On average, the highest nodule dry weight (190.5 mg/plant) was recorded in the plot where soybean seeds were inoculated with *Bradyrhizobium* before sowing and the lowest dry weight of 5.5 mg/plant was recorded in un-inoculated lentil.

Seed and stover yields varied significantly with the legume irrespective of whether inoculation took place with rhizobia. These yields were higher when the legume was inoculated (Figure 1), with the highest seed yield (2.1 t/ha) being recorded in inoculated soybean, and the lowest (0.8 t/ha) in un-inoculated lentil. Increases in seed yield associated with inoculation were respectively 69.4, 41.3 and 44.0 percent for soybean, mungbean and lentil. Inoculation also had a significant effect on stover yields, with the average highest yield (4.5 t/ha) being recorded from inoculated soybean and the un-inoculated mungbean showing the lowest yield (1.6 t/ha). Stover yield increases associated with inoculation were 50.8, 53.4 and 62.5 percent, respectively in lentil, mungbean and soybean. On average the total yield (seed plus stover) increased by 42, 37 and 61 percent in the crops lentil, mungbean and soybean, respectively.

Effect of rhizobial inoculation on legume nitrogen content

The experimental results showed that the N content in legume seed and stover were influenced significantly by the type of legume and inoculant (Table 2). The highest N content was in inoculated soybean (111.6 and 113.7 kg/ha during 2008–09 and 2009–10, respectively) and increased by 89.3, 76.3 and 78.8 percent in lentil, mungbean and soybean, respectively compared to un-inoculated conditions. The maximum stover N content (72.0–81.9 kg/ha) was obtained from inoculated soybean while the minimum N content (17.5–18.7 kg/ha) was obtained from un-inoculated mungbean. The highest average total legume N (189.6 kg N/ha) was found in inoculated soybean and this differed significantly from other legumes, with the lowest amount (41.0 kg N/ha) of total legume N being recorded in un-inoculated mungbean. Due to inoculation, the total legume N uptake increased by 77.4, 71.0 and 98.4 percent, respectively in lentil, mungbean and soybean plants.

TABLE 1. Nodulation in different legumes as affected by rhizobial inoculant

Legume x Inoculant	Nodules per plant			Nodule dry wt. (mg/plant)		
	2008-09	2009-10	Mean	2008-09	2009-10	Mean
Lentil un-inoculated	10.4b	05.7c	08.1	04.7e	06.2e	05.5
Lentil inoculated	15.5b	15.2b	15.4	15.3d	11.7d	13.5
Mungbean un-inoculated	09.8b	06.6c	08.2	22.5c	26.6c	24.6
Mungbean inoculated	12.0b	16.5b	14.3	55.4b	55.3b	55.3
Soybean un-inoculated	03.2c	02.0c	02.6	14.6d	12.2d	13.4
Soybean inoculated	25.5a	41.3a	33.4a	183.3a	197.7a	190.5
% CV	23.8	20.3	—	38.0	17.6	—

Values in a column under a factor/ interaction treatment having same letter do not differ significantly at 5 percent level of probability.

Table 2. Nitrogen content in different legumes as affected by rhizobial inoculant

Legume × Inoculant	Nitrogen in legume seed (kg/ha)			Nitrogen in legume stover (kg/ha)		
	2008-09	2009-10	Mean	2008-09	2009-10	Mean
Lentil un-inoculated	26.07e	26.84e	26.46	22.98c	18.30c	20.64
Lentil inoculated	50.01c	50.17c	50.09	36.44b	30.40b	33.42
Mungbean un-inoculated	22.98e	22.93e	22.96	18.68c	17.49c	18.09
Mungbean inoculated	38.10d	42.86d	40.48	32.12b	27.25b	29.69
Soybean un-inoculated	62.56b	63.77b	63.17	33.18b	31.66b	32.42
Soybean inoculated	111.56a	113.73a	112.65	81.94a	72.03a	76.99
%CV	7.35	3.60	—	3.49	5.46	—

Values in a column under a factor/ interaction treatment having same letter do not differ significantly at 5 percent level of probability.

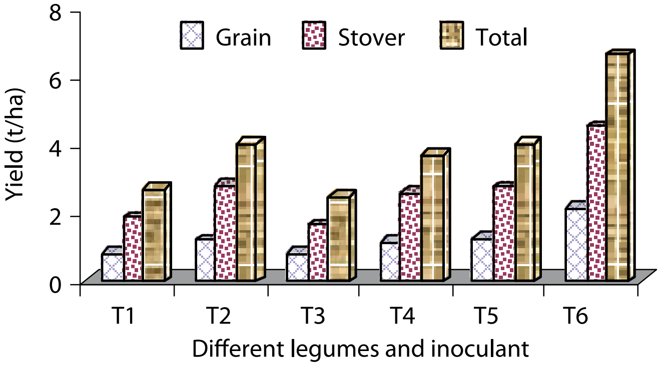


FIGURE 1. Seed, stover and total yield of legumes as affected by rhizobial inoculant (average of results from two years). T_1 — lentil un-inoculated; T_2 — lentil inoculated; T_3 — mungbean un-inoculated; T_4 — mungbean inoculated; T_5 — soybean un-inoculated; and T_6 — soybean inoculated.

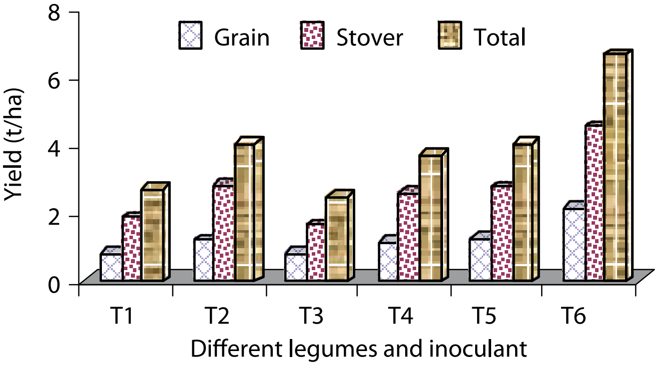


FIGURE 2. Total N, Ndfa, Ndfs, and Ndff of legumes as affected by rhizobial inoculant (average of two years' results). T_1 — lentil un-inoculated; T_2 — lentil inoculated; T_3 — mungbean un-inoculated; T_4 — mungbean inoculated; T_5 — soybean un-inoculated; and T_6 — soybean inoculated.

TABLE 4. N derived from different sources in legume stover as affected by rhizobial inoculant

Legume x Inoculant	N derived from different sources in legume stover (kg/ha)					
	Ndfa		Ndfs		Ndff	
	2008-09	2009-10	2008-09	2009-10	2008-09	2009-10
Lentil un-inoculated	14.22d	12.35e	6.90c	4.54c	1.86c	1.41c
Lentil inoculated	29.18b	25.30b	5.72d	3.87e	1.54c	1.20c
Mungbean un-inoculated	11.15e	11.20e	5.93d	4.80c	1.60c	1.49c
Mungbean inoculated	25.63c	21.59c	5.11d	4.32d	1.38c	1.34c
Soybean un-inoculated	16.22d	17.34d	13.37a	10.93a	3.60a	3.39a
Soybean inoculated	67.45a	60.78a	11.42b	8.59b	3.07b	2.67b
%CV	3.84	5.13	5.81	4.39	5.75	7.48

Values in a column under a factor/ interaction treatment having same letter do not differ significantly at 5 percent level of probability.

TABLE 3. N derived from different sources in legume seed as affected by rhizobial inoculant

Legume x Inoculant	N derived from different sources in legume seed (kg/ha)					
	Ndfa		Ndfs		Ndff	
	2008-09	2009-10	2008-09	2009-10	2008-09	2009-10
Lentil un-inoculated	16.87d	18.31d	7.16c	6.47c	2.04c	2.06c
Lentil inoculated	41.38b	42.36b	6.71c	5.93c	1.93c	1.86d
Mungbean un-inoculated	14.50d	15.00d	6.60c	6.02c	1.88c	1.91d
Mungbean inoculated	31.00c	35.05c	5.52c	5.93c	1.57d	1.89d
Soybean un-inoculated	33.63c	35.08c	22.51a	21.77a	6.42a	6.93a
Soybean inoculated	94.30a	96.71a	13.43b	12.91b	3.83b	4.11b
%CV	8.60	4.54	10.46	4.34	9.80	4.65

Values in a column under a factor/interaction treatment having same letter do not differ significantly at 5 percent level of probability

Quantity of legume nitrogen derived from different sources

Legume N (in seed and stover) and the relative proportions derived from different sources (atmosphere [Ndfa], soil [Ndfs] and fertilizer [Ndff]) were estimated using the ^{15}N tracer technique (Tables 3 and 4 and Figure 2). The quantity of legume N derived from different sources varied and was influenced significantly by the legume species and inoculation. Maximum Ndfa values for both legume seed and stover were obtained for inoculated soybean and minimum values for un-inoculated mungbean. With inoculation, soybean derived the highest N from the atmosphere (84.1 percent of its N amounting 159.6 kg N/ha), followed by lentil (82.7 percent and 69.1 kg N/ha) and mungbean (80.7 percent and 56.6 kg N/ha). The lowest N (25.9 kg N/ha) derived from atmosphere was recorded from un-inoculated mungbean (Figure 2).

Legumes received a considerable amount of N from the soil under un-inoculated conditions although some inefficient natural rhizobia were present. The highest amount of Ndfs (34.3 kg N/ha) was recorded in un-inoculated soybean plots (in both seed and stover) and the lowest amount was observed in inoculated mungbean (10.4 kg N/ha). The mean total Ndfs for the un-inoculated soybean plot was about more than three times greater than the total Ndfs found from inoculated mungbean (Figure 2). Due to inoculation a considerable amount of total N was obtained from the soil (32.4 percent in inoculated soybean, 10.6 percent in inoculated mungbean and 11.2 percent in inoculated lentil).

All legume plants also received more N from fertilizer sources under un-inoculated compared with inoculated conditions. The highest total Ndff was recorded from the un-inoculated soybean plot (10.2 kg/ha), whereas the lowest value was obtained from the inoculated mungbean plot (3.1 kg/ha).

DISCUSSION

Total N content differed significantly between legumes and with and without inoculant, with the highest and lowest N uptakes being recorded in inoculated soybean and un-inoculated mungbean, respectively. With inoculation, the average plant N content increased by 70.9–98.3 percent, with the greatest increase being obtained in soybean (98.3 percent) followed by lentil (77.3 percent) and mungbean (70.9 percent). Results of a similar nature have been reported previously by other authors for soybean (Hoque, Sattar and Dutta, 1995; Jensen, 1997; Molla *et al.,* 2001; Hungria *et al.,* 2003; Shaha, 2007), lentil (Bremer *et al.,* 1990; Whitehead *et al.,* 2000; Shaha, 2007), chickpea and bean (Duc, Mariotti and Amarger, 1988; Rupela, 1994).

Soybean fixed the most N from the atmosphere followed by lentil and mungbean. When the seeds of soybean were treated properly before sowing with *Bradyrhizobium* inoculant, N derived from the atmospheric increased by 2.7–fold in seed and 3.8-fold in stover compared with un-inoculated soybean. Comparatively lower amounts of N were derived by inoculated lentil (2.4 and 2.1 times more for seed and stover, respectively) and mungbean (2.2 and 2.1 times more for seed and stover, respectively) than in un-inoculated plants. Most interesting were the differences recorded between legumes in N_2 fixation, inoculated soybean being the best fixer, followed by the lentil, with mungbean being the poorest.

Selection of legumes with higher yields and N_2 fixation ability is a potential approach for increasing the contribution of N from atmosphere into soil–plant systems. The results from this study clearly demonstrate this. For example, the contribution of biological N_2 fixation from these three inoculated legumes would range from 56.6 kg N/ha for mungbean to 159.6 kg N/ha for soybean, i.e. an almost 2.8–fold increase by substituting inoculated soybean for inoculated mungbean, or a 22.1 percent increase by substituting inoculated lentil for mungbean. Sattar, Islam and Hossain (2000), Alves, Boddey and Urquiaga (2003) and Tien *et al.* (2002) showed that between 56 percent and 89 percent of the N requirements of soybean could be met from the atmosphere if inoculated, while the corresponding figures for inoculated chickpea, lentil and mungbean were 65–75 percent, 66–73 percent and 45–76 percent, respectively, while un-inoculated legumes could meet only between 44 percent and 67 percent of their N requirements from the atmosphere.

The infectiveness and efficiency in N_2 fixation and thus the response to inoculation by commercial *Rhizobium leguminosarum* inoculant showed a high level of plant genus–rhizobium specificity. In this study, lentil and mungbean showed moderate responses under un-inoculated conditions due to the presence of natural rhizobia in the soil while soybeans, by contrast showed little response when not inoculated. Oberson *et al.* (2007) showed that at maturity the total amount of N_2 fixed by soybean was 150–260 kg N/ha and during maturity the amount of Ndfa was 102 kg N/ha, equivalent to 47 percent of the total N assimilated (Zapata *et al.,* 1987).

ACKNOWLEDGEMENT

We gratefully acknowledge the financial support of IAEA for establishing the ^{15}N analyser at the BNF Laboratory, Soil Science Division, BINA, Bangladesh.

REFERENCES

Alves, B.J.R., Boddey, R.M. & Urquiaga, S. 2003. The success of BNF in soybean in Brazil. *Plant Soil*, 252: 1–9.

Bremer, E., Van Kessel, C., Nelson, L., Rennie, R.J. & Rennie, D.A. 1990. Biomedical and life sciences and earth and environmental science. *Plant Soil*, 122: 47–56.

Duc, G., Mariotti, A. & Amarger, N. 1988. Measurement of genetic variability for symbiotic dinitrogen fixation in field-grown faba been (*Vicia faba* L.) using a low level ^{15}N-tracer technique. *Plant Soil*, 106: 269–276.

Fedorova, M., Denton, M., Schulze J., Liu, J., Graham, M., Allan, D., Samac, D.A. & Vance, C.P 2005. Improving the legume-rhizobium symbiosis. Can genomics help? *In* I. Tikonovich, ed. *Molecular plant microbe interactions: New bridges between past and future,* pp. 29–31. Dordrecht, Kluwer Academic Publishers.

Hoque, M.F., Sattar, M.A. & Dutta, R.K. 1995. Nodulation, chlorophyll and leghaemoglobin content of soybean as affected by *Bradyrizobium japonicum* and sulphur molybdenum supplementation, *Bangladesh J. Bot.*, 28: 42–52.

Hungria, M., Campo, R.J., Mendes, I.C. & Graham P.H. 2003. Contribution of biological nitrogen fixation to the N nutrition of grain crops in the tropics. *Aust. J. Plant Physiol.,* 14: 689–694.

IAEA. 1990. Use of ^{15}N methodology to assess biological nitrogen fixation, by G. Hardarson, & S.K.A. Danso. In *Use of nuclear techniques in studies of soil-plant relationships,* Training Course Series No. 2, pp. 129–160. Vienna.

IAEA. 2002. *Semi-micro Kjeldahl analysis for plant materials,* by Martina Aigner. IAEA Laboratories at Seibersdorf, Austria.

Jensen, E.S. 1997. *The role of grain legume N_2 fixation in the nitrogen cycling of temperate cropping systems.* RISO National Laboratory, Roskilde. 107pp.

Keeney, D.R. & Nelson, D.W. 1982. Nitrogen – inorganic forms. In A.L. Page, R.H. Miller & D.R. Keeney, eds. *Methods of soil analysis, Part 2. Chemical and microbiological properties.* Agronomy Series No. 9, pp. 643–698. Madison, WI, American Society of Agronomy.

Molla, A.H., Shamsuddin, Z.H., Halini, M.S., Morziah, M. & Puteh, A.B. 2001. Potential for enhancement of growth and nodulation of soybean co-inoculated with *Azospirillum* and *Bradyrhizobium* in laboratory systems. *Soil Biol. Biochem*., 33: 457–463.

Morgan, T. 1997. Symbiotic effectiveness of *Rhizobium leguminosarum* bv. trifolii collected from pastures in south- western Victoria. *Aust. J. Exp. Agric.,* 37: 623-630.

Oberson, A., Nanzer, S., Bosshard, C., Dubois, D., Mader, P. & Frossard, E. 2007. Symbiotic N_2 fixation by soybean in organic and conventional cropping systems estimated by ^{15}N dilution and ^{15}N natural abundance. *Plant Soil*, 290: 64–83.

Rupela, O.P. 1994. *Screening for intracultivaral variability for nodulation of chickpea and pigeon pea,* pp. 75–83. Patancheru, Andhra Pradesh, India, ICRISAT.

Sattar, M.A, Islam, M.Z. & Hossain, M.B. 2000. Enhancing residual benefits of legumes through rhizobial inoculation on productivity of succeeding rice. *Nuclear Sci. Appl.,* 9: 33–36.

Shaha, B.K. 2007. *Nodulation, yield biochemical constituents and N uptake by lentil and soybean as affected by different rhizobial strains.* Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh. (M.S. Thesis).

Tien, H.H., Hien, T.M., Son, M.T. & Herridge, D. 2002. Rhizobial inoculation and N_2 fixation of soybean and mungbean in the eastern region of South Vietnam. *In* D.E. Herridge, ed. *Inoculant and nitrogen fixing legumes in Vietnam.* ACIAR Proceedings 109e, pp. 29–36. Canberra, ACIAR.

Toomsan, B., Donagh, M.J.H., Limpinuntana, V. & Giller, K.E. 1995. Nitrogen fixation by groundnut and soybean and residual nitrogen benefits to rice farmers field in Northeast Thailand. *Plant Soil,* 175: 45–56.

Wani, S.P., Rupela, O.P. & Lee, K.K. 1995. Sustainable agriculture in the semi arid tropics through biological nitrogen fixation in grain legumes. *Plant Soil*, 174: 29–49.

Whitehead, S.J., Summerfield, R.J., Muehlbauer, F.J., Coyne, C.J., Ellis, R.H. & Wheeler, T.R. 2000. Crop improvement and the accumulation and partitioning of biomass and nitrogen in lentil. *Crop Sci.,* 40: 110–120.

Zapata, F., Danso, S.K.A., Hardarson, G. & Fried, M. 1987. Time course of nitrogen fixation in field-grown soybean using N-15 methodology. *Agron. J.,* 79: 172–176.

Bio-solubilization of Rock Phosphate and Plant Growth Promotion by *Aspergillus niger* TMPS1 in Ultisol and Vertisol

M. Shrivastava[1,*] and S.F. D'Souza[1]

ABSTRACT

The effectiveness of the phosphate solubilizing fungus *Aspergillus niger* strain TMPS1 isolated from the rhizospheric soil of mangrove was evaluated for wheat grown on Ultisol and Vertisol soil types under greenhouse conditions using the phosphorus-32 (^{32}P) isotope dilution technique. The fungus was identified using 18S ribosomal ribonucleic acid (18S rRNA). Two low grade Indian rock phosphates (RP) from Lalitpur (LRP) and Purulia (PRP) were used as phosphorus (P) sources. Plants were grown for six weeks. In both soils dry matter yield and P uptake of wheat increased significantly with fungus inoculation and rock phosphate fertilizer addition compared with the unfertilized control and un-inoculated RP fertilizer treatments. This demonstrates solubilization of the RPs by fungus which increases the relative contribution to plant P uptake. The relative contribution to plant P uptake from the RPs was comparatively higher in Ultisol than in Vertisol, implying higher solubilization of the RPs by fungus in the former. Phosphate derived from the LRP was greater than from PRP in fungus inoculated treatments in both soil types. This demonstrates the potential of *Aspergillus niger* strain TMPS1 isolate as a phosphate solubilizer in Ultisols and Vertisols. Further, the advantages were shown of using ^{32}P to distinguish the contributions of bio-available native soil P and P from RPs to P nutrition in plant–microbe interactions.

Key words: *18S rRNA, Aspergillus niger, ^{32}P, rock phosphates, Ultisol, Vertisol.*

INTRODUCTION

Phosphorus (P) is an important plant nutrient and high P absorbing capacity of soil, P immobilization and/or fixation of P to insoluble mineral complexes results in low availability of this macronutrient for plant uptake (Marschner, 1995). Vertisol and Ultisol soil types constitute a considerable proportion of the cultivable area in India and P deficiency is one of the major stress factors for crop production in both types of soil (Velayutham and Bhattacharyya, 2000).

Precipitation with calcium (Ca) ions in calcareous Vertisols, and with iron (Fe) and aluminium (Al) ions in acidic Ultisols are the main causes of reduced P availability in these soils (Hisinger, 2001). For this reason, the optimal development of crops demands costly inputs of P fertilizers, part of which is utilized by plants and the remainder converted into insoluble fixed forms (Omar, 1998; Vassileva, Vassilev and Azcon, 1998).

The direct application of rock phosphate (RP) is considered as an agronomic and economically attractive alternative to the use of more expensive water-soluble P fertilizer sources (Hammond, Chien and Mokwunye, 1986). In India, it is estimated that about 260 million tons (t) of phosphatic rock deposits are available but most of this material is not suitable for phosphatic fertilizer production due to its low reactivity and the presence of impurities. These RPs could, however, be applied directly for crop production with or without modification (Shrivastava, Bhujabal and D'Souza, 2007).

Sustainable P supply in agroecosystems can be achieved by using RP in conjunction with phosphate solubilizing microorganisms (Vandermeer, 1995). The use of phosphate solubilizing microorganisms, including arbuscular mycorrhizal (AM) fungi, has been proposed as a low cost and low energy approach to help increase the agronomic effectiveness of RP fertilizers (Gyaneshwar *et al*., 2002). Fungi in soil are able to traverse distances more easily than bacteria and thus may be more important for P solubilization in soils (Kucey, 1983), and RP solubilization by various fungi have been reported (Vassilev *et al*., 1998; Goenadi, Siswanto and Sugiarto, 2000; Reddy, Kumar and Khosla, 2002).

The effectiveness of solubilization of phosphate-bearing materials under soil conditions is unclear because of the possible re-fixation of phosphate ions on their way to the root surface. Phosphorus-32 (^{32}P) radiotracer techniques have been used to evaluate the exchange rates between the solution and solid phases of soil, and to measure the bioavailability of P from RP materials (Zapata and Axmann, 1995; Shrivastava *et al.,* 2007). Isotopic techniques can also be applied to determine the extent of microbial biotransformation of RPs and unavailable soil P into bioavailable soil P (Gianinazzi-Pearson and Gianinazzi, 1989; Toro, Azcón and Barea, 1997).

In the present study, the capacity of the fungus *Aspergillus niger* strain TMPS1 isolated from mangrove rhizosphere as phosphate solubilizing bio-fertilizer was assessed under greenhouse conditions using the ^{32}P isotope dilution technique.

MATERIALS AND METHODS

Isolation of phosphate solubilizing fungus

Phosphate solubilizing fungus was isolated from the rhizospheric soils of mangroves after serial dilution of the soil solution on Pikovskaya's agar plates (Pikovskaya, 1948). Formation of a clear halo around the fungal growth after five days (d) of incubation indicated phosphate solubilizing ability. This fungus was selected on the basis of the

---

[1] Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai-400085, India

[*] E-mail address of corresponding author: manojshrivastava31@gmail.com

L.K. Heng, K. Sakadevan, G. Dercon and M.L. Nguyen (eds), Proceedings — International Symposium on Managing Soils for Food Security and Climate Change Adaptation and Mitigation. Food and Agriculture Organization of the United Nations, Rome, 2014: 73–77

diameter of the halo (≥ 15 mm) around the fungus. The culture was maintained on Pikovskaya agar slants at 4–6°C and sub-cultured every month.

Molecular identification of phosphate solubilizing fungus

Fungal spores were harvested by washing the slants with sterile saline. For the molecular characterization of the fungus, genomic DNA from fungal isolate was extracted using the method of Latha *et al*., 2002. The extracted DNA was used as a template to amplify the 18S rRNA genes using the polymerase chain reaction (PCR). Universal primers (NS5 [5'-AACTTAAAGGAATTGACGGAAG-3'] and NS8 [5'-CCGCAGGTTCACCTACGGA-3']) for the 18S rRNA as described by White *et al.* (1990) were used to amplify a DNA sequence of TMPS1 560bp in length. Primers were synthesized by MWG-Biotech Ltd. (Ebersdorf, Germany). Polymerase chain reaction amplifications were carried out in 50 mL reaction volumes with a Primus thermocycler (Eppendorf) using the following steps: initial denaturation at 94°C for 1 min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 40°C for 1 min, and extension at 72°C for 2 min followed by a final extension phase at 72°C for 10 min. The purified PCR products were sequenced in both directions. The nucleotide sequences of strain TMPS1 was deposited in the NCBI GenBank database under accession number DQ316605.

Greenhouse study

Isotope dilution technique

The ^{32}P isotope dilution technique (IAEA, 1990) was used to evaluate RP solubilization by *Aspergillus niger* strain TMPS1 under greenhouse conditions. In this method, ^{32}P measures the exchange rates governing the equilibrium between liquid and solid phase phosphate. The specific activity (SA), i.e. the ^{32}P: phosphorus-31 (^{31}P) ratio is determined in the plant tissue to ascertain the effect of fungus inoculation on RP solubilization and in turn plant P uptake. A lowering of the SA with fungus inoculation compared with no inoculation would indicate that the plant is receiving extra ^{31}P released a result of RP solubilization by the fungus. Therefore, the isotope-based technique can be used to determine the extent of the transformation of the unavailable soil P pool into bioavailable P by the metabolic activity of the phosphate solubilizing microorganisms.

Soils

Greenhouse pot culture experiments were conducted using two soils, an Ultisol from Karnataka and a Vertisol from Maharashtra, India. The characteristics of the Utisol were as follows: $pH_{1:2}$ (soil: water), 4.8; texture: sandy clay; available P (Bray I) 5.9 mg/kg; organic carbon, 20.4 g/kg, total N, 3.9 g/kg; cation exchange capacity, 8 cmol (p^+)/kg; phosphorus fixing capacity, 700 g/kg, and 0.02 M $CaCl_2$ -extractable Al, 2.58 mg/kg. The Vertisol had: pH (water), 8.2; texture clayey; available P (Olsen), 3.7 mg/kg; organic carbon, 5.1 g/kg; total N, 0.6 g/kg; cation exchange capacity, 52 cmol (p^+)/kg, free calcium carbonate, 3.6 percent; and phosphorus fixing capacity, 620 g/kg. Both soils were deficient in plant available P content.

Rock phosphates

Two Indian RPs from Lalitpur (LRP) and Purulia (PRP) containing 22.1 and 33 percent total P_2O_5, respectively, were used in this study. Both were low grade and had low reactivity.

Experimental

The soils were sieved (2 mm) and 2 kg were placed in plastic pots. Both RPs were applied as finely ground (100 mesh) natural products at a rate 500 mg P/kg soil. Soil labelling was done by thoroughly mixing the soil with 100 mL/pot of the solution containing an activity of 10 megabecquerel (MBq) ^{32}P. Soil was inoculated with 25 mL/pot spore suspension of *Aspergillus niger* TMPS1 containing 1×10^7 spores/mL.

All pots were kept at field capacity moisture content and left to equilibrate for two d. In all, there were seven treatments for each soil type: (i) soil alone (absolute control), (ii) soil + ^{32}P (radioactive control), (iii) soil + fungus + ^{32}P, (iv) soil + LRP, + ^{32}P, (v) soil + LRP + fungus + ^{32}P, (vi) soil + PRP + ^{32}P, and (vii) soil + PRP + fungus + ^{32}P. All the treatments were laid out in quadruplicate in a completely randomized block design.

Wheat (*Triticum aestivum* L. cv. PBW343) seeds were sterilized by soaking in 5 percent sodium hypochlorite (NaOCl) solution for 10 min. and washed three times with distilled water before sowing. Ten sterilized seeds were sown in each pot. The pots were weighed and watered to field capacity daily. After one week of germination plants were thinned to eight plants per pot. Plants were harvested after six weeks of growth.

Shoot dry weights were recorded after drying at 70°C to constant weight. Dried plant samples were digested in di-acid mixture (HNO_3: $HClO_4$, 5:1 v/v) and analysed for total P content by the yellow phospho-vanado-molybdate complex method at 420 nm (Koeing and Johnson, 1942). The ^{32}P activity was measured by liquid scintillation counting (Packard Tri-Carb 2100) using the Cerenkov effect. Counts were corrected for counting efficiency (54 percent) and expressed in Bq (disintegrations per second). The specific activity (SA) of P was then calculated by considering the radioactivity per amount of P in the plant material and expressed in Bq/mg P.

Calculations

Isotopic parameters were calculated as follows and as described by IAEA (1990) and Zapata and Axmann (1995):

Total P uptake by plant (mg·P/pot) U_{TP} = DMY (mg/pot) x (%P/100) x DMY

Specific activity (Bq mg/P) = Bq/g plant/mg P/g plant

$$\%\mathrm{PdfS} = \frac{\mathrm{SA\ in\ plant(Bq \cdot mg\,P)\ in\ presence\ of\ RP}}{\mathrm{SA\ in\ plant(Bq\ \ mg\,P)\ in\ absence\ \ of\ RP}} \times 100$$

where Pdfs is P derived from soil.

$$\%\mathrm{PdfRP} = 1 - \frac{\mathrm{SA\ in\ plant(Bq \cdot mg\,P)\ in\ presence\ of\ RP}}{\mathrm{SA\ in\ plant(Bq \cdot mg\,P)\ in\ absence\ of\ RP}} \times 100$$

where PdfRP is P derived from rock phosphate
P uptake from labelled soil (mg·P/pot) = U_{TP} x (%PdfS/100)
P uptake from rock phosphate (mg P/pot) = U_{TP} × (%PdfRP/100)

Statistical analyses

All the data were analysed by analysis of variance (ANOVA) and the means compared with least significant difference (LSD) at $p < 0.05$ level (Hoshmand, 1993).

Table 1. Effect of fungus inoculation on shoot dry matter yield (g/pot) and P uptake of wheat in Ultisol and Vertisol

Group	Treatments	Dry matter yield (g/pot)		P uptake (mg/pot)	
		Ultisol	Vertisol	Ultisol	Vertisol
1	Control (No P)	0.40	0.93	0.48	1.35
2	Soil+ ^{32}P	0.42	0.95	0.54	1.36
3	Soil+ TMPS1+ ^{32}P	0.69	1.90	0.88	2.77
4	Soil + LRP+^{32}P	0.81	0.99	1.03	1.38
5	Soil + LRP + TMPS 1+^{32}P	1.36	2.88	1.81	4.12
6	Soil + PRP + ^{32}P	0.73	0.97	0.95	1.37
7	Soil+ PRP+ TMPS 1 +^{32}P	1.12	2.90	1.51	4.14
LSD ($p < 0.05$)		0.23	0.47	0.24	0.61

Table 2. Effect of fungus inoculation on specific activity (Bq mg/P) of wheat in Ultisol and Vertisol

Group	Treatments	Specific activity (Bq mg/P)	
		Ultisol	Vertisol
1	Soil + ^{32}P	255.3	429.1
2	Soil + TMPS1 + ^{32}P	224.0	370.3
3	Soil + LRP + ^{32}P	230.7	422.6
4	Soil + LRP + TMPS1 + ^{32}P	149.4	300.0
5	Soil + PRP + ^{32}P	243.4	426.0
6	Soil + PRP+ TMPS1 + ^{32}P	157.5	306.2
LSD ($p < 0.05$)		22.0	20.2

RESULTS AND DISCUSSION

Molecular identification of phosphate solubilizing fungus

The complete 18S rRNA sequence of the isolate used in this study was obtained and compared with sequences available in the GenBank (NCBI, USA) database. This fungal strain showed a 98 percent similarity with *Aspergillus niger*. The 18S rRNA sequence of this isolates has been deposited at GenBank viz. accession no. DQ316605. The culture has been designated as Trombay mineral phosphate solubilizer 1 (TMPS1).

Dry matter yield and P uptake

In both soil types, dry matter yield and P uptake were significantly higher in fungus inoculated soil with or without RP treatments as compared with the un-fertilized control and un-inoculated RP fertilizer treatments (Table 1). The increases in plant biomass yield and P uptake could be partially due to increased RP solubilization by the fungus TMPS1.

The increased solubilization of P from RP and subsequent P uptake by plants could be attributed to chelation reactions whereby organic acid released by the fungus chelate the cations present in RP and release the P for plant uptake (Whitelaw, 2000). Greenhouse studies with corn (*Zea mays L.*) showed that corn growth increased with the addition of oxalic or citric acid, suggesting that organic acids have potential as amendments for increasing plant-available P in PR-treated soils (Kpomblekou-A and Tabatabai, 2003). Organic acids secreted from the fungus probably bind the free Al^{3+} present in Ultisol and reduce the phytotoxicity of Al and enhance the plant growth. In the case of Vertisols, the high content of exchangeable Ca and alkaline pH affect nutrient availability negatively and thereby influence soil fertility. When water-soluble phosphatic fertilizers (e.g. single super phosphate or di-ammonium phosphate etc.) are applied to Vertisols, they are converted rapidly into insoluble non-available forms of phosphates. Organic acids produced by this fungus solubilized the RP and native insoluble soil P and formed a complex with the free Ca present in the soil, preventing re-fixation of the P. This illustrates the plant growth promotion as well as the phosphate solubilizing abilities of this fungus in two distinct soils types. Various studies have shown that the dry matter yield of wheat plants increased significantly after inoculation of the RP solubilizing fungi *Aspergillus niger, Aspergillus awamori, Penicillium digitatum, Penicillium citrinum* and *Penicillium bilaji* under greenhouse as well as field conditions (Kucey, Jansen and Leggett, 1989; Omar, 1998).

Isotopic data

Specific activity

Results of the SA measurements in wheat plants are presented in Table 2.

Results show that the SA in the Ultisol was significantly lower in RP treatments that were both un-inoculated and inoculated with TMPS1 than those not receiving RPs, and that the reductions in SA were more pronounced in fungus inoculated treatments. In the case of the Vertisol, significant reduction in plant SA was observed only in the treatment of RP inoculated with TMPS1 compared with the control. The acidic nature of Ultisols helps in the solubilization of RP and inoculation of the fungus further enhanced the RP solubilization process, which in turn reduced the SA in plant shoots. The TMPS1 fungus released phosphate ions either from the soil or added RPs, which reduced the ^{32}P:^{31}P ratio (SA) in the plants. Lower ^{32}P:^{31}P ratios of plants due to microbial inoculation were also reported by Toro, Azcón and Barea (1997).

Phosphorus derived from labelled soil (bioavailable P) and rock phosphate

Plant P derived from labelled soil (bioavailable) and RPs are presented in Tables 3 and 4. In both un-inoculated soils, the plant absorbed predominantly the bioavailable endogenous soil P and percentage share of this P was lower under fungus inoculated treatments. Phosphorus derived from the labelled source (bioavailable soil P) and RPs was significantly higher in both soils inoculated with fungus. This demonstrates the solubilization of RP by the fungus which increases the relative contribution to plant P uptake. This was comparatively

Table 3. Effect of fungus inoculation on percentage of P derived from labelled (bioavailable) soil (PdfS) and rock phosphates (PdfRP) in Ultisol

Group	Treatments	Ultisol			
		PdfS		PdfRP	
		%	mg P/pot	%	mg P/pot
1	Soil + LRP + ^{32}P	90.4	0.93	9.6	0.10
2	Soil + LRP + TMPS 1 + ^{32}P	66.7	1.21	33.3	0.60
3	Soil + PRP + ^{32}P	95.3	0.91	4.7	0.04
4	Soil + PRP + TMPS1 + ^{32}P	70.3	1.06	29.7	0.45
LSD ($p < 0.05$)			0.13		0.08

Table 4. Effect of fungus inoculation on percentage of P derived from labelled (bioavailable) soil (PdfS) and rock phosphates (PdfRP) in Vertisol

Group	Treatments	Vertisol			
		PdfL		PdfRP	
		%	mg P/pot	%	mg P/pot
1	Soil + LRP + ^{32}P	98.5	1.35	1.5	0.02
2	Soil + LRP + TMPS 1 + ^{32}P	81.1	3.34	18.9	0.78
3	Soil + PRP + ^{32}P	99.3	1.36	0.7	0.01
4	Soil + PRP + TMPS1 + ^{32}P	82.7	3.42	17.3	0.72
LSD ($p < 0.05$)			0.82		0.09

higher in the Ultisol, which implies greater solubilization of the RPs by fungus in the Ultisol.

Possible mechanisms for RP solubilization in soil by this fungus are production of low molecular weight organic acids such as gluoconic, malic, citric and oxalic acids (Cerezine, Nahas and Banzatto, 1988; Reyes *et al*., 1999), and the release of protons accompanying respiration and/or $NH^+{}_4$ assimilation (Illmer and Schinner, 1995; Ahuja, Ghosh and D'Souza, 2007). Low molecular weight organic acids (LMWOAs) possess one or more carboxyl and hydroxyl functional groups that can form complexes with metals in soils and thus play an important role in soil processes such as mineral weathering, nutrient mobilization and Al detoxification (Hue, Caddock and Adams, 1986; Bolan *et al.,* 1994). Kpomblekou-A and Tabatabai (1994) studied the ability of 19 LMWOAs to release P from PRs and showed that reactions involved in the P release process were not only pH-dependent but also related to the structural characteristics of the organic acids.

CONCLUSIONS

The results of the present study demonstrate the potential use of an *Aspergillus niger* TMPS1 isolate as a phosphate solubilizer in Ultisol and Vertisol soil types. In general, RP solubilization in alkaline Vertisols does not occur, but this fungus solubilized native unavailable soil P as well as RP in such a soil. The advantages of using ^{32}P to distinguish the contributions of bioavailable native soil P and bioavailable P from RPs to P nutrition in plant–microbe interactions were also shown. Future work is required to determine the phosphate solubilizing and plant growth promoting potentials of this isolate under field condition in different agro-climatic regions for different crops.

REFERENCES

Ahuja, A., Ghosh, S.B. & D'Souza, S.F. 2007. Isolation of a starch utilizing, phosphate solubilizing fungus on buffered medium and its characterization. *Biores. Technol*., 98: 3408–3411.

Bolan, N.S., Naidu, R., Mahimairaja, S. & Baskaran, S. 1994. Influence of low-molecular-weight organic acids on the solubilization of phosphate. *Bio. Fertil. Soils*, 18: 311–319.

Cerezine, P.C., Nahas, E. & Banzatto, D.A. 1988. Soluble phosphate accumulation by *Aspergillus niger* from fluorapatite. *Appl. Microbiol. Biotechnol*., 29: 501–505.

Gianinazzi-Pearson, V. & Gianinazzi, S. 1989. Phosphorus metabolism in mycorrhizas. *In* L. Boddy, R. Marchant & D.J. Read, eds. *Nitrogen, phosphorus and sulphur utilization by fungi*. Cambridge, England, Cambridge University Press.

Goenadi, D.H., Siswanto, S.Y. & Sugiarto, Y. 2000. Bioactivation of poorly soluble phosphate rocks with a phosphorus solubilizing fungus. *Soil Sci. Soc. Am. J.*, 64: 927–932.

Gyaneshwar P, Kumar, G.N., Parekh, L.J. & Poole, P.S. 2002. Role of soil microorganisms in improving P nutrition of plants. *Plant Soil*, 245: 83–93.

Hammond, L.L., Chien, S.H. & Mokwunye, A.U. 1986. Agronomic value of unacidulated and partially acidulated phosphate rocks indigenous to the tropics. *Adv. Agr*., 40: 89–140.

Hisinger, P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: A review. *Plant Soil*, 237: 173–195.

Hoshmand, A.R. 1993. *Experimental research design and analysis: A practical approach for agricultural and natural sciences*. Boca Raton, Fl, USA, CRC Press.

Hue, N.V., Caddock, G.R. & Adams, F. 1986. Effects of organic acids on aluminum toxicity in subsoils. *Soil Sci. Soc. Am. J.*, 50: 28–34.

IAEA. 1990. Isotope techniques in soil fertility and plant nutrition studies, by F. Zapata. *In* Hardarson, G. ed. *Use of nuclear technique in studies of soil plant relationships* pp. 55–59. Vienna.

Illmer, P. & Schinner, F. 1995. Solubilization of inorganic calcium phosphates- solubilization mechanisms. *Soil Biol. Biochem*., 27: 257–263.

Koeing, R.A. & Johnson, C.R. 1942. Colorimetric determination of phosphorus in biological materials. *Ind. Eng. Chem. Anal*., 14: 155.

Kpomblekou-A.K. & Tabatabai, M.A. 2003. Effect of low-molecular weight organic acids on phosphorus release and phytoavailability of phosphorus in phosphate rocks added to soils. *Agric. Ecosys. Environ.*, 100: 275–284.

Kpomblekou-A.K. & Tabatabai, M.A. 1994. Effect of organic acids on release of phosphorus from phosphate rocks. *Soil Sci*., 158: 442–453.

Kucey, R.M.N. 1983. Phosphate-solubilizing bacteria and fungi in various cultivated and virgin Alberta soils. *Can. J. Soil Sci.*, 63: 671–678.

Kucey, R.M.N., Jansen, H.H. & Leggett, M.E. 1989. Microbially mediated increases in plant available phosphorus. *Adv. Agron*., 42: 199–228.

Latha, J., Mathur, K., Mukherjee, P.K., Chakrabarti, A., Rao, V.P. & Thakur, R.P. 2002. Morphological, pathogenic and genetic variability amongst sorghum isolates of *Colletotrichum graminicola* from India. *Indian J. Phytopath*., 55: 19–25.

Marschner, H. 1995. *Mineral nutrition of higher plants*. London, Academic Press.

Omar, S.A. 1998. The role of rock-phosphate-solubilizing fungi and vesicular-arbusular-mycorrhiza (VAM) in growth of wheat plants fertilized with rock phosphate. *World J. Microbiol. Biotech*., 14: 211–218.

Pikovskaya, R.I. 1948. Mobilization of phosphorus in soil connection with the vital activity of some microbial species. *Microbiologiya,* 17: 362–370.

Reddy, M.S., Kumar, S. & Khosla, B. 2002. Biosolubilization of poorly soluble rock phosphates by *Aspergillus tubingensis* and *Aspergillus niger. Bioresour. Tech*., 84: 187–89.

Reyes, I., Bernier, L., Simard, R.R. & Antoun, H. 1999. Effect of nitrogen source on the solubilization of different inorganic phosphates by an isolate of *Penicillium rugulosum* and two UV-induced mutants. *FEMS Microbiol. Ecol*., 28: 291–295.

Shrivastava, M., Bhujabal, B.M. & D'Souza, S.F. 2007. Agronomic efficiency of Indian rock phosphates in acidic soils employing radiotracer A-value technique. *Commun. Soil Sci. Plant Anal*., 38: 461–471.

Toro, M., Azcón, R. & Barea, J.M. 1997. Improvement of arbuscular mycorrhiza development by inoculation of soil with phosphate solubilizing rhizobacteria to improve rock phosphate bioavailability (^{32}P) and nutrient cycling. *Appl. Environ. Microbiol*., 63: 4408–4412.

Vandermeer, J. 1995. The ecological basis of alternative agriculture. *Ann. Rev. Ecol. Syst.,* 26: 201–224.

Vassileva, M., Vassilev, N. & Azcon, R. 1998. Rock phosphate solubilization by *Aspergillus niger* on olive cake-based medium and its further application in a soil-plant system. *World J. Microbiol. Biotech*., 14: 281–284.

Velayutham, M. & Bhattacharyya, T. 2000. Soil resource management. In *Natural resource management for agricultural production in India*. New Delhi, Indian Society of Soil Science, 135 pp.

White, T.J., Bruns, T., Lee, S. & Talor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: M.A. Innis, D.H. Gelfand, J.J. Sninsky & T.J. White, eds. *PCR protocols: A guide to methods and applications,* pp. 315–322. San Diego, California, USA, Academic Press.

Whitelaw, M.A. 2000. Growth promotion of plants inoculated with phosphate solubilizing fungi. *Adv. Agron*., 69: 99–151.

Zapata, F. & Axmann, H. 1995. ^{32}P isotopic techniques for evaluating the agronomic effectiveness of rock phosphate materials. *Fertil. Res*., 41: 189–195.

Country-wide Agro-ICT Infrastructure for Supporting Assessment of Soil Carbon Sequestration, Soil Nutrient Balance, Soil Water Status and Appropriate Crop Rotation

W.H. Mayer[1,*]

ABSTRACT

High returns on investment in agriculture and forestry come from integrating the newest technologies and cooperation between public and private stakeholders. With the help of ortho-images, a network of agro-sensors (weather, soil), geographic information systems (GIS), a land parcel information system (LPIS), farm and/or forest management systems, advisory services covering regions or countries farmers can be supported in terms of logistics, precision, or virtual farming needs and good agricultural practices (GAP). These integrated technologies can also help to implement environmental risk management tasks. In general, integration of local information with large-scale images, agro-sensor stations and information and communication technology (ICT) that provide tools for managing agriculture and forestry requires 2 to 3 yr for implementation. Technology integration with geographic information systems and stakeholder cooperation is a win-win model for a bright agro-forest future supporting food/feed production, biomass for energy, environmental caretaking and risk management.

Keywords: *GIS, maps, agro applications, information and communication technology, precision farming, business model.*

INTRODUCTION

Based on the use of precise ortho-images such as those available from Microsoft Bing^{TM} Maps, geographic information systems (GIS)-based agro-information and communication technology (ICT) of PROGIS, data from agro-sensor technology and rural area management consulting services, the AGRO-ICT-Backbone® concept was developed. It provides not only the necessary information technology (IT) tools but is also a holistic model to establish an agricultural infrastructure throughout a whole country for fostering better agricultural development. This information technology tool includes:

- The production of a high resolution 30 cm ortho-image for the whole country as a basis for further planning and control with an update frequency of 3–4 yr.
- The setup, or if available as in Europe, the upgrade of existing land parcel information (LPIS) systems — or a cultivation register and/or a rural open street map (OSM), based on ortho-images and PROGIS GIS software WinGIS® .
- The implementation of a sophisticated farm management information system (FMIS) which supports farm advisory (extension) services and serves the ministry for regional or country-wide statistical needs.
- The installation and integration of a logistic system including mobile solutions to support farmers and their chain partners such as the industry, for timely delivery of seeds, fertilizer, harvested products and for traceability needs.
- The installation of agro-sensor networks, consisting of agro-weather stations and soil sensors for decision support and guidance.
- Value added services for needs like precision and virtual farming, land consolidations, environmental management, carbon calculation, risk management to support the 2013 Common Agricultural Policy (CAP) needs etc. including consulting if needed. A special training concept enables users to develop their own on-top applications for solving local needs.
- Apps for mobile phone solutions for Windows phone with Bing maps, (but also iPhone with Google maps, or Android with Google maps), such as:
  - GIS applications for field identification;
  - access to the logistic system for automated order processing;
  - access to farm management tools for sending and receiving specific orders for cultivation (including precision farming maps via advisors);
  - access to the software developer component for personal and local GIS-based developments;
- Capacity-building including education and training models enables local experts to be ready for a rollout.
- The intelligent business model enables the owner of the ICT infrastructure (public, private or private-private partnership), to generate return on investments (ROI) by supporting stakeholders such as banks, insurance companies, large farms, large forest enterprises, chain partners like the food industry, suppliers of farm equipment, agro-chemicals and other agro-resources as well as international investors.

Beneficiaries are farmers and forest holders, also smallholder enterprises, groups of farmers, cooperatives, advisory/extension services, other service providers, affiliated industries, ministries, banks and insurance companies, researchers, rural populations and the public as a whole.

---

[1] PROGIS Software GmbH, Postgasse 6, A-9500 Villach, Austria
[*] E-mail address of corresponding author: mayer@progis.com; www.progis.com

L.K. Heng, K. Sakadevan, G. Dercon and M.L. Nguyen (eds), Proceedings — International Symposium on Managing Soils for Food Security and Climate Change Adaptation and Mitigation. Food and Agriculture Organization of the United Nations, Rome, 2014: **79–82**

Solutions from PROGIS

The implementation of this agro-ICT backbone has to be realized within a large-scale project together with a range of local partners and experts. It can be done in a public, public-private or private project and is partitioned into the following steps:

Ortho-image

The production of 30 cm ortho-images with a vertical digital surface model (DSM) of <1.5 m resolution and a 60 cm infrared image. Examples of technical specifications of compliant ortho-images are given in Microsoft (MS). "Global Ortho: Rapid, High Efficiency Ortho Update Technologies" (available at office@progis.com).

Preparation of LPIS

The first task is to implement the GIS system WinGIS® and on the base of MS images, set up the LPIS (Figure 1) or cultivation register including the assignment of owners or leaseholders to individual plots and thereby build up a country-wide land parcel database. An OSM technology can be integrated. The LPIS systems are already implemented in most countries of the European Community (EC) and updates can be done directly by farmers or farm advisors to increase precision and reduce land administration costs by data transfer to the existing LPIS/IACS system (see details later).

GIS services

Providing GIS services for non-specialists was a primary aim of PROGIS when developing WinGIS®. It is easy to learn and use GIS software running on a personal computer, with extensive geographic application possibilities and facilities. Due to the ability to integrate online map data such as Microsoft Bing Maps as an "embedded module", the access to worldwide available geographic data like satellite and aerial images, road maps and address databases is already part of the software package. Import and export interfaces support the most common GIS/CAD file formats like the ESRI™ shape files, the AutoCAD™ DXF, MapInfo™ MIF and also text based file formats like CSV or GPX for data import from, e.g. global positioning system (GPS) devices. In a few steps external spatial data can be loaded into the user´s project.

By using the developer component, application developers have the possibility to link their application with WinGIS® in order to visualize, edit and administer any data with a geographic relation. This is very relevant for realizing suggestions to implement local integrated agricultural control system (IACS) applications, to monitor good agricultural practices (GAP)/common agriculture policy (CAP) compliance or for supporting consultancy applications.

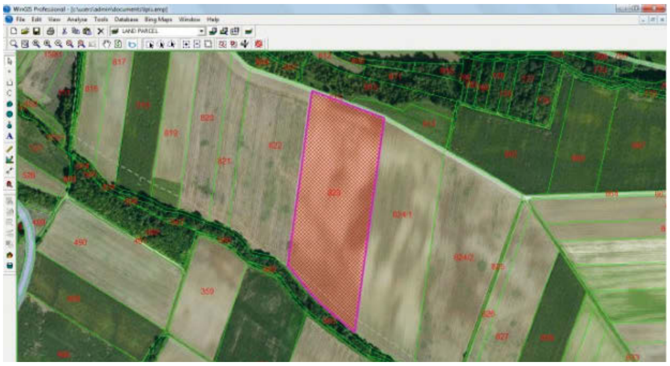


Figure 1. LPIS polygons on ortho-image.

With the help of such a software development kit (SDK), local IT experts managing the IACS system of an EC country can easily implement an application to generate a subsidy form out of the farm management information system (FMIS) and transfer it via Internet to the government homepage. The effect would be a "one stop shop software", managed by a trained farmer or by an advisor that in parallel with the subsidy form also manages the business calculation, a nutrient balance, a carbon balance, integrates data for other future documentation needs like food traceability, a business plan, insurance data or after 2013, CAP´s ICT needs. Not only would governments save money, but farmers will save travel and time costs from driving to a subsidy centre. Within a similar timeframe, much more output can be realized on one side and if advisors are supporting farmers within a region (in all negotiations about a CAP reform new advisory concepts are asked for) much more can be achieved in all sectors where single farmers alone can´t reach the targets but in groups they would be able to do so. These targets concern mainly the environment, landscapes and natural risks, but also logistics, precision farming, land consolidation missions etc. are served This is also something the new GAP regulations will support (see later).

Implementation of FMIS

When the European Union (EU) launched the CAP reform to increase food quality and safety for the welfare of its citizens, PROGIS developed DokuPlant™ on top of the GIS software tools for farmers and advisors to manage the many needs which this new legislation brought along. This integrates expert databases (all agricultural data and cultivation recommendations sustainably supported by local experts) and a perpetual calendar and documentation tool, and facilitates planning, calculation, control and traceability. With this, extension officers/advisors are able to aggregate the data from fields, farms or a whole region and to prepare them for a Ministry or other public authority for statistical use or for projects.

The following information is generated from every field and can be accumulated countrywide:

- Activity management;
- Crop rotation;
- Cost calculation;
- Nutrient balance and carbon balance;
- All input/resource needs;
- Harvest estimations

PC-GIS, real time management and the expert data base are integrated. The mapping of plots/fields is supported and a perpetual calendar enables the display of any performed activity: what–when–where (Figure 2). The integrated database is filled with agro-expert data, generated in close cooperation with local agro-forest-environmental scientists/experts and contains (example: agro-Germany) 2 500 agro-machine data (KTBL, costs, time, …), data on thousands of mineral and organic-fertilizers, 850 herbicides with contents, crops including varieties and 400 plants with average yield and seed needs. The complete working process for a year with all activities and relevant data are pre-defined for all crops and enables planning with one click: Where (plot in the map) do I plan what (select crop from the expert data bank). This database is consequently also a knowledge base and know-how transfer from scientists to the base, the farmers and foresters – is carried out daily and sustainably. After planning, the data entry can be done manually or automatically.

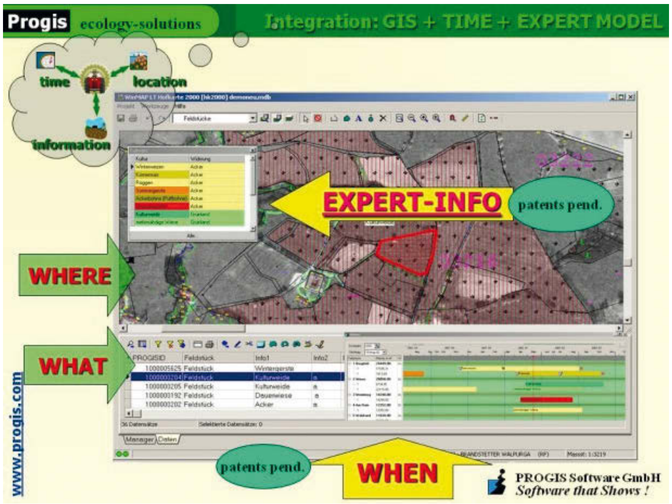


FIGURE 2. Farm management: where– what– when– expert-data.

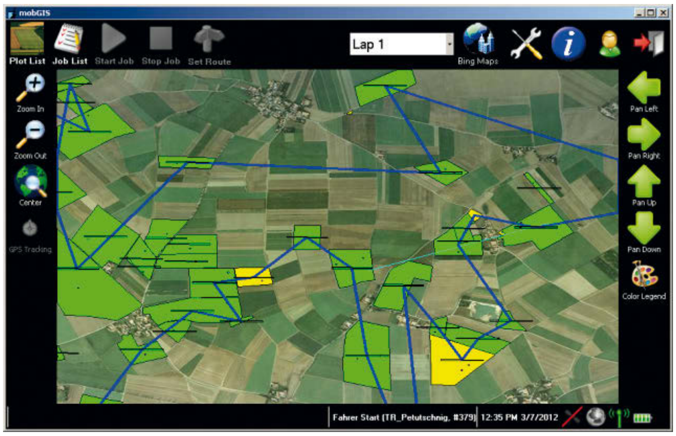


FIGURE 3. Logistics – where to do what.

Forest management

ForestOffice is FMIS for forest enterprises. It deals with sustainable forestry planning, forest facilities, forest management and forest logistics; the expert database contains local growth tables of different trees. Both agricultural and forest expert data have to be modified by local experts working within a "farmer/forester–advisor–expert" business model.

Logistic services

The protection of the environment and of natural resources is on everyone's lips. Within the agricultural sector group management, activity- based planning and sharing of production facilities contribute to reach these targets. PROGIS developed a smart logistic solution to solve these needs. The base data are those accumulated from the FMIS, and farmers, foresters and the industry deduce therefrom their planning, processing and time optimization answering queries like: "where to deliver what" or "where to pick up what and when" and how to come to a location (with the help of the rural OSM) supports all process related partners (Figure 3).

The system leads to an optimization of daily and seasonal routing, accurate information of harvest status, GPS position data visualization, online two-way communication (GPRS/UMTS) between central and mobile terminals and order processing. The system consists of a central station and a number of mobile units ("mobGIS"). It handles crops for food/feed or biomass production, liquid manure deposits, forest harvesting or any other logistic task. Up to 30 percent cost reductions or even more can be achieved. Environmental pollution is far smaller than with conventional methods and due to the recordings ongoing improvements may occur.

FIGURE 4. Agro-sensor network.

FIGURE 5. Apps for Windows phone.

Agro-sensor networks

Sustainable cultivation and protection of soils depend a lot on the application of fertilizers, pesticides and water. Agro-sensor stations help to take decisions and to optimize rates. A network of agro-climate sensors (Figure 4) – one station for every microclimate – and soil moisture sensors are needed. Based on the data and a tool set, experts can provide farmers with tailor made recommendations (e.g. forecasts for weather situations), but also obtain protocols of the climate situation of the past and the related impact for the future; for example, mass reproduction of a fungus or a beetle with an SMS induced decision "start spraying".

The expert models, e.g. based on meteorological conditions collected during the last four weeks concerning which fungi or beetle will tend to outbreak, have to be adjusted or developed and fine-tuned from local phytopathology experts.

With the soil moisture sensors, which are also available in different depths, all necessary data for irrigation can be collected and used to support an automatic controlled irrigation system.

Mobile phone solutions

A range of apps has been developed for "mobile agriculture" to support farm and land management via mobile phones and to develop its own supportive GIS-based apps (Figure 5). It is already possible to digitize, edit and delete polygons, to record GPS positions, to cluster them and to send all recorded positions and digitized polygons via E-mail and import the data into the WinGIS® software for further processing. Also the access to DokuPlant and Logistics is a great advantage for advisors to communicate with their clients and advise them on field-related activities via mobile phone and thereby bridge local distances.

Organizational components

New business models

In the same manner as ICT has supported many other sectors throughout the last decades, ICT is able to support agriculture, but enabling structures and a new form of cooperation are needed. Farmers will also be able to support the new requirements of the CAP reform, but they need better support, assisted by new advisory structures focusing on farmers' needs and not only on the needs of other stakeholders. The farmer is the integrative factor within the food/feed, bioenergy or even environmental or natural risk chain-management and he/she has to be supported. Then all other chain members will also benefit from the ICT structure.

New business models are necessary — and available — that take care of the leverage effect due to integration of technologies and cooperation of structures! Less group egoism in agro-forestry chain management is a must!

A prerequisite to start such an agro-solution is a local infrastructure comprizing local hardware, communication technologies and the whole appropriate personnel organizational structures. It contains both the hardware and software for aggregating data at Ministry level, the countrywide structure for LPIS and farm advisory system (FAS)), the mobile solutions and the communication layout. Access to ortho-images and weather data supporting all farmers' needs is a must in the future. Making data available like Finland´s Cadaster Department did recently is a must, with private ortho-image suppliers like MS-BING being anideal option for future cooperation.

Beneficiaries who are the stakeholders in such a concept are described in the following section and to all mentioned there, the ICT-backbone can provide valuable services. For these services a great deal of ROI finance can be acquired due to the benefits delivered by the ICT, but it always remains a political decision to which extent the Ministry will support the achieved benefits or how much beneficiaries from the use of this ICT backbone will have to pay. (On request ROI calculations can be carried out for single sectors).

The business models may be different: public, private or public-private. A model is imaginable, where public (Ministry of Agriculture) and private (banks, insurance, and investors) share the investment and set up a common structure to support the different beneficiaries with information against a fee.

Beneficiaries

A crucial effect of this agricultural ICT backbone concept is that data will be generated displaying the whole current situation, and for planning the future situation of agriculture and forestry in a country. At a certain point after implementation, the empirical knowledge derived from the storage of the history together with latest R&D leads to further actions. Many stakeholders are interested in these data and need them for their daily work. With an appropriate model for data sharing, this can bring benefits to several businesses. It can be taken for granted that chain partners will be ready to contribute financially to obtain access to this information. The model in detail has to be worked out together with local structures and representatives from different stakeholders, based on a trust centre concept that respects the ownership of information.

A public–private run ICT infrastructure consisting of new ortho-images for the country covering GIS and IT solutions for rural area management in connection with land management and extension services, agriculture management and logistics can be used by different governmental organizations and also by private structures and can support:

- The Ministry of Agriculture's needs for organizing subsidies;
- The Ministry responsible for landscape changes or for the cadaster and ground tax;
- Consultants in their advisory work;
- Food chain partners for traceability and for the documentation of production,
- Logistic service experts to undertake the right actions on the right field, to find the right roads to the field and be there at the appropriate time as well as deliver goods to the food industry "just in time";
- Agro control organization for subsidies;
- Bankers to draw up a business plan for financing farmers/forest holders and obtain output from the LPIS including a calculation of the growth period (costs and expected return);
- Insurance companies to develop the appropriate policies for the relevant crops and fields since an output can also be obtained from an LPIS system that informs on which farmers have which crops and how many ha are grown including a map;
- Ecology experts and natural risk managers for the appraisal of the risks related to field or ecological coherences;
- Medical experts to judge the influence of a farm activity on the public at large;
- And last but not least, support farmers by giving them tools for economic calculations.

CONCLUSIONS

Specialized GIS-software (Geographic Information Systems - WinGIS) and ICT tools for the management of agriculture, forestry, the environment and natural risks can be developed to assist farmers/foresters on their investment in agriculture and forestry. In addition, through a range of apps developed for mobile phone, it enables the management of farm and land via mobile phones. Telemetry and agro-sensors like weather stations or soils sensors offers advisory services and consultation for many rural area management solutions with the help of local experts.

Acquisition Efficiency by Maize Landraces in Acid Soils of Mexico

J.S. Bayuelo-Jiménez[1,*], L.C. Paredes-Gutiérrez[2], J. Pineda-Pineda[3], J.C. Patrón-Ibarra[4] and J.J. Adu-Gyamfi[5]

ABSTRACT

A main constraint to agricultural productivity in the central Mexican highlands is low available soil phosphorus (P) exacerbated by high P sorption capacity of the Andisols. Therefore, substantial amounts of P fertilizers must be applied to obtain optimum crop yields. One cost-effective strategy for this type of soil is to enhance the plant's efficiency to acquire inorganic phosphorus (P_i) from soil and/or to use P more efficiently. The present study was conducted to evaluate genotypic variation in both root architecture and plant growth traits associated with P acquisition efficiency (PAE) and/or P utilization efficiency (PUE) of maize landraces in a P-deficient soil. The results showed that genotypes differed greatly in plant growth, grain yield, root morphology, P uptake, PAE, PUE, and P efficiency defined as growth with sub-optimal P availability. Phosphorus-efficient genotypes not only had greater biomass per unit of absorbed P, but also developed larger root systems, produced more nodal and lateral roots, and had greater root hair density and P uptake per unit root weight than did the P-inefficient genotypes under P deficiency. Genotypes with enhanced nodal rooting and dense root hairs had greater P uptake and growth under low P. The ^{32}P isotope dilution technique was employed to assess the ability of the genotypes tested to utilize P from different P sources. The P-responsive genotypes showed increased P acquisition from fertilizer, whereas P-efficient genotypes accessed soil P not available to P-inefficient ones. These results indicate that maize landraces exhibit variation for several root traits that may be useful for genetic improvement of P acquisition efficiency in maize.

Key words: *^{32}P maize, P acquisition efficiency, P utilization efficiency, root architecture and morphology*

INTRODUCTION

Phosphorus (P) is one of the least available mineral nutrients in many cropping environments (Schaffert *et al*., 1999). Phosphorus deficiency is more critical in the highly weathered soils common in many developing regions, which may also have limited access to intensive fertilization (Hinsinger, 2001). This has led to the search for more environmentally and economically feasible strategies for improving crop production in low P soils. One approach is to enhance the plant's efficiency to acquire inorganic phosphorus (P_i) from soil and/or use P more efficiently (Lynch, 2007). Nutrient use efficiency of a crop is defined as the ability of a genotype to acquire nutrients from the soil and/or to utilize them for growth and yield (Fageria, 2008). Phosphorus use efficiency can be divided into P acquisition efficiency (PAE) and P utilization efficiency (PUE) (Wissuwa and Ae, 2001). PAE refers to mobilizing P from poorly soluble sources or to take up the soluble P available in the soil solution, while PUE is the ability to produce biomass or yield efficiently using the acquired P. Enhancing P use efficiency by plants can be achieved through improving P acquisition and/or utilization of low available P (Manske *et al*., 2000; Richardson *et al*., 2011).

Phosphorus availability for plants is typically greater in the topsoil, so root traits that enhance topsoil foraging tend to enhance P acquisition (Lynch, 2011). Plants have an array of adaptations to low P availability, including increased relative biomass allocation to roots (Bayuelo-Jiménez *et al*., 2011), architectural traits that enhance topsoil foraging, traits that reduce the metabolic cost of soil exploration such as root cortical aerenchyma (Lynch, 2011), increased production and secretion of P–mobilizing root exudates (Richardson *et al*., 2011), and increased proliferation and elongation of root hairs (Ma *et al.*, 2001). Plants with improved internal P utilization efficiency (more plant yield per unit of P uptake) can directly reduce the amount of P fertilizer required for agricultural production (Richardson *et al.*, 2011). Greater P utilization is mainly attributed to efficient translocation and use of stored P and low internal P requirements (Akthar, 2007), as well as reduced P-export from farms at harvest (Rose *et al*., 2010).

The deployment of root architectural traits in plant breeding programmes has great potential to alleviate P deficiency. Evaluation of P-efficient germplasm among existing Mexican landraces is of interest in this regard since Mexico is the global centre of maize genetic diversity (Sánchez, Goodman and Stuber, 2000). In particular, landraces from Michoacan State are well adapted to low P environments and possess traits not common in elite germplasm (Bayuelo-Jiménez *et al*., 2011). One of the most important traditional maize growing areas in this region is the P'urhépecha Plateau. Over 60 percent of the total arable land is P-deficient in this region (Alcalá, Ortiz and Gutiérrez, 2001).

---

[1] Instituto Nacional de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, Km 9 Carretera Morelia-Zinapécuaro. 58800. Tarímbaro. Michoacán, México
[2] Instituto Nacional de Investigaciones Nucleares, Carretera México-Toluca s/n, La Marquesa, C.P. 52750, Ocoyoacac, México
[3] Universidad Autónoma Chapingo, Departamento de Suelos, km 38.5 Carretera México Texcoco. 56230, Chapingo, Estado de México
[4] Benemérita Universidad Autónoma de Puebla. Departamento de Agroecología y Medioambiente, 14 Sur No. 6301, Ciudad Universitaria. 72570. Puebla, México
[5] Soil Science Unit, International Atomic Energy Agency, Wagramer Strasse 5, A-1400 Vienna, Austria
[*] E-mail address of corresponding author: bayuelo@umich.mx

L.K. Heng, K. Sakadevan, G. Dercon and M.L. Nguyen (eds), Proceedings — International Symposium on Managing Soils for Food Security and Climate Change Adaptation and Mitigation. Food and Agriculture Organization of the United Nations, Rome, 2014: 83–90

Andisols contain considerable amounts of P but a large proportion is bound to different soil constituents, forming complexes of limited bioavailability (FAO, 2001). This type of soil is commonly referred to as a P-fixing soil and the concentration of P in the soil solution is suboptimal for crop production. A common strategy for soils with low total P content is regular amendment with small doses of P fertilizer. However, in soils with high total P content that fix most of the P, fertilizer P will also be fixed. In these types of soils, plants respond to P fertilizer application but annual applications of water-soluble superphosphate or ammonium phosphate fertilizers are required to sustain crop yields (Richardson *et al*., 2011). Plants differ greatly in their ability to grow on low P soils because they have developed specific physic-chemical mechanisms to utilize P compounds in these low P fertility soils (Lynch, 2011). Evaluating and identifying crop plants for their ability to access and utilize sparingly soluble P forms in soils has been proposed as a practical means for overcoming P deficiency and optimizing P fertilizer use in cropping systems where P availability is limited (Naruzzaman, Lambers and Bolland, 2006).

The use of phosphorus-32 (^{32}P) isotope as a tracer applied to the soil with P fertilizer permits the detection of exchangeable phosphate ions in the solution and those absorbed by the plant (IAEA, 1990). Isotopic dilution techniques have been widely used to assess the availability of nutrients such as soil P to plants. The objective of this study was to evaluate the adaptation of native landraces to low P soils in the P´urhépecha Plateau, and the expression of root traits that could be important for P acquisition and utilization efficiency. This study also aimed to evaluate the ability of selected maize genotypes grown in an Andisol to use fertilizer P by using the ^{32}P isotope dilution technique.

TABLE 1. Properties of the topsoil (0–20 cm) of the Andisol in Ponzomaran, Juan Tumbio, and Bonilla locations, Michoacán, Mexico

Environmental variables	Pontzomaran	Juan Tumbio	Bonilla
Latitude (N)	19° 24´	19° 31´	19^0 30´
Longitude (W)	101° 38´	101° 36´	101° 41´
Altitude (m.a.s.l.)	2 280	2140	2 240
Rainfall (mm)	800–1 000	700–1 400	900–1 100
Sand (%)	38.6	48.1	55.1
Clay (%)	38.5	18.9	11.6
Silt (%)	22.9	36.0	33.3
Apparent density (g/cm)	0.86	1.08	0.89
pH	5.5	6.1	6.1
Organic matter (%)	7.9	6.2	4.2
Cation exchange capacity (cmol/kg)	18.6	14.7	15.3
Exchangeable aluminium (cmol/kg)	0.09	0.04	0.03
K (mg/kg)	131	444.7	454
Ca (mg/kg)	1 225	1 450	2 254
Inorganic N (mg/kg)	35	27	17.4
Available P Bray 1 (mg/kg)	1.20	4.75	2.74
Low P	1.07	3.06	1.39
High P	3.84	8.69	2.24

MATERIALS AND METHODS

Field studies to assess the genetic variability of maize landraces for root traits that enhance P acquisition in a low P soil

Three experiments were carried out under low and high P fertilization and rain-fed conditions in farmers' fields in Pontzomaran, San Juan Tumbio and Bonilla, in the central highlands of Michoacan, Mexico during the 2008 and 2009 growing season. Soils in the study sites are vitrands (FAO, 2001) and their characteristics are shown in Table 1.

Experiments were arranged in a randomized complete block design with four replications in a split-plot arrangement of treatments where P level was the main plot and genotypes the sub-plots. Each experimental unit consisted of five 5-m long rows for each accession. Experiments were conducted in P-deprived soils. The low (LP) and high (HP) treatments consisted of 23 $kg \cdot P_2O_5 \cdot ha^{-1}$ and 97 $kg \cdot P_2O_5 \cdot ha^{-1}$, applied as single super phosphate at seeding. All plots were supplemented additionally with 60 kg nitrogen $(N) \cdot ha^{-1}$ as urea at the seedling and silking stages. Maize genotypes were seeded within the optimum sowing dates (around April 17–23, after the beginning of the 2008 and 2009 rainy season).

Plant material

Fifty local maize genotypes were grown. All genotypes were originally from the P'urhépecha region, which had been used recently by the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) maize breeding programme; they differed in yield, P responses to fertilization, stalk lodging susceptibility and grain type (floury to flint corn). They were represented by three maturity types according to the number of days (d) required to reach the silking stage: 12 early [(E) 75–85 d], 22 intermediate [(I) 85–95 d] and 14 late [(L) 95–105 d], and by four breeding groups within each maturity type: landraces (C), advanced landraces (AC), hybrids x landraces (HxC) and synthetic hybrids (S). Landraces in the advanced generation were derived from advanced breeding lines of landrace types collected in low P soils from the P'urhépecha Plateau and crossed with an advanced generation of commercial hybrids.

Plant measurements

Root crowns were excavated at the same growth stage by removing a soil cylinder of 30 to 40 cm soil depth and 50 cm from the shoot base between 78 and 89 d after planting (DAP), at the silking stage (R1). The root crowns were immersed in water with P-free soap for about 8 minutes in order to facilitate soil removal. Four crown root traits were scored visually: nodal root length and number, nodal root branching and angle. A rating scale of 1–4 was used to rank root branching and root angle where 1 = first order lateral branching and 4 = multiple lateral branches with up to four orders of branching. For nodal root angle, one indicates shallow root angles (1 = 0° – 22.5°); 2 = 22.5° – 45°; 3 = 45° – 67.5° and four indicates steep root angles (4 = 67.5° – 90°). For root hair evaluation, root fragments were dyed in 0.05 percent trypan blue. Root hairs were evaluated visually using a rating scale of 1–9 to rank the density/length as follows: 1 — no root hairs; 3 — low root hair density/length; 5 — intermediate root hair density/length, 7 —between 5 and 9 rating scale; 9 — abundant root hairs (Vieira, Jochua and Lynch, 2007).

Plant growth and tissue analyses

One plant per plot was harvested between 78 and 89 DAP. Shoot and root biomass were determined after drying at 60°C to constant weight. Root and shoot dry tissue samples were ground and analysed for P concentration after Murphy and Riley (1962). Phosphorus acquisition efficiency (PAE) is a measure of the P uptake per unit root dry weight (DW) (mg·P/g root DW), and was calculated from total P per plant divided by root dry mass in grams (Fageria, 2008). Phosphorus utilization efficiency (PUE) is a measure of DW return per unit P uptake (g·DW·mg^{-1}·P), and was calculated as total plant DW divided by P content per plant.

Statistical analysis

Phosphorus efficiency of the maize genotypes was determined by the P efficiency index or PEI (Pan *et al.*, 2008) and assessed using principal component analysis (PCA) of standardized values for plant growth and grain yield parameters at low P, and relative values at low P to those obtained under high P supply. The principal component analysis was computed using the SAS FACTOR procedure with the PRIN option and the VARIMAX method for orthogonal rotation (SAS, 2000) on all 50 maize genotypes. The relative weight of each principal component was determined by the corresponding contribution rate accounting for variation of all growth traits. Consequently, PEI values of different genotypes were calculated according to the retained principal components and their relative weights, namely PEI = $\sum_{i=1}^{50} PC_i \times RW_i$. The criterion used for classification of maize genotypes was determined by the method of cluster analysis. Genotypes were divided into three categories according to the P efficiency index and four categories according to P efficiency index in combination with growth potentials (shoot DW at high P). A distance matrix was developed with the standardized data using the dissimilarity Euclidian distance coefficient and a Ward´s minimum variance clustering method (Romersburg, 1988).

Controlled environmental studies to identify genotypes for superior P acquisition and/or utilization using ^{32}P isotope dilution technique

This study was conducted at the National Nuclear Research Institute (ININ), Mexico, in a greenhouse with a temperature between 14.3 and 26.5°C, a relative humidity of 50 to 70 percent, and a fall photoperiod (13-h day and 11-h night). Artificial light for 12 hour (h) per d from cool-white fluorescent tubes supplemented the fall daylight. Plants were grown in pots with 6 kg of vitric Andisol soil of low available soil P, which was obtained from a plot on Laderas de Bonilla, Michoacan, where maize landraces are cultivated (Table I).

The experiment was set up simultaneously for non-labelled (without ^{32}P) and labelled (with ^{32}P) on two levels of P supply (low and medium available P). The design was completely randomized with a factorial arrangement of (3 × 6) treatments, i.e. three levels of P supply for six genotypes. There were four replications for a total of 72 units. Each experimental unit was composed for one pot and included P additions (ammonium phosphate [$(NH_4)_2HPO_4$] at rates of 0.0, 0.22 and 0.44 g·P/pot. These rates are equivalent to 0, 25 and 50 kg·P_2O_5/ha. The Andisol soil was labelled with $(NH_4)_2H^{32}PO_4$ by applying a high activity (10 mC_i). The tracer was added to the soil at a depth of 10 cm at the plant root zone at concentrations of 0, 0.395 and 0.791 mC_i/pot in 300 mL of water at pH 6.0 to ensure uniform labelling. Plants were irrigated with distilled water applied to the soil to field capacity (33 kPascal) to avoid leaching of the radioactive ^{32}P-labeled marker and other nutrients. Two seeds were planted in every pot. Five d after emergence, seedlings were thinned to one plant per pot. Nitrogen was added as a solution of ammonium nitrate (NH_4NO_3) at 60 kg·N·ha^{-1} to the pots in which maize was grown.

Plant material

The efficiency of P uptake was determined for five maize genotypes represented by two early (Gregorio and M-I-04), two intermediate (PICH-4 and Zacapu), and one late (DP x Tromba) emergence/maturity genotypes, and a commercial variety (Leopardo) was used as a check. These genotypes differed in efficiency and P responsiveness at the early vegetative stages (Table 2).

Plant measurements

One plant from each pot was sampled at 39 d after sowing. The plant shoots and roots were oven-dried at 70°C for two d, ground and digested in a mixture of concentrated nitric (HNO_3) and perchloric ($HClO_4$) acids (4:1). The concentration of total P in the digest was determined as described by Murphy and Riley (1962). The ^{32}P activity in the digest was determined by liquid scintillation counting using a Beckman LS-60000LL liquid scintillation system. Once the activity was quantified, isotopic variables were determined.

Based on the isotopic dilution method, the proportions of P taken up by plants from the fertilizer and soil were calculated according to IAEA (1990):

- The percentage of P derived from the fertilizer (% Pddf) was obtained by dividing the specific activity (SA) in the plant by SA in the fertilizer × 100.
- The percentage of P derived from the soil (% Pdds) was obtained by difference, i.e. 100– (% Pdds).
- The amount of P fertilizer taken up by the crop (P-fertilizer yield) was calculated by multiplying the total P yield by the percentage of P derived from the fertilizer, i.e. P-fertilizer yield (mg·P/pot) = (total P yield x % Pddf)/100.
- The P-fertilizer extracted by the plant relative to the dose of P applied (efficiency P-fertilizer) is known as P use efficiency, i.e. P-fertilizer efficiency (%) = (P – fertilizer yield/dose of P applied) × 100.
- The P content in the plant was calculated by multiplying the P concentration by the dry weight [P concentration in plant (mg·P/g) x dry matter yield (g·P/pot) /100.

Statistical analysis was performed for a completely randomized design and a factorial arrangement: P supply, genotype and interaction P supply x genotype. The average effect of each treatment was determined using the Tukey test (p = 0.05) with SAS (2000) software.

RESULTS AND DISCUSSION

Genetic variability for P efficiency and P responsiveness

Substantial variation in growth for low P soil was recorded among maize landraces from the central Mexican highlands. Genotypes were grouped into three categories of P efficiency based on shoot growth, PAE and PUE parameters at LP and their relative values to those at HP. The results indicated that 12 genotypes across locations had the lowest growth and the highest levels of P efficiency (PEI > 0.54) under low P (Table 2).

When the combination of PEI with P responsiveness at HP is considered, SHUI-2, Paramuén, ZR-6, CB-II, Paso del Muerto, CCHEDE, Macho III-05, M-I-04, and M-IV-03 were the most efficient genotypes for the P-deficient acidic soil of this region (Table 2). These genotypes were categorized as the most P-efficient under LP and as the most responsive to increased P availability. Applications of 97 kg·P_2O_5·ha^{-1} (equivalent to 41.7 kg·P·ha^{-1}) increased the shoot biomass and grain yields of the genotypes. However, the increase was nearly equal to the difference between genotypes in the low P soil (23 kg·P_2O_5·ha^{-1}, equivalent to 9.9 kg·P·ha^{-1}). Thus, in low P soil the P-efficient

TABLE 2. Shoot dry weight, P acquisition efficiency (PAE), and P utilization efficiency (PUE) of 50 common maize genotypes grown in a P-deficient soil with low P (LP) or high P addition (HP), in 2008 and 2009

ID	Genotypes	M	BG[b]	Shoot dry weight (g/plant)					PAE (mg·P ·g^{-1} root DM)				PUE (g·DM·mg^{-1} P)			
				LP	HP	PEI[c]	SdSh[d]	PR[e]	LP	HP	SdPAE	PR	LP	HP	SdPUE	PR
40	Paramuén	Late	C	154	196	0.75	3.38	ER	17.2	18.2	0.55	ER	0.83	0.87	1.24	ER
109	Paso del Muerto	Late	AC	155	174	0.66	1.70	ER	21.8	19.7	0.44	ER	0.72	0.88	-0.16	ENR
78	CB-II	Middle	C	106	134	0.37	0.21	ER	17.4	19.8	0.92	ER	0.78	0.92	-0.48	ENR
236	M-II-03	Early	S	116	139	0.30	0.63	ER	16.2	15.4	-0.18	ENR	0.84	0.91	-0.52	ENR
117	A-7545	Late	HxC	109	132	0.22	0.19	ER	21.8	15.7	-0.43	ENR	0.82	0.94	-0.32	ENR
233	M-I-03	Early	S	105	147	0.20	0.98	ER	18.4	16.3	-0.02	ENR	0.76	0.87	0.31	ER
239	M-IV-03	Late	S	112	132	0.10	0.10	ER	16.2	17.7	-0.01	ENR	0.81	0.89	0.19	ER
75	ZR-6	Late	C	142	147	0.05	1.03	ER	18.7	17.7	0.27	ER	0.90	0.89	0.46	ER
234	M-I-04	Early	S	141	138	0.04	0.58	ER	22.2	20.0	1.05	ER	0.93	0.95	-0.63	ENR
182	M-III-05	Middle	S	130	128	0.64	-0.43	ENR	20.0	19.2	0.57	ER	0.84	0.86	0.45	ER
144	San Gregorio	Early	AC	102	107	0.60	-1.14	ENR	19.5	14.4	-0.62	ENR	0.85	1.18	-2.56	ENR
79	PICH-4	Middle	C	136	124	0.54	-0.12	ENR	17.4	14.9	-0.29	ENR	0.87	0.86	0.03	ER
6	SHUI-2	Late	C	102	117	0.15	-0.45	ENR	15.4	20.2	1.18	ER	0.92	0.89	0.99	ER
127	DP x Tromba	Late	HxC	97	138	-0.95	0.37	NER	23.8	16.9	0.40	NER	1.18	0.85	0.18	NER
215	Zacapu	Late	AC	91	104	-0.36	-1.70	NENR	15.4	17.8	-0.15	NENR	1.10	0.86	0.21	NER
	Average			115	128				17.9	16.7			0.92	0.88		
	LSD (0.05) [a]			52	53				9.00	7.9			0.23	0.21		

a — to compare paired values among genotypes; b BG — breeding groups: landrace (C), advanced landrace (AC), hybrids x landrace (H x C), and synthetic (S); C — P efficiency index (PEI) obtained from the principal component analysis; d — standardized value of shoot dry weight (SdSh), P acquisition efficiency (SdPAE), and P utilization efficiency (SdPUE) under high P conditions. Data from the three individual experiments were standardized by dividing relative values by the standard deviation of the trial. Phosphorus responsiveness (PR) is expressed by shoot biomass under HP level using four categories: efficient and responsive (ER), non-efficient and responsive (NER), non-efficient and non-responsive (NENR), and efficient and non-responsive (ENR).

genotypes seemed to produce the same amount of dry matter and grain yield as genotypes with higher P fertilizer applications (97 $\text{kg·P}_2\text{O}_5\text{·ha}^{-1}$). These results directly support the added advantage of selection and breeding for P efficiency for maintaining productivity in low P soils.

Higher P efficiency in plants can be achieved by improving P acquisition and/or internal P utilization (Fageria, 2008). Comparisons between common maize genotypes in three soil environments and maturity types showed that differences in P acquisition were due to large variations in plant growth, P content and root architecture (data not shown). In general, genotypes with increasing P efficiency at LP had greater root and shoot dry weights, root to shoot ratios and root and shoot P contents. Genotypes of the P-efficient group had more nodal rooting (64 to 81) and root branching (two orders of branching) under LP, whereas root hair density and length of nodal first-order laterals were significantly greater under LP than in HP (on a rating scale of 5 to 7).

Mechanism involved in P efficiency: root architecture and morphology

Root system architecture and morphology are key traits for optimizing P acquisition, and thus their P use efficiency and responsiveness (Manske, 2000). Architectural traits associated with enhanced topsoil foraging include shallower growth angles of axial roots, enhanced adventitious rooting, a greater number of axial roots and greater dispersion of lateral roots (Lynch, 2007). Genotypes with increased or sustained elongation of axilar roots and lateral root development under P deficiency demonstrated superior ability to acquire P and maintain growth ($r^2 = 0.53$–0.82). This study confirmed that enhanced nodal rooting and greater nodal branching (nodal root laterals) were indeed important for plant adaptation to low P in maize (Table 3).

Nodal rooting was correlated significantly with plant growth and P uptake in the field among contrasting genotypes (Table 3). Efficient genotypes with greater nodal rooting and lateral branching at low P had greater biomass and P uptake efficiencies ($r^2 = 0.53$– 0.94) than did inefficient genotypes with reduced nodal root formation and lateral branching ($r^2 = 0.18$–0.23). The greater nodal rooting of P-efficient genotypes under low P could be explained by the greater overall biomass and the weak allometric relationship between plant biomass and root biomass ($r^2 = 0.04$). Therefore, if nodal roots in the topsoil are advantageous for acquiring P under limited P availability as suggested by Lynch (2007), this weak allometric relationship would facilitate the selection of efficient genotypes with high nodal rooting in the field.

Several lines of evidence show that root hairs contribute to P acquisition (Jungk, 2001). Low P availability increases the length and density of root hairs (Ma *et al.*, 2001). In this study large variations were noted for the ability to develop root hairs along the nodal first-order laterals (Table 3). Whereas several inefficient genotypes had

TABLE 3. Relationships between root traits: nodal root length (Nod_RL), number of nodal roots (Nod_No), nodal branching (Nod_Br), root hair density, (RHD_mnr) and length, (RHL_mnr) from the middle region of nodal roots and measures of P efficiency: shoot dry weight (SDW), grain yield (GY) and P acquisition efficiency (PAE) of P-efficient genotypes across locations

Parameter		SDW (g/plant^{-1})			GY (kg/ha)			PAE (mg·P·g^{-1}·root)		
	P	Early	Middle	Late	Early	Middle	Late	Early	Middle	Late
Nod_RL	HP	0.49[a]	0.02	-0.85	-0.08	-0.01	-0.09	-0.04	-0.02	-0.54
	LP	0.50	0.01	0.76	-0.72	0.67	0.88	-0.96	0.25	0.11
Nod_No	HP	0.01	0.13	0.19	-0.21	0.02	0.00	-0.27	0.79	0.35
	LP	0.94	0.53	0.75	-0.21	0.32	0.56	-0.89	0.02	0.43
Nod_Br	HP	0.05	0.28	0.73	0.46	-0.23	0.04	0.52	0.06	0.42
	LP	0.57	0.82	0.31	-0.90	0.35	0.04	-0.82	0.22	0.73
RHD_mnr	HP	0.03	-0.25	-0.26	-0.11	-0.03	0.60	-0.15	-0.14	-0.34
	LP	0.31	-0.52	0.34	0.47	-0.24	-0.30	0.02	-0.05	0.97
RHL_mnr	HP	-0.53	-0.12	0.79	-0.94	-0.04	0.20	-0.97	-0.07	0.55
	LP	0.74	-0.12	0.81	0.46	-0.16	-0.82	0.19	-0.12	0.69

a — regression coefficients at the 5 percent level

shorter and fewer root hairs, efficient genotypes developed much longer and denser hairs on nodal roots. The presence of denser root hairs of nodal first-order laterals ($r^2 = 0.95$), was associated with plant performance at low P, grain yield and root P acquisition at LP (Table 3). Late P-efficient genotypes can be selected showing a significant relationship between root hairs and root P acquisition, suggesting that the P acquisition ability of genotypes is a decisive factor in expression of high P efficiency.

Phenotypic traits conferring P utilization efficiency

Phosphorus utilization efficiency represents the amount of dry matter produced per unit of P absorbed (g·DM·mg^{-1} P) (Fageria, 2008). Any species able to maintain metabolic activities at low tissue P concentration and produce more dry matter per unit of P absorbed is considered efficient in P utilization. In this study, there were genotypes in which the P content in the shoot had a highly significant correlation with root ($r = 0.54^{**}$) and shoot dry weights ($r = 0.78^{***}$) suggesting that genotypes with higher root dry weight accumulated greater amounts of P in the shoot and produced higher shoot dry matter at LP. Thus, under P stress, better P acquisition and PUE by the P-efficient genotypes for biomass synthesis collectively formed the basis of higher shoot dry matter production, evidence that P uptake and PUE are significant plant traits for selecting low P-tolerant genotypes.

Variations in P uptake and use efficiency by maize using ^{32}P

Due to the very low P available soil used in this study (2.2 mg·P·kg^{-1} soil), typical of Mexican volcanic soils, the response of the maize to fertilizer application was evident (Figure 1). From ANOVA data, P levels, genotypes and P × genotype interactions were significantly different ($p = 0.01$). Phosphorus-responsive San Gregorio, M-I-04, and PICH-4 produced the highest dry weight yields (7.7 and 5.9 g/plant) and Zacapu and DP x Tromba had the lowest yields (3.4 g/plant). With regard to the P treatments, the highest value was obtained in the HP treatment (5 g/plant) followed by LP (2.8 g/plant) and the control (only soil P) treatment (1.9 g/plant). The root and shoot P concentrations and P uptakes by the six maize genotypes under the three P treatments are shown in Figure 1. According to the ANOVA results, the interaction P levels x genotypes was not significant, only the effect of P and genotypes. All maize genotypes grown in the Andisol soil had low root P concentrations (<1.1 mg·g^{-1}·P), suggesting a strong P limitation in the soil (Figure 1b-e). The P concentrations in shoot tissue of the P-responsive Gregorio, Macho I-04, PICH-4 and Leopardo genotypes (0.87–1.0 mg·g^{-1}·P) were low compared with the P-efficient accession DP x Tromba (1.6 mg·g^{-1}·P), suggesting that maize has a higher ability to take up more P from low available P soils than P-responsive ones (Figure 1e).

The P uptake for the HP level (5.9 mg·P·plant^{-1}; 338.6 mg·P/pot) was significantly higher than that for the LP treatment (3 mg·P·plant^{-1}; 164.5 mg·P/pot). Thus, maize genotypes were able to take up significant amounts of P from fertilizer. The P uptake for the P-responsive genotypes was about 2.5-fold greater than that of P-efficient ones. This response to the high application rate confirms the great P demand of this crop and the significance of uptake and usage mechanisms. The data in Table 4 show the proportions of P in samples derived from the P fertilizer treatment (%Pdff) and soil (%Pdds). For the %Pdff the effects of genotypes, P levels and the P x genotype interactions were significant. Comparing the genotypes at each P level, the P-responsive San Gregorio was significantly higher than the other genotypes. From the comparisons among the LP level for each genotype, San Gregorio and PICH-4 showed the lowest %Pdd values. In other words, they were more dependent on the water-soluble fertilizer for their nutrition. By contrast, the P-efficient DP x Tromba with its greater % Pdf value (most efficient in P uptake from soil) had a lower %Pddf.

The P use efficiency of added $(NH_4)_2HPO_4$ ranged respectively from 3.4 to 51.7 percent and from 0.7 to 13.9 percent in the HP and LP levels (Table 5). From the ANOVA, significant differences were found for the effects of treatment or the P x genotype interactions. The range of P use efficiency of the P-responsive genotypes (11.9 to 51.7 percent) was higher than for the P-efficient genotypes (3.4 to 5.8 percent). The P use efficiency from P fertilizer was relatively high for a high P-fixing volcanic soil but maize is a high P demanding crop (Schaffert *et al.*, 1999), and the experiment was conducted under well-controlled conditions for a relatively short period (36 d). On the other hand, the P use efficiency from P-efficient genotypes was low in both P treatments, thus confirming the ability of DP x Tromba to take up significantly greater amounts of P from non-labile P in the Andisol. The mean specific activity of P in DP x Tromba was lower than in all other genotypes which had the highest value under HP

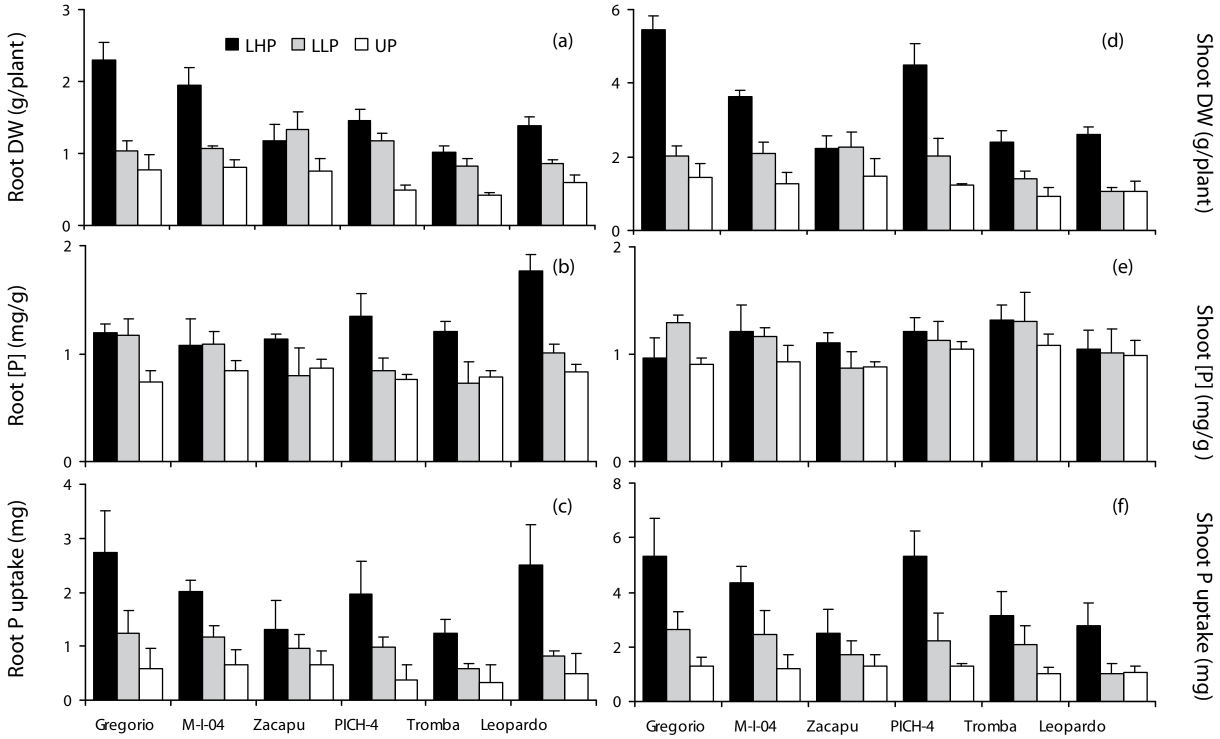


FIGURE 1. Root dry weight (a), root P concentration (b), root P uptake (c), shoot dry weight (d), shoot P concentration (e) and shoot P uptake (f) for maize grown in a soil labelled (LHP, LLP) and unlabelled (UP) with ^{32}P. The error bar represents standard error from the mean (n).

TABLE 4. Effects of the P-fertilizer treatments on total P yield, specific activity and % P derived from fertilizer (%PDFF) in maize

				Total P yield (mg·P·pot^{-1})				Specific activity (Bq·mg^{-1} P)				%Pdff			
				Root		Shoot		Root		Shoot		Root		Shoot	
ID	Accession	M [b]	BG [c]	HP [d]	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP
144	San Gregorio	E	AC	40.9	43.2	466.1	125.5	4 410	5016	26 320	6984	6.2	7.1	37.3	9.9
234	M-I-04	E	S	40.0	24.5	208.7	84.4	5 807	3125	14 362	4911	8.2	4.4	20.3	7.0
215	Zacapu	I	HXC	11.8	14.2	125.6	45.1	2 615	2124	15 037	3672	3.7	3.0	21.3	5.2
79	PICH-4	I	C	37.8	40.9	179.3	84.1	5 760	6182	9 577	5388	8.2	8.8	13.6	7.6
127	DP x Tromba	L	HXC	23.1	11.6	95.7	22.8	5 532	2954	8 903	1847	7.8	4.2	12.6	2.6
247	Leopardo	I	cv	21.5	13.7	74.7	11.2	2 776	2438	8 016	1638	3.9	3.5	11.3	2.3
	LSD $_{0.05}$ [a]			16.8	14.2	105.0	50.9	1 192	1503	5 214	1464	1.7	2.1	7.1	2.1

and LP conditions (Figure 1a). If all the genotypes drew their P from the same pool of available P, then the specific ^{32}P activity of the P in their shoots should be comparable, although concentrations of P in the shoots and the amounts of P accumulated may differ (Larsen, 1952). Consequently, the lower specific activity of the P taken up by DP x Tromba indicates that it was able to access P in the soil that was less available to all other genotypes.

In acid soils, P ions precipitate as iron (Fe) and aluminium (Al) phosphates such as strengite ($FePO_4{\cdot}2H_2O$) and variscite ($AlPO_4{\cdot}2H_2O$), due to the high concentration of trivalent Fe and Al in the soil solution. These types of P compounds are commonly assigned to stable P pools that are considered sparingly available to plants. However, evidence has accumulated that some plant species are efficient in utilizing certain sparingly soluble P sources. For example, Wang *et al*. (2011) found that wheat (*Triticum aestivum* L.) was efficient in using $AlPO_4$ due to its ability to develop high root length density, and canola (*Brassica napus* L.) can access $FePO_4$ due to its ability to excrete protons from the root (Pearse, 2011). Also, recent studies have identified genotypic differences between maize landraces in P efficiency (Bayuelo-Jiménez *et al*., 2011), particularly that the development of an extensive root system with dense root hairs was one of the main strategies of acquiring soil P for P-efficient genotypes. Phosphorus-efficient landraces of the Mexican highlands can therefore be beneficial for improving the use of native soil P and P fertilizers.

TABLE 5. Effects of the P-fertilizer treatments on P derived from soil (%PDFS), fertilizer P yield, and efficiency P fertilizer in maize

				%Pdfs				Fertilizer P yield (mg·P·pot^{-1})				EPF (%)			
				Root		Shoot		Root		Shoot		Root		Shoot	
ID	Accession	M [b]	BG [c]	HP [d]	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP
144	San Gregorio	E	AC	93.8	92.9	62.7	90.1	2.5	3.1	178.8	12.5	0.6	1.5	43.2	6.0
234	M-I-04	E	S	91.8	95.6	79.7	93.0	3.5	1.1	42.7	6.1	0.8	0.5	10.3	2.9
215	Zacapu	I	HXC	96.3	97.0	78.7	94.8	0.4	0.6	26.4	2.6	0.1	0.3	6.4	1.3
79	PICH-4	I	C	91.8	91.2	86.4	92.4	3.1	3.7	25.8	7.0	0.7	1.8	6.2	3.4
127	DP x Tromba	L	HXC	92.2	95.8	87.4	97.4	1.8	0.5	12.3	0.6	0.4	0.2	3.0	0.3
247	Leopardo	I	cv	96.1	96.5	88.7	97.7	0.8	0.5	8.5	0.3	0.2	0.2	2.0	0.1
	$LSD_{0.05}$ [a]			1.6	2.1	7.1	2.0	1.4	1.6	51.9	5.4	0.3	0.8	12.5	2.6

a — to compare paired values among genotypes; b — maturity: early (E), intermediate (I), late (L); c — BG: d — breeding groups: landrace (C), advanced landrace (AC), hybrids x land race (H × C), and synthetic (S); [d] — P additions as $(NH_4)_2HPO_4$ at rates of 0.22 g·P/pot (LP) and 0.44 g·P/pot (HP).

This study demonstrated the ability of maize to absorb significant amounts of P from the inorganic soil P pools. The use of the ^{32}P isotopic technique enabled quantitative measurement of P uptake from P fertilizer sources and differences in P acquisition among genotypes. Although these differences were small, the genotype DP x Tromba showed increased P acquisition from inorganic soil P pools. Phosphorus-efficient genotypes in combination with a high P use efficiency from fertilizers are therefore an attractive strategy for sustainable agricultural production in Mexican Andisols.

ACKNOWLEDGEMENTS

The authors are grateful to the International Atomic Energy Agency (IAEA), Vienna, Austria for financial support under Research Contract MEX-13800.

REFERENCES

Alcalá de Jesús, M., Ortíz-Solorio, C.A. & Gutiérrez-Castorena, M.C. 2001. Clasificación de los suelos de la Meseta Tarasca, Michoacán. *Terra*., 19: 217–239.

Akthar, M.S., Oki, Y., Adachi, T., Murata, Y. & Khan, M.H.R. 2007. Relative phosphorus utilization efficiency, growth response, and P uptake kinetics of *Brassica* cultivars under a phosphorus stress environment. *Commun. Soil Sci. Plant Anal*., 38: 1061–1085.

Bayuelo-Jiménez, J.S., Pérez-Decelis, V.A., Magdaleno-Armas, M.L., Gallardo-Valdéz, M., Ochoa, I. & Lynch, J.P. 2011. Genetic variation for root traits of maize (*Zea mays* L.) from P'urhépecha Plateau, under contrasting phosphorus availability. *Field Crops Res*., 121: 350–362.

Fageria, N.K. 2008. The role of nutrient efficient plants in improving crop yields in the twenty first century. *J. Plant Nut*., 31: 1121–1157.

FAO. 2001. *Lecture notes on the major soils of the world*, by P. Driessen, J. Deckers, O. Spaargaren & F. Nachtergaele, eds. World Soil Resources Report, Rome. 334 pp.

Hinsinger, P. 2001. Bio-availability of soil inorganic P in the rhizosphere as affected by root induced chemical changes: A review. *Plant Soil*, 237: 173-195.

IAEA. 1990. *Use of nuclear techniques in studies of soil-plant relationships*: Manual, IAEA Training Courses Series 2. Vienna. 220 pp.

Jungk, A. 2001. Root hairs and the acquisition of plant nutrients from soil. *J. Plant Nut. Soil Sci*., 164: 121–129.

Larsen, S. 1952. The use of ^{32}P in studies on the uptake of phosphorus by plants. *Plant Soil*, 4: 1–10.

Lynch, J.P. 2007. Roots of the second green revolution. *Aust. J. Bot*., 55: 493–512.

Lynch, J.P. 2011. Root phenes for enhanced soil exploration and phosphorus acquisition: Tools for future crops. *Plant Physiol*., 156: 1044–1049.

Ma, Z., Bielenberg, D.G., Brown, K.M. & Lynch, J.P. 2001. Regulation of root hair density by phosphorus availability in *Arabidopsis thaliana*. *Plant Cell Environ*., 24: 459–467.

Manske, G.G.B., Ortíz-Monasterio, J.I., Ginkel, M.V., González, R.M., Rajarm, S., Molina, E. & Vlek, P.L.G. 2000. Traits associated with improved P-uptake efficiency in CIMMYT´s semi-dwarf bread wheat grown on an acid Andisol in Mexico. *Plant Soil*, 221: 189–204.

Murphy, J. & Riley, J.P. 1962. A modified single solution method for the determination of phosphate in nature waters. *Anal. Chim. Acta*, 27: 31–36.

Naruzzaman, M., Lambers, H. & Bolland, M.D.A. 2006. Distribution of carboxylates and acid phosphatase and depletion of different phosphorus fractions in the rhizosphere of a cereal and three grain legumes. *Plant Soil*, 281: 109–120.

Pan, X.W., Li, W.B., Zhang, Q.Y., Li, Y.H., & Liu, M.S. 2008. Assessment of phosphorus efficiency characteristics of soybean genotypes in phosphorus-deficient soils. *Agric. Sci. China*, 7: 958-969.

Pearse, S.J. 2011. Why does the musketeer approach to phosphorus acquisition from sparingly soluble forms fail: All for one, but not one for all? *Plant Soil*, 348: 81–83.

Richardson, A.L., Lynch, J.P., Ryan, P., Delhaize, E., Smith, A., Smith, S.E., Harvey, P.R., Ryan, M., Veneklaas E. J., Lambers, H., Oberson, A. & Culvenor, A. 2011. Plant and microbial strategies to improve the phosphorus efficiency of agriculture. *Plant Soil*, 349: 127–156.

Romersburg, H.C. 1988. *Cluster analysis for researchers*. Belmont, CA, Lifetime Learning Publications. 330 pp.

Rose, T.J., Patriasca-Tanaka, J., Rose, M.T., Fukuta, Y. & Wissuwa, M. 2010. Genotypic variation in grain phosphorus concentration, and opportunities to improve P-use efficiency in rice. *Field Crops Res*., 119: 154–160.

Sánchez, G.J.J., Goodman, M.M. & Stuber, C.W. 2000. Isozymatic and morphological diversity in the races of maize of Mexico. *Econ. Bot*., 54: 43–59.

Schaffert, R.E., Alves, V.M.C., Parentoni, S.N. & Raghothama, K.G. 1999. Genetic control of phosphorus uptake and utilization efficiency in maize and sorghum under marginal conditions. In J.M. Ribaut &

E. Poland, eds. *Molecular approaches for the genetic improvement of cereals for stable production in water-limited environments,* pp. 79–85. El Batan. Mexico, CIMMYT.

SAS. 2000. *SAS user´s guide: Statistics*. Cary, NC, SAS Institute.

Vieira, R.F., Jochua, C.N. & Lynch, J.P. 2007. Method for evaluation of root hairs of common bean genotypes. *Pesq. Agropec. Bras.*, 42: 1365–1368.

Wang, X.J., Guppy, C.N., Watson, L., Sale, W.G. & Tang, C. 2011. Availability of sparingly soluble phosphorus sources to cotton (*Gossypium hirsutum* L.), wheat (*Triticum aestivum* L.) and white lupin (*Lupinus albus* L.) with different forms of nitrogen as evaluated by a ^{32}P isotopic dilution technique. *Plant Soil*, 348: 85–98.

Wissuva, M. & Ae, N. 2001. Genotypic variation for tolerance to phosphorus deficiency in rice and the potential for its exploration in rice improvement. *Plant Breed.*, 120: 43–48.

Phosphorus Acquisition from Sparingly Soluble Forms by Maize and Soybean in Low – and Medium – P Soils using ^{32}P

J.J. Adu-Gyamfi[1,*], M. Aigner[1], S. Linic[1] and D. Gludovacz[2]

ABSTRACT

A glasshouse pot experiment was conducted to evaluate the differential ability of maize (*Zea mays*) and soybean (*Glycine max*) to utilize soil phosphorus (P) for plant growth from total P, available P and inorganic (Ca–P, Al–P and Fe–P) soil P pools using a carrier-free ^{32}P solution. A maize variety (DK 315) and a soybean variety (TGX 1910-4F) were grown in pots containing 1 kg of a low available P soil (Hungarian) or a medium available P (Waldviertel) soil labelled with ^{32}P for 42 d or without ^{32}P (unlabelled) for 42 and 60 d. The shoot and root biomass of maize and soybean were significantly greater when grown on the Waldviertel than on the Hungarian soils. The shoot P concentrations were higher for soybean (1.7–2.2 g/kg) than for maize (1.1–1.4 g/kg). The total radioactivity (dpm x 10^6) was higher in plants grown in Waldviertel than in Hungarian soil and the values reflected in the plant P uptake and shoot biomass of soybean and maize. The L-values ($\mu g \cdot P \cdot g \cdot soil^{-1}$) of maize and soybean were higher in Waldviertel (72–78) than in Hungarian (9.6–20) soil. No significant differences in L-values were observed for maize and soybean grown on the Waldviertel soil, but for the Hungarian soil, the L-values were higher for maize (20.0) than for soybean (9.6), suggesting that in this low P soil, maize was more efficient than soybean in taking up soil P. The available P (Bray II) and the Ca–P were the fractions most depleted by plants followed by the Fe–P fractions in the two soils, but differences between the crops were not significant. When soil P is limited, maize and soybean are able to access P mainly from the available P (Bray II), and the sparingly soluble Fe– and Ca–P fractions, and not Al–P from the soil.

Key words: *aluminium-P, calcium-P, Hungarian soil, iron–P, maize, soybean, sparingly soluble phosphorus, Waldviertel soil*

INTRODUCTION

Soils characterized by poor phosphorus (P) availability are widespread globally (Raghothama and Karthikeyan, 2005) and for these soils to be agriculturally productive, they require regular application of water-soluble superphosphate or ammonium phosphate fertilizers to either maintain the soil P status of fertile soils or increase that of soils with inherently low P fertility. Soluble phosphates applied to P deficient soils is retained by iron (Fe), aluminum (Al) and calcium (Ca) ions and are virtually unavailable to most plant species.

Plants differ greatly in their ability to grow on low P soils because they have developed specific physico-chemical mechanisms/processes to utilize P compounds in these low P fertility soils. These mechanisms include: (i) alterations (morphological and physiological) to root systems, i.e. mycorhizal plants have better water uptake and Al tolerance in acid soils (Hiradate *et al.,* 2007), (ii) secretion of low molecular weight organic compounds (exudates production), i.e. malonic, oxalic, citric, malic and piscidic acids secreted by roots of pigeon pea help to release low-soluble P compounds in soils (Ishikawa *et al*., 2002), (iii) secretion of enzymatic compounds, i.e. phosphatases, and (iv) molecular changes such as enhanced expression of P transporters (Naruzzaman *et al*., 2006). Intra-specific variations in a crop's ability to use sparingly soluble forms (P associated with Al, Fe and Ca) in low-P available soils have been well documented for pigeonpea (Ae *et al*., 1990) and for soybean, cowpea and maize (Nwoke *et al*., 2007).

Radio-isotopic P techniques, using the principle of isotopic exchange, allow measurement of the amount of orthophosphate that can be transferred from the soil solid to the solution over a given time, and can thus provide a powerful alternative means for characterizing soil P availability and the sources of P, with minimum modifications of soil P forms compared with conventional extraction methods. The technique has been used to measure the quantity of available P in soils for determining the E-value or exchangeable P (Larsen, 1952), the L-value or labile P (Fried and Dean, 1952), using plants grown in a soil labelled with carrier-free ^{32}P or ^{33}P-orthophosphate and the A-value or available P. This study aimed to evaluate the differential ability of maize and soybean to access and utilize P from different soil pools using two soils, one low- and one medium-P availability. The experiment aimed to test the hypothesis that P uptake from sparingly soluble P forms (Al–P, Fe–P and Ca–P) by different crops can be used as a criterion to evaluate and possible select crop plants tolerant to low available P soils.

MATERIALS AND METHODS

Plant growth conditions

Two experiments were set up simultaneously with maize (cereal) and soybean (legume) as test crops to include two treatments consisting of non-labelled (without ^{32}P) and labelled (with ^{32}P) imposed on soils with low and medium available P in a factorial design with four replications. In total, there were 20 pots for the radioisotope (including

---

[1] Soil and Water Management & Crop Nutrition Laboratory, International Atomic Energy Agency, Seibersdorf, Austria
[2] Nuclear Material Laboratory, Safeguard Analytical Services, International Atomic Energy Agency, Seibersdorf, Austria
[*] E-mail address of corresponding author: J.Adu-gyamfi@iaea.org

L.K. Heng, K. Sakadevan, G. Dercon and M.L. Nguyen (eds), Proceedings — International Symposium on Managing Soils for Food Security and Climate Change Adaptation and Mitigation. Food and Agriculture Organization of the United Nations, Rome, 2014: 91–95

TABLE 1. Physical and chemical properties of the soils

Properties†	Hungarian soil	Waldviertel soil
Sand (%)	83	27.3
Silt (%)	8.8	58.2
Clay (%)	8.2	14.5
Bulk density (g/cm)	–	1.29
Saturated water content (%)	—	47
pH (H_20/KCl)	5.5/4.6	6.5/6.0
Total P (mg/kg)	302	502
Available P (Bray/Olsen) (mg?kg)	21/13.3	44/12.8
Inorganic P (Ca–Al–Fe) (mg/kg)	36–85–65	56–144–68
EC (25°C) (µS/cm)	—	166
Total nitrogen(N) (g/kg)	0.83	1.21
Organic C (g/kg)	7.91	20
Ca (cmol/kg)	1.82	13.69
Mg (cmol/kg)	0.61	3.13
K (cmo/kg)	0.09	0.15
Na (cmol/kg)	0.04	0.06
CEC (cmol/kg)	2.66	23.59

†EC — electrical conductivity; Ca, Mg, K and Na were determined using cobalthexamine; CEC — cation exchange capacity

four pots without plants as controls) and 36 pots (including four pots without plants and two sampling periods) for the unlabelled treatment. A low-P soil from Hungary and a medium-P soil (Waldviertel) from Austria were used. The physical and chemical characteristics of the two soils are given in Table 1.

A maize variety (DK 315) from Austria and a soybean variety (TGX 1910-4F) from IITA, Nigeria, were grown in plastic pots (one plant per pot) containing 1 kg of soil in a naturally lit glasshouse with a temperature of 34/21°C for day/night and relative humidity of 40–70 percent. Each pot received basal fertilizer equivalent to 200 kg·N·ha^{-1} as ammonium sulphate and 50 kg·K·ha^{-1} as potassium chloride. Prior to planting, the weight and P concentration of soybean and maize seeds used for the experiment were determined. The amount of P in seed was 0.98 mg·P·kg^{-1} (0.35 g with 2.8 mg·kg^{-1}·P) for maize and 3.48 mg·P·kg^{-1} (0.59 g with 5.9 mg kg^{-1} P) for soybean. Phosphorus-32 labelled $K_2H^{32}PO_4$ (specific activity 40.7 GBq/mmol) was applied to the pots. A total of 250 ml for the Waldviertel and 150 ml for the Hungarian containing 12.4 MBq (335 µCi) of a $K_2H^{32}PO_4$ solution was applied to each of the 20 pots containing 1 kg soil. Pre-germinated maize and soybean seeds were sown at one per pot immediately after the addition of ^{32}P. Twenty ml of inoculum (*Bradyrhizobium japonicum*) mixture were added to all the soils. Nitrogen was applied at 100 kg·N·ha^{-1} as ammonium sulphate to the maize.

Plant and soil sampling and analyses

The first plant sampling for the labelled and the non-labelled treatments was done at 42 d after sowing (DAS) whereas the non-labelled treatments were allowed to grow until 60 DAS. Soil samples (10–12 g) were taken with a special soil auger (inner diameter 8 mm, outside diameter 10 mm and length 25 cm) at 0, 1, 5, 42 and 60 DAS, oven-dried at 70°C for 18 h, milled and a portion used for analysis. Plants were harvested and separated into shoots (radioisotope-labelled) and shoot and roots (non-radioisotope), chopped into small pieces, oven-dried, weighed, and ground. Total P in soils was determined using the colorimetric method (Murphy and Riley, 1962) after acid digestion, and available P (Bray P2 and Olsen) determined by the colorimetric method after extraction. The inorganic soil P fractions were measured according to a fractionation scheme based on the method described by Sekiya (1983). The ^{32}P radioactivity in all the fractions (total P, available P, Ca–P, Al–P and Fe–P) was measured by liquid scintillation spectrometry (Packard 2000). Phosphorus in the maize

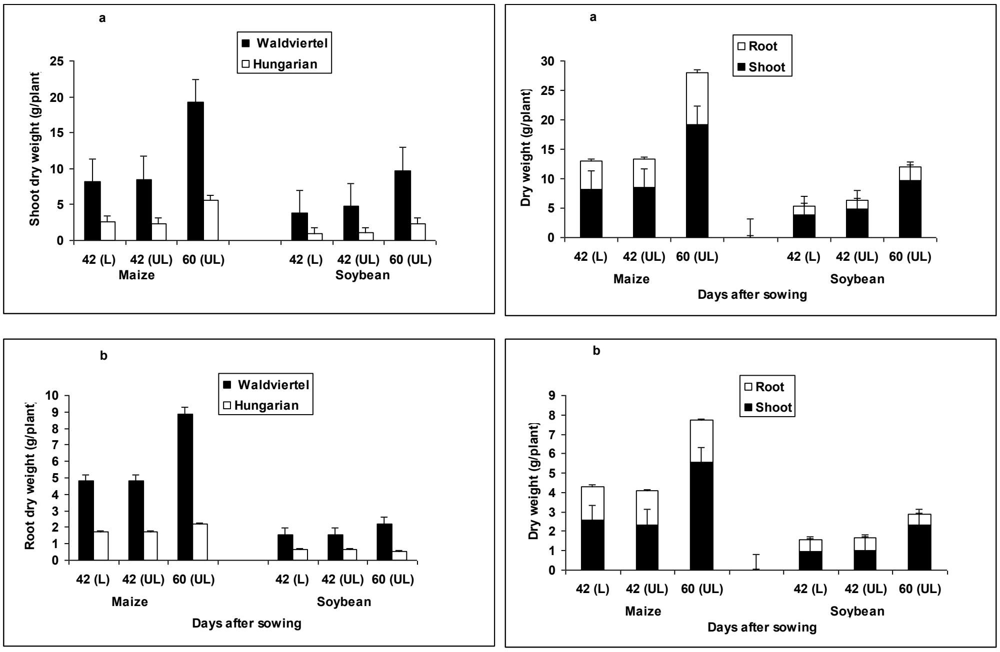


FIGURE 1. Dry weight of shoot (a) and root (b) for maize and soybean grown in Waldviertel and Hungarian soils labelled (L) and unlabelled (UL) with ^{32}P.

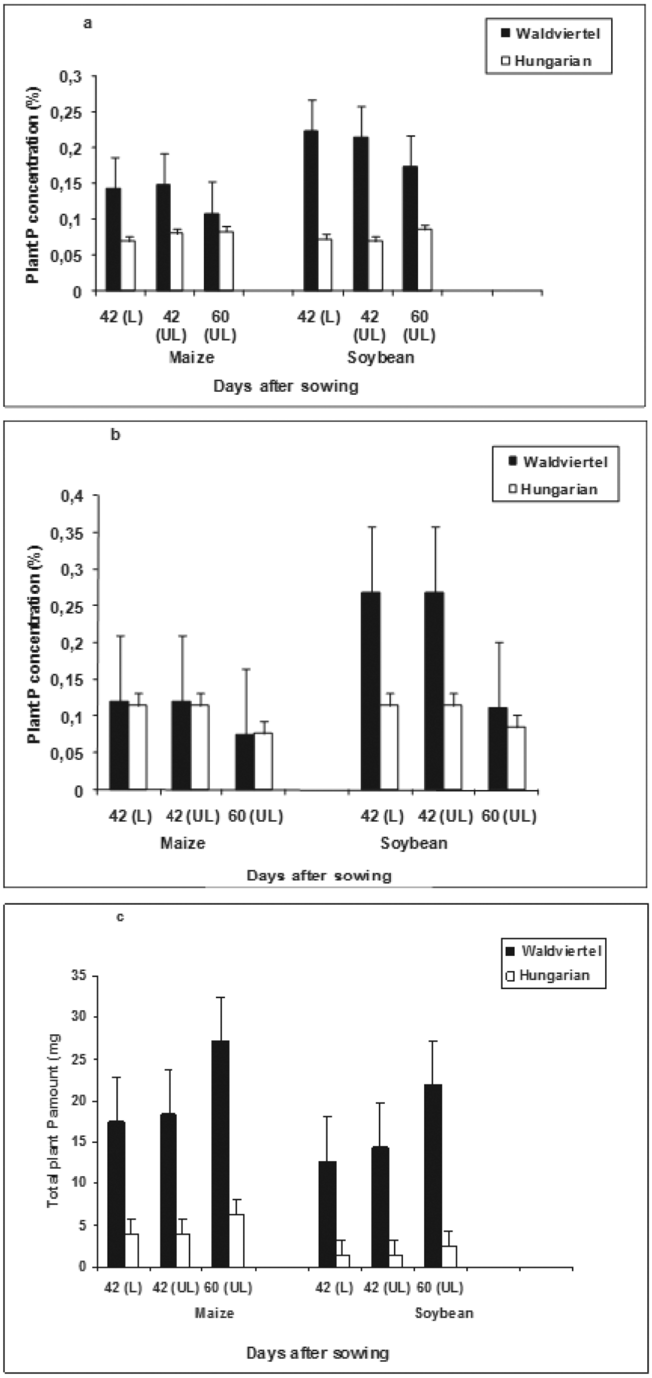


FIGURE 2. Phosphorus concentration of shoot (a) and root (b) and plant P amount (c) for maize and soybean grown in two soils. For legend see Figure 1.

and soybean seeds was determined after five seed samples, each of 100 mg, were ground and acid digested.

RESULTS

Plant growth and P uptake

The shoot and root biomass of both maize and soybean were significantly greater in Waldviertel than in Hungarian soil and there was a significant increase in shoot dry weight per plant from 42 to 60 DAS (Figure 1). Shoot weight of maize increased from 8.1 g at 42 DAS to

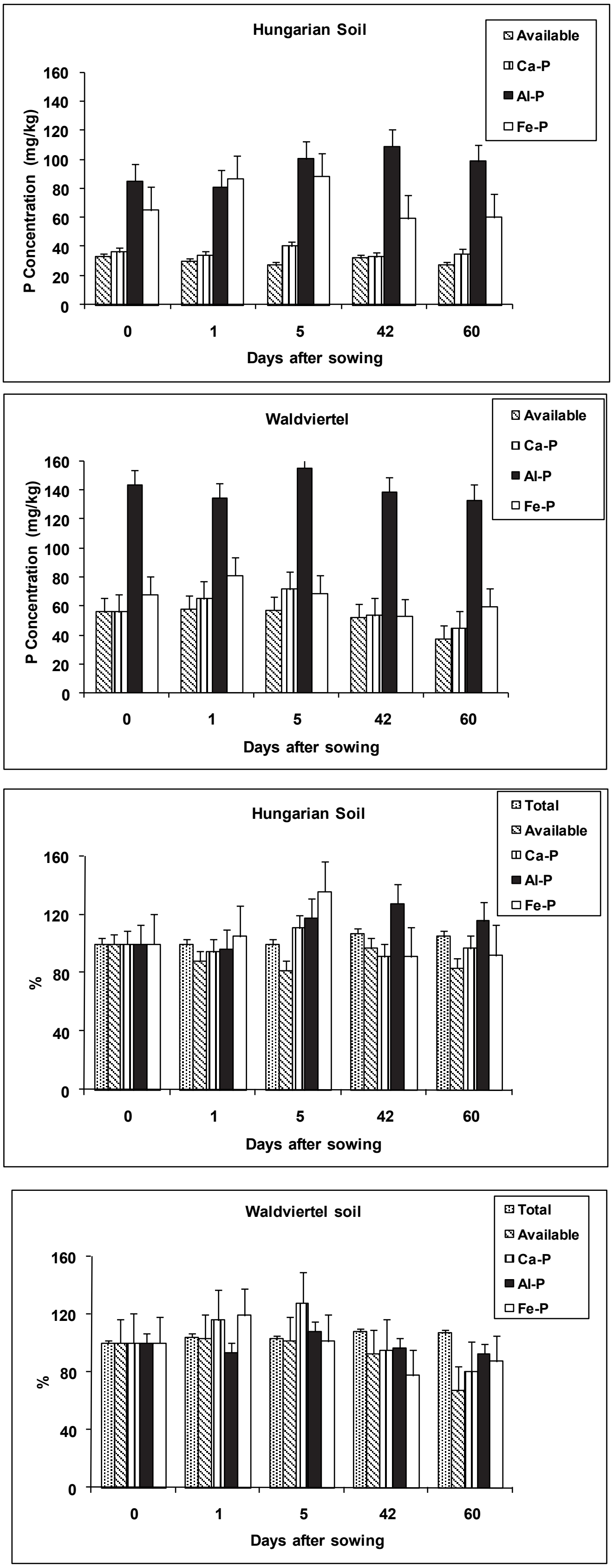


FIGURE 3. Inorganic P pools (Fe–P, Al–P and Ca–P) and available P extracted from the two soils at 0, 1, 5, 42 and 60 DAS.

19.1 g/plant at 60 DAS in Waldviertel, and from 2.2 g at 42 DAS to 5.7 g/plant in the Hungarian soil. For soybean, there was an increase from 4.1 g at 42 DAS to 9.8 g/plant for the Waldviertel and from 1.0 g at 42 DAS to 2.3 g/plant in the Hungarian soil.

The shoot P concentrations were higher for soybean (1.8–2.2 mg/g) than for maize (1.1–1.4 mg/g) and decreased with plant age for maize but not significantly for soybean (Figure 2).

Although the plant P concentrations were high in soybean compared with maize, the total P amount (mg P) was significantly higher in maize than in soybean, reflecting the higher yield in maize than in soybean. The values for maize increased in Waldviertel from 17.6 mg P at 42 DAS to 27.3 mg P at 60 DAS, while for soybean values increased from 13 mg P at 42 DAS to 22 mg P at 60 DAS (Figure 2).

In the Hungarian soil, there was no significant change in Ca–P but a slight decrease in Fe–P and a substantial increase in Al–P (85–98 mg/kg) from 0 to 60 DAS (Figure 3). In the Waldviertel soil, Ca–P decreased (from 56 to 45 mg/kg), Al–P (from 144 to 133 mg /kg) and Fe–P (from 68 to 59.5 mg/kg) between 0 and 45 DAS (Figure 3).

Radioactivity in plants and L-values.

The total radioactivity was higher in plants grown in Waldviertel than in Hungarian soil and the values reflected the plant P uptake and shoot biomass of soybean and maize. To assess the amount of isotopically exchangeable P, the L-value was estimated using the following equation:

$$L = (^{31}P\ \text{shoot} - {}^{31}P\ \text{seed}) \times {}^{32}P\ \text{added to soil} / (^{32}P\ \text{shoot})$$

where L is the L-value (µg·P·g $^{-1}$soil), the initial applied dose of ^{32}P (Bq·kg^{-1}soil); ^{32}P shoot is activity of shoot mass (Bq·g^{-1} DM); ^{31}P shoot is the total amount of P in shoot biomass (mg·P·g^{-1} DM).

The L-values (µg P g^{-1} soil) in maize and soybean were higher in the Waldviertel (> 70.0) than in Hungarian soil (< 20.0). No significant differences in L-values were observed for maize and soybean grown on the Walviertel soil, but for the Hungarian soil, the L-values were higher for maize (20.0) than in soybean (9.6) suggest ing that in this soil, maize was more efficient in taking up P than soybean (Figure 4). Recovery of radioactivity in shoot was higher for maize (14.5 percent) than for soybean (10.3 percent) and was four times higher in Waldviertel (mean recovery 12.4 percent) than in the Hungarian soil (mean recovery 2.5 percent) (Figure 4).

DISCUSSION

Plant growth and P uptake

Maize and soybean grown in the Hungarian soil had low shoot P concentrations (<1 mg·g^{-1}·P) suggesting a strong P limitation. The P concentration in shoot tissue of maize (1.1–1.5 mg·g^{-1}·P) was low compared with that in soybean (1.8–2.2 mg·g^{-1}·P) and this is attributed to a dilution effect as biomass increased (see Figures 2 and 3). However, in the low available P soil, total P accumulation in shoots was higher in maize than in soybean suggesting that maize has a greater ability to take more P from low available P soils than soybean. The Hungarian soil had 21 mg/kg (Bray II) and 13.5 mg/kg Olsen) P, and for 1 kg soil, more than 12 mg P is expected to be available to the plant. This suggests that the available P extraction using Bray II may contain other P forms that are not easily available to maize and soybean. In addition, the fact that nodules were not observed on soybean grown in the Hungarian soil despite the *Rhizobium* inoculation, while soybean grown on the Waldviertel was well nodulated (0.6 mg·DM·plant^{-1}) suggests that nodule formation in the soybean was severely impaired by P deficiency, thereby contributing to the poor growth (Adu-Gyamfi *et al.,* 1989).

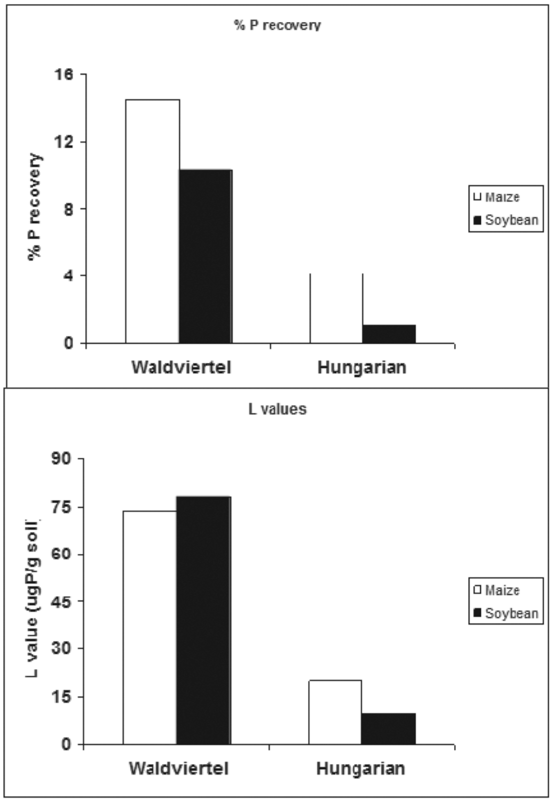


FIGURE 4. Percentage P recovery in plant shoots and the L-values calculated for maize and soybean grown on two soils.

Dynamics of P fractions

The Bray P2 and the Ca–P were the fractions depleted by plants followed by the Fe–P fractions in the two soils, and differences observed between the crops were not significant. The results suggest that more P was released in the soil solution (labile) from the available and Ca–P fractions for plant uptake than from the Fe– and Al–P fractions. Among the inorganic pools, Al–P, Fe–P and Ca–P are the three major fractions in the soil. The results reported here imply that when P is not supplied, maize and soybean are able to access mainly Fe– and Ca–P but not Al–P from the soil.

Isotopically exchangeable parameters and P uptake by maize and soybean in low and medium P soils

The high total radioactivity in maize compared with soybean suggests that plant P uptake from soil was greater by maize than by soybean irrespective of the soil used. Whereas there was a 1.4-fold increase in radioactivity in maize over soybean in the Waldviertel soil, there was a 4.3-fold increase by maize over soybean in the Hungarian soil. These data suggest that maize could take up more P under conditions of low P availability than soybean. This is supported by the finding that the L-value (with seed P uptake correction factor) of maize was double that of soybean in the low P Hungarian soil. Maize and soybean grown in medium P (Waldviertel) soil had lower specific radioactivities (dpm x 10^{3} mg^{-1} P or kBq x 10^{3} mg^{-1} P) in shoot than those grown on the low P Hungarian soil, and the values were lower in maize than in soybean. Low specific radioactivity indicates that plants were using otherwise unavailable P sources.

CONCLUSIONS

The main finding from this study was that maize was more efficient in taking up P from sparingly soluble inorganic-P sources than soybean in the medium P (Waldviertel) soil; as indicated by the low specific radioactivity (dpm × 10^3 mg^{-1}·P or kBq × 10^3 mg^{-1}·P) in shoots. The L-values of maize were double those of soybean in the low P (Hungarian) soil suggesting the superiority of maize to access sparingly soluble P from soils compared with soybean. When P was not supplied, maize and soybean were able to access P mainly from the available P (Bray P2), Fe– and Ca–P sparingly soluble fractions, but not Al–P from the soil. Maize and soybean showed severe P deficiency when grown on the Hungarian soil and the P concentration in plants was below 1 mg/g suggesting that Bray P2 overestimated the available/labile P fractions in the soil.

REFERENCES

Ae, N., Arihara, J., Okada, K., Yoshihara, T. & Johansen, C. 1990. Phosphorus uptake by pigeonpea and its role in cropping systems of the Indian subcontinent. *Science,* 248: 447–480.

Adu-Gyamfi, J.J., Fujita, K. & Ogata, S. 1989 Phosphorus absorption and utilization efficiency of pigeon pea (*Cajanus cajan* (L) Millsp.) in relation to dry matter production and dinitrogen fixation. *Plant Soil,* 110: 315–24.

Fried, M. & Dean, L.A. 1952. A concept concerning the measurement of available soil nutrients. *Soil Sci.*, 73: 263–271.

Hiradate, S., Ma, J.F. & Matsumoto, H. 2007. Strategies of plants to adapt to mineral stress in problem soils. *Adv. Agron.*, 96: 66–133.

Ishikawa, S., Adu-Gyamfi, J.J., Nakamura, T., Yoshihara, T., Watanabe, T. & Wagatsuma, T. 2002 Genotypic variability in phosphorus solubilizing activity of root exudates by pigeonpea grown in low-nutrient environments. *Plant Soil,* 245: 71–81.

Larsen, S. 1952. The use of P in studies on the uptake of phosphorus by plants. *Plant Soil,* 4: 1–10.

Murphy, J. & Riley, J.P. 1962. A modified single solution method for the determination of phosphorus in natural waters. *Anal. Chim. Acta,* 27: 31–36.

Naruzzaman, M., Lambers, H., Bolland, M.D.A. & Veneklass, E.J. 2006. Distribution of carboxylates and acid phosphatase and depletion of different phosphorus fractions in the rhizosphere of a cereal and three grain legumes. *Plant Soil,* 281: 109–120

Nwoke, O.C., Diels, J., Abaidoo, R.C. & Sanginga, N. 2007. Utilization of phosphorus from different sources by genotypes of promiscuous soybean and cowpea in a low phosphorus savanna soil. *African J. Agric. Res.,* 2: 150–158

Raghothama, K.G. & Karthikeyan, A.S. 2005 Phosphate acquisition. *Plant Soil,* 274: 37–49

Sekiya, K. 1983. Phosphorus. *In* Dojou Youbun Bunsekihou, ed. *Methods of soil analysis,* pp. 225–257. Ministry of Agriculture, Forestry and Fisheries, Youkendou, Tokyo.