ICRISAT

FAO

IAEA

Genetic Manipulation of Crop Plants to Enhance Integrated Nutrient Management in Cropping Systems— 1. Phosphorus

International Crops Research Institute for the Semi-Arid Tropics

Abstract

Citation: Johansen, C., Lee, K.K., Sharma, K.K., Subbarao, G.V., and Kueneman, E.A. (eds.). 1995. Genetic manipulation of crop plants to enhance integrated nutrient management in cropping systems—1. Phosphorus: proceedings of an FAO-ICRISAT Expert Consultancy Workshop, 15-18 Mar 1994, ICRISAT Asia Center, Patancheru, India. (In En. Summaries in En, Fr.) Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 184 pp. ISBN 92-9066-296-4. Order code: CPE 094.

This Workshop forms part of an overall endeavor to establish a global consortium of researchers focusing their efforts on improving the ability of crop plants to acquire phosphorus, particularly through sources from which it is only sparingly available. These sources include bound forms of soil phosphorus and such fertilizers or amendments as rock phosphate. This volume explains the overall procedures followed, presents the formal papers prepared for the Workshop, and highlights the outcome of the deliberations in the form of a preliminary draft proposal for a global project. A background paper covers the possibilities of favorably manipulating phosphorus acquisition, which include modifying root absorption area by improving rooting characteristics or mycorrhizal associations, manipulating ion absorption mechanisms, and modifying the rhizosphere through root exudations or effects of microorganisms. Position papers prepared by invited specialists examine in detail the prospects for favorable manipulation of specific components of phosphorus acquisition. During the Workshop, research areas with prospects for impact on agriculture in the medium term were identified and appropriate outputs and activities proposed. The promising research areas chosen were modification of root morphology, manipulation of root exudates, enhancement of mycorrhizal effects through crop management practices, and optimization of cropping systems approaches. The Workshop laid the foundation for subsequent activities of formulating detailed project proposals and soliciting support from potential donors for the proposed global consortium.

Résumé

Manipulation génétique des cultures visant l'amélioration de la gestion intégrée des éléments nutritifs dans les systèmes culturaux—I. Phosphore: comptes rendus d'un Atelier FAO-ICRISAT d'experts-conseils, 15–18 mars 1994, Centre ICRISAT pour l'Asie, Patancheru, Inde. Cet atelier fait partie d'un effort intégral pour établir un consortium mondial de chercheurs oeuvrant sur l'amélioration de la capacité des plants à obtenir du phosphore, surtout à partir des sources à réserves très limitées. Ces sources comprennent des formes liées du phosphore du sol et des engrais tel que du phosphate naturel. L'ouvrage précise les procédures suivies, présente les communications de l'Atelier, et souligne les résultats des discussions sous la forme d'une proposition préliminaire pour mettre sur pied un projet mondial. Une communication de base traite des possibilités de manipuler favorablement l'obtention du phosphore dont la modification de la région d'absorption racinaire en améliorant les caractéristiques d'enracinement ou les associations mycorhiziennes, la manipulation des mécanismes d'absorption de l'ion et la modification de la rhizosphère par les exsudations racinaires ou les effets des microorganismes. Des communications présentées par des spécialistes examinent les perspectives de manipulation favorable des composantes spécifiques de l'absorption du phosphore. Au cours de l'Atelier, on a identifié des domaines de recherche à l'impact potentiel sur l'agriculture au moyen terme et proposé des objectifs et des activités. Les domaines prometteurs choisis ont été la modification de la morphologie racinaire, la manipulation des exsudats racinaires, l'augmentation des effets mycorhiziens à travers des pratiques culturales et l'optimisation des approches de systèmes culturaux. L'Atelier a fondé la base des activités ultérieures sur la formulation détaillée des projets et des demandes aux donateurs potentiels des contributions financières à l'intention du consortium mondial proposé.

Cover: DNA sequence of iron deficiency-specific clone 1 (*ids*-1) and predicted amino acid sequence of its product.

Genetic manipulation of crop plants to enhance integrated nutrient management in cropping systems—1. Phosphorus: proceedings of an FAO-ICRISAT Expert Consultancy Workshop

---

15—18 Mar 1994
ICRISAT Asia Center, Patancheru, India

Edited by
C Johansen, K K Lee, K K Sharma,
G V Subbarao, and E A Kueneman

FAO Food and Agriculture Organization of the United Nations
Via delle Terme de Caracalla, 00100 Rome, Italy

IAEA International Atomic Energy Agency
P O Box 100, 1400 Vienna, Austria

ICRISAT International Crops Research Institute for the Semi-Arid Tropics
Patancheru 502 324, Andhra Pradesh, India

1995

Contents

Acknowledgments

The Organizing Committee of this Workshop, which comprises the editors of these Proceedings, wishes to thank FAO, IAEA, and ICRISAT for their moral and financial support of the Workshop in particular, and of this global consortium concept in general. For his efforts in organization and conduct of the Workshop, we are grateful to R Narsing Rao, who acted as Secretary to the Workshop Organizing Committee. We also thank P Sai Ranga Rao, P Nageswara Rao, and B Nagappa for their clerical support, particularly during and after the Workshop. Other staff in the Legume Physiology Section of Agronomy Division, ICRISAT Asia Center who assisted with various aspects related to this Workshop are also gratefully acknowledged. We are most grateful for the professional and reliable support given to the Workshop by various service units of ICRISAT, including Housing and Food Services, Travel Unit, ICRISAT Delhi Office, Transport Unit, Visitors Services, Security, Information and Management Exchange Program, and Farm and Engineering Services Program.

Finally, we thank all participants for their valuable inputs and enthusiastic support in moving towards developing a global research project on enhancing phosphorus acquisition capabilities of crop plants.

Introduction

Background

Research undertaken as part of a Government of Japan Special Project at ICRISAT Asia Center, India, during 1985-89 highlighted the role of root exudates of chickpea (*Cicer arietinum* L.) and pigeonpea *[Cajanus cajan* (L.) Millsp.] in solubilizing phosphorus (P) from sparingly available sources in the soil, sources not normally available to other crop plants. The findings are presented in an ICRISAT publication [Johansen, C., Lee, K.K., and Sahrawat, K.L. (eds.). 1991. Phosphorus nutrition of grain legumes in the semi-arid tropics. Patancheru, A.P. 502 324, India: ICRISAT. 264 pp. ISBN 92-9066-200-X] as well as in several other publications. These findings kindled interest among several persons and groups concerned with developing and promoting sustainable agricultural production systems, particularly in the tropics where resource degradation is an increasingly serious threat. Particularly attractive was the concept of being able to access forms of P not normally available to crop plants and to incorporate this P in labile forms in the nutrient cycles of cropping systems, thereby potentially reducing the need for application of soluble phosphate fertilizers. The unavailable forms of P targeted include bound forms of native soil P and sparingly available forms in such unprocessed fertilizers and amendments as rock phosphate.

While focusing on the possible role of root exudates in a cropping systems context, it became apparent that newly emerging concepts and techniques in cellular and molecular biology may have a role in enhancing integrated nutrient management (INM) at the cropping systems level. There is a wealth of information at the component level on how mineral nutrients are taken up and utilized in plants. Recent and ongoing research is unraveling the mechanisms of genetic control over these processes, and techniques are being developed to transfer relevant genes across genotypes and species to enhance aspects of their mineral nutrition favorably. It is also apparent that practical exploitation of this established and emerging component-based knowledge will require a systems orientation and a broad multidisciplinary approach.

With these considerations, we thought it appropriate to develop the concept of a global consortium of researchers focusing on practically-oriented genetic manipulation of the ability of crop plants to acquire P from sparingly available sources. Other essential nutrients could also be approached in this way but, in the first instance, we decided to address P; hence this effort is considered as 'Part 1'. The overall objective of the global consortium was thus to develop and execute a global project aimed at enhancing integrated P management in cropping systems by

favorable genetic manipulation of a plant's ability to acquire and utilize P efficiently.

Objectives of the Workshop

As part of the overall objective of the global consortium, the particular objectives of this Workshop were as follows.

- Assemble a group of specialists actively involved in this research area.
- Examine and evaluate possible mechanisms of enhancing P acquisition by roots of crop plants, e.g., root morphology, uptake mechanisms, root exudates, and mycorrhizae.
- Estimate the impact and agroecological consequences of favorable manipulation of the most promising of these mechanisms.
- Select the most appropriate candidate mechanisms for enhancement through a concerted, global, time-bound research effort.
- Elaborate the research agenda with respect to such factors as biochemical pathways, physiological control, genetic control, and genetic manipulation.
- Consider the feasibility of genetic transfer of desirable mechanisms within and between crop species.
- Formulate work plans with adequate detail so that they can form the substantive component of project proposals to be used to solicit appropriate funding for execution of the project.

Procedure

The following steps were taken to implement the global consortium on P acquisition.

- Identification of participants best able to contribute to such an approach. Criteria included research and standing in relevant subject areas, association with institutions likely to support such an approach, and availability and interest to participate in the exercise.
- Identification of funding to support the Workshop. This was ultimately provided by the Food and Agriculture Organization of the United Nations (FAO), the International Atomic Energy Agency (IAEA), and ICRISAT.
- Preparation of a background paper to help assemble and focus subject matter to be discussed at the Workshop (this paper is included in these proceedings). The paper was sent to participants prior to the Workshop in time for them to consider it in preparing their position papers.
- Request participants to prepare position papers on selected topics for presentation at the Workshop. These papers are included in the proceedings.
- Conduct of the Workshop (15-18 Mar 1994).

1. Presentations of introductory, explanatory, and position papers on the first day.

2. Identification and prioritization of subject areas worthy of inclusion in a global, multidisciplinary, time-bound research effort on the second day.

3. For the remainder of the Workshop, grouping of participants into chosen subject-area working groups to formulate project details. These included specification of objectives, outputs, activities, and responsible researchers and institutions. The format used for UNDP project application was adopted. The draft document compiled during the Workshop is included in these proceedings.

4. A plenary session summarizing the progress made during the Workshop and circulation to participants of the draft proposal.

- Subsequent to the Workshop, continued work on project development, including details of budgeting, by selected participants.
- Preliminary approaches to potential donors, with the intention of assembling a global consortium of donors to support project activities.

Opening address

J G Ryan[1]

Welcome to ICRISAT. This Workshop falls between weeks of intensive activities in trying to make ICRISAT's newly evolved research project system operational. ICRISAT's Medium Term Plan (MTP) for 1994-98 foreshadowed a change in the way ICRISAT is organized and managed in order to implement the Plan effectively.

A fundamental change is that ICRISAT's research will be packaged with the *project*—not the *program*—as the basic unit of operation and management.

A portfolio of global research projects is being established. These projects are defined in relation to the priority needs and research opportunities within a set of specific *production systems* identified in the four major regions of the semi-arid tropics (Asia, Western and Central Africa, Southern and Eastern Africa, and Latin America and the Caribbean). This production system concept provides a means of integrating and targeting our research, and for better linkages between ICRISAT and the national programs and regional networks.

Each global research project will have a designated team and clearly defined objectives and milestones. The team will be accountable for the development, conduct, management, resources, reporting, and impact assessment of the project. The composition of each project team will be multidisciplinary. Once the responsibility for conducting a project, or part of a project, has devolved to a staff member, that person carries that responsibility and authority.

To facilitate the definition, development, management, and conduct of projects, an organizational framework employing a tandem matrix was developed. There are two dimensions to the matrix. The x-axis consists of four geographic regions. Each region has an *Executive Director* responsible for the management and support of the agreed production systems within the region. The y-axis consists of seven research disciplines, each led by a *Division Director* responsible for providing leadership and ensuring quality. These research divisions have global responsibilities. The axes of the matrix are designed to emphasize shared responsibilities, goals, and outcomes through development and delivery of a relevant global research project portfolio.

In addition to the four Executive Directors and the seven Division Directors, the new management framework includes two new positions—the Associate Directors General:

1. International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, Andhra Pradesh, India.

- The *Associate Director General for Research* is responsible for the global research portfolio, aided by the Executive Directors of the regions and the Division Directors.
- The *Associate Director General for Finance and Administration* is responsible for corporate functions related to finance, administration, human resources, purchasing, computer services, and donor relations.

All these new jobs have been filled from within the ranks. No new staff have been recruited.

However, it is important to remind you that there are several features of ICRISAT that have not changed:

- ICRISAT *still* has a global mandate to improve staple food crops that sustain the world's poorest people, and to seek ways to manage the fragile natural resources of the semi-arid tropics.
- Our partners are *still* the national programs of the countries in which these people live.
- The targets of our work are *still* the farmers and consumers of these countries.
- Our work is *still* important to hundreds of millions of people throughout the world.

Details of projects will be finalized over the coming months such that they will become fully operational in 1995. It is emphasized that all funding, staffing, allocation of other resources, accountability, and staff evaluation will now be focused on these projects. Those projects rated as of the highest priority, on the basis of MTP calculations, will be favored for CGIAR core funding, whereas those of lesser priority would depend on provision of supplementary funding for their viability. However, we think that arranging our projects in this way should package them such that they would appear generally attractive to donors. There is also scope for fitting in externally-funded special projects into the new ICRISAT project portfolio, so as to gain synergies. Many donors recognize the established networking advantages that ICRISAT has to offer.

With the extensive efforts over the previous weeks and months of many ICRISAT scientists in this room in attempting to formulate this new ICRISAT project portfolio, this Workshop should be well served with ideas of how to formulate viable project proposals on the topic at hand. It does seem that the objectives and activities of this Workshop and those of ICRISAT over this period are identical: to clearly document how existing and emerging scientific knowledge can best be channeled to address ever increasing global problems of agricultural production, and sustainability of that production, keeping in mind a depleting resource base and escalating demands from humanity. This documentation needs to be in a manner that will attract sufficient and continuing donor support such that these problems can be effectively addressed.

More specifically, it now seems appropriate to remind you of some of the earlier research at ICRISAT directed towards phosphorus (P), such that it can be drawn upon in the course of this Workshop. Early studies showed relatively minor responses of the mandate pulses, chickpea and pigeonpea, to P application in Indian Alfisols and Vertisols, compared to those of other crops and in view of the relatively low soil P test values. This apparent enigma was the basis for a special project conducted at ICRISAT Asia Center from 1985 to 1989, in cooperation with the Ministry of Agriculture, Forestry and Fisheries, Government of Japan (GOJ). The GOJ provided funding for employment and support of three scientists to work at ICRISAT Asia Center to tackle this research problem, two of which are attending this Workshop-Ae and Arihara. Such special projects allowed more in-depth study of particular problems than we perhaps could have managed with core resources and, importantly, introduced alternative scientific approaches. This project culminated in a Workshop in December 1989 and a subsequent book based on it, entitled *Phosphorus Nutrition of Grain Legumes in the Semi-Arid Tropics.*

The findings of this project relating to effects of root exudates on release of sparingly soluble soil P stimulated widespread scientific interest and raised questions as to the extent to which this phenomenon could be favorably exploited. One of the interested parties was FAO, which prompted and largely funded the efforts that have culminated in the present Workshop.

I should also at this point mention some of the other P studies conducted at ICRISAT over the years.

- P exchange characteristics in Indian soils.
- Residual effects of P on sorghum and pigeonpea.
- P as a component of organic matter cycling.
- P fertilizer sources, responses, and cycling in the Sahel.
- Crop genotypic differences in P response.
- Mycorrhizal effects on P nutrition.

Finally, I would like to wish you every success in your endeavors to chart a viable, global research agenda in your topic area. We at ICRISAT will be most interested in the outcome of your deliberations, given our present preoccupation with experimenting on how to develop viable, attractive, global research projects.

Genetic manipulation of crop plants to enhance integrated nutrient management in cropping systems—the case of phosphorus

C Johansen[1], G V Subbarao[2], K K Lee[1], and K K Sharma[1]

Abstract

Increasing demands for agricultural production without undue degradation of the resource base and total environment require optimal use of phosphorus (P) in the soil-crop-animal systems. This paper overviews possibilities of enhancing the ability of agriculturally important plants to acquire P, particularly from sparingly available sources. These possibilities include modifying root absorptive area by improving rooting characteristics or mycorrhizal associations, manipulating ion absorption mechanisms, and modifying the rhizosphere through root exudation or effects of microorganisms. Present understanding of how these factors are regulated by environmental, physiological, and biochemical influences is described and prospects for their genetic manipulation explored. Possibilities of harnessing newly emerging concepts and techniques in cellular and molecular biology are examined in detail A major shortcoming of present knowledge is inadequate quantification of the effects of various adaptive mechanisms for acquiring P. Such quantification is necessary to calculate by how much a proposed manipulation will enhance P acquisition, and thus estimate returns on research investment. A case is made for an integrated, global approach to enhancing P acquisition abilities of cultivated species.

Introduction

Ever-increasing demands of agricultural production throughout much of the tropics and subtropics are having inevitable adverse effects on mineral nutrient cycles and thus on sustainability of those production systems. This is not only occurring on

1. ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India.
2. Present address: National Institute of Agro-Environmental Sciences, Tsukuba, 305 Japan.

ICRISAT Conference Paper no. CP 971.

Johansen, C., Subbarao, G.V., Lee, K.K., and Sharma, K.K. 1995. Genetic manipulation of crop plants to enhance integrated nutrient management in cropping systems—the case of phosphorus. Pages 9-29 *in* Genetic manipulation of crop plants to enhance integrated nutrient management in cropping systems—1. Phosphorus: proceedings of an FAO/ICRISAT Expert Consultancy Workshop, 15-18 Mar 1994, ICRISAT Asia Center. India (Johansen, C., Lee, K.K., Sharma, K.K., Subbarao, G.V., and Kueneman, E.A., eds.). Patancheru 502 324, Andhra Pradesh. India: International Crops Research Institute for the Semi-Arid Tropics.

such marginal lands as where natural vegetation has been recently replaced by cropping, but also on lands considered as better-endowed (FAO 1993). A key factor in such resource degradation is a decline in soil organic carbon levels, with consequent declines in available and sparingly available levels of mineral nutrients associated with soil organic carbon and a loss of the general buffering effect of soil organic carbon (e.g., soil organic carbon influences soil pH, which in turn largely controls mineral nutrient availability to plants).

The World Phosphate Institute classified 65% of 500 soil samples collected from 42 countries in the tropics as acutely deficient in phosphorus (P); only 8% of the samples were classified as not deficient (Koala et al. 1988). Based on extensive surveys in India, it was concluded that 45.7% of the soils were low in available P, 49.7% were medium, and only 4.6% were considered as high in P (Tandon 1987). Application of processed mineral fertilizers or amendments cannot be relied upon to reasonably redress such nutrient depletion because of economic, logistic, and many other reasons. It would thus be desirable for crop plants to access a greater proportion of the total soil nutrient pool than is otherwise available to them. For example, total soil P is often 100-fold more than the fraction of soil P normally available for uptake by crop plants (Al-Abbas and Barber 1964). However, it is recognized that more effective mining of total soil nutrient reserves is only a medium-term solution and that long-term sustainability will require some compensation of nutrients removed from the system in agricultural produce or through other losses.

In many tropical and subtropical areas, there are options to use less-processed fertilizers and amendments. An example is the use of unprocessed rock phosphate to supply P, which can be particularly effective in acid soils (Khasawneh and Doll 1978). However, it is desirable to identify plant species or genotypes that can best solubilize nutrients from sparingly soluble fertilizer sources, and thus introduce available forms of nutrients into the nutrient cycle of the cropping systems at an earlier stage. Crop species differ in their ability to utilize rock phosphate (Khasawneh and Doll 1978, Flach et al. 1987, Hoffland et al. 1992).

There are three broad categories by which plants can increase their access to native or applied soil nutrients: (1) increasing absorptive area, (2) favorably modifying the absorption mechanisms to increase uptake from low ambient concentrations, and (3) rhizosphere modification to increase nutrient availability. Options in the first category include such increases in root surface area as achieved through root proliferation or root hair development, or mycorrhizal associations that allow capture of nutrients beyond the rhizosphere. With regard to the second category, plant species and genotypes have been shown to differ in their abilities to extract nutrients from low ambient concentrations, i.e., their threshold levels (e.g., Barber 1979, Itoh 1987). Thirdly, many possible mechanisms have been recorded by which root exudates, ranging from protons to complex organic molecules,

influence nutrient availability and uptake. These can be direct effects or through microbial inhabitants of the rhizosphere. A recently reported example in this category is the exudation of piscidic acid by pigeonpea roots, which facilitates release of phosphorus (P) from iron-bound soil P (Fe-P) which is not normally available to plants (Ae et al. 1991).

Further, there have been recent advances in the understanding of genetic control of these nutrient acquisition mechanisms (e.g., Bassam et al. 1990, Randall et al. 1993, Barrow 1993). This has opened up hitherto unrealized possibilities for favorable genetic manipulation of these mechanisms, either through standard plant breeding procedures or through new opportunities unfolding in the field of molecular biology. The latter suggests possibilities of identifying the genes controlling nutrient ion uptake mechanisms and root exudation processes and transferring them between genotypes and even species. Thus, opportunities have now arisen for applying advances in molecular biology to tackle problems of integrated nutrient management of agricultural production systems.

There are many possible candidate crops, nutrients, uptake mechanisms, and root exudates to consider. Although this paper focuses on the effects of root exudates on P nutrition of legume crops, which are discussed against the background of the recent literature in this area, we have also tried to compare the prospects for favorable genetic manipulation among all other possible means of enhancing P acquisition. We concentrate particularly on the prospects of using emerging molecular biological techniques to effect desirable gene transfers. This information is directed towards examining the feasibility of establishing a collaborative project to manipulate P acquisition so as to enhance integrated nutrient management.

Methods of enhancing phosphorus acquisition

Absorptive area

Phosphorus acquisition can be enhanced by either increasing contact of root surface area with soil or through symbioses with mycorrhizae, which can acquire P from regions that lie beyond the rhizosphere.

Root surface/soil contact. The primary means of increasing exposure of root tissue to the soil solution is root proliferation, either within a given soil volume or into otherwise untapped soil volumes. However, there is an upper limit to nutrient uptake achievable by root proliferation in a given soil volume as nutrient depletion zones eventually overlap (Nye and Tinker 1977). Deep-rooting species can access nutrients in soil zones that shallow-rooted species cannot reach. This is a suggested benefit of agroforestry systems, where a deep-rooting perennial can transfer nutrients acquired from the region below the rooting zone of annual crops (Irizarry

and Rivera 1983). It is claimed that such deep-rooting annual crops as pigeonpea also extract nutrients from deeper soil layers and make them available for recycling through surface soil and through subsequent crops (Chauhan 1993). However, there has been little reliable quantification of these effects.

Root morphology, for a given amount of root biomass, is important in determining the extent of contact between the root surface and soil. Roots with higher specific root length (SRL), i.e., roots with smaller diameters, are more effective in this regard (Fitter 1991). Root hair development is a well-known strategy for increasing the absorptive surface area. A variation of this strategy is development of proteoid roots, or rootlet clusters, in which the rootlets are covered with root hairs (Marschner et al. 1987). This is obviously an adaptation to low-nutrient and drought prone soils (Lamont 1982) but it is also found in such grain legume species as *Lupinus* (Gardner et al. 1981).

Mycorrhizae. The value of vesicular-arbuscular mycorrhizae (VAM) in extending the nutrient absorptive area of crop species has been thoroughly documented (e.g., Mosse 1981, Bolan 1991, Jacobson et al. 1992). Plant growth stimulation with mycorrhizal infection is normally attributed to enhanced P uptake, although uptake of other nutrients in limiting supply may also be increased (e.g., Cooper and Tinker 1978). Many, if not most, plant species are natural hosts for VAM, and there is a general inverse relationship between extent of VAM colonization and ability to produce root hairs and proteoid roots (Thompson 1991). Many crop plants show mycorrhizal dependency, defined by Gerdemann (1975) as 'the degree to which a host relies on the mycorrhizal condition to produce maximum growth at a given level of soil fertility'. Many cultivated legumes fall into this category (Thompson 1991).

Mycorrhizal infection is very much determined by rhizosphere conditions, which are in turn influenced by the root exudations discussed below. Infection and growth of mycorrhizae are stimulated by P deficiency, which also results in increased exudation of reducing sugars and amino acids (Graham et al. 1981). The functioning of the symbiosis depends on supply of photosynthates from the shoot, which can be considered as an investment in extended absorptive area. Similarly, extension of root surface area (as discussed in the previous section) relies on appropriate partitioning of photosynthates from shoots to roots. Most research on VAM is directed towards their favorable manipulation either through inoculation with superior strains of VAM or by adjusting agronomic practices to favor VAM multiplication and infection of target crops (Lee and Wani 1991, Thompson 1991). However, this paper focuses on possible manipulations of the host plant to develop optimal symbioses with VAM.

Absorption mechanisms

Uptake of P into the cytoplasm of root cells is dependent upon metabolic energy generated by roots (Clarkson and Grignon 1991). There is normally a concentration gradient of inorganic P (Pi), in the form of $H_2PO_4^-$, between the soil solution adjacent to the root and the cytoplasm to be overcome. This is thought to be mediated by H^+-translocating ATPases, which create a pH gradient across the plasma membrane (Clarkson and Grignon 1991). Anions, including H_2PO_4-, are then co-transported with H^+, either as HVanion symports or OH^-/anion and HCO_3^-/anion antiports. Ions are thought to move passively, in response to an electrochemical gradient, through ion channels in the plasma membrane (Tester 1990). Ion selectivity in such channels is controlled by amino acid configurations in protein pores (Tyerman and Schachtman 1993).

Similar to P uptake into root tissue, P uptake into mycorrhizal hyphae must be against an electrochemical potential gradient as there is a Pi concentration gradient of about 1000:1 between hyphae and soil solution (Smith and Gianinazzi-Pearson 1988). Following P uptake by hyphae, polyphosphate is accumulated in the vacuoles of the VAM hyphae. Synthesis and breakdown of polyphosphate in vacuoles and transport of Pi across the tonoplast serve to regulate cytoplasmic Pi levels. From the hyphal cytoplasm, Pi moves to roots through a diffusion gradient (Smith and Gianinazzi-Pearson 1988).

Clarkson and Grignon (1991) have assembled the evidence demonstrating feedback mechanisms that control rates of P uptake in higher plants. Rates may be high in young or P-starved tissue but revert to lower values as internal Pi levels increase. This could be achieved by increased P efflux or reduced activity of P transporters. These data demonstrate that, at least for the crop plants studied, there is already a large potential for P transport into root cells, which is modulated by the environment internal and external to the plasma membrane. Therefore, the scope for increasing the potential to actively transport P appears to be limited, as the rate of P transport is already greater than that necessary for satisfying the long-term P requirements for growth.

However, increasing the ability of roots to absorb P from very low ambient concentrations (C_{min}) offers a way to manipulate P absorption mechanisms favorably. Values for C_{min} for P, as determined in solution culture studies, are in the range 0.01-0.1 uMP, depending on plant species and genotypes within a species (Fist et al. 1987, Itoh 1987).

Rhizosphere modifications

Microorganisms. Noninfecting rhizosphere microorganisms (i.e., excluding mycorrhizae and nodule-forming rhizobia) can have marked effects on plant uptake of P and other nutrients by influencing growth and development of roots, nutrient availability in the rhizosphere, and the nutrient uptake process (Rovira

et al. 1983, Tinker 1984). Microbial composition and populations in the rhizosphere are determined by root exudates as well as mucilage and sloughed-off cells and tissues. Conversely, presence of microorganisms in the rhizosphere can significantly increase root exudation, as shown in studies comparing nonsterile and sterile soil (Barber and Martin 1976). Such microorganisms influence nutrient availability to plants in many ways, ranging from acting as a competitive sink for nutrients to producing and releasing metabolites that increase nutrient availability in the rhizosphere. It has long been recognized that microorganisms can solubilize plant-unavailable soil P by producing such low molecular weight organic acids such as citric, succinic, lactic, and malic acids (Chhonkar and Subba Rao 1967). These microorganisms are normally more numerous in the rhizosphere than in bulk soil. Though not necessarily involved directly in increasing nutrient availability, it should be noted that rhizosphere microorganisms can produce such growth regulators as indoleacetic acid (IAA) and gibberellins (Bottini et al. 1989, Fallik et al. 1989). These hormones can stimulate root growth, thereby increasing the capture of P and other nutrients. Rhizosphere microorganisms can markedly influence rhizosphere pH and redox potential, with consequent effects on nutrient availability.

Direct exudate effects. It is difficult to differentiate direct effects of root exudates on nutrient availability from effects of microorganisms, due to the intimate interaction of these factors. Nevertheless, some distinct direct effects have been identified.

Changes in pH. It is generally accepted that the motive force of active ion transport is charge separation across membranes, mediated by proton pumps in the form of membrane-bound ATPases (Poole 1978). This rhizosphere pH is influenced greatly by the relative proportions of cations and anions absorbed by the root (Marschner 1986, van Beusichem et al. 1988, Haynes 1990). A well-known example is a decrease in ambient pH when NH_4- is the main form of N uptake and an increase when NO_3^- is the main form (Darrah 1993). The influence of solution pH on the concentrations of available forms of nutrient ions is well known; for example, the proportion of H_2PO_4-, and P uptake by plants, decline concomitantly as pH increases above 6.0 (Hendrix 1967).

Rhizosphere pH can also be influenced by excretion of HCO_3~, evolution of CO_2 by respiration, and exudation of organic acids, but net movements of H^+ and HCO_3- have most influence on rhizosphere pH (Marschner 1986). Plant species differ markedly in their ability to alter rhizosphere pH; for example, chickpea is more effective than maize in lowering rhizosphere pH (Marschner and Rdmheld 1983). Genotypic variation in ability to change the degree of rhizosphere pH has been reported in soybean and maize (Romheld and

Marschner 1984). These differences are reflected in cation/anion uptake ratios. Therefore, the utilization of P from such sparingly soluble sources as rock phosphate can vary among plant species according to their cation/anion uptake ratio (Bekele et al. 1983).

Specific compounds. The amount of organic carbon released into the rhizosphere may constitute up to 30% of total plant dry matter, at least in young plants (Marschner 1986). The released material comprises mucilage, sloughed-off cells and tissues, and various organic compounds of low molecular weight. As previously mentioned, this serves as a substrate for a considerable microbial population in the rhizosphere, which in turn can influence nutrient availability and plant uptake. We are concerned here with possible direct effects of low-molecular-weight compounds on nutrient availability that cannot be attributed entirely to pH effects.

Marschner (1986) has conveniently summarized these direct exudate effects as follows.

- Mechanism I. This involves a reduction of MnO_2 by organic acids (e.g., malic acid) and subsequent chelation of Mn^{2+} (thus making it available for root uptake).
- Mechanism II. A reduction of Fe(III) oxide by citrate, phenolics, or amino acids and subsequent chelation of Fe, which facilitates transport to uptake sites at the plasmalemma.
- Mechanism III. Solubilization of sparingly soluble inorganic phosphates by organic acids. This is achieved by both lowering the pH and desorption of phosphate from sesquioxide surfaces by anion exchange. Citric and malic acids can form relatively stable chelates with Fe(III) and Al(III), thereby increasing solubility of P and alleviating toxic effects of Al(III). This is a particularly important mechanism of P acquisition by *Eucalyptus* spp (Mulette et al. 1974), which are normally adapted to acid mineral soils of very low available P status.
- Mechanism IV. This refers to the effect of citrate reacting with Fe(III) phosphate to produce ferric hydroxyphosphate citrate polymers that diffuse to the root surface. This mechanism is believed to operate in lupins, where citrate acts as a rhizosphere shuttle for the mobilization of iron phosphates for P uptake; *Lupinus albus* can release large quantities of citrate, amounting to 1.5-12% of the root dry weight (Gardner et al. 1983). Marschner (1986) points out that root surface area interacts positively with the ability of root exudates to solubilize nutrients.

The relative lack of responsiveness of pigeonpea to P fertilizer in soils where iron-bound P (Fe-P) was predominant (Alfisols) has been attributed to root exudation of piscidic acid by pigeonpea (Ae et al. 1990, 1991, 1993). In a manner similar to Mechanism III of Marschner (1986), they proposed that the alcoholic hydroxyl and carboxyl groups in the tartaric portion of piscidic acid are involved in chelation of Fe(III) of Fe-P, thus releasing P for plant uptake and immobilizing Fe. Arihara et al. (1991) further demonstrated, in pot studies, that this ability of pigeonpea to release P from Fe-P could also result in greater P availability to maize subsequently grown in the same pots. This indicates the possibility of pigeonpea being able to introduce otherwise unavailable P into the nutrient cycles of cropping systems, an effect attributable to a specific root exudate.

Environmental, physiological, and biochemical regulation

Root systems

Root systems of most higher plant species possess a high degree of phenotypic plasticity, which is an understandable adaptation in view of the normal high heterogeneity, both in space and time, of the rooting zone of soils (Fitter 1991). This poses difficulties in assessing genetic differences between root systems, due to the usual large environmental influence and genotype x environment (G x E) interaction. This high adaptability of root systems to various and varying environments is mediated by hormonal control, which in turn responds to such environmental factors as nutrient imbalance, soil aeration, and mechanical impedance (Marschner 1986). For example, auxins derived from the shoot promote formation of root laterals whereas cytokynins derived from apical meristems of roots inhibit it. Indoleacetic acid strongly influences root hair formation (Tien et al. 1979), which is generally stimulated by conditions of low nutrient supply or factors inhibiting root extension.

Not only root morphology but also root/shoot ratios can vary considerably with resource availability; a greater edaphic resource limitation causes higher root/shoot ratios. This raises the question of the costs of investing a greater absorptive root area to enable procurement of limiting nutrients and other resources. For roots, this usually implies the costs of photosynthates for both construction and maintenance of root systems (Fitter 1991). It is sometimes argued that enhanced ability to invest in root biomass jeopardizes yield potential of above-ground portions. However, the large degree of phenotypic plasticity would minimize the threat of unnecessary partitioning to roots and, further, in environments where edaphic factors are limiting, growth yield potential cannot in any case be reached and thus greater investment in efforts to access such limiting resources should take precedence.

Mycorrhizae

The degree of infection, and thus additional absorptive area for nutrients, is affected by soil inoculum level and such soil factors as pH and temperature. Infection increases with an increase in the content of soluble carbohydrates in roots (Same et al. 1983) and root exudation of sugars (Azcon and Ocampo 1984). As mycorrhizae depend on photosynthates for growth and function, unfavorable above-ground environmental conditions reflect in depressed mycorrhizal growth (Same et al. 1983). Phosphorus deficiency in particular stimulates mycorrhizal proliferation, presumably by increasing the rate of exudation of reducing sugars and amino acids (Graham et al. 1981). However, it is the P concentration in the plant rather than that in the soil solution that enhances exudation and mycorrhizal development (Marschner 1986). As for increasing root absorptive area, increased mycorrhizal infection requires increased supply of photosynthates, which is considered an investment in accessing immobile soil nutrients in limiting supply.

Absorption mechanisms

Although the details of ATP metabolism are well understood, there is only limited understanding of the nature and synthesis of ion transport channel proteins. As discussed in the section on absorption mechanisms, active absorption of P and other nutrients is regulated by feedback mechanisms. An understanding of their operation will depend on further knowledge of how channel proteins are synthesized.

Exudates

Metabolic pathways for most of the amino acids, reducing sugars, and organic acids (e.g., malic and oxalic acids) that are excreted from roots are well understood. It is to be noted that exudation of these compounds is normally stimulated by stress. The composition and quantity of these exudates are modulated by the internal P levels. Deficiencies of certain mineral nutrients increase not only the total quantity of root exudates, but also the qualities of particular constituents. Potassium deficiency induces higher levels of organic acids in root exudates (Kraffczyk et al. 1984), whereas P deficiency leads to higher amounts of sugars, amino acids, and citric acid (Lipton et al. 1987, Dinkelaker et al. 1989). In alfalfa, Fe-deficiency stimulated the release of a novel compound, namely, 2-(3,5-dihydroxyphenyl)-5, 6-dihydroxybenzofuran, in root exudates, which had 62 times more capability of dissolving ferric phosphate than root exudates under Fe-sufficient conditions (Masaoka et al. 1993). Toxic aluminum (Al) concentrations in the ambient solution induce malic acid excretion, which in turn forms complexes with Al and prevents its uptake and also interferes with uptake of other ions (Delhaize et al. 1993). Thus, root exudation in general appears to be an adaptation

to stress. It is also a prerequisite to establishing effective symbioses with microorganisms. Metabolic pathways and their regulation by internal and external factors are yet to be determined for low molecular weight phenolic compounds exuded from roots and thought to be influencing P uptake, such as piscidic acid in the case of pigeonpea.

Genetic control and manipulation

This section examines the scope for genetic manipulation of root systems, mycorrhizal symbioses, P absorption mechanisms, and root exudates to enhance P acquisition by agriculturally important plants (with emphasis on legumes). A prerequisite to favorable genetic manipulation is the existence of, or potential to create (e.g., by mutation), adequate genetic variability for traits of interest. Desirable traits can then be utilized through traditional plant breeding approaches or by recently evolved molecular and cellular biological techniques for gene transfer. These options will be examined for their potential in enhancing P acquisition from sparingly available sources.

Root systems

Despite the large G x E interactions characteristic of plant root systems, it is possible to quantify genotypic variation in root systems (O'Toole and Bland 1987). This variation is largely multi- or polygenic, which makes it difficult to transfer desirable root characteristics into appropriate agronomic backgrounds through traditional breeding approaches (Tanksley et al. 1989, Zobel 1991). This is especially so because of the difficulties in screening for genotypic differences in root characteristics, whether in the field or under more controlled and uniform situations. Zobel (1991) suggested that problems of breeding for improved root systems could be minimized by using recessive single-gene root mutants, which are more amenable to genetic manipulation. However, he cautioned that pleiotropic effects are usually associated with such mutants.

Over half a century ago, it was demonstrated that genotypes absorbing higher amounts of P were characterized by a higher rate of root branching, and that both P absorption and root branching behaved as dominant traits in crosses (Smith 1934, Lyness 1936). Root hair length in *Trifolium repens* was found to be heritable, and it was positively correlated with phosphate uptake and plant growth in a P-deficient soil (Caradus 1979). It was subsequently demonstrated that P uptake is greater in plants selected for longer root hairs, but this effect was overridden in the presence of mycorrhizal infection (Caradus 1982). Hochmuth (1986) demonstrated that P uptake in tomato was greater in a hairy root mutant, due to increased numbers of root hairs. Factors affecting P accumulation were found to reside on chromosome 9 of maize (Naismith et al. 1974) and on three different chromosomes in rye (Graham 1982).

Although many genotypic differences in root morphology seem under multi- or polygenic control, Zobel (1991) suggested that this may be because of our incomplete understanding of the mechanism involved, and that such mechanisms could be under identifiably simpler genetic control. For example, root morphology is controlled by various plant growth hormones (e.g., auxin-mediated control of the formation of lateral roots) and detection of genes controlling particular steps of hormonal synthetic pathways may result in increased opportunities to manipulate root morphology.

Such molecular markers as isozymes, restriction fragment length polymorphism (RFLP), and the more recently described random amplified polymorphic DNA (RAPD), all have excellent potential for being used as tools for gene mapping of root characteristics or other traits associated with P uptake. The molecular maps produced by these markers provide the opportunity to resolve complex traits into their individual genetic components, making it feasible to identify, map, and measure the effects of genes underlying quantitative traits (Tanksley et al. 1989). Each quantitative trait loci (QTL) can be studied as a discrete entity, and its individual and interactive properties measured. Following this approach, linkage relationships of 9 isozymes, 1 RFLP marker, and 43 RAPD markers have been reported in an attempt to develop a complete gene map for *Vicia faba* (Torres et al. 1993). RFLPs have also been used to map QTLs in tomato for water use efficiency (Martin et al. 1989) and tolerance to low-P stress (Reiter et al. 1991). Isozyme-based analysis of QTLs has also been reported for maize (Kahler and Wehrhahn 1986, Edwards et al. 1987). Indirect selection for marker loci linked to QTLs of interest can enhance selection efficiency and help in the eventual cloning of loci underlying quantitative trait variation using chromosome-walking strategies (Paterson et al. 1991).

The possibility of inserting foreign DNA into plants provides a functional assay for gene activity and, with the development of large-scale genetic transformation techniques, many clones can potentially be screened for genetic activity (Potrykus 1991, Kung and Wu 1993). The dramatic progress made recently in the development of gene transfer systems for both dicots and monocots makes it possible to produce numerous transgenic plants containing desirable genes. Following this strategy, many genes have been cloned and transferred to diverse genotypes (Dale et al. 1993). These include marker genes, genes for herbicide resistance, insect resistance, virus cross-protection through viral coat protein genes, fungal disease resistance, environmental stress tolerance, and other genes involved in growth and development.

Mycorrhizae

The extent of root colonization by VAM varies with genotype (Smith et al. 1993), but the variation has not as yet been related to specific genes (Smith et al. 1992).

For example, Krishna et al. (1985) reported that root colonization by indigenous VAM fungi differed among genotypes of pearl millet and that the trait for VAM colonization is heritable. Assuming that the extent of VAM colonization correlates with enhanced uptake of P or other nutrients, the degree of VAM colonization can be used as an indicator of the level to which the VAM symbiosis can be manipulated, until specific genes exerting control over colonization are identified.

Certain biochemical activities correlated with VAM, such as acid phosphatase activity, may be potential criteria to breed for higher root colonization by VAM (Kesava Rao et al. 1990), but there are reports that VAM colonization and acid phosphatase activity are not always correlated (e.g., Rubio et al. 1990).

Absorption mechanisms

It is only recently that the molecular structure of ion transport systems in cell membranes is being elucidated. For example, it is now becoming possible to identify gene sequences associated with proteins of ion channels and H^+-ATPase (Smith et al. 1993, Tyerman and Schachtman 1993). However, Clarkson and Hawkesford (1993) point out that, as potential rates of operation of ion transporters are higher than their normal rates, genetic manipulation to enhance uptake of ions across the plasma membrane would serve little purpose. Enhanced uptake rates at ambient P concentrations approaching the threshold level, or operation of P transporters below existing thresholds, may present options for improvement. But this too could be of little overall significance as it would merely increase the rate of depletion of rhizosphere P to insignificant levels, thus hastening inevitable P deficiency.

Exudation

Genetic manipulation of root exudates to enhance P uptake holds promise because of the following reasons.

- Phosphorus is relatively immobile in the soil and any extension of rhizosphere limits, and increased solubilization within the rhizosphere, should favor P acquisition.
- There are many candidate exudates to choose from.
- In many cases, metabolic pathways of these exudates are known, and their genetic control is, or shows promise of being, understood.
- There are large differences among and within plant species in the type and amount of different compounds exuded (e.g., Hale et al. 1971).
- There is a possibility of manipulating root exudates to favor rhizosphere microorganisms beneficial to P mobilization.

Most recent work on characterizing genetic control of exudates affecting uptake of nutrient ions has been concerned with iron (Fe) acquisition (Romheld and Marschner 1984). For example, Okumura et al. (1991) isolated and characterized cDNA clones involved in Fe acquisition that include genes for mugineic acid-family phytosiderophores (MA) and Fe(III)-MAs. One such cDNA clone was named *Ids1* (iron deficiency-specific clone 1), which encodes a protein of 74 residues (7500 Da) and contains two cysteine-rich regions. The *Ids1* clone has been proposed to have a function at the regulatory region of MAs synthetic genes or Fe(III)-MAs transporter gene by conjugating with Fe^{2+}, similar to the Fur protein of Mori and Nishizawa (1987) and Shojima et al. (1990). The Fur protein is known to have a function at the promotor region of the synthetic genes of microbial siderophores or transporter gene of Fe(III)-siderophore conjugating with Fe^{2+} (Neilands 1990).

More recently, Mori et al. (1993) have constructed a λZAPII-cDNA library from Poly(A)$^+$-RNA isolated from Fe-deficient roots of *Hordeum vulgare.* Differential screening of barley roots grown with and without Fe with this cDNA library resulted in seven clones that hybridized specifically with the probe for Fe deficiency. One of these clones, which was completely sequenced and named *Ids2,* resembled genes for the 2-oxyglutarate-dependent dioxygenase, which needed ascorbate and Fe^{2+} as co-factors for the dehydration and oxidation of its substrates. The proposed role of *Ids2* gene is in the hydroxylation of deoxymugineic acid to mugineic acid or hydroxylation of mugineic acid to epihydroxy (or hydroxy) mugineic acid. This gene also has a Cu regulatory cis element of yeast MT gene, which is expressed under excess Cu. It has been speculated that *Ids2* in barley roots is also induced by the endogenous Cu, which is indirectly increased if Fe is deficient (Mori et al. 1993).

There are many options for genetic modification of rhizosphere pH. One is to alter relative uptake of such major cations and anions as NH_4^+ and NO_3^-, which could be done by modifying the channels for these ions. For example, Gahoonia et al. (1992) showed for rye grass that NH_4^+ nutrition, as compared to NO_3^- nutrition, favored mobilization of P in the rhizosphere. Rhizosphere pH could also be modified by adjusting exudation rates of organic or amino acids or by influencing H^+-ATPase activity.

To date, little is known concerning the genetic control of exudates that may specifically affect P mobilization, such as piscidic acid.

Towards a systematic research agenda

Fundamental to considering how best to approach favorable genetic manipulation of P acquisition by crop plants is reasonable quantification of candidate mechanisms. For example, Fitter (1991) discussed relative advantages of different root system configurations; Smith et al. (1993) indicated how to assess mycorrhizal

contribution to P acquisition; and Bar-Yosef (1991) calculated quantities of P released from soil through exudation of such specific organic acids as citrate. However, Bar-Yosef cautioned that improved quantification of P solubilization by root exudates would depend on further work on the following aspects.

- Better definition of the root exudate composition of different crops and control of exudate release in relation to phenology, physiology, and environmental influence.
- Better understanding of the interactions among root exudates, soil microorganisms, and soil constituents.
- Development of appropriate simulation models to evaluate the relative importance of chemical, physical, and biological factors in determining P availability to plants.

Once a quantitative basis is established, a 'marginal rates of return' approach is necessary to calculate by how much a proposed manipulation will actually increase P uptake and, ultimately, reflect in the economic value of a crop, or that of a subsequent crop if a residual effect of increasing P availability is envisaged. Most existing quantifications of root systems or exudate effects are restricted to the level of a single plant. However, it is necessary to scale up to the levels of a crop community, cropping system, and region. This will allow economic calculation of relative advantages of investments in P fertilizer production and distribution vis-a-vis research to increase the capability of crop plants to acquire sparingly soluble soil P.

Having established the potential gains from increasing P acquisition capabilities, and with appropriate accounting for costs and detrimental effects, the relative ease of genetic manipulation among candidate mechanisms needs to be evaluated. To alter root systems, traditional breeding approaches are difficult due to probable polygenic control, screening difficulties, and G x E effects. However, such breeding may enhance the probability and extent of infection by mycorrhizae (i.e., the reverse of screening for resistance to fungal diseases) or increase the production of an easily measurable root exudate. Use of genetic markers could alleviate some of the problems of breeding for root traits, but regular and reliable physiological characterization of root traits will continue to be necessary. Prospects for using gene transfer techniques will depend on such factors as the extent of genetic mapping achieved and the ability to transfer genes to target plants. In all cases, rigorous means of field validation of any intended improvements are essential.

Prioritization according to the scope for quantitative improvement and most promising methodology would also define the target crops and suggest laboratories best capable of undertaking a concerted research effort. Priorities should favor those crops normally grown in P-deficient soils but to which optimum rates of soluble fertilizers are unlikely to be added. In the case of

legumes, alleviation of P deficiency should normally also increase contributions of fixed N to the cropping system, thus making a double-edged contribution to macronutrient sustainability.

Most of the research referred to in this paper is component-research, done by separate laboratories with limited immediate research aims, even if a long-term aim of practical contribution to integrated nutrient management (INM) may have been a guiding force. In view of the increasing problems related to INM in cropping systems virtually throughout the world, and of this plethora of prospects for favourable manipulation of nutrient acquisition mechanism, the time now seems opportune for a concerted, time-bound effort to test the hypothesis that genetic manipulation can significantly contribute to INM. Thus, a consortium-approach involving interested laboratories with maximum comparative advantage is envisaged.

References

Ae, N., Arihara, J., Okada, K., Yoshihara, T., and Johansen, C. 1990. Phosphorus uptake by pigeon pea and its role in cropping systems of the Indian subcontinent. Science 248: 477-480.

Ae, N., Arihara, J., and Okada, K. 1991. Phosphorus uptake mechanisms of pigeonpea grown in Alfisols and Vertisols. Pages 91-98 *in* Phosphorus nutrition of grain legumes in the semi-arid Tropics. (Johansen, C., Lee, K.K., and Sahrawat, K.L., eds.). Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Ae, N., Arihara, K., Okada, K., Yoshihara, T., Otani, T., and Johansen, C. 1993. The role of piscidic acid secreted by pigeonpea roots grown in an Alfisol with low P-fertility. Pages 279-288 *in* Genetic aspects of plant mineral nutrition (Randall, P.J., Delhaize, E., Richards, R.A., and Munns, R., eds.). Dordrecht, The Netherlands: Kluwer Academic.

Al-Abbas, A.H., and Barber, S.A. 1964. A soil test for phosphorus based upon fractionation of soil phosphorus: I. Correlation of soil phosphorus fractions with plant-available phosphorus. Soil Science Society of America Proceedings 28: 218-221.

Arihara, J., Ae, N., and Okada, K. 1991. Root development of pigeonpea and chickpea and its significance in different cropping systems. Pages 183-194 *in* Phosphorus nutrition of grain legumes in the semi-arid tropics (Johansen, C., Lee, K.K., and Sahrawat, K.L., eds.). Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Azcon, R., and Ocampo, J.A. 1984. Effect of exudation on VA mycorrhizal infection at early stages of plant growth. Plant and Soil 82: 133-138.

Barber, D.A., and Martin, J.K. 1976. The release of organic substances by cereal roots into soil. New Phytologist 76: 69-80.

Barber, S.A. 1979. Growth requirement for nutrients in relation to demand at the root interface. Pages 5-20 *in* The soil root interface (Harley, J.L., and Scott-Russell, R., eds.). London: Academic Press.

Barrow, N.J. ed. 1993. Plant nutrition—from genetic engineering to field practice. Dordrecht, The Netherlands: Kluwer Academic. 806 pp.

Bar-Yosef, B. 1991. Root excretions and their environmental effects: influence on availability of phosphorus. Pages 529-557 *in* Plant roots: the hidden half (Waisel, Y., Eshel, A., and Kafkafi, U., eds.). New York, USA: Marcel Dekker.

Bassam, N.El., Dambroth, M., and Loughman, B.C. (eds.). 1990. Genetic aspects of plant mineral nutrition. Dordrecht, The Netherlands: Kluwer Academic. 560 pp.

Bekele, T., Cino, B.J., Ehlert, P.A.I., van der Maas, A.A., and van Diest, A. 1983. An evaluation of plant borne factors promoting the solubilization of alkaline rock phosphates. Plant and Soil 75: 361-378.

Bolan, N.S. 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant and Soil 134: 189-207.

Bottini, R., Fulchieri, M., Pearce, D., and Pharis, R.P. 1989. Identification of gibberellins A, A_3 and iso A_3 in cultures of *Azospirillum lipoterum.* Plant Physiology 90: 45-47.

Caradus, J.R. 1979. Selection for root hair length in white clover *(Trifolium repens* L.). Euphytica 28: 489-494.

Caradus, J.R. 1982. Genetic differences in the length of root hairs in white clover and their effect on phosphorus uptake. Pages 84-88 *in* Proceedings of the 9th International Plant Nutrition Colloquium (Scaife, A., ed.). Warwick, UK: Warwick University.

Chauhan, Y.S. 1993. Pigeonpea. Pages 79-91 *in* Rooting pattern of tropical crops (Salam, A., and Wahid, W.A., eds.). New Delhi, India: Tata McGraw Hill.

Chhonkar, R.K., and Subba Rao, N.S. 1967. Phosphate solubilization by fungi associated with legume root nodules. Canadian Journal of Microbiology 13: 749-753.

Clarkson, D.T., and Grignon, C. 1991. The phosphate transport system and its regulation in roots. Pages 49-62 *in* Phosphorus nutrition of grain legumes in the semi-arid tropics (Johansen, C., Lee, K.K., and Sahrawat, K.L., eds.). Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Clarkson, D.T., and Hawkesford, M.J. 1993. Molecular biological approaches to plant nutrition. Pages 23-33 *in* Plant nutrition—from genetic engineering to field practice (Barrow, N.J., ed.). Dordrecht, The Netherlands: Kluwer Academic.

Cooper, K.M., and Tinker, P.B. 1978. Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizae. II. Uptake and translocation of phosphorus, zinc and sulphur. New Phytologist 81: 43-52.

Dale, P.J., Irwin, J.A., and Scheffler, J.A. 1993. The experimental and commercial release of transgenic crop plants. Plant Breeding 111: 1-22.

Darrah, P.R. 1993. The rhizosphere and plant nutrition: a quantitative approach. Pages 3-22 *in* Plant nutrition—from genetic engineering to field practice (Barrow, N.J., ed.). Dordrecht, The Netherlands: Kluwer Academic.

Delhaize, E., Ryan, P.R., and Randall, P.J. 1993. An aluminium tolerance mechanism in wheat. Abstract no.26 *in* Abstracts of the Australian Society of Plant Physiologists 33rd Annual General Meeting, 27-30 Sep 1993. Perth, Australia: The University of Western Australia.

Dinkelaker, B., Romheld, V., and Marschner, H. 1989. Citric acid secretion and precipitation of calcium citrate in the rhizosphere of white lupin *(Lupinus albus* L.). Plant, Cell and Environment 12: 285-292.

Edwards, M.D., Stuber, C.W., and Wendel, J.F. 1987. Molecular-marker-facilitated investigations of quantitative trait loci in maize; 1. Numbers, genomic distribution and types of gene action. Genetics 116: 113-125.

Fallik, E., Okon, Y., Epstein, E., Goldman, A., and Fisher, M. 1989. Identification and quantification of IAA and IBA in *Azospirillum brasilense* inoculated maize root. Soil Biology and Biochemistry 21: 147-153.

FAO (Food and Agricultural Organization of the United Nations). 1993. Report of the regional expert consultation on the sustainability of the rice-wheat production system in different agro-ecological settings in Asia. RAPA (Regional office for Asia and Pacific) Report 1993/20. Bangkok, Thailand.

Fist, A.J.., Smith, F.W., and Edwards, D.G. 1987. External phosphorus requirements of five tropical grain legumes in flowing solution culture. Plant and Soil 99: 75-84.

Fitter, A.H. 1991. Characteristics and functions of root systems. Pages 3-25 *in* Plant roots: the hidden half (Eshel, A., and Kafkafi, U., eds.). New York, USA: Marcel Dekker.

Flach, E.N., Quak, W., and van Diest, A. 1987. A comparison of the rock phosphate mobilizing capacities of various crop species. Tropical Agriculture (Trinidad) 64: 347-352.

Gahoonia, T.S., Claassen, N., and Jungk, A. 1992. Mobilization of phosphate in different soils by ryegrass supplied with ammonium or nitrate. Plant and Soil 140: 241-248.

Gardner, W.K., Parbery, D.G., and Barber, D.A. 1981. Proteoid root morphology and function in *Lupinus albus.* Plant and Soil 60: 143-147.

Gardner, W.K., Barber, S.A., and Parbery, D.G. 1983. The acquisition of phosphorus by *Lupinus albus* L. III. The probable mechanism by which phosphorus movement in the soil/ root interface is enhanced. Plant and Soil 70: 107-124.

Gerdemann, J.W. 1975. Vesicular-arbuscular mycorrhizae. Pages 575-591 *in* The development and function of roots (Torrey, J.G., and Clarkson, D.T., eds.). New York, USA: Academic Press.

Graham, R.D. 1982. Breeding for nutritional characteristics in cereals. Advances in Plant Nutrition 1: 57-102.

Graham, J.H., Leonard, R.T., and Menge, J.A. 1981. Membrane mediated decrease in root exudation responsible for phosphorus inhibition of vesicular arbuscular mycorrhizae formation. Plant Physiology 68: 548-552.

Hale, M.G., Foy, C.L., and Shay, F.J. 1971. Factors affecting root exudation. Advances in Agronomy 23: 89-109.

Haynes, R.J. 1990. Active ion uptake and maintenance of cation anion balance: A critical examination of their role in regulating rhizosphere pH. Plant and Soil 126: 247-264.

Hendrix, J.E. 1967. The effect of pH on the uptake and accumulation of phosphate and sulfate ions by bean plants. American Journal of Botany 54: 560-564.

Hochmuth, G J. 1986. A gene affecting tomato root hair production in solution culture. Reports of Tomato Genetic Cooperative 36: 4-6.

Hoffland, E., Van den Boogard, R., Nelemans, J., and Findenegg, G. 1992. Biosynthesis and root exudation of citric and malic acids in phosphate starved rape plants. New Phytologist 122: 675-680.

Irizarry, H., and Rivera, E. 1983. Nutrient uptake and dry matter accumulation by intensively managed pigeonpeas grown on a corozal clay, an Ultisol. Journal of Agricultural Science (Puerto Rico) 67: 188-196.

Itoh, S. 1987. Characteristics of phosphorus uptake of chickpea in comparison with pigeonpea, soybean, and maize. Soil Science and Plant Nutrition 33: 417-422.

Jacobson, I., Abbott, L.K., and Robson, A.D. 1992. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. New Phytologist 120: 371-380.

Kahler, A.L., and Wehrhahn, C.F. 1986. Association between quantitative traits and enzyme loci in the F_2 population of a maize hybrid. Theoretical and Applied Genetics 72: 15-26.

Kesava Rao, P.S., Tilak, K.V.B.R., and Arunachalam, V. 1990. Genetic variation for VA mycorrhiza-dependent phosphate mobilisation in groundnut *(Arachis hypogaea* L.). Plant and Soil 122: 137-142.

Khasawneh, F.E., and Doll, E.C. 1978. The use of phosphate rock for direct application of soils. Advances in Agronomy 30: 159-206.

Koala, S., Sims, J.R., El-Altar, H., and El-Halfawi, M. 1988. Phosphorus deficiency in the semi-arid tropics and implications for grain legume production. Pages 205-216 *in* World crops: cool season legumes (Summerfield, R.J., ed.). London, UK: Kluwer Academic.

Kraffczyk, I., Beringer, H., and Trolldenier, G. 1984. Soluble root exudates of maize: Influence of potassium supply and rhizosphere microorganisms. Soil Biology and Biochemistry 16: 315-322.

Krishna, K.R., Shetty, K.G., Dart, P.J., and Andrews, D.J. 1985. Genotypic dependant variation in mycorrhizal colonization and response to inoculation of pearl millet. Plant and Soil 86: 113-125.

Kung, S.D., and Wu, R. 1993. Transgenic plants, vol. 1. Engineering and utilization. London, UK: Academic Press. 383 pp.

Lamont, B. 1982. Mechanisms for enhancing nutrient uptake, with particular reference to Mediterranean South Africa and Western Australia. Botanical Review 48: 597-689.

Lee, K.K., and Wani, S.P. 1991. Possibilities for manipulating mycorrhizal associations in crops. Pages 107-116 *in* Phosphorus nutrition of grain legumes in the semi-arid tropics (Johansen, C., Lee, K.K., and Sahrawat, K.L., eds.). Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Lipton, D.S., Blanchar, R.W., and Blevins, D.G. 1987. Citrate, malate and succinate concentration in exudates from P-sufficient and P-starved *Medicago sativa* L. seedlings. Plant Physiology 85: 315-317.

Lyness, A.S. 1936. Varietal differences in the phosphorus feeding capacity of plants. Plant Physiology 11: 665-688.

Marschner, H. 1986. Mineral nutrition of higher plants. London, UK: Academic Press. 456 pp.

Marschner, H., and Romheld, V. 1983. *In-vivo* measurement of root induced pH changes at the soil root interface: effects of plant species and nitrogen source. Zeitschrift fur Pflanzenphysiologie 111: 241-251.

Marschner, H., Rdmheld, V., and Kissel, M. 1987. Localization of phytosiderophore release and of iron uptake along intact barley roots. Physiologia Plantarum 71: 157-162.

Martin, B., Neinhuis, J., King, G., and Schaefer, A. 1989. Restriction fragment length polymorphism associated with water use efficiency in tomato. Science 243: 1725-1728.

Masaoka, Y., Kojima, N., Sugihara, S., Yoshihara, T., Koshino, M., and Ichihara, A. 1993. Dissolution of ferric phosphate by alfalfa *(Medicago sativa* L.) root exudates. Pages 79-82 *in* Plant nutrition—from genetic engineering to field practice (Barrow, N.J., ed.). Dordrecht, The Netherlands: Kluwer Academic.

Mori, S., and Nishizawa, N. 1987. Methionine as a domain precursor of phytosiderophores in Graminaceae plants. Plant and Cell Physiology 28: 1081-1092.

Mori, S., Okumura, N., Nakanishi, H., Umehara, Y., Chino, M., and Nishizawa, N.K. 1993. Expression of a gene *(Ids 2)* induced by Fe deficiency treatment in the roots of *Hordeum vulgare* L. Pages 147-150 *in* Plant nutrition—from genetic engineering to field practice (Barrow, N.J., ed.). Dordrecht, The Netherlands: Kluwer Academic.

Mosse, B. 1981. Vesicular arbuscular mycorrhizae research for tropical agriculture. Research Bulletin of Hawaii Institute of Tropical Agriculture and Human Resources. Honolulu, Hawaii, USA: University of Hawaii.

Mulette, K.L., Hannon, N.J., and Elliott, A.G.L. 1974. Insoluble phosphorus usage by Eucalyptus. Plant and Soil 41: 199-205.

Naismith, R.W., Johnson, M.W., and Thomas, W.I. 1974. Genetic control of relative calcium, phosphorus and manganese accumulation on chromosome 9 in maize. Crop Science 14: 845-849.

Neilands, J.B. 1990. Molecular aspects of regulation of high affinity iron absorption in microorganisms. Pages 63-90 *in* Metal-ion induced regulation of gene expression (Eichhoru, G.L. and Marzilli, L.G., eds.). Amsterdam, The Netherlands: Elsevier.

Nye, P.H., and Tinker, P.B. 1977. Solute movement in the soil-root system. Oxford, UK: Blackwell. 342 pp.

Okumura, N., Nishizawa, N.K., Umehara, Y., and Mori, S. 1991. An iron deficiency-specific cDNA from barley roots having two homologous cysteine-rich MT domains. Plant Molecular Biology 17: 531-533.

O'Toole, J.C., and Bland, W.L. 1987. Genotypic variation in crop plant root systems. Advances in Agronomy 41: 91-141.

Paterson, A.H., Tanksley, S.D., and Sorrells, M.E. 1991. DNA markers in plant improvement. Advances in Agronomy 46: 40-90.

Poole, R J. 1978. Energy coupling for membrane transport. Annual Review of Plant Physiology 29: 437-460.

Potrykus, I.1991. Gene transfer to plants: Assessment of published approaches and results. Annual Review of Plant Physiology and Molecular Biology 42: 205-225.

Randall, P J., Delhaize, E., Richards, R.A., and Munns, R. 1993. Genetic aspects of plant mineral nutrition. Dordrecht, The Netherlands: Kluwer Academic. 416 pp.

Reiter, R.S., Coors, J.G., Sussman, M.R., and Gabelman, G.H. 1991. Genetic analysis of tolerance to low phosphorus stress in maize using restriction fragment length polymorphism. Theoretical and Applied Genetics 82: 561-568.

Romheld, V., and Marschner, H. 1984. Plant induced pH changes in the rhizosphere of Fe-efficien and Fe-inefficient soybean and corn cultivars. Journal of Plant Nutrition 7: 623-630.

Rovira, A.D., Bowen, G.D., and Foster, R.C. 1983. The significance of rhizosphere microflora and mycorrhizas in plant nutrition. Pages 61-89 *in* Encyclopedia of Plant Physiology, New Series Vol. 15A (Lauchli, A., and Bieleski, R.L., eds.). Berlin, Germany: Springer-Verlag.

Rubio, R., Moraga, E., and Borie, F. 1990. Acid phosphatase activity and vesicular arbuscular mycorrhizal infection associated with roots of four wheat cultivars. Journal of Plant Nutrition 13: 585-598.

Same, B.I., Robson, A.D., and Abbott, L.K. 1983. Phosphorus, soluble carbohydrates and endomycorrhizal infection. Soil Biology and Biochemistry 15: 593-597.

Shojima, S., Nishizawa, N.K., Fushiya, S., Nozoe, S., Irifune, T., and Mori, S. 1990. Biosynthesis of phytosiderophores. *In vitro* biosynthesis of 2'-deoxy-mugineic acid from 1-methionine and nicotianamine. Plant Physiology 93: 1497-1503.

Smith, S.N. 1934. Response of inbred lines and crosses in maize to variations of nitrogen and phosphorus supplied as nutrients. Journal of the American Society of Agronomy 26: 785-804.

Smith, S.E., and Gianinazzi-Pearson, V. 1988. Physiological interactions between symbionts in vesicular arbuscular mycorrhizal plants. Annual Review of Plant Physiology and Plant Molecular Biology 39: 221-244.

Smith, S.E., Robson, A.D., and Abbott, L.K. 1992. The involvement of mycorrhizas in assessment of genetically dependent efficiency of nutrient uptake and use. Plant and Soil 146: 169-179.

Smith, F.W., Hawkesford, M J., Prosser, I.M., and Clarkson, D.T. 1993. Approaches to cloning genes encoding for nutrient transportation in plants. Pages 151-154 *in* Plant nutrition—from genetic engineering to field practice (Barrow, N.J., ed.). Dordrecht, The Netherlands: Kluwer Academic.

Tandon, H.L.S. 1987. Phosphorus research and agricultural production in India. New Delhi, India: Fertilizer Development and Consultation Organization. 160 pp.

Tanksley, S.D., Young, N.D., Paterson, A.H., and Bonierbale, M.W. 1989. RFLP mapping in plant breeding: new tools for an old science. Biotechnology 7: 257-264.

Tester, M. 1990. Plant ion channels: whole cell and single channel studies. New Phytologist 114: 305-340.

Thompson, J.P. 1991. Improving the mycorrhizal condition of the soil through cultural practices and effects on growth and phosphorus uptake by plants. Pages 117-137 *in* Phosphorus nutrition of grain legumes in the semi-arid tropics (Johansen, C., Lee, K.K., and Sahrawat, K.L., eds.). Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Tien, T.M., Gaskins, M.H., and Hubbell, D.H. 1979. Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet *(Pennisetum americanum* L.). Applied and Environmental Microbiology 37: 1016-1024.

Tinker, P.B. 1984. The role of microorganisms in mediating and facilitating the uptake of plant nutrients from soil. Plant and Soil 76: 77-91.

Torres, A.M., Weeden, N.F., and Martin, A. 1993. Linkage among isozyme, RFLP and RAPD markers in *Vicia faba.* Theoretical and Applied Genetics 85: 937-945.

Tyerman, S.D., and Schachtman, D.P. 1992. The role of ion channels in plant nutrition and prospects for their genetic manipulation. Plant and Soil 146: 137-144.

van Beuscichem, M.L., Kirleby, E.A., and Baas, R. 1988. Influence of nitrate and ammonium nutrition on the uptake, assimilation, and distribution of nutrients in *Ricinus communis.* Plant Physiology 86: 914-921.

Zobel, R.W. 1991. Genetic control of root systems. Pages 27-38 *in* Plant roots: the hidden half (Waisel, Y., Eshel, A., and Kafkafi, U., eds.). New York, USA: Marcel Dekker.

Genotypic differences in phosphate uptake

P J Randall[1]

Abstract

Phosphorus (P) is a key nutrient in agricultural production systems. Phosphorus is a valuable and scarce resource and must be used efficiently. Also, excess P accumulation can cause detrimental effects in some ecosystems. While improved management and agronomy are important, there appear to be opportunities to select and breed plants that utilize P more efficiently. This paper discusses some plant characteristics that might influence P acquisition by plants, namely, physical and P uptake characteristics of the roots and the influence of roots on P availability in the rhizosphere. It explores the question—Do crop and pasture species and cultivars differ in the efficiency with which they acquire P?—and examines the extent of genetic variation.

Experimental evidence and models of P uptake indicate the importance of root properties that affect the surface area of the root for absorption and the volume of soil available for depletion. Root hairs are important for P uptake but their effect can be negated by mycorrhizae. Characteristics that determine uptake kinetics of the roots are probably of little importance when the level ofP in the soil solution is high, but their importance at low P is still controversial. Plant processes that modify the rhizosphere and, either directly or through microbial action, alter the concentration of P in soil solution may have a considerable influence on P uptake, particularly if they increase the solubilization rate of P-containing compounds in the soil. Modifications to the rhizosphere include changes in pH and exudation of chelates, organic acids, and other organic molecules including phosphatases. Research is needed to quantify the effects of rhizosphere modification on P availability. An understanding of the mechanisms and the genetic controls involved is required to allow manipulation and selection in plant improvement programs.

1. CSIRO Division of Plant Industry, G P 0 Box 1600, Canberra, ACT 2601, Australia.

Randall, P.J. 1995. Genotypic differences in phosphate uptake. Pages 31-47 *in* Genetic manipulation of crop plants to enhance integrated nutrient management in cropping systems—1. Phosphorus: proceedings of an FAO/ICRISAT Expert Consultancy Workshop, 15-18 Mar 1994, ICRISAT Asia Center, India (Johansen, C., Lee, K.K., Sharma, K.K., Subbarao, G.V., and Kueneman, E.A., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Introduction

Soils vary greatly in the levels of phosphorus (P) in the soil solution, the fraction directly accessible to plants. They vary both in the rates at which the solution P is replenished from labile forms following plant uptake, and in the quantities of P present in those labile forms. Some soils supply adequate P for plant growth due to inherently high available P levels or because of the residual value of past applications of P-containing materials. Farmers blessed with such soils can ignore the P problem and concentrate on optimizing the supply of other nutrients. Unfortunately, P supply is a key issue for productivity for most farmers (Stangel and von Uexkull 1990).

On soils inherently low in plant-available P, the preferred option is to correct the deficiency by supplying P-containing fertilizer to boost production of crops and pastures. In legume-based systems, application of P is doubly beneficial in terms of increasing yields and enhanced nitrogen (N) fixation by the legume, thus improving the N status of the system. However, fertilizer P application represents a considerable input cost to farmers and in many agricultural systems its use is precluded on economic grounds.

The use of P-efficient plants to improve yields on P-deficient soils is a second option. An example of such an approach is reported by Ae et al. (1993), who showed that the 'efficient' pigeonpea could acquire P and produce a reasonable yield on a soil where the 'inefficient' sorghum failed without P fertilizer. Clearly, such an approach involving continued removals of nutrients in produce without replacement cannot be sustained indefinitely. However, plant-available P is generally a small fraction of total soil P and the amount of P removed in produce is a smaller fraction still. Graham (1978) illustrated this point with calculations for wheat-growing soils which, in their top 1-m layer, contained P in quantities that were equivalent to those removed in the grain of 1250 wheat crops and yet were highly P-responsive. In fertilized soils, part of the unavailable P is derived from previous fertilizer applications that have reacted with soil minerals. For example, the P budget for Australian farms shows that the P removed in produce is about one-third that applied annually in fertilizer (McLaughlin et al. 1991), indicating that P is accumulating. There appears to be scope for seeking agricultural plants with superior abilities to acquire normally unavailable P. Their use should be combined with agronomic measures to recycle and conserve P and minimize the removal of P in produce.

Even where P fertilizer application is economic, P-efficient plants may allow farmers to optimize productivity with lower annual or cumulative applications of P, or to use low-grade P fertilizer materials. This would have immediate economic benefits and would conserve resources of high-grade phosphate rock. It is also important to recognize the potential damage to fragile ecosystems in certain

catchments (Hodgkin and Hamilton 1993) that can arise from excessive use of P fertilizers on agricultural land.

There has been considerable work at the physiological level aimed at understanding the complex of factors that determine P efficiency in plants (reviews by Gerloff and Gabelman 1983, Blair 1993). Plant breeding and selection programs for P efficiency are in progress, e.g., for clover (Dunlop et al. 1992) and for beans (Thung 1992). Recently, mutants affecting P uptake and transport have been sought in the model plant *Arabidopsis thaliana* (Delhaize et al. 1993a). Comparisons of such mutants with the 'wild type' should further enhance our understanding of the physiology and genetics of P efficiency. Understanding P efficiency at the physiological and molecular levels (Goldstein 1991) should assist in developing and refining selection criteria for plant improvement programs. At the genetic level, there is information to suggest that progress may be possible in breeding for characters conferring P efficiency (Caradus, these proceedings). This paper explores the question of P efficiency with emphasis on the ability of different plant species and cultivars to acquire P from the soil.

Definitions of P efficiency

There is no general agreement as to what constitutes P-efficiency in agriculture, because any definition is likely to be specific to particular agricultural systems, soils, and plant products.

The efficient use of P in the broad agricultural context may depend on a multitude of factors such as better prediction of P fertilizer requirements, the form of P fertilizer applied, and whether ammonium fertilizer is applied at the same time, method of application (banding, placement, or broadcast), the elimination of nutrient and other limitations to growth, interactions with soil moisture, the timing of P fertilizer application in the rotation, the retention of crop residues, grazing intensity of pastures, and the rate of cycling of P in soils. The relative importance of these factors is likely to vary markedly with soil characteristics, climate, fertilizer history, and the agricultural system. However, in high input systems, agronomic management must also take into account the differences in P requirement of different agricultural plants; in low-input systems such differences may be crucial.

An agronomic definition of plant P efficiency relates plant yield to the nutrient supply (Craswell and Godwin 1984):

P efficiency = plant yield/P supply

Defined in this way, P efficiency depends on two interrelated groups of plant factors that determine uptake efficiency: uptake of P relative to supply (the subject of this paper) and utilization efficiency (dry matter production per unit of P taken up). Efficiency should be distinguished from responsiveness (the capacity to

increase uptake and yield as nutrient supply increases). Responsive plants would be most desirable in fertilized, high-input systems, while P-efficient plants, which produce high yields at low levels of P, are likely to be most valuable in low-fertility situations. Combining responsiveness and efficiency characteristics in one genotype is, however, a realistic breeding objective (Blair 1993).

There are considerable difficulties in devising specific selection criteria for P efficiency in breeding programs. This is partly because the definition, even for a single crop species, will vary depending on the context in which the plant is to be grown, as illustrated for wheat by Jessop et al. (1983). In addition, while the general principles governing P acquisition and utilization have been described, the relative importance of particular processes under different circumstances is unclear. Some of the difficulties in reconciling various aspects of P efficiency were discussed by Blair (1993), who listed 18 operational definitions of aspects of P efficiency including efficiency of both uptake and utilization. He used them to rank seven ecotypes and cultivars of white clover. The rank position of any genotype varied considerably depending on the definition. The problem of developing effective selection criteria to distinguish P efficiency from general plant vigor is discussed by Gourley (1994), who suggests comparisons of lines at nonlimiting nutrient supply levels as well as under low-nutrient conditions.

Do crop and pasture species and cultivars differ in the efficiency with which they acquire phosphorus?

It is a truism that different plant species require different amounts of P to achieve maximum yields. There is abundant evidence (Fohse et al. 1988) to show that marked differences exist between species in external P requirement (P applied to produce 90% maximum yield). In some experiments, species have been shown to maintain their relative differences at both high and low P supply. For example, McLachlan (1976) found that buckwheat obtained more P than rye, crimson clover, or subterranean clover at all levels of available P in two soils. In other cases, relative efficiency varies with P supply level or the soil. Caradus (1980) reported that pasture grasses were in general more productive than legumes at low soil P, while legumes were more responsive to P and tended to outyield grasses at higher P. Ae et al. (1991) recorded that on a low-P Alfisol, pigeonpea was superior in P uptake to chickpea or sorghum but on a low-P Vertisol, pigeonpea took up less soil P.

As well as differences among species, substantial variation among genotypes within a species for P uptake is known to occur, e.g., among cultivars of barley (Table 1).

Table 1. P uptake in 60 days by barley cultivars growing in the field.

Cultivar	P uptake (kg ha^{-1})
Salka	11.5
Nurenberg	11.0
Lofa	9.8
Mona	6.5
Zita	6.5
Rupal	5.5

Source: Nielsen and Schjorring (1983).

However, in many cases, differences among cultivars appear to be smaller than those among species, and errors associated with measurement of P efficiency, especially in soil experiments, often make it difficult to demonstrate the differences among cultivars experimentally. Spencer et al. (1980), in a detailed study, found some numerically large differences in external P requirement among eight genotypes each of Caucasian clover and white clover but the differences were not significant. Nevertheless, considerable differences have been found in studies involving other crops and a selection of these is summarized in Table 2.

General considerations affecting acquisition of P by plants from soils

The factors affecting P acquisition by plants have been well reviewed (Clarkson 1985) and the processes described and analyzed by mathematical models (Barber and Silberbush 1984, Amijee et al. 1991). Figure 1 shows a schematic diagram of the rhizosphere illustrating some of the major factors and processes to be considered.

The soil solution is the immediate source of P for plants (Figure 1). Soil solution concentrations of phosphate (Pi) are generally low, in the order of 1-5 μM. The mean diffusion rate of Pi in the soil solution is probably one-hundredth the rate of a mobile nutrient such as nitrate (Amijee et al. 1991). Uptake at the root surface rapidly depletes the Pi in the adjacent soil solution, creating a diffusion gradient in the rhizosphere. The rate of uptake is then controlled by the effective diffusion rate to the root. The P in the soil solution is in dynamic equilibrium with labile P fractions consisting mainly of phosphates adsorbed to surfaces of clay minerals, Fe and Al minerals, hydrous oxides, and carbonates. Microbes in the rhizosphere also compete for Pi in the soil solution and may solubilize unavailable soil P. The role of organically bound P in supplying P to plants may also be important.

Genotypic differences in ability to acquire soil P may arise from (1) differences in the effective absorbing area, (2) differences in absorption characteristics at the

Table 2. Selected information on genotypic differences in P acquisition efficiency in a range of crop and pasture plants.

Species	Genotypes	Measure of efficiency	Differences	Reference
Crop/pasture species				
Caucasian clover White clover	8 ecotypes of each species	Applied P (mg kg^{-1}) level for 90% maximum yield (soil in pots)	Differences not significant	Spencer et al. (1980)
White clover Lucerne	2 lines, 2 cultivars	External P for 80% maximum yield (solution)	19-32 um 15-29 urn	Gourley (1994)
Wheat	semi-dwarf	dry wt (soil in pots)	5.0-10.6 mg g^{-1} at low P	Jones(1989)
Barley	7 cultivars	Net P influx rate, plant dry wt basis (soil)	190-260 pmols s^{-1} g^{-1}	Nielsen and Schjorring (1983)
Lettuce	15 cultivars	P in shoots g^{-1} root dry wt (sand/alumina)	No differences found	Buso and Bliss (1988)
Maize	12 inbred and hybrid lines	Net P influx rate, root length basis (solution)	0.31-0.68 pmole s^{-1} cm^{-1}	Baligar and Barber (1979)
Wild species				
Plantago major	3 popula-tions, with and without VAM	P uptake rate, root fresh weight basis (soil)	1.7-2.0, 0.8-1.0 umoles $d^{-1}g^{-1}$	Baas and van Beusichem (1990)
Arabidopsis thaliana	25 genotypes	p uptake rate, root length basis (solution)	0.13-0.24 nmoles h^{-1} cm	Krannitz et al. (1991)

root surface, or (3) differences in the way roots interact with soil to alter the P supply.

Genotypic differences in physical root characteristics

Length and branching

Because of the low mobility of P, larger root systems with a greater surface are advantageous for acquiring P, especially from low-P soils (Barber and Silberbush 1984). For most agricultural crops, morphological characteristics and rates of growth of roots are likely to be the primary determinants of P uptake from such soils. Sensitivity analysis using mechanistic models has shown the importance of root growth rate and root radius in P acquisition (Barber and Silberbush 1984), and this has been confirmed in many studies. For example, differences in root length accounted for a large part of the differences in P uptake among six barley cultivars (Nielsen and Schjorring 1983), referred to in Table 1. Fawole et al. (1982) found

highly heritable differences in root weight and root/shoot ratio among lines of *Phaseolus,* with a P-efficient line able to maintain large roots under both low- and high-P conditions. More recent work at the Centro Internacional de Agricultura Tropical (CIAT) in Colombia has indicated that 'root growth and architecture may be quite variable in beans and are significantly related to P acquisition' (CIAT 1989).

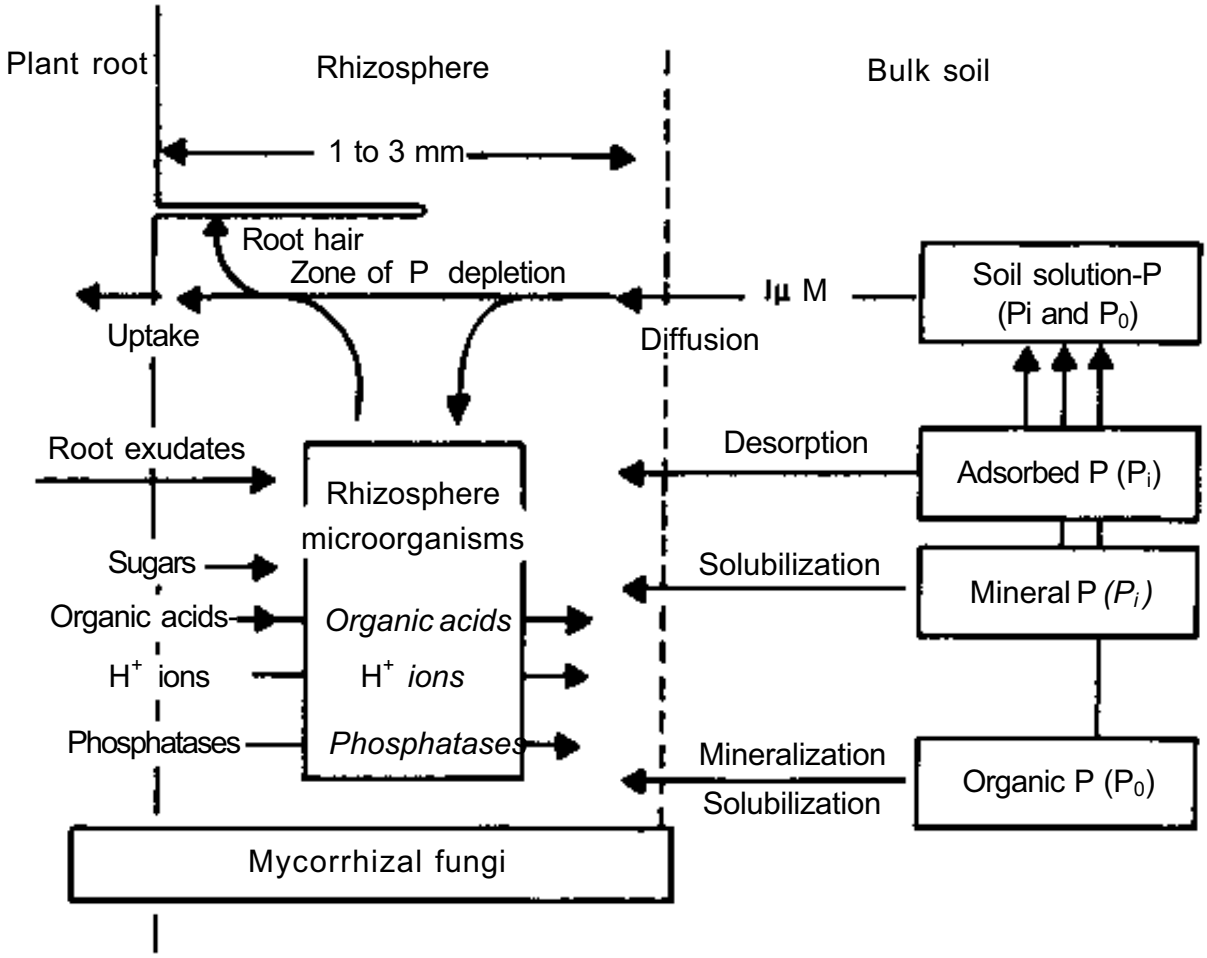


Figure 1. Schematic diagram of the rhizosphere illustrating some of the processes affecting phosphorus acquisition by plants (adapted from Richardson 1994).

Root hairs

The importance of root hairs has been shown in simulation models of P uptake (Barber and Silberbush 1984). Fohse et al. (1991) argued that root hairs made a large contribution to P uptake due to (1) increased total surface area of the root, (2) increased volume of soil exploited, and (3) their small radius, which increases the amount of nutrient in close proximity to the absorbing surface. Consequently, Pi concentrations at the surface of root hairs would be depleted more slowly than those at the root surface, allowing higher P influx rates. Differences among species

in maximum influx rates would thus become more significant the greater the proportion of P taken up via root hairs. We may have to consider root uptake characteristics when uptake through root hairs becomes significant and soils are low in P.

Using selections from white clover with different root-hair-lengths, Caradus (1981) showed that the line with longer root hairs had higher shoot P contents but lower P uptake/unit root weight when the roots were nonmycorrhizal. Infection with mycorrhizae tended to even out differences between the selections.

Interactions between P supply, mycorrhizae, and root morphology

Evaluation of genotypic differences in root architecture on P uptake is complicated by marked interactions with environmental factors. The pattern of distribution of roots in soil may be of little consequence if P is distributed uniformly. However, localized higher concentrations of Pi will occur around fertilizer granules and mineralizing dung and plant material, and root systems tend to proliferate in moist P-rich soil zones with marked effects on plant performance (Simpson and Pinkerton 1989).

Hackett (1968) showed that low P caused barley to increase its root/shoot ratio, decrease number and volume of root axes, and develop five times the number of second-order laterals. Some plants experiencing P stress may redirect resources to root growth in a well regulated manner (Smith et al. 1990), which may be a crucial factor in determining P efficiency.

The importance of mycorrhizae in P nutrition is well documented (Thompson 1991, Smith et al. 1993). Mycorrhizae can increase P uptake 3- to 4-fold or more in soils with low available P and mycorrhizal status must be taken into account when comparing plants for P efficiency (Smith et al. 1993). For example, six cultivars of *Medicago sativa* differed in their response to P when infected with mycorrhizae but were similar in their response to P when they were nonmycorrhizal and the effect of mycorrhizae changed with P level (Lambert et al. 1980). Mycorrhizae override the advantage of longer root hairs in P uptake in white clover (Caradus 1981). The level of infection depends on P status and the infection propagule density in the soil, which in turn is affected by previous crops in the rotation (Thompson 1991). Previous cropping and fallow management can thus influence P efficiency through their effects on mycorrhizae.

Root uptake characteristics

The capacity of the root to take up P considerably exceeds the diffusion rate towards the root, and differences in root uptake characteristics usually have little practical effect on P acquisition. Phosphorus uptake is normally around 20% of the

potential rate (Clarkson and Grignon 1991), rising to full capacity as plant P status declines. Sensitivity analysis using models also shows that differences in uptake characteristics are likely to have only minor influence (Amijee et al. 1991). However, we may have to reconsider the importance of differences in uptake characteristics when considering uptake by root hairs (Fohse et al. 1991), as discussed earlier.

Substantial differences among genotypes have been shown in many studies for the uptake parameters, namely, maximum influx rate (Imax), Michaelis-Menten constant (Km), and the minimum solution concentration at which influx is balanced by efflux (Cmin). An example is given in Table 3, which shows a 10-fold or more range in the three parameters measured for maize lines and hybrids grown in nutrient solution. Difficulties in interpreting measured values for such kinetic parameters have been discussed by Rengel (1993). Values are strongly dependent on the method of measurement, and they vary with temperature and nutrient status of the plant. Their significance for P efficiency is difficult to test in uptake models for such a diffusion-limited nutrient as P, as discussed above.

Table 3. Data for selected plant P uptake parameters for 12 maize genotypes.

Parameter	Units	Range	Mean
Maximum influx rates (Imax)	pmole s^{-1} cm	0.09- 0.89	0.27
Michaelis-Menten constants (Km)	mmole m^{-3}	1.0 -11.6	4.1
Minimum concentrations for net uptake (Cmin)	mmole m^{-3}	0.2 - 3.5	1.8

Source: Baligar and Barber (1979).

Rhizosphere nutrient mobilization by roots

Changes in rhizosphere pH

Plants absorbing excess cations over anions lower the pH of their rhizosphere, which in turn affects the solubility of P and its uptake by plants (Hedley et al. 1983). A further consequence of excess cation uptake could be the depletion of soil solution Ca which, in soils containing calcium phosphates or rock phosphates, could increase solution P concentrations (Bekele et al. 1983).

McLachlan (1976) found that P uptake and total cation uptake were well correlated. The slope of the regression line was similar for two genotypes each of clover and rye but was different for buckwheat, which was more efficient at taking up both cations and P and acidifying its rhizosphere. Recently, Fohse et al. (1991) reported no correlation between P uptake and excess cations or Ca uptake in seven species grown in an alkaline soil. There have been few examinations of differences

within species in excess cation content in relation to its effects on P uptake. Jarvis and Robson (1983) grew 10 cultivars of subterranean clover in an acid soil. They showed that the cultivars differed significantly in excess cation uptake and this was negatively correlated ($r = -0.82$, $P < 0.01$) with soil pH at harvest. Total P uptake increased with increasing cation excess and calculations from their data showed a significant correlation ($r = -0.57$, $P < 0.05$) (Figure 2). Further studies are warranted to assess the likely magnitude of intraspecific differences in cation excess and effects on dissolution of poorly soluble P sources, e.g., rock phosphate (Bekele et al. 1983).

Organic acid excretion

Organic excretions from roots may increase P uptake by increasing Pi in the soil solution through effects on desorption of P or through complexing cations that co-precipitate with P. The effects will depend on the forms of labile P present in the soil, soil buffer capacity, and the chemical properties and concentration of the exudate. Bar-Yosef (1991) provides a comprehensive discussion of the likely effects of different classes of root exudate.

The most extensively studied example of enhanced P uptake correlated with organic acid excretion is that of white lupin. Gardner et al. (1983) and Marschner et al. (1986) showed that white lupin develops cluster roots in low-P conditions and that citrate excretion from these structures can solubilize P in the rhizosphere and increase its uptake by the plant. Wheat plants growing adjacent to the lupin plants also benefitted from the increased P availability (Gardner and Boundy 1983). Localized excretion of citrate and malate from P-deficient roots of oilseed rape can solubilize the P bound to Ca in rock phosphate (Hoffland 1992). Piscidic acid is thought to assist in the acquisition of P bound to Fe in Alfisols (Ae et al. 1993).

Organic exudates from plant roots can stimulate nitrogen fixation (Christiansen-Weniger et al. 1992) and other microbiological processes in the rhizosphere (Richardson 1994) that may indirectly affect P availability to plants.

Intraspecific differences in organic acid excretion are known; e.g., wheat lines that differ in excretion of malic acid from the root tips in response to Al. The character is inherited effectively as a single gene and near-isogenic lines have been described (Delhaize et al. 1993b). But there appears to be little work on intraspecific variation in organic acid excretion in relation to P availability.

Phosphatases

There is mounting evidence that root phosphatases play a part in P acquisition. For example, Tarafdar and Claassen (1988) showed that roots of clover hydrolyzed phytate and other organic P compounds at rates exceeding that of P uptake by the root In addition to releasing Pi from soil organic P, they may hydrolyze ester-P

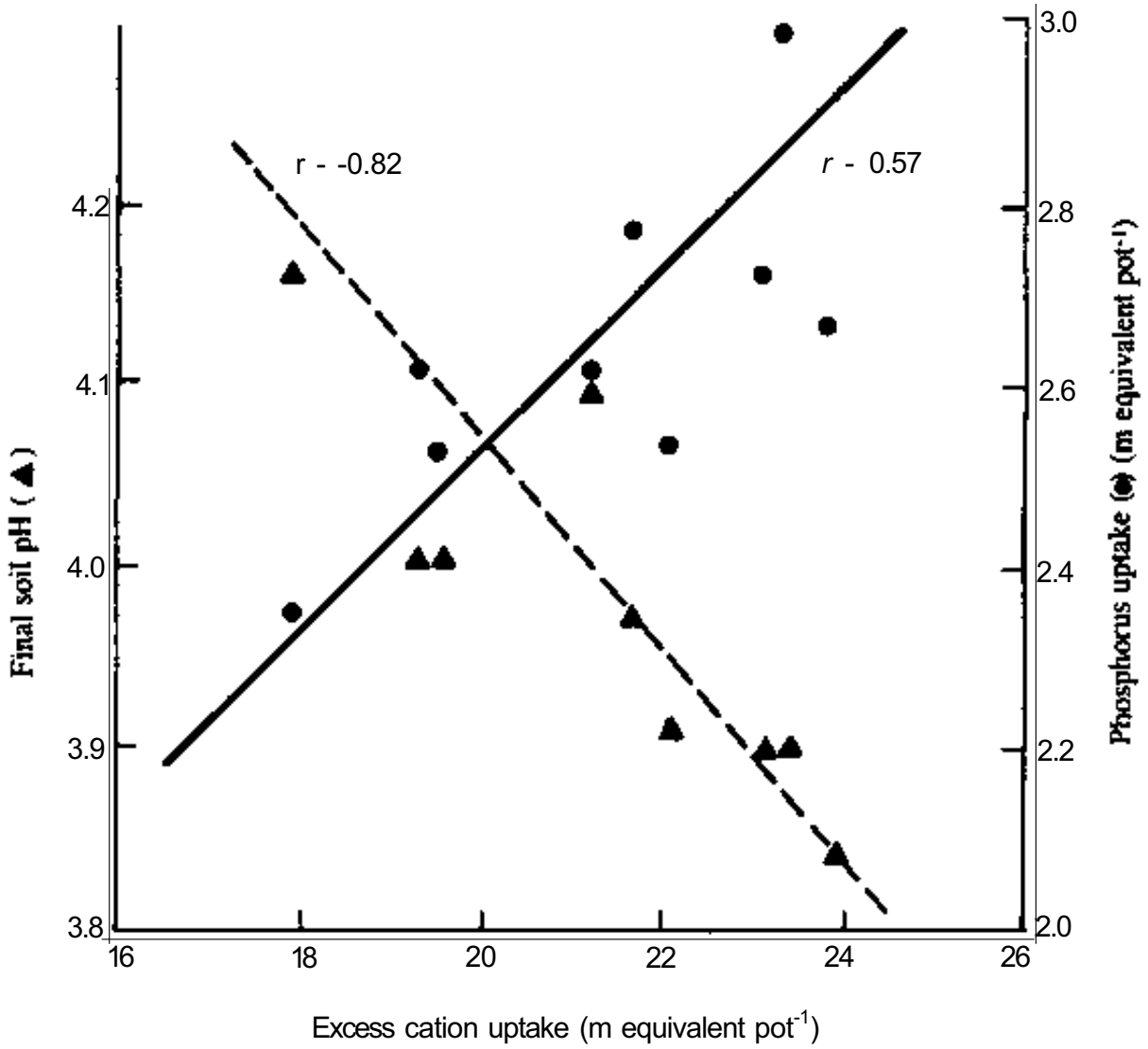


Figure 2. The relationship between excess cation over anion uptake by nine subterranean cultivars and final soil pH (left axis) and total phosphorus uptake (right axis). Data calculated from Jarvis and Robson (1983).

leaking from roots to allow its re-absorption as Pi (Barrett-Lennard et al. 1993).

While species differences in root phosphatase are known (McLachlan 1976), as yet there are few reports relating intraspecific differences in root phosphatase to P uptake. Helal (1990) showed a strong positive relationship between uptake of P from phytate and root external phosphatase activity among four bean varieties. Cultivars of wheat varied 2- to 3-fold in external phosphatase activity (McLachlan and De Marco 1982) but the significance of this for P uptake from organic forms was not tested and differences may have reflected plant P status rather than genetics; plant phosphatase activity is elevated by P deficiency (Bieleski 1973). Genotypic differences in ability to increase root phosphatase at low P may be more important than levels measured in P-adequate plants. Ecotypes of *Aegilops peregrina* differed in level of external root phosphatase, and activity increased at low P with an ecotype from a high-P soil showing greater response than that from a low-P site (Silberbush et al. 1981), indicating possible differences in regulation or,

alternatively, in internal P status between ecotypes. Prospects for manipulating plant phosphatases are mentioned in the paper by Delhaize (these proceedings).

Do different plants draw on different pools of P in the soil?

Smith (1981) grew buffel grass and four tropical pasture legumes *(Stylosanthes hamata, S. guianensis, Macroptilium atropurpureum,* and *Desmodium intortum)* in two alkaline clay soils and compared P uptake and ability to obtain P from different soil P pools using an isotopic dilution technique. Although the species differed considerably in the rate of removal of P and total P uptake, L-values did not differ significantly. He concluded that these species drew on the same pools of P and that differences in P acquisition were due to differences in ability to locate soluble P. Similar conclusions were reached by Armstrong et al. (1993) in a comparison of maize, sorghum, mung bean, cowpea, and soybean supplied with amorphous Fe and Al phosphates, the crystalline forms strengite and variscite, and potassium phosphate. After synthesis of the P sources, they were uniformly labeled with ^{32}P by neutron irradiation. Further comparisons using the same technique showed that differences in P uptake by four rangeland grasses *(Cenchrus ciliaris, Aristida armata, Digitaria ammophila,* and *Thyridolepis mitchelliana)* could not be explained by differences in the ability to use sparingly soluble forms of P (Armstrong and Helyar 1993). Although such experiments should be interpreted with caution because of the possibility of isotopic exchange between different fractions (Bolan et al. 1984), these two lines of evidence point to both P-efficient and P-inefficient plants drawing on the same pools of soil P.

In contrast, Ae et al. (1993) found that while maize, sorghum, millet, soybean, and chickpea obtained more P from Ca-phosphate than from Fe- or Al-phosphates, pigeonpea took up P equally well from Ca- or Fe-bound P. Differences among species in the ability to obtain P from insoluble phosphate rocks are known (e.g., Bekele et al. 1983) and at the cultivar-level, Caradus et al. (1990) showed that white clover cultivars differed in response to P depending on the solubility of the P fertilizer.

It will be interesting to examine this question further by applying the labeling techniques mentioned above to a greater range of species and cultivars including those known to excrete significant quantities of organic acid and other compounds into their rhizospheres.

Acknowledgments

I am grateful to Dr C Johansen and the Organizing Committee for the invitation and support to attend the FAO-ICRISAT Expert Consultancy Workshop, and to Denis De Marco for useful discussions on the paper.

References

Ae, N., Arihara, J., and Okada, K. 1991. Phosphorus uptake mechanisms of pigeonpea grown in Alfisols and Vertisols. Pages 91-98 *in* Phosphorus nutrition of grain legumes in the semi-arid tropics (Johansen, C., Lee, K.K., and Sahrawat, K.L., eds.). Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Ae, N., Arihara, J., Okada, K., Yoshihara, T., Otani, T., and Johansen, C. 1993. The role of piscidic acid secreted by pigeonpea roots grown on an Alfisol with low-P fertility. Pages 279-288 *in* Genetic aspects of plant mineral nutrition (Randall, P.J., Delhaize, E., Richards, R.A., and Munns R., eds.). Dordrecht, The Netherlands: Kluwer Academic.

Amijee, F., Barraclough, P.B., and Tinker, P.B. 1991. Modeling phosphorus uptake and utilization by plants. Pages 63-75 *in* Phosphorus nutrition of grain legumes in the semi-arid tropics (Johansen, C., Lee, K.K., and Sahrawat, K.L., eds.). Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Armstrong, R.D., and Helyar, K.R. 1993. Utilisation of labelled mineral and organic phosphorus sources by grasses common to semi-arid Mulga shrub lands. Australian Journal of Soil Research 31: 271-283.

Armstrong, R.D., Helyar, K.R., and Prangnell, R. 1993. Direct assessment of mineral phosphorus availability to tropical crops using ^{32}P labelled compounds. Plant and Soil 150: 279-287.

Baas, R., and van Beusichem, M.L. 1990. Genetic differentiation in *Plantago major* L. in growth and P uptake under conditions of P limitation. Plant and Soil 123: 185-192.

Baligar, V.C., and Barber, S.A. 1979. Genotypic differences of corn for ion uptake. Soil Science Society of America Proceedings 71: 870-873.

Barber, S.A., and Silberbush, M. 1984. Plant root morphology and nutrient uptake. Pages 65-87 *in* Roots, nutrient and water influx, and plant growth. American Society of Agronomy Special Publication Number 49 (Barber, S.A., and Bouldin, D.R., eds.). Madison, Wisconsin, USA: American Society of Agronomy.

Barrett-Lennard, E.G., Dacup, M., and Greenway, H. 1993. Role of extracellular phosphatases in the phosphorus nutrition of clover. Journal of Experimental Botany 44: 1595-1600.

Bar-Yosef, B. 1991. Root excretions and their environmental effects. Influence on availability of phosphorus. Pages 529-557 *in* Plant roots, the hidden half (Waisel, Y., Eshel, A., and Kafkafi, U., eds.). New York, USA: Marcel Dekker.

Bekele, T., Cino, B.J., Ehlert, P.A.I, van der Maas, A.A., and van Diest, A. 1983. An evaluation of plant-borne factors promoting the solubilisation of alkaline rock phosphates. Plant and Soil 75: 361-378.

Bieleski, R.L. 1973. Phosphate pools, phosphate transport and phosphate availability. Annual Review of Plant Physiology 24: 225-252.

Blair, G. 1993. Nutrient efficiency—what do we really mean? Pages 205-214 *in* Genetic aspects of plant mineral nutrition (Randall, P.J., Delhaize, E., Richards, R.A., and Munns R., eds.). Dordrecht, The Netherlands: Kluwer Academic.

Bolan, N.S., Robson, A.D., Barrow, N.J., and Aylmore, L.A.G. 1984. Specific activity of phosphorus in mycorrhizal and non-mycorrhizal plants in relation to the availability of phosphorus to plants. Soil Biology and Biochemistry 16: 299-304.

Buso, G.S.C., and Bliss F.A. 1988. Variability among lettuce cultivars grown at two levels of available phosphorus. Plant and Soil 111: 67-73.

Caradus, J.R. 1980. Distinguishing between grass and legume species for efficiency of phosphorus use. New Zealand Journal of Agricultural Research 23: 75-81.

Caradus, J.R. 1981. Effect of root hair length on white clover growth over a range of soil phosphorus levels. New Zealand Journal of Agricultural Research 24: 353-358.

Caradus, J.R., Mackay, A.D., and Wewala, G.S. 1990. Responses to phosphate fertilizers of differing solubilities by white clover cultivars. Pages 73-76 *in* Genetic aspects of plant mineral nutrition (El Bassam, N., Dambroth, M., and Loughman, B.C., eds.). Dordrecht, The Netherlands: Kluwer Academic.

Christiansen-Weniger, C, Groneman, A.F., and van Veen, J.A. 1992. Associative N_2 fixation and root exudation of organic acids from wheat cultivars of different aluminium tolerance. Plant and Soil 139: 167-174.

CIAT (Centro Internacional de Agriculture Tropical). 1989. Page 145 *in* Annual report 1989. Cali, Colombia: CIAT.

Clarkson, D.T. 1985. Factors affecting mineral nutrient acquisition by plants. Annual Review of Plant Physiology 36: 77-115.

Clarkson, D.T., and Grignon, C. 1991. The phosphate transport system and its regulation in roots. Pages 49-62 *in* Phosphorus nutrition of grain legumes in the semi-arid tropics (Johansen, C., Lee, K.K., and Sahrawat, K.L., eds.). Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Craswell, E.T., and Godwin, D.C. 1984. The efficiency of nitrogen fertilizers applied to cereals in different climates. Advances in plant nutrition 1: 1-55.

Delhaize, E., Randall, P.J., Wallace, P.A., and Pinkerton, A. 1993a. Screening *Arabidopsis* for mutants in mineral nutrition. Plant and Soil 155/156: 131-134.

Delhaize, E., Ryan, P.R., and Randall, P.J. 1993b. Aluminum tolerance in wheat *(Triticum aestivum* L.). II. Aluminium stimulated excretion of malic acid from root apices. Plant Physiology 103: 695-702.

Dunlop, J., Lambert, M.G., Van Den Bosch, J., Caradus, J.R., Hart, A.L., Wewela, G.S., Mackay, A.D., and Hay, M J.M. 1992. A program to breed a cultivar of *Trifolium repens* L. for more efficient use of phosphate. Pages 547-552 *in* Genetic aspects of plant mineral nutrition (El Bassam, N., Dambroth, M, and Loughman, B.C., eds.). Dordrecht, The Netherlands: Kluwer Academic.

Fawole, I., Gabelman, W.H., and Gerloff, G.C. 1982. Genetic control of root development in beans *(Phaseolus vulgaris* L.) grown under phosphorus stress. Journal of the American Society of Horticultural Science 107: 98-100.

Fohse, D., Claassen, N., and Jungk, A. 1988. Phosphorus efficiency of plants. 1. External and internal P requirement and P uptake efficiency of different plant species. Plant and Soil 110: 101-109.

Fohse, D., Claassen, N., and Jungk, A. 1991. Phosphorus efficiency of plants. 2. Significance of root radius, root hairs and cation-anion balance for phosphorus influx in seven plant species. Plant and Soil 132: 261-272.

Gardner, W.K., Barber, D.A., and Parberry, D.G. 1983. The acquisition of phosphorus by *Lupinus albus* L. III. The probable mechanism by which phosphorus movement in the soil/ root interface is enhanced. Plant and Soil 70: 107-124.

Gardner, W.K., and Boundy, K.A. 1983. The acquisition of phosphorus by *Lupinus albus* L. IV. The effect of interplanting wheat and white lupin on the growth and mineral composition of the two species. Plant and Soil 70: 391-402.

Gerloff, C.G., and Gabelman, W.H. 1983. Genetic basis of inorganic plant nutrition. Pages 453-480 *in* Encyclopaedia of plant physiology New Series vol. 15B Inorganic plant nutrition (Lauchli, A., and Bieleski, R.L., eds.). Berlin, Germany: Springer-Verlag.

Graham, R.D. 1978. Nutrient efficiency objectives in cereal breeding. Pages 165-170 *in* Plant nutrition 1978. New Zealand Department of Scientific and Industrial Research (DSIR). Information series 134. (Ferguson, I.R., Bieleski, R.L., and Ferguson, I.B., eds.). Wellington, New Zealand: DSIR.

Goldstein, A.H. 1991. Plant cells selected for resistance to phosphate starvation show enhanced P use efficiency. Theoretical and Applied Genetics 82: 191-194.

Gourley, C.J.P. 1994. Plant nutrient efficiency: a comparison of definitions and suggested improvement. Plant and Soil 158: 29-37.

Hackett, C. 1968. A study of the root system of barley. 1. Effects of nutrition on two varieties. New Phytologist 67: 287-299.

Hedley, M.J., Nye, P.H., and White, R.E. 1983. Plant induced changes in the rhizosphere of rape *(Brassica napus* var. Emerald) seedlings. IV. The effect of rhizosphere phosphorus status on the pH, phosphatase activity and depletion of soil phosphorus fractions in the rhizosphere and on cation-anion balance in the plants. New Phytologist 95: 69-82.

Helal, H.M. 1990. Varietal differences in root phosphatase activity as related to the utilisation of organic phosphates. Plant and Soil 123: 161-163.

Hodgkin, E.P., and Hamilton, B.H. 1993. Fertilizers and eutrophication in south western Australia: setting the scene. Fertilizer Research 36: 95-103.

Hoffland, E. 1992. Quantitative evaluation of the role of organic acid exudation in the mobilisation of rock phosphate by rape. Plant and Soil 140: 279-289.

Jarvis, S.C., and Robson, A.D. 1983. A comparison of the cation/anion balance of ten cultivars of *Trifolium subterraneum* L., and their effects on soil acidity. Plant and Soil 75: 235-243.

Jessop, R.S., Jones, G.P., and Blair, G.J. 1983. Performance of 22 wheat varieties under low and high phosphorus conditions and implications for selection of P-efficient varieties. Pages 445-454 *in* Proceedings of the 3rd International Congress on Phosphorus Compounds, Brussels. Casablanca, Morocco: Institut Mondial du Phosphate.

Jones, G.D.P. 1989. Phosphorus efficiency in wheat—a useful selection criterion? Field Crops Research 21: 257-264.

Krannitz, P.G., Aarssen, L.W., and Lefebvre, D.D. 1991. Correction for non-linear relationships between root size and short term Pi uptake in genotype comparisons. Plant and Soil 133: 157-167.

Marschner, H., Romheld, V., Horst, WJ., and Martin P. 1986. Root induced changes in the rhizosphere: Importance for the mineral nutrition of plants. Zeitschrift fur Pflanzenernahrung und Bodenkunde 149: 441-456.

Lambert, D.H., Cole, H., and Baker, D.E. 1980. Variations in the response of alfalfa clones and cultivars to mycorrhizae and phosphorus. Crop Science 20: 615-618.

McLachlan, K.D. 1976. Comparative phosphorus response in plants to a range of available phosphorus situations. Australian Journal of Agricultural Research 27: 323-341.

McLachlan, K.D., and De Marco, D.G. 1982. Acid phosphatase activity of intact roots and phosphorus nutrition of plants. III. Its relation to phosphorus garnering by wheat and a comparison with leaf activity as a measure of phosphorus status. Australian Journal of Agricultural Research 33: 1-12.

McLaughlin, M.J., Fillery, I.R., and Till, A.R. 1991. Operation of the phosphorus, sulfur and nitrogen cycles. Pages 67-116 *in* Australia's renewable resources: sustainability and global change (Gifford, R.M., and Barson, M.M., eds.). Canberra, Australia: Bureau of Rural Resources.

Nielsen, N.E., and Schjorring, J.K. 1983. Efficiency and kinetics of phosphorus uptake from soil by various barley genotypes. Plant and Soil 72: 225-230.

Rengel, Z. 1993. Mechanistic simulation models of nutrient uptake : A review. Plant and Soil 152: 161-173.

Richardson, A.E. 1994. Soil micro-organisms and phosphorus availability. Pages 50-62 *in* Soil biota—management in sustainable farming systems (Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R., and Grace, PR., eds.). Melbourne, Australia: Commonwealth Scientific and Industrial Research Organization.

Silberbush, M., Shomer-Ilan, A., and Waisel, Y. 1981. Root surface phosphatase activity in ecotypes of *Aegilops peregrina.* Physiologia Plantarum 53: 502-504.

Simpson, J.R., and Pinkerton, A. 1989. Fluctuations in soil moisture, and plant uptake of surface applied phosphate. Fertilizer Research 20: 101-108.

Smith, F.W. 1981. Availability of soil phosphate to tropical pasture species. Pages 282-285 *in* Proceedings of XIV International Grassland Congress, Lexington, USA (Smith, A.J., and Hays, V.W., eds.). Boulder, Colorado, USA: Westview Press.

Smith, F.W., Jackson, W.A., and Vanden Berg, P.J. 1990. Internal phosphorus flows during development of phosphorus stress in *Stylosanthes hamata.* Australian Journal of Plant Physiology 17: 451-464.

Smith, S.E., Robson, A.D., and Abbott, L.K. 1993. The involvement of mycorrhiza in assessment of genetically dependent efficiency of nutrient uptake and use. Pages 221-231 *in* Genetic aspects of plant mineral nutrition (Randall, P.J., Delhaize, E., Richards, R.A., and Munns, R., eds.). Dordrecht, The Netherlands: Kluwer Academic.

Spencer, K., Govaars, A.G., and Hely, F.W. 1980. Early phosphorus nutrition of eight forms of two clover species, *Trifolium ambiguum* and *T. repens.* New Zealand Journal of Agricultural Research. 23: 457-475.

Stangel, P.J., and Von Uexkull, H.R. 1990. Regional food security: demographic and geographic implications. Pages 21-43 *in* Phosphorus requirements for sustainable agriculture in Asia and Oceania. Los Banos, Philippines: International Rice Research Institute.

Tarafdar, J.C., and Claassen, N. 1988. Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and micro-organisms. Biology and Fertility of Soils 5: 308-312.

Thompson, J.P. 1991. Improving the mycorrhizal condition of the soil through cultural practices and effects on growth and phosphorus uptake by plants. Pages 117-137 *in* Phosphorus nutrition of grain legumes in the semi-arid tropics (Johansen, C., Lee, K.K., and Sahrawat, K.L., eds.). Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Thung, M. 1992. Phosphorus: a limiting nutrient in bean *(Phaseolus vulgaris* L.) production in Latin America and field screening for efficiency and response. Pages 501-521 *in* Genetic aspects of plant mineral nutrition (El Bassam, N., Dambroth, M., and Loughman, B.C., eds.). Dordrecht, The Netherlands: Kluwer Academic.

Genotype selection for improved phosphorus utilization in crop plants

B S Ahloowalia, K S Kumarasinghe, B Sigurbjoemsson, and M Maluszynski[1]

Abstract

Phosphorus (*P*) *deficiency reduces leaf-expansion, axillary bud growth, and shoot canopy, thereby reducing photosynthetic surface area and carbohydrate utilization. Hence, selection for morphological characters associated with an increased photosynthetic surface area and deep rooting will be an effective method to overcome reduced availability of P.*

Introduction

Phosphorus (P) nutrition exerts a significant influence on plant growth and development. Phosphorus deficiency reduces leaf-expansion, axillary bud growth, and shoot canopy, thereby reducing photosynthetic surface area and carbohydrate utilization. The availability of P and uptake of P are also reduced in soils with high aluminum (Al) and low pH. It should be possible to overcome P deficiency by selecting genotypes that perform better on low-P soils. To obtain genotypes tolerant of low soil P status, screening of germplasm from regions with low-P soils is an important approach. In this paper, we report the differences among genotypes of wheat and cowpea in their tolerance to low P availability in soils in Africa. This research was carried out under an FAO/IAEA/SIDA Coordinated Research Programme, namely, 'The use of isotope studies on increasing and stabilizing plant productivity in low phosphate and semi-arid and sub-humid soils of the tropics and sub-tropics'. This paper presents some of the results from three African countries involved in the study, namely, Sierra Leone, Nigeria, and Egypt. The primary objectives of this program were as follows.

- To identify genotypes of crop and tree species with a high efficiency for uptake and use of phosphate and water.
- To identify the morphological and physiological parameters in the plant responsible for high efficiency in uptake and use of phosphate and water.

1. Plant Breeding and Genetic Division, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. International Atomic Energy Agency. Wagramer Strasse 5, P 0 Box 100. A-1400 Vienna. Austria.

Ahloowalia, B.S., Kumarasinghe, K.S., Sigurbjoemsson, B., and Maluszynski, M. 1995. Genotype selection for improved phosphorus utilization in crop plants. Pages 49-54 *in* Genetic manipulation of crop plants to enhance integrated nutrient management in cropping systems—1. Phosphorus: proceedings of an FAO/ICRISAT Expert Consultancy Workshop, 15-18 Mar 1994. ICRISAT Asia Center, India (Johansen, C., Lee. K.K.. Sharma, K.K., Subbarao, G.V., and Kueneman, E.A., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

- To assess the yield potentials of the efficient crop and tree genotypes under different agroecological conditions and make recommendations for their use, multiplication, and distribution in the appropriate countries.

Results

In Sierra Leone, 11 genotypes of cowpea were screened under field conditions for phosphate use efficiency in a soil (Njala soil series) that was low in available P (4 mg kg^{-1} available P). In a study conducted at two levels of P (low-P = 0 kg ha^{-1} and high P = 15 kg ha^{-1}), significant differences were observed in grain yield of cowpea (Figure 1) at the different levels of applied P. Three genotypes (3, 6, and 10) were identified as superior in grain yield at both low and high P levels. However, high phosphate use efficiency, in terms of above-ground dry matter per unit of P in above-ground parts, did not always translate into high yield (Figure 2). Therefore, the potential of genetic improvement to match high yielding cultivars with high phosphate use efficiency appears to be very high.

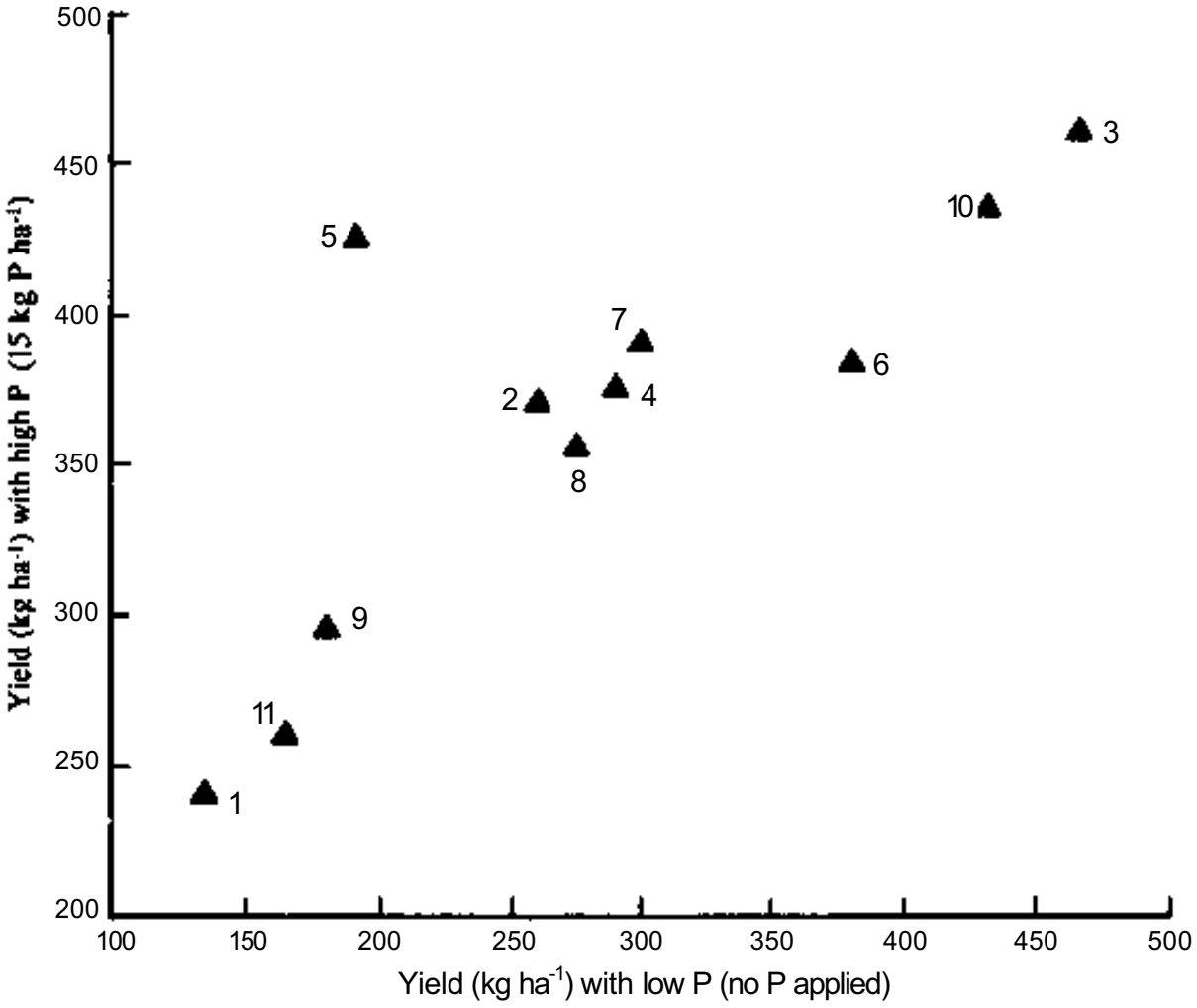


Figure 1. Grain yield of cowpea at low and high phosphorus levels, Sierra Leone, 1991/92.

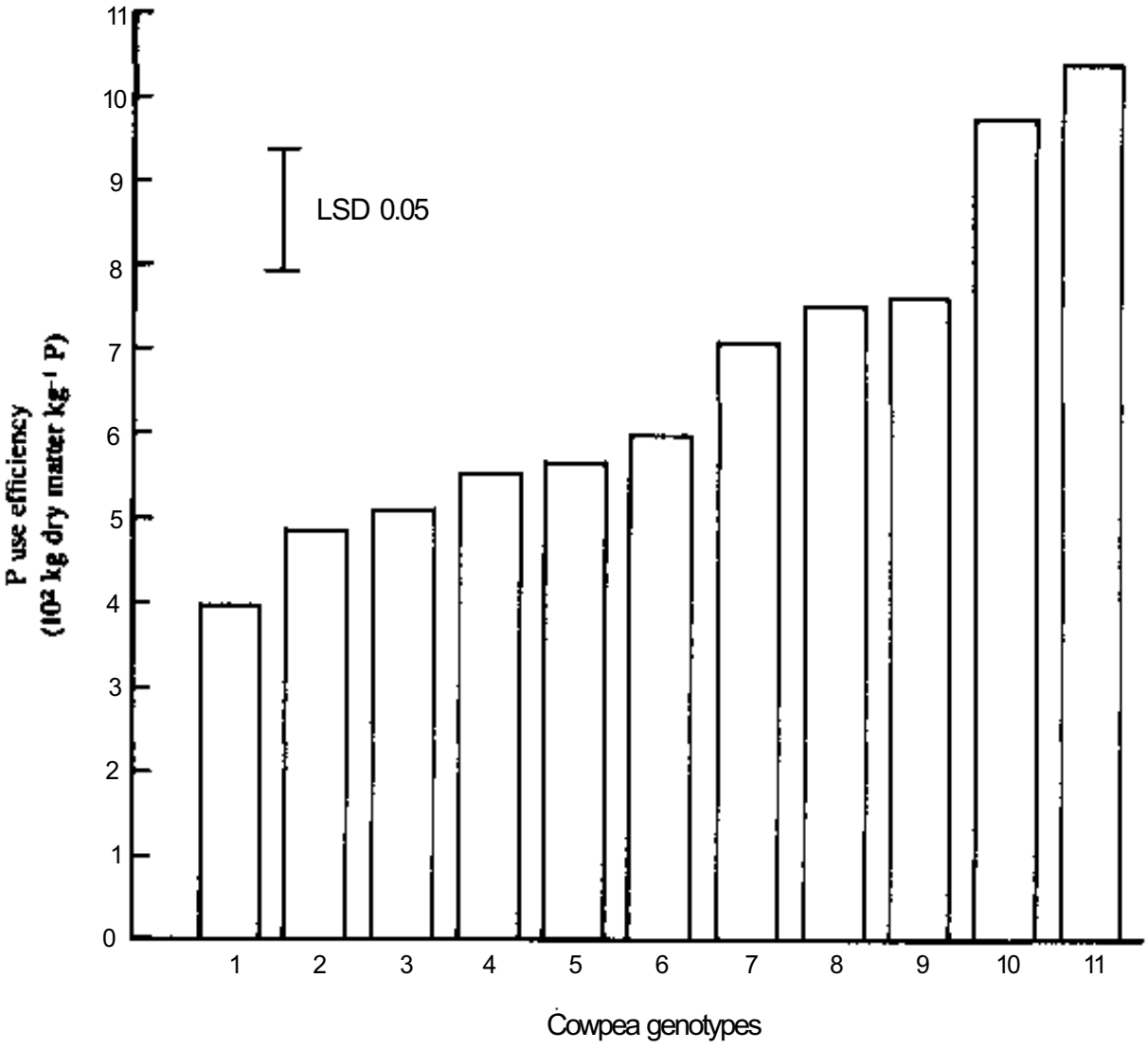


Figure 2. Genotypic differences in phosphate use efficiency of cowpea, Sierra Leone, 1989/90.

In Nigeria, 20 genotypes of cowpea were screened in a severely P-deficient soil under low P (no P applied) and high P (60 kg P ha^{-1}) conditions. There were three-fold differences in grain yield at low P (Figure 3). Phosphorus application had little effect on increasing the yield of genotypes that produce high yields at low P, but showed positive effects on those that produce low yields at low P levels. The root system appears to play a major role in enabling plants to grow well in P-deficient soils: high yielding genotypes had a tendency to produce bigger root systems than those of the low-yielders (Figure 3). Application of P in these soils had little effect on plant growth mainly because of the soil's high capacity for P fixation.

In Egypt, 13 wheat cultivars were tested for phosphate use efficiency, in terms of grain yield per unit of P in above-ground parts, on a loamy sandy soil (5 mg kg^{-1} available P) at three levels of applied P (low P = 0 kg P ha^{-1}, medium P = 24 kg P ha^{-1}, and high P = 75 kg P ha^{-1}). Response of the cultivars to P application was not

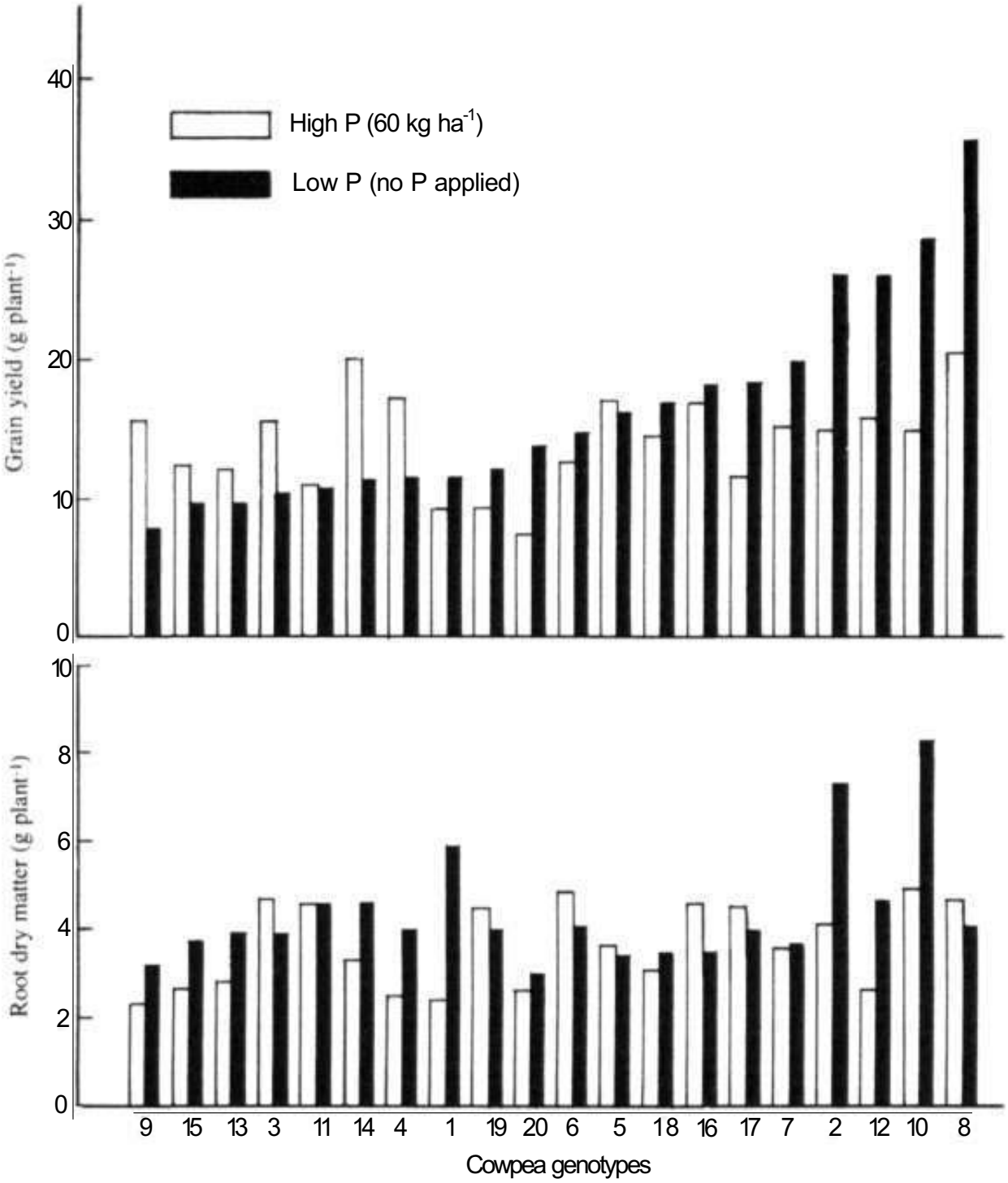


Figure 3. Genotypic differences in grain yield and root dry matter in cowpea, Nigeria, 1990-92.

consistent. Whereas some cultivars reached maximum biomass production and grain yield at the highest level of P, other cultivars had their growth suppressed by P application. Biomass production and grain yield differed significantly at low P levels, revealing differences in phosphate use efficiency, but these differences disappeared as the level of P applied increased (Figure 4). Mycorrhizal infection of roots was generally low (about 15%); however, the cultivars with a high phosphate

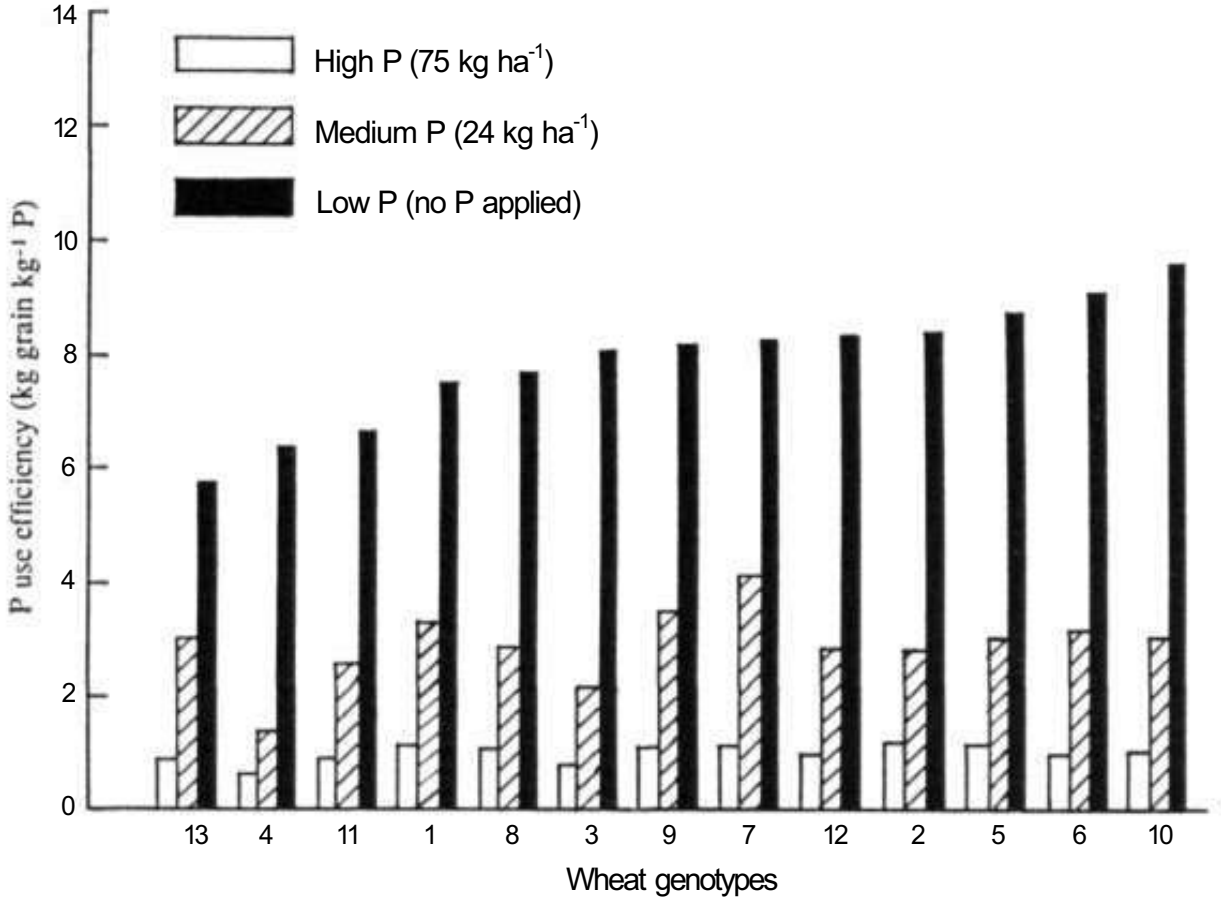


Figure 4. Genotypic differences in phosphate use efficiency of wheat at different phosphorus levels, Egypt, 1991/92.

use efficiency showed the highest infection rates. Also, there were large differences in root length densities in the 0-30 cm soil layer. Phosphate-efficient cultivars were amongst those with the highest root length densities.

Discussion

Phosphorus deficiency can be overcome in several ways. An efficient and economic procedure is be to breed varieties that can grow and yield on P-deficient soils. As a first step, there is need to screen germplasm for tolerance to low P availability, as shown in the studies in African soils. There arc two basic approaches to maximizing yield under conditions of low P supply: increasing biomass or grain yield for a given unit of P within the plant (P use efficiency) and increasing the root system's ability to acquire P. It is possible to combine these approaches to genetically manipulate plants such that they have not only a lower internal demand for P but also a greater ability to scavenge P from sparingly available sources.

The present results indicate that such morphological characters as increased root length and density of roots are associated with improved plant performance of some genotypes on low-P soils. Hence, selection for such traits should enhance a

plant's ability to survive and perform on low-P soils. It has recently been found that certain genotypes are able to release the bound phosphates by producing root exudates. For example, in rice, chemical root exudates from cultivar 'Oryzica Sabana 6' are released in the soil (Anonymous 1993). These exudates include citric acid, which binds with Al to alleviate Al toxicity and at the same time releases P. Such genotypes have the multiple advantages of tolerance to acidic soil, release of P, and prevention of Al toxicity. These exudates need to be analyzed and the genes responsible for producing them identified. Thus, P nutrition may also be enhanced by selecting genotypes that can release bound P in soils. Perhaps this approach could be used for *in vitro* screening and pre-selection for Al tolerance of cells and plants. If a dye could be associated with increased exudation, then experiments for *in vitro* selection for root exudates could be carried out much more efficiently, allowing mutations in the loci involved to be tracked.

Uptake of P by plants in P-deficient soils is substantially improved by mycorrhizal symbiosis (Harley and Smith 1983). In potato *(Solanum tuberosum* L.), inoculation of plants grown on P-deficient or low-P soil with three vesicular-arbuscular mycorrhizal (VAM) fungi *(Glomus* spp) stimulated P uptake into roots (McArthur and Knowles 1993). This in turn stimulated uptake of nitrogen, potassium, magnesium, iron, and zinc. Species differ in symbiotic effectiveness, and the resultant enhanced plant growth and development. It is likely that there are compatible genes in the host and the VAM symbiont in the same manner as the *nod* genes and *nif* genes in the legume/rhizobium interaction, and genes for resistance/virulence in host/pathogen interactions in disease development. The identification, isolation, mutation, and cloning of such genes would be a first step in the engineering of new genotypes for improved P nutrition in crops. Modern molecular techniques should allow such experiments with the genotypes already identified for increased ability for P acquisition under P-deficient conditions.

References

Anonymous. 1993. Why can some rice grow in acid soils? CIAT International 12: 3.

Harley, J.L., and Smith, S.E. 1983. Mycorrhizal symbiosis. London, UK: Academic Press. 483 pp.

McArthur, D.A.J., and Knowles, N.R. 1993. Influence of species of vesicular-arbuscular mycorrhizal fungi and phosphorus nutrition on growth, development, and mineral nutrition of potato (*Solanum tuberosum* L.). Plant Physiology 102: 771-782.

Genetic control of phosphorus uptake and phosphorus status in plants

J R Caradus[1]

Abstract

Genetic variation in plant characters associated with enhancing nutrient management of cropping systems has been identified in many species. However, except for such characters as root size and to a lesser extent root morphology, there has been little emphasis placed upon determining heritability, mode of inheritance, or the effect of selection for these characters. In reviewing this topic, it was found that narrow sense heritabilities for root size had a median of approximately 0.5; for root growth characters, generally less than 0.3; for mycorrhizal colonization, less than 0.5; and for such morphological characters of roots as diameter, greater than 0.6 (but for root branching, it was less than 0.5). Selection studies have been successful in manipulating root size, root hair length, and degree of root branching. Phosphorus (P) uptake as measured by plant P concentration and dry weight increase per unit of applied P (i.e., P response) are heritable and amenable to selection. However, there have been no studies to determine the heritability or mode of genetic control for mechanisms associated with solubilization of sparingly soluble soil P. These include root exocellular acid phosphatase activity, root exudation, and root-induced pH change.

Introduction

Several options are open to plant breeders for enhancing nutrient management in cropping systems. The first broad categorization of choices has been between (1) selection for specific plant characters of adaptive significance and (2) selection for whole plant adaptation. The next decision is whether to use artificial environments or field environments while screening for genetic variation. Several studies and reviews have focused on this choice (Fox 1978, Wiersum 1981, Caradus and Snaydon 1986d). The aim here is to determine the genetic control of specific plant characters that are associated with improved phosphorus (P) uptake by crop plants. Once this is understood, it is possible to determine the probability of success of

1. AgResearch. Grasslands Research Centre, Tennent Drive, Fitzherbert West, Private Bag 11008, Palmerston North, New Zealand.

Caradus, J.R. 1995. Genetic control of phosphorus uptake and phosphorus status in plants. Pages 55-74 *in* Genetic manipulation of crop plants to enhance integrated nutrient management in cropping systems—1. Phosphorus: proceedings of an FAO/ICRISAT Expert Consultancy Workshop, 15-18 Mar 1994, ICRISAT Asia Center, India (Johansen, C., Lee, K.K., Sharma, K.K., Subbarao, G.V., and Kueneman, E.A., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

selection programs based on these characters. Even with this understanding, however, selection for a single plant character can often have the deleterious effects of (1) ignoring the general agronomy of the crop, which may result in poor field adaptation, and (2) concomitant changes in other plant characters of adaptive significance.

Two principles must be recognized before determining direction of genetic manipulation to enhance nutrient management.

- Plant growth is rarely limited by the ability of roots to absorb inorganic P. Phosphorus uptake is limited in most cases by the supply of inorganic P to the absorbing mechanism.
- The amount of P that can be removed from the available P in soil solution is largely determined by the amount of absorptive surface area.

Recognition of these two principles assists in prioritizing a long list of plant characters that might be amenable to genetic manipulation to improve plant access to soil P, whether applied as fertilizer or not. Determining the genetic control of root characters associated with increasing the absorptive surface area therefore becomes a major priority. This would include characters that influence root size, root distribution, root morphology, and mycorrhizal association. Secondly, plant characters that increase the solubility of sparingly soluble soil P are also important. Such characters include root exudates, root exocellular phosphatases, and root-induced pH changes. In addition to these, several other associated plant characters need consideration since they too can influence the rate of P uptake. These include the P status of plant tissues, plant growth rate, and (in the case of leguminous crops) nodulation and nitrogen fixation.

This review examines the genetic control of plant characters associated with P uptake and P status in an attempt to identify plant characters that breeders should consider as feasible selection criteria in programs aimed at enhancing P management. To achieve this, the review focuses on studies that have involved either selection and progeny testing or estimates of heritability for the characters concerned.

Genetic control of absorption area

At high levels of P supply, root size is less important for P uptake in such species as white clover (Caradus and Snaydon 1986a) and wheat (Romer et al. 1989) since high P uptake intensities per unit root and time enable sufficient P to be absorbed even when the root system is relatively small. For P-deficient plants grown at a low P level, root size is of greater significance in determining the amount of P absorbed. Jones et al. (1989) conclude from a study of P efficiency measures in wheat that P uptake in shoots per unit root dry weight, which describes the ability of the plant to obtain P from the soil, was a far more beneficial measure for use in breeding programs than either grain yield per unit P uptake or grain P content as a percentage of total P uptake.

In solution culture, P uptake per plant is related to root size, which itself is a function of whole plant size (Caradus and Snaydon 1986a). In soil, however, the growth of roots into 'new' soil may be the only means of obtaining a continued and adequate supply of P (Darrah 1993). Hence, root extension rate and the production of new roots need consideration.

Root system size

Measures of root system size include root weight, root length, root number, and root volume. Heritabilities for root weight have been estimated frequently (Table 1), with a median narrow sense heritability of 0.53 (mean 0.52 ± 0.05). Heritabilities for root volume, number, and length were also generally high. Heritabilities increased with depth of rooting for root number and root surface area (Lehman and Engelke 1991). Median narrow sense heritability for root/shoot ratio was 0.52 (mean 0.52 ± 0.12). Root growth rate narrow sense heritabilities were less than 0.4 except for root extension rate of *Agrostis stolonifera* (Lehman and Engelke 1991) (Table 1).

Selection for extremes of root size has generally been successful. For example, in alfalfa, selection for high and low root weight was successful in four of six cultivars (Pederson et al. 1984) (Table 2). In perennial ryegrass, selection for large root dimension successfully increased root length, volume, weight, root/shoot ratio, shoot weight, and leaf number (Table 3). Selection for the longest root length has also been successful in white clover (Ennos 1985). There has been some inconsistency among studies that have examined the importance of additive and dominance effects on root size. Additive gene effects indicate that a genetic trait is altered by each additional allele, while dominant gene effects indicate gene action deviating from an additive condition, such that the heterozygote is more like one parent than like another. In maize, both additive and dominance components of genetic variation were important for root number, while only dominant gene action was important for root length (Sharma and Bhalla 1990). Additive and dominance gene effects controlled root length and weight in spring wheat (Kalashnik et al. 1982), root weight and root volume in peas (Saleh and Gritton 1988), and root length, root number, root/shoot ratio, and root volume in rice [Armenta-Soto et al. (1983) (Table 4) and Ekanayake et al. (1985) (Table 5)]. However, Ochesanu and Cabulea (1989) found that additive gene effects predominated in the control of root length, root number, root volume, and root weight in maize. Das et al. (1991) also found that only additive gene effects were important for root length and diameter in rice. However, dominant genes for high values of both root volume and root dry weight outnumbered the recessives in all the hybrid families of wheat studied, though the coefficient of dominance for both characters was less than unity, indicating partial dominance (Kuburovic 1984).

Table 1. Heritability of characters describing root system size and morphology (h_B = broad sense, h_N = narrow sense, and h_R = realized heritability).

Character	Species	Heritability	Reference
Root system size			
Root dry mass	*Lycopersicon esculentum*	$h_N = 0.27$	Troughton and Whittington 1969
	Triticum aestivum	$h_N = 0.68$	
	Ipomoea batatas	$h_N = 0.41$	Jones et al. 1969
	Pisum sativum	$h_B > 0.68$	Ali-Khan and Snoad 1977
	Oryza sativa	$h_N = 0.43$	Chang et al. 1982
	Vigna radiata	$h_B = 0.59$	Sree Rangasamy and Shanmugam 1984
	Pinus radiata	$h_N = 0.56$	Nambiar et al. 1982
	Phaseolus vulgaris	$h_B = 0.69 - 0.90$	Fawole et al. 1982a
	Gossypium hirsutum	$h_N = 0.37$	da Silva 1984
	Triticum aestivum	$h_N = 0.48 - 0.53$	Kuburovic 1984
	Oryza sativa	$h_N = 0.56^1, 0.92^2$	Ekanayake et al. 1985
	Pisum sativum	$h_B = 0.41 - 0.81$	Saleh and Gritton 1988
	Vigna unguiculata	$h_B = 0.24$	Singh et al. 1988
	Phleum pratense	$h_B = 0.40$	Schapendank and de Vos 1988
	Trifolium repens	$h_N = 0.56$	Woodfield and Caradus 1990
		$h_B = 0.28$	
		$h_B = 0.22, 0.24$	Caradus and Woodfield 1990
	Medicago sativa	$h_R = 0.05 - 0.38$	Saindon et al. 1991
Root volume	*Tsuga heterophylla*	$h_B = 0.90$	Foster et al. 1984
	Triticum aestivum	$h_N = 0.52 - 0.57$	Kuburovic 1984
	Oryza sativa	$h_N = 0.18^1$, $h_N = 0.55^2$	Ekanayake et al. 1985

Continued

Table 1. *Continued*

Character	Species	Heritability	Reference
	Pisum sativum	$h_B = 0.44 - 0.71$	Saleh and Gritton 1988
	Vigna unguiculata	$h_B = 0.23$	Singh et al. 1988
Root number	*Lolium perenne*	$h_B = 0.07, 0.29$	Troughton and Whittington 1969
	Oryza sativa	$h_N = 0.49$	Chang et al. 1982
		$h_N = 0.44$	Armenta-Soto et al. 1983
	Hordeum vulgare	$h_B = 0.75$	Omara and Hussein 1987
	Agrostis stolonifera	(10-cm depth) $h_N = 0.08$	Lehman and Engelke 1991
		(20-cm depth) $h_N = 0.14$	
		(30-cm depth) $h_N = 0.31$	
		(40-cm depth) $h_N = 0.61$	
Root length	*Oryza sativa*	$h_N = 0.61$	Chang et al. 1982
	Trifolium repens	$h_N = 0.42 - 0.84$	Ennos 1985
	Tsuga heterophylla	$h_B = 0.87$	Foster et al. 1984
	Vigna radiata	$h_B = 0.62$	Sree Rangasamy and Shanmugam 1984
	Hordeum vulgare	$h_B = 0.67$	Omara and Hussain 1987
Root surface area	*Agrostis stolonifera*	(10-cm depth) $h_N = 0.07$	Lehman and Engelke 1991
		(20-cm depth) $h_N = 0.23$	
		(30-cm depth) $h_N = 0.27$	
		(40-cm depth) $h_N = 0.43$	
		(50-cm depth) $h_N = 0.82$	
Root system "size"	*Medicago sativa*	$h_N = 0.48$	Chloupek 1984
		$h_N = 0.36$	Chloupek and Rod 1985
		$h_B = 0.60$	Chloupek and Samánek 1985

Continued

Table 1. *Continued*

Character	Species	Heritability	Reference
Root/shoot ratio	*Lolium perenne*	$h_B = 0.50, 0.81$	Troughton and Whittington 1969
	Oryza sativa	$h_N = 0.52$	Chang et al. 1982
	Pinus radiata	$h_B = 0.53$	Nambiar et al. 1982
	Oryza sativa	$h_N = 0.61$	Armenta-Soto et al. 1983
	Vigna unguiculata	$h_B = 0.21$	Singh et al. 1988
	Trifolium repens	$h_B = 0.22, 0.24$	Caradus and Woodfield 1990
		$h_B = 0.20$	Woodfield and Caradus 1990
		$h_N = 0.22$	
Root growth rate			
Mass	*Pinus radiata*	$h_N = 0.34$	Nambiar et al. 1982
Root volume	*Lolium perenne*	$h_B = 0.23, 0.28$	Troughton and Whittington 1969
Root number	*Lolium perenne*	$h_B = 0.02, 0.25$	Troughton and Whittington 1969
Number of new apices	*Pinus radiata*	$h_N = 0.25$	Nambiar et al. 1982
Root extension rate	*Agrostis stolonifera*	$h_N = 0.62 - 0.76$	Lehman and Engelke 1991
Length of new roots	*Pinus radiata*	$h_N = 0.36$	Nambiar et al. 1982
Mycorrhizal colonization			
Roots infected (%)	*Medicago sativa*	$h_N = 0.19$	Lackie et al. 1988
	Vigna unguiculata	$h_N = 0.46$	Mercy et al. 1990

Continued

Table 1. *Continued*

Character	Species	Heritability	Reference
Root morphology			
Diameter	*Oryza sativa*	$h_N = 0.62$	Chang et al. 1982
	Pinus radiata	$h_N = 0.28$	Nambiar et al. 1982
	Oryza sativa	$h_N = 0.62$	Armenta-Soto et al. 1983
		$h_N = 0.61^1, 0.80^2$	Ekanayake et al. 1985
Root hair length	*Trifolium repens*	(long) $h_R = 0.33$ (short) $h_R = 0.44$	Caradus 1979
Primary root length	*Oryza sativa*	$h = 0.53 - 0.82$	Hajra et al. 1988
Adventitious root score	*Trifolium pratense*	$h_N = 0.30$	Montpetit and Coulman 1991
Branching			
Score	*Medicago sativa*	$h_N = 0.02$, $h_N = 0.13$	McIntosh and Miller 1980
Proportion fibrous roots	*Trifolium repens*	$h_B = 0.26, 0.34$	Caradus and Woodfield 1990
		$h_B = 0.44$ $h_N = 0.48$	Woodfield and Caradus 1990
First-order roots	*Pinus taeda*	$h_B = 0.77$	Kormanik et al. 1990
Number of laterals	*Pisum sativum*	$h_B = 0.54$	Ali-Khan and Snoad 1977
Rooting intensity	*Vigna radiata*	$h_B = 0.64$	Sree Rangasamy and Shanmugam 1984
Root length density	*Oryza sativa*	$h_N = 0.44^1$, $h_N = 0.77^2$	Ekanayake et al. 1985

1. Parent-progeny regression.
2. From analysis of F_3 progeny line.

Table 2. Effect of selection in alfalfa for high and low root-mass on root diameter, branching, and dry weight of progeny.

Source cultivar	Root mass class	Root branching score[1]	Root dry mass (mg)
Mesa Sirsa	High	2.92	318
	Low	2.63	273
Moapa	High	2.98	335
	Low	2.35	276
Lahontan	High	2.73	310
	Low	2.90	298
	High	2.87	267
	Low	2.79	299
Grimm	High	2.72	318
	Low	2.68	288
Teton	High	2.90	274
	Low	3.08	277
$LSD_{0.05}$		0.14	14
Mean	High	2.85	304
	Low	2.74	285
$LSD_{0.05}$		0.06	6

1. Scored as 1 = a single tap root, 5 = many lateral branches.

Source: Pederson et al. (1984).

Table 3. Effect of two cycles of recurrent selection (1.3% selection intensity) for large root dimension on root and shoot characteristics of *Lolium perenne* L.

Plant character	Unselected	Selected	P[1]
Root length (cm)	17.1	21.4	**
Root volume (cm^3)	0.96	1.33	**
Root dry mass (mg)	43	72	**
Number of leaves	9.1	11.7	**
Shoot dry mass (mg)	110	140	**
Root/shoot ratio	0.39	0.51	**

1.** $P < 0.01$.

Source: Veronesi (1990).

Table 4. Genetic components[1], their ratios, and levels of significance in the F_1 population of rice.

Character	D	H,	H_2	$(H_3/D)^{1/2}$	h^2/H_2
Maximum root length	86.40*2	176.96*	120.65*	1.43	1.65
Root-tip thickness	0.08**	0.11**	0.08**	1.16	3.28
Root/shoot ratio	6448.53*	21614.55*	13091.97*	3.35	2.10
Root number	296.81*	282.44*	264.07*	0.97	29.86

1. D = component of variation due to the additive effect of the genes.
   H_1 = component of variation due to the dominance effect of the genes.
   $H_2 = H_1 [1 - (u - v)^2]$, where u - proportion of positive genes in the parents and v - proportion of negative genes in the parents and where u + v = 1.
   h^2 - dominance effect.
   $(H_1/D)^{1/2}$ = mean degree of dominance.
   h^2/H_2 = number of genes or groups of genes showing dominance.
2. * $P< 0.05$. ** $P< 0.01$.

Source: Armenta-Soto et al. (1983).

Table 5. Estimate of genetic parameters for different root characters of rice.

Parameter	Root diameter	Root volume	Root length density	Root dry mass
Mid parent effect	1.15*[1]	19.0**	2.48**	0.7**
Additive effect	0.50**	13.5**	0.13ns[2]	0.4**
Dominance effect	0.15**	5.6**	2.60**	0.6**
Potence ratio	0.003	0.03	9.69	0.42

1. * *P<0.05,** P<0.01.*

Source: Ekanayake et al. (1985).

Attempts have been made to combine useful plant characters relating to enhancement of nutrient management of crops with good agronomic adaptation. PI 206002, an agronomically poor line of bean but known to have an extensive root system, was crossed with an adapted cultivar Sanilac, using an inbred-backcross method (Gabelman et al. 1986). More than 10% of the lines derived from this program had plants with root size similar to that of PI 206002 when grown in solution culture. In the field, lines with larger root system and lower shoot P concentration, as identified in solution culture, were associated with increased P uptake in a soil marginally deficient in P. In this case, dominance variance was more important than additive variance (Fawole et al. 1982a).

Few estimates have been made of the number of genes controlling root size parameters. Armenta-Soto et al. (1983) used derived statistics to determine that at

least two genes or groups of genes influence dominance for root length and root/ shoot ratio, whereas for root number at least 30 are involved (Table 4).

Root morphology

The fineness of root systems in P-deficient soils is important because the greater volume of soil close to a fine root results in a slower depletion of P than that which occurs from soil close to a coarser root (Fohse et al. 1991). While species with relatively thin roots may have greater plasticity in root growth and greater capacity for water and nutrient uptake, they may also have reduced root longevity and less mycorrhizal dependence (Eissenstat 1992). Less mycorrhizal dependence may be of some advantage since it has been estimated that approximately 10% of photosynthetically fixed carbon may be used to support the mycorrhizal symbiosis (Lambers 1987).

Bhat et al. (1976) observed that there is a considerable widening of the rhizosphere due to the presence of root hairs—up to three times in rapeseed. Root hair length is heritable (Table 1) and can be easily manipulated by selection (Caradus 1979). Selection for long root hairs in white clover resulted in a 14% increase in one cycle of selection (Table 6). Selection for increased root branching in alfalfa was generally successful but depended upon cultivar and nitrogen source (Table 7). While genetic variation in root system size tends to correspond to genetic control of shoot size, special genes have been found mat control such specific traits as the 'cottony' root in tomato, which has a high capacity for P uptake (Chloupek and Rod 1992).

The formation of highly branched roots, often referred to as proteoid roots, is a specialized response to P deficiency in Proteaceae (Lamont 1982) and lupin species (Gardner et al. 1983). Rhizosphere soil of these roots becomes strongly acidified and, compared to rhizosphere soil of 'normal' roots, contains more reductants and chelators (Grierson and Attiwell 1989).

Table 6. Selection for root hair length in white clover.

	Root hair length (urn)		
	Parent plants		
Selection	Original selection	Retest	Progeny test
Unselected (cv Tamar)	405± 5[1]	n.m.[2]	334 ± 11
Short	278± 6	2 3 8 ± 6	278 ± 7
Long	545 ± 6	4 4 2 ± 1 9	380 ± 8

1. Standard error.
2. Not measured.

Source: Caradus (1979).

Table 7. Selection in alfalfa for degree of root branching (based on scores 1 = no laterals to 5 = no defined tap root).

	Cultivar	
	Apica	Iroquois
Rhizobium inoculated		
	1.48 c[1]	1.62 bc
Progeny from selection for high branching	2.09 a	1.93 ab
Progeny from selection for low branching	1.36 c	1.33 c
Nitrogen fed		
Parent	2.20 bc	2.07 cd
Progeny - high	2.55 ab	2.74 a
Progeny - low	2.67 a	1.43 e

1. Within columns and nitrogen treatments, means followed by a different letter are significantly different.

Source: Saindon et al. (1991).

Mycorrhizae

The effectiveness of mycorrhizae in utilizing soil P can be explained by the fineness and length of the hyphae, not by any special P absorption characteristics of their surfaces (Silberbush and Barber 1983). Vesicular-arbuscular (VA) mycorrhizae extend the nutrient absorptive area of roots through extension into as yet 'unexplored' soil. Selection of plant genotypes that are conducive to colonization by efficient VA mycorrhizae has been suggested as a possible approach to manipulating VA mycorrhizal associations (Lee and Wani 1991), particularly when plants rely on rock phosphate as a source of P (Daft 1991). Genetic variation in extent of root colonization by mycorrhizae has been demonstrated in many species (Smith et al. 1992). Selection for high incidence of mycorrhizal infection is realistic and achievable, particularly if it is based on recent heritability estimates, as was done recently in *Vigna unguiculata* (Table 1). Additionally, Lackie et al (1988) considered that even with a narrow sense heritability for percentage of root colonized by VA mycorrhizae of 0.19 and an expected response to selection, if the top 10% of the population was selected, of 2.2% per cycle, there was good potential for improving the extent of VA mycorrhizae colonization in the population.

Genetic control of solubilizing mechanisms

Plant roots can substantially change the microenvironment in the surrounding soil to promote the release of soil minerals including P (Clarkson 1985). This can be

through exudation of polysaccharides that increase soil-root contact, enzyme release, or acid secretion.

While a few studies identified genetic variation in root exocellular acid phosphatase activity (Caradus and Snaydon 1987, Helal 1990), there have been no reports of heritability estimates or mode of genetic control for this plant character. Root exudates of such organic acids as citric acid have been associated with solubilization of phosphate in soil for *Lupinus albus* L. (Dinkelaker et al. 1989), but again there are no reports of mode of genetic control or heritability for either root-induced pH changes or root exudation.

Associated effects

Growth rate and plant phosphorus status

There is now considerable evidence, and I believe general acceptance, that enhanced uptake of mineral nutrients including P is a correlated genetic consequence of enhanced growth (Wild and Breeze 1981, Caradus 1990, Vakhmistrov and O En Do 1992). This relationship is influenced by the minimum tissue P concentrations necessary to sustain maximum growth (Koide 1991).

Instantaneous P uptake per unit of root is related to the P status of the plant, being higher for P-deficient plants than for plants with an adequate or optimal level of tissue P (Lefebvre and Glass 1982). The demonstration in reciprocal grafting experiments that shoot growth regulates root growth and P uptake per plant (Caradus and Snaydon 1986c) would suggest that shoot factors rather than root factors regulate P uptake per unit root size. Removing a part of the shoot, by cutting, reduces P uptake per unit root weight, but removal of half of the root system from P supply, either by splitting root systems or root pruning alone, had, in the short term (approximately 3 days), no effect on P uptake per unit root weight (Caradus and Snaydon 1986b). Phosphorus uptake is regulated primarily by the inorganic P concentration of the root cell (de Jager 1979), which largely reflects the P status of the shoot (de Jager and Posno 1979). Clarkson and Hawkesford (1993) have shown in a recent review that the genes for transporters of ions across membranes can be identified by using the techniques of molecular biology. However, they concede that the absorption rates of major nutrients, including P, are quite strictly regulated by biochemical factors which vary with the rate at which nutrients are used in growth.

Intraspecific variation for P uptake can be equated with observed differences in plant P concentration for maize (Baker et al. 1970), alfalfa (Hill and Jung 1975, Hill and Barnes 1977, Miller et al. 1987), and wheat (Saric et al. 1987). Selections for high and low shoot P concentrations have been successful (Baker et al. 1971, Hill and Lanyon 1983, Melton et al. 1989) with realized heritabilities for increased P concentration of up to 0.36 for alfalfa (Miller et al. 1987) and narrow sense

heritabilities of 0.42 for wheat (Kolmakova et al. 1983). In maize, concentration of P in the ear leaf has been estimated to be controlled by three major genes (Gorsline et al. 1968), probably located on chromosome 9 (Naismith et al. 1974). In wheat, P utilization (the inverse of P concentration) was controlled predominantly by additive genes (Kolmakova et al. 1983) while in sorghum, dominant effects were more important (Furlani et al. 1987) and in beans, epistatic effects (i.e., gene interaction where one gene interferes with the phenotypic expression of another nonallelic gene/s) were important (Fawole et al. 1982b).

Intraspecific differences in growth rate per unit of P applied (i.e., P response) has been observed in several species, e.g., sorghum (Clark 1990) and white clover (Mackay et al. 1990). In white clover, genotypes were identified that combined both tolerance to low P (i.e., high yields at low P) and an ability to respond to added P (Caradus et al. 1992a). Inheritance studies have shown that high P response (higher dry weight increase per unit of P applied) was dominant over low P response (lower dry weight increase per unit of P applied), and that narrow sense heritabilities for P-response were moderate (0.33 to 0.46) (Caradus et al. 1992b). The ratio of dominant to recessive genes in all parents was approximately 2 for P response. An estimate of the number of effective factors, which may be synonymous with the number of genes or groups of genes which exhibit some degree of dominance, showed that at least four individual or groups of genes are involved in P response (Table 8).

Selection for yield at low P has also been used to identify adapted strains of tomato (Coltman et al. 1987). Broad sense heritability for yield at low P varied from 0.61 to 0.67 depending upon generation. Dominance effects were more important than additive genetic variance in the expression of low-P tolerance.

Table 8. Estimates of genetic components for P response in white clover determined by linear (p) and quadratic (p^2) coefficients of fitted quadratic responses curves.

	P response measures	
Derived values	P	P^2
Dominance ratio	1.05	1.22
Ratio of dominant to recessive alleles	2.24	2.57
Number of effective factors	3.68	3.96

Source: Caradus et al. (1992b).

Conclusion

There is considerable genetic variation for most morphological and physiological characters that have been associated with enhanced P management by plants.

Additionally, heritabilities for these characters are generally moderate to high and suggest that selection programs would be successful. Schettini et al. (1987) have shown that quantitative traits related to P nutrition can be successfully transferred into agronomically useful germplasm. However, for parameters other than root size and morphology, there have been relatively few studies examining the heritability or mode of inheritance of such characters.

References

Ali-Khan, S.T., and Snoad, B. 1977. Root and shoot development in peas. I. Variability in seven root and shoot characters of seedlings. Annals of Applied Botany 85: 131-136.

Armenta-Soto, J., Chang, T.T., Loresto, G.C., and O'Toole, J.C. 1983. Genetic analysis of root characters in rice. SABRAO Journal 15: 103-116.

Baker, D.E., Jarrell, A.E., Marshall, L.E., and Thomas, W.I. 1970. Phosphorus uptake from soils by corn hybrids selected for high and low phosphorus concentration. Agronomy Journal 62: 103-106.

Baker, D.E., Wooding, F.J., and Johnson, M.W. 1971. Chemical element accumulation by populations of corn (*Zea mays* L.) selected for high and low accumulation of P. Agronomy Journal 63: 404-406.

Bhat, K.K., Nye, P.H., and Baldwin, J.P. 1976. Diffusion of phosphate to plant roots in soil. IV. The concentration distance profile in the rhizosphere of roots with root hairs in low-P soil. Plant and Soil 44: 63-72.

Caradus, J.R. 1979. Selection for root hair length in white clover *(Trifolium repens* L.). Euphytica 28: 489-494.

Caradus, J.R. 1990. Mechanisms improving nutrient use by crop and herbage legumes. Pages 253-311 *in* Crops as enhancers of nutrient use (Baligar, V.C. and Duncan, R.R., eds.). San Diego, USA: Academic Press.

Caradus, J.R., and Snaydon, R.W. 1986a. Plant factors influencing phosphorus uptake by white clover from solution culture. I. Population differences. Plant and Soil 93: 153-163.

Caradus, J.R., and Snaydon, R.W. 1986b. Plant factors influencing phosphorus uptake by white clover from solution culture. II. Root and shoot pruning and split-root studies. Plant and Soil 93: 165-174.

Caradus, J.R., and Snaydon, R.W. 1986c. Plant factors influencing phosphorus uptake by white clover from solution culture. III. Reciprocal grafting. Plant and Soil 93: 175-181.

Caradus, J.R., and Snaydon, R.W. 1986d. Response to phosphorus of populations of white clover. 3. Comparison of experimental techniques. New Zealand Journal of Agricultural Research 29: 169-178.

Caradus, J.R., and Snaydon, R.W. 1987. Aspects of the phosphorus nutrition of white clover populations. II. Root exocellular acid phosphatase activity. Journal of Plant Nutrition 10: 287-301.

Caradus, J.R., and Woodfield, D.R. 1990. Estimates of heritability for, and relationships

between, root and shoot characters of white clover. I. Replicated clonal material. Euphytica 46: 203-209.

Caradus, J.R., Mackay, A.D., Wewala, S., Dunlop, J., Hart, A.L., Lambert, M.G., van den Bosch, J., and Hay, M.J.M. 1992a. Heritable differences in white clover for response to phosphorus: new prospects for low input pastoral systems. Proceedings of the New Zealand Grassland Association 53: 59-66.

Caradus, J.R., Mackay, A.D., Wewala, S., Dunlop, J., Hart, A.C., van den Bosch, J., Lambert, M.G., and Hay, MJ.M. 1992b. Inheritance of phosphorus response in white clover. Plant and Soil 146: 199-208.

Chang, T.T., Loresto, G.C., O'Toole, J.C., and Armenta-Soto, J.L. 1982. Strategy and methodology of breeding rice for drought prone areas. Pages 217-244 *in* Drought resistance in crops with emphasis on rice. Los Baftos, Philippines: International Rice Research Institute.

Chloupek, 0.1984. Root system size as selection criteria in lucerne breeding. Pages 197—202 *in* Proceedings of the *Medicago sativa* Group of Eucarpia, 27-30 Aug 1984, Brno, Czechoslovakia. Brno, Czechoslovakia: Czechoslovak Science and Technology Society.

Chloupek, O., and Rod, J. 1992. The root system as a selection criteria. Plant Breeding Abstracts 62: 1337-1341.

Chloupek, O., and Samaneck, J. 1985. [Evaluation of above ground parts and roots of lucerne under frequent cutting.] (In Cz. Summaries in Ru, En.). Sbornik UVTIZ, Genetika a Slechteni 21: 61-70.

Clark, R.B. 1990. Physiology of cereals for mineral nutrient uptake, use and efficiency. Pages 131-209 *in* Crops as enhancers of nutrient use (Baligar, V.C., and Duncan, R.R., eds.). San Diego, USA: Academic Press.

Clarkson, D.T. 1985. Factors affecting mineral nutrient acquisition by plants. Annual Review of Plant Physiology 36: 77-115.

Clarkson, D.T., and Hawkesford, MJ. 1993. Molecular biological approaches to plant nutrition. Pages 23-33 *in* Plant nutrition—from genetic engineering to field practice (Barrow, N.J., ed.). Dordrecht, The Netherlands: Kluwer Academic.

Coltman, R.R., Gabelman, W.H., Gerloff, G.C., and Barta, S. 1987. Genetics and physiology of low-phosphorus tolerance in a family derived from two differentially adapted strains of tomato *(Lycopersicon esculentum* Mill.). Pages 309-315 *in* Proceedings of the second international symposium on genetic aspects of plant mineral nutrition, 16-20 Jun 1985, Wisconsin, Madison, USA (Gabelman, H.W., and Loughman, B.C., eds.). Dordrecht, The Netherlands: Martinus Nijhoff.

Daft, M.J. 1991. Influences of genotypes, rock phosphate and plant densities on mycorrhizal development and the growth responses of five different crops. Agriculture, Ecosystems and Environment 35: 151-169.

Darrah, P.R. 1993. The rhizosphere and plant nutrition: a quantitative approach. Pages 3-22 *in* Plant nutrition—from genetic engineering to field practice (Barrow, N.J., et.). Dordrecht, The Netherlands: Kluwer Academic.

Das, R.K., Miah, N.M., and Loresto, G.C. 1991. Genetic variability in root and shoot characters of selected rice genotypes. International Rice Research Newsletter 16:5.

da Silva., J.V. 1984. [Application of fundamental studies to breeding drought resistant plants.] (In Fr, Summary in En.). Bulletin de la Societe Botanique de France, Actualites Botaniques 131: 51-57.

de Jager, A. 1979. Localised stimulation of root growth and phosphate uptake in *Zea mays* L. resulting from restricted phosphate supply. Pages 391-404 *in* The soil-root interface: proceedings of an international symposium (Harley, J.L., and Russell, R.S., eds). London, UK: Academic Press.

de Jager, A., and Posno, M. 1979. A comparison of the reaction to a localised supply of phosphate in *Plantago major, P. lanceolata* and *P. media.* Acta Botanica Neerlandica 28: 479-489.

Dinkelaker, B., Romheld, V., and Marschner, H. 1989. Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (*Lupinus albus* L.). Plant Cell and Environment 12: 285-292.

Eissenstat, D.M. 1992. Costs and benefits of constructing roots of small diameter. Journal of Plant Nutrition 15: 763-782.

Ekanayke, I.J., O'Toole, J.C., Garrity, D.P., and Masajo, T.M. 1985. Inheritance of root characters and their relations to drought resistance in rice. Crop Science 25: 927-933.

Ennos, R.A. 1985. The significance of genetic variation for root growth within a natural population of white clover (*Trifolium repens*). Journal of Ecology 73: 615-624.

Fawole, I., Gabelman, W.H., and Gerloff, G.C. 1982a. Genetic control of root development in beans (*Phaseolus vulgaris* L.) grown under phosphorus stress. Journal of the American Society of Horticultural Science 107: 98-100.

Fawole, I., Gabelman, W.H., Gerloff, G.C, and Nordheim, E.U. 1982b. Heritability of efficiency in phosphorus utilization in beans (*Phaseolus vulgaris* L.) grown under phosphorus stress. Journal of the American Society of Horticultural Science 107: 94-97.

Fohse, D., Claassen, N., and Jungk, A. 1991. Phosphorus efficiency of plants. II. Significance of root radius, root hairs and cation-anion balance for phosphorus influx in seven plant species. Plant and Soil 132: 261-272.

Foster, G.S., Campbell, R.F., and Adams, W.T. 1984. Heritability, gain, and C effects in rooting of western hemlock cuttings. Canadian Journal of Forest Research 14: 628-638.

Fox, R.H. 1978. Selection for phosphorus efficiency in corn. Communications in Soil Science and Plant Analysis 9: 13-37.

Furlani, A.M.C., Clark, R.B., Ross, W.M., and Maranville, J.W. 1987. Differential phosphorus uptake, distribution and efficiency by sorghum parents and their inbreds. Pages 287-298 *in* Proceedings of the second symposium on genetic aspects of plant mineral nutrition, 16-20 Jun 1985, Madison, USA (Gabelman, W.H., and Loughman, B.C., eds.). Dordrecht, The Netherlands: Martinus Nijhoff.

Gabelman, W.H., Gerloff, G.C., Schettini, T., and Coltman, R. 1986. Genetic variability in root systems associated with nutrient acquisition and use. HortScience 21: 971-973.

Gardner, W.K., Barber, D.A., and Parbery, D.G. 1983. The acquisition of phosphorus by *Lupinus albus* III. The probable mechanism by which phosphorus movement in the soil/root interface is enhanced. Plant and Soil 70: 107-124.

Gorsline, G.W., Thomas, W.I., and Baker, D.E. 1968. Major gene inheritance of Sr-Ca, Mg, K, P, Zn, Cu, B, Al-Fe, and Mn concentration in corn. Pennsylvania State University Agriculture Experiment Station Bulletin 746. 47 pp.

Grierson, P.F., and Attiwell, P.M. 1989. Chemical characteristics of proteoid root mat of *Banskia integrifolia.* Australian Journal of Botany 37: 137-143.

Hajra, N.G., Hajra, S.G., and Bairagi, P. 1988. Heritability of primary root length. International Rice Research Newsletter 13: 7.

Helal, H.M. 1990. Varietal differences in root phosphatase activity as related to the utilization of organic phosphates. Plant and Soil 123: 161-163.

Hill, R.R., and Barnes, R.F. 1977. Genetic variability for chemical composition of alfalfa. II. Yield and traits associated with digestibility. Crop Science 17: 948-952.

Hill, R.R., and Jung, G.A. 1975. Genetic variability for chemical composition of alfalfa. I. Mineral elements. Crop Science 15: 652-657.

Hill, R.R., and Lanyon, L.E. 1983. Phosphorus fertilizer response in experimental alfalfa selected for different phosphorus concentrations. Crop Science 23: 973-976.

Jones, A., Steinbauer, C.E., and Pope, D.T. 1969. Quantitative inheritance of 10 root traits in sweet potatoes. Journal of American Society of Horticultural Science 94: 271-275.

Jones, G.P.D., Blair, G J., and Jessop, R.S. 1989. Phosphorus efficiency in wheat—a useful selection criteria? Field Crops Research 21: 257-264.

Kalashnik, N.A., Gamzikova, O.I., Kolmakova, I.R., Gamsikova, O.I., and Kolmakowa, I.P. 1982. [Effect of nutrient regime on combining ability and heritability of the primary root system in spring wheat.] (In Ru, Summary in En.) Genetika 18: 139-144.

Koide, R.T. 1991. Nutrient supply, nutrient demand and plant response to mycorrhizal infection. New Phytologist 117: 365-386.

Kolmakova, I.R., Gamzikova, O.I., Kalashnik, N.A., and Gamzikov, G.P. 1983. [Combining ability and heritability for phosphorus fertilizer utilization in varieties of spring wheat (from data of studies using ^{32}P)]. (In Ru. Summary in En.) Genetika 19: 808-814.

Kormanik, P.P., Ruehle, J.L., and Muse, H.D. 1990. Frequency distribution and heritability of first-order lateral roots in loblolly pine seedlings. Forest Science 36: 802-814.

Kuburovic, M. 1984. [Inheritance of features of the development of the root system in wheat.] (In Cr, Summaries in En.) Poljoprivredna Znanstvena Smotra 66: 347-360.

Lackie, S.M., Bowley, S.R., and Peterson, R.L. 1988. Comparison of colonization among half-sib families of *Medicago sativa* L. by *Glomus versiforme* (Daniels and Trappe) Berch. New Phytologist 108: 477-482.

Lambers, H. 1987. Growth, respiration, exudation and symbiotic associations: the fate of carbon translocated to the roots. Pages 125-145 *in* Root development and function (Gregory, J., Lake, J.V., and Rose, D.A., eds.). New York, USA: Cambridge University Press.

Lamont, B. 1982. Mechanisms of enhancing nutrient uptake in plants, with particular reference to Mediterranean, South Africa and Western Australia. Botanical Review 48:597-689.

Lee, K.K, and Wani, S.P. 1991. Possibilities for manipulating mycorrhizal associations in crops. Pages 107-116 *in* Phosphorus nutrition of grain legumes in the semi-arid tropics (Johansen, C., Lee, K.K., and Sahrawat, K.L., eds.). Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Lefebvre, D.D., and Glass, A.D.M. 1982. Regulation of phosphate influx in barley roots: Effects of phosphate deprivation and reduction of influx with provision of orthophosphate. Physiologia Plantarum 54: 199-206.

Lehman, V.G., and Engelke, M.C. 1991. Heritability estimates of creeping bentgrass root systems grown in flexible tubes. Crop Science 31: 1680-1684.

Mackay, A.D., Caradus, J.R., Dunlop, J., Wewala, S., Mouat, M.C.H., Lambert, M.G., Hart, A.L., and van den Bosch, J. 1990. Response to phosphorus of a world collection of white clover cultivars. Pages 553-558 *in* Genetic aspects of plant material nutrition (El Bassam, N., Dambroth, M., and Loughman, B.C., eds.). Dordrecht, The Netherlands: Kluwer Academic.

Mcintosh, M.S., and Miller, D.A. 1980. Development of root-branching in three alfalfa cultivars. Crop Science 20: 807-809.

Melton, B., Carrier, C., McCaslin, B., Miller, D., Waissman, N., and Olivares, E. 1989. Registration of P-3 alfalfa germplasm. Crop Science 29: 832-833.

Mercy, M.A., Shivashankar, G., and Bagyaraj, D.J. 1990. Mycorrhizal colonization in cowpea is host dependent and heritable. Plant and Soil 121: 292-294.

Miller, D.J., Waissman, N., Melton, B.A., Currier, C., and McCaslin, B.D. 1987. Selection for increased phosphorus in alfalfa and effects on other characteristics. Crop Science 27: 22-26.

Montpetit, J.M., and Coulman, B.E. 1991. Responses to divergent selection for adventitious root growth in red clover *(Trifolium pratense* L.). Euphytica 58: 119-127.

Naismith, R.W., Johnson, M.W., and Thomas, W.I. 1974. Genetic control of relative calcium, phosphorus, and manganese accumulation on chromosome 9 in maize. Crop Science 14: 845-849.

Nambiar, E.K.S., Cotterill, P.P., and Bowen, G.D. 1982. Genetic differences in the root regeneration of radiata pine. Journal of Experimental Botany 33: 170-177.

Ochesanu, C., and Cabulea, I.1989. [Studies on the genetic control of the root system in maize] (In Ro. Summary in En.) Cercetari de Genetica Vegetala si Animala 1: 61-80.

Omara, M.K., and Hussein, M.Y. 1987. Genetic variability in root characteristics in relation to yield under drought in early maturing barley. Assiut Journal of Agricultural Sciences 18: 319-332.

Pederson, G.A., Kendall, W.A., and Hill, R.R. 1984. Effect of divergent selection for root weight on genetic variation for root and shoot characters in alfalfa. Crop Science 24: 570-573.

Romer, W., Augustin, J., and Schilling, G. 1989. The relationship between phosphate absorption and root length in nine wheat cultivars. Pages 123-125 *in* Structural and functional aspects of transport in roots: proceedings of Third International Symposium on

Structure and Function of Roots, 3-7 Aug 1987, Nitra, Czechoslovakia (Loughman, B.C., Ga§parikova, O., and Kolek, J., eds.). Dordrecht, The Netherlands: Kluwer Academic.

Saindon, G., Michaud, R., and St Pierre, C.A. 1991. Breeding for root yield in alfalfa. Canadian Journal of Plant Science 71: 727-735.

Saleh, G.B., and Gritton, E.T. 1988. Genetic control of root weight, root volume and root to shoot weight ratio in peas. Pertanika 11: 165-173.

Saric, M.R., Krstic, B., and Stankovic, Z. 1987. Genetic aspects of mineral nutrition of wheat. I. Concentrations of N, P, K., Ca and Mg in leaves. Journal of Plant Nutrition 10: 1539-1549.

Schapendank, A.H.C., and de Vos, A.L.F. 1988. A selection strategy for persistency testing of grasses on hydroponics. Pages 193-202 *in* Natural selection and breeding for adaptation: Proceedings of the Eucarpia Fodder Crops Section Meeting, 22-24 Sept 1987, Lusignan, France. Lusignan, France: Station d'Amelioration des Plantes Fourrageres, INRA.

Schettini, T.M., Gabelman, W.H., and Gerloff, G.C. 1987. Incorporation of phosphorus efficiency from exotic germplasm into agriculturally adapted germplasm of common bean *(Phaseolus vulgaris* L.). Plant and Soil 99: 175-184.

Sharma, J.K., and Bhalla, S.K. 1990. Genetics of some drought tolerant traits in maize. Crop Improvement 17: 144-149.

Silberbush, M., and Barber, S.A. 1983. Sensitivity of simulated phosphorus uptake to parameters used by a mechanistic-mathematical model. Plant and Soil 74: 93-100.

Singh, N.B., Dube, P.S., and Singh, A.K. 1988. Genetic variability in root characteristics in chickpea. International Chickpea Newsletter 18: 35-37.

Smith, S.E., Robson, A.D., and Abbott, L.K. 1992. The involvement of mycorrhiza in assessment of genetically dependent efficiency of nutrient uptake and use. Plant and Soil 146: 169-179.

Sree Rangasamy, S.R., and Shanmugam, A.S. 1984. Variability and correlation analysis in seedling characters in greengram. Indian Journal of Agricultural Sciences 54: 6-9.

Troughton, A., and Whittington, W.J. 1969. The significance of genetic variation in root systems. Pages 296-314 *in* Root growth: Proceedings of the Fifteenth Easter School in Agricultural Science, University of Nottingham, UK (Whittington, W.J., ed.). London, UK: Butterworths.

Vakhmistrov, D.B., and O En Do. 1992. [Relation between growth and absorption activities of the genotype]. (In Ru. Summary in En., UK.) Fiziologiya i Biokhimiya kul'turnykh Rastenii 24: 260-269.

Veronesi, F. 1990. Responses to selection for root dimension in a South European variety of perennial ryegrass. Plant Breeding 105: 321-326.

Wiersum, L.K. 1981. Problems in soil fertility characterization by means of plant nutrient requirements. Plant and Soil 61: 259-268.

Wild, A., and Breeze, V.G. 1981. Nutrient uptake in relation to growth. Pages 331-344 *in* Physiological processes limiting plant productivity: Proceedings of the Thirtieth Easter School in Agricultural Science, University of Nottingham, UK. London, UK: Butterworths.

Woodfield, D.R., and Caradus, J.R. 1990. Estimates of heritability for, and relationships between, root and shoot characters of white clover. II. Regression of progeny on mid-parent. Euphytica 46: 211-215.

The role of rhizosphere microorganisms in influencing phosphorus uptake, and prospects for favorable manipulation

J F Loneragan[1]

Abstract

This paper briefly summarizes published observations and assesses the potential of genetic manipulation to favor rhizosphere microorganisms that improve the ability of plants to utilize soil phosphorus (P) normally not available to them.

It is concluded that the present level of understanding of the relationships among soil, plant, and rhizosphere microorganisms offers little scope for genetic manipulation of nonsymbiotic rhizosphere microorganisms. Unless they are shown to have some unique mechanism denied to plant roots, it is also unlikely that they will be more effective targets for genetic manipulation than the plant roots themselves.

The control of pathogens, parasites, and pests, which can adversely affect root development and P uptake, appears a promising area for genetic manipulation but lies outside the scope of this paper.

In the vesicular-arbuscular mycorrhizae (VAM) symbiosis, manipulation of fungal genes is not warranted until existing variation has been assessed and methods for introducing superior strains into field crops have been developed. Manipulation of plant genes requires a better definition of the determinants of P efficiency in VAM-infected field crops. Breeding programs aimed at maximizing efficient P uptake from soils should include mycorrhizae since they may drastically change the comparative ranking of genotypes within species; such programs would have the additional benefit of retaining potentially valuable genetic variability.

Introduction

That nonsymbiotic rhizosphere microorganisms might be used to enhance the ability of plants to absorb P from low-P soils was suggested at an earlier FAI-FAO seminar held in India (Rangaswami 1975, cited by Kalpage 1979), and on many

1. School of Biological and Environmental Sciences, Murdoch University, Perth, Western Australia 6150, Australia.

Loneragan, L.F. 1995. The role of rhizosphere microorganisms in influencing phosphorus uptake, and prospects for favorable manipulation. Pages 75-78 *in* Genetic manipulation of crop plants to enhance integrated nutrient management in cropping systems—1. Phosphorus: proceedings of an FAO/ICRISAT Expert Consultancy Workshop, 15-18 Mar 1994,ICRISAT Asia Center, India (Johansen. C., Lee, K.K., Sharma, K.K., Subbarao, G.V., and Kueneman, E.A., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

other occasions too (e.g., Gerretson 1948, Kalpage 1979, Kunishi and Bandel 1991). Indeed, when he visited the Soviet Union in 1958, Cooper (1959) found that agronomists were recommending 'phosphobacterin' *(Bacillus megatherium* var *phosphaticum)* inoculation of field crop seeds as a standard procedure to supplement fertilizer P. He found universal faith but no hard evidence to support claims of an average 10% yield increase in 50-70% of crops. Nor did he find any evidence to support the assumption that phosphobacterin increased the availability of organic P.

Yet there is indisputable evidence that, under some conditions, rhizosphere microorganisms can affect the utilization of soil P by plants. They do so by competing with them for P or by affecting the release of soil P from unavailable forms or the efficiency of the root system for absorbing P. While some of the mechanisms are specific to P, many are nonspecific, affecting the utilization of all nutrients and being especially important for any limiting nutrient.

But the relationships among soils, plants, and rhizosphere microorganisms are very complex and poorly understood (Bowen and Rovira 1991). Even the identification and quantification of the rhizosphere microorganisms remains a major problem (Darrah 1993). Moreover, the system is very sensitive to environmental and biological variables so that experimental results are frequently contradictory (Barber et al. 1976) and field results variable. Hence, while many nonsymbiotic rhizosphere microorganisms have a potential to affect the P nutrition of plants, few, if any, of them will be used for improving utilization of soil P by crops until their activities in the rhizosphere are better understood.

By contrast, mycorrhizal fungi in symbiotic association with plant roots have already been used successfully to improve P uptake from soils in many crop plants.

This paper briefly summarizes published observations and assesses the potential of genetic manipulation to favor rhizosphere microorganisms that improve the ability of plants to utilize soil P normally not available to them.

Availability of phosphorus

In soils with supplies of P that are normally not available to plants, rhizosphere microorganisms may act to release P from both organic and inorganic forms.

Inorganic phosphorus

As early as 1948, Gerretson (1948) showed that plant roots inoculated with rhizosphere microorganisms absorbed more P from insoluble calcium phosphate than sterile roots did. Bacterial isolates from the rhizoplane of wheat roots were more effective than isolates from the bulk soil or rhizosphere soil (Katznelson and Bose 1959). When grown on glucose media, some strains of *Pseudomonas fluorescens* and other bacteria isolated from the rhizosphere of crop plants

dissolved silicate and phosphate minerals, including natural and synthetic apatites and iron and aluminum hardpans from soils. This group constituted a higher proportion of the bacteria in the rhizosphere than in the soil; the most active dissolvers produced large yields of 2-ketogluconic acid, which was thought to act through acidification and chelation (Duff et al. 1963). Subsequent workers (Moghimi and Tate 1978) have shown that 2-ketogluconate has a negligible calcium stability constant, and attribute its ability to dissolve hydroxyapatite to its strength as an acid. Wheat seedlings efficient in obtaining P from ^{32}P-labeled synthetic hydroxyapatite produced significant amounts of 2-ketogluconic acid but no other acid in their rhizosphere (Moghimi et al. 1978a, 1978b); the 2-ketogluconic acid was thought to be produced by microbial conversion of glucose secreted from the roots.

However, the contribution of these rhizosphere microorganisms to the P nutrition of crop plants in the field has not been assessed, so that it is impossible to assess the potential value of their manipulation. Nor does a logical basis exist for their manipulation since the factors governing their activities have not been determined. Those researchers who have attempted to quantify it are highly skeptical of its significance (Barber et al. 1976, Nye and Tinker 1977) or consider the information too limited to arrive at any judgement (Darrah 1993). Hayman (1975) suggested that nonsymbiotic rhizosphere microorganisms would be enormously disadvantaged for their carbon supplies compared with mycorrhizae, while Tinker (1984) considered that the overriding factor against them having a significant role 'is that these effects could mostly be produced by the plant itself, and in some cases such as pH change, to a much larger extent than any conceivable microbiological process'.

Organic phosphorus

There is also evidence that rhizosphere microorganisms may contribute to P uptake in crop plants through release of P from organic P compounds in the soil. Organic P compounds constitute an appreciable proportion of the total P in most top soils (Dalai 1977), but have generally been considered a poor source of P to plants. Re-examination of this view has shown rapid transformation of organic P (Helal and Sauerbeck 1984) and considerable mobilization of P from insoluble organic P in the rhizosphere (Helal and Dressier 1989). Seeling and Jungk (1992, cited by Jungk et al. 1993) also estimated that organic P contributed one-third of the P taken up by crop plants in a field experiment.

Phosphorus in the soil solution as organic P is mobile and, on reaching the root, may be released there by phosphatase activity, making it available for plant uptake. Phosphatase activity has been shown to be much higher in the rhizosphere than in the bulk soil (Tarafdar and Jungk 1987, Dinkelaker and Marschner 1992). Plant roots, such fungi as ectomycorrhizae, and bacteria may all contribute, with roots

and fungi producing acid phosphatase and bacteria alkaline phosphatase (Tarafdar and Claassen 1988). In nonmycorrhizal roots of many plant species, phosphatase activity at the root surface was associated with the plant root itself and not with the microorganisms; it increased substantially under conditions of P deficiency (McLachlan 1980). Further support for root phosphatase being more important than microbial phosphatase comes from experiments where washed plant roots and separated, nonsterile soil both had appreciable phosphatase activity; with decreasing adequacy of P supply, the root activity increased substantially whereas that of the soil decreased, possibly as a result of decreasing microbial biomass (Helal and Sauerbeck 1991).

On soils with low levels of available P and appreciable contents of organic P, increasing phosphatase activity in the rhizosphere could improve the P nutrition of crop plants. If genetic manipulation to increase phosphatase activity is considered worthwhile, it would appear far better to target the plant directly rather than indirectly through rhizosphere microorganisms.

Efficiency of the root system

The efficiency of a root system for P uptake depends upon both the activity and the extent of its absorbing surfaces. Rhizosphere microorganisms affect both profoundly.

In most cases, the rhizosphere microorganisms do so in ways that are not specific to P but impact on all nutrients, being especially significant for the most limiting nutrient. But where P *is* the limiting nutrient, the effects of rhizosphere microorganisms on root development and activity have a particularly strong impact because the low mobility of P in soils renders P uptake especially dependent on root extension into the soil (Darrah 1993).

Rhizosphere microorganisms which affect root development and activity include the following:

- mycorrhizae
- microorganisms that stimulate the development of proteoid roots
- other plant-growth-promoting rhizobacteria
- pathogens, parasites, and pests.

Mycorrhizae

The nature of symbiotic associations of mycorrhizae with plant roots and their role in P nutrition of plants is well known (Wilcox 1991, Krikun 1991, O'Dell et al. 1993). Mycorrhizae are so widespread that the typical nutrient absorbing organ for most agronomic crop plants is a symbiotic association of a VAM with a plant root; for most tree crops, it is a symbiotic association of an ectomycorrhiza with a plant root.

VAM symbioses are nonspecific, with a single plant species capable of associating with many, and possibly all, VAM fungi and each fungus capable of infecting a wide range of host species. Colonization of roots with VAM increases the efficiency of P uptake by plants in low-P soils, probably by extending the root system into a greater volume of soil. The beneficial effects to plants of P uptake by VAM may be partially offset by an unexplained effect on P utilization, evidenced by higher critical concentrations of P in tissues of infected plants compared to nonmycorrhizal plants.

In reviewing the impact of VAM on the efficiency of P uptake by plants, Smith et al. (1992) consider early and rapid spread of colonization to be as important as persistence and efficiency in P uptake. All of these attributes respond to environmental factors and vary with the plant and fungal species and cultivars or strains. Among environmental factors, the P status of plants is particularly important, with mild P deficiency enhancing all attributes, and adequacy and severe deficiency depressing them. But the extent to which P status affects colonization, and probably other attributes, varies greatly with the plant, high soil P having little effect over a wide range of soil P in some species and effectively eliminating colonization in others.

Plant genes exert strong control over VAM colonization, which varies widely among genotypes in some species. Fungal species and strains also vary widely in their capacity to develop effective mycorrhizal associations with plants. Abbott and Robson (1992) consider that the existing variation among the over 120 fungal species described so far needs to be assessed before any genetic engineering is undertaken to provide further variation. They also point out that our present poor understanding of the factors affecting the formation and functioning of mycorrhizae in field soils limits the use that can be made of any superior fungus for field crops.

The plant's control of VAM colonization of its roots offers a more immediate prospect for profitable genetic manipulation. But although rapid progress is being made in the identification of the plant genes involved, the determinants of P efficiency in VAM-infected field crops need further definition before genetic manipulation can be effectively targeted. In the meantime, breeding programs aimed at maximizing efficient P uptake from soils should include mycorrhizae; such programs would have the additional benefit of retaining potentially valuable genetic variability (Smith et al. 1992).

Proteoid roots

Proteoid roots, or 'root clusters', are not as well known nor as widespread among plant genera as are mycorrhizae. They were first identified in Proteaceae where they consist of clusters of short and extremely hairy lateral roots, which can make up to 80% of the root mass in the decomposing litter layer and be so dense as to

form a mat. They develop only under conditions of low nutrient supply and in response to the presence of noninfective rhizosphere microorganisms (Malajczuk and Bowen 1974). Proteoid roots enhance nutrient absorption, and inoculation of plants with soil microorganisms to form proteoid roots has doubled plant growth in low-P soil. Similar roots have been identified in genera of other families including a range of legumes (Bowen 1983, Lamont 1993).

In *Lupinus albus,* rhizosphere microorganisms were not essential for the formation of proteoid roots but stimulated their formation considerably (Gardner et al. 1982a). In this species, proteoid roots enhanced the excretion of reductants and chelating agents and the breakdown of adjacent iron and aluminum phosphates (Gardner et al. 1982b, 1982c). Large amounts of citric, malic, and aconitic acids have also been found in water leachates of the proteoid root layer of a mature stand of *Banksia integrifolia* (Grierson 1992). In both species, the excretion of citric acid by proteoid roots was thought to be primarily responsible for their ability to enhance P uptake (Gardner et al. 1983, Grierson 1992).

The factors restricting proteoid roots to particular species are not known. Nor are the identity and mode of action of the rhizosphere microorganisms responsible for the stimulation of proteoid root formation in those genera in which they do occur. Furthermore, if, as hypothesized, their efficacy in enhancing P uptake is related to excretion of citric acid, they will only be effective in plants with this inherent capacity. So long as these limitations exist, rhizosphere microorganisms capable of enhancing proteoid root formation are nonstarters as candidates for genetic manipulation to improve P uptake from soils.

Other plant-growth-promoting rhizobacteria

This grouping includes those rhizosphere microorganisms that have promoted plant growth in ways not already discussed. It includes *Azospirillum brasilense* and *Pseudomonas putida,* which have been shown to affect root morphology and P uptake, as well as a group of rhizosphere microorganisms that inhibit the detrimental activities of soilborne pathogens, parasites, and pests.

Inoculating sorghum, wheat, and maize with *A. brasilense* increased P uptake, probably as a result of increasing root surface area (Lin et al. 1983, Kapulnik et al. 1983, 1985) and inoculating tomato seedlings with a species of *Azospirillum* promoted root hair growth markedly (Kapulnik 1991). In addition, a strain of *P. putida* increased both root length and ^{32}P uptake by canola seedlings (Lifshitz et al. 1987).

However, as all these observations were made on plants grown in artificial, and often inadequate, media, their relevance to plants grown in nonsterile soil is

questionable. To be effective in soils, microorganisms need to be able to colonize, grow, and persist in the soil rhizosphere in competition with other organisms (Bolton et al. 1993). These desirable properties have been shown by a number of organisms with the capacity to inhibit the pathogenic activity of harmful organisms. Their inhibitory mechanisms include competition and excretion of antibiotic and chelating compounds. Unfortunately, field trials have given inconsistent results. Further discussion lies outside the scope of this paper. Several reviews have been published recently (Kapulnik 1991, Arshad and Frankenberger 1993, Baker and Dickman 1993, Kloepper 1993).

Pathogens, parasites, and pests

In many situations, pathogens (Katan 1991), parasites, especially nematodes [Cohn and Spiegel (1991), Dickman (1993)], and such pests as arthropods (Gerson 1991) dominate the ability of plants to obtain soil P. They must be considered in any broad strategy for better nutrient utilization. However, they are more properly studied within their own disciplines and do not feature in this workshop. But we should note that genetic manipulation of the plant has considerable potential to moderate their effects and we should support work to this end.

Conclusions

The present level of understanding of the relationships among soil, plant, and rhizosphere microorganisms offers little scope for genetic manipulation to enhance the activities of nonsymbiotic rhizosphere microorganisms in improving the ability of crop plants to obtain their P requirements from soils. Moreover, unless they are shown to have some unique mechanism denied to plant roots, it is also unlikely that they will be more effective targets for genetic manipulation than the plant roots themselves.

The control of pathogens, parasites, and pests, which can adversely affect root development, and P uptake appear more promising for genetic manipulation but are outside the scope of this paper.

The VAM symbiosis also offers prospects for genetic manipulation to improve the efficiency of P uptake from soils. Manipulation of fungal genes is not warranted until existing variation has been assessed and methods for introducing superior strains into field crops have been developed. Successful manipulation of plant genes requires a better definition of the determinants of P efficiency in VAM-infected field crops. In the meantime, breeding programs aimed at maximizing efficient P uptake from soils should include mycorrhizae since they may drastically change the comparative ranking of genotypes within species; such programs would have the additional benefit of retaining potentially valuable genetic variability.

References

Abbott, L.K., and Robson, A.D. 1991. Field management of VA mycorrhizal fungi. Pages 354-362 *in* The rhizosphere and plant growth: papers presented at a symposium, 8-11 May 1991, Beltsville Agricultural Research Center, Beltsville, Maryland, USA (Keister, D.L., and Cregan, P.B., eds.). Dordrecht, The Netherlands: Kluwer Academic.

Arshad, M., and Frankenberger, W.T., Jr. 1993. Microbial production of plant growth regulators. Pages 307-347 *in* Soil microbial ecology: application in agricultural and environmental management (Metting, F.B., ed.). New York, USA: Marcel Dekker.

Baker, R., and Dickman, M.B. 1993. Biological control with fungi. Pages 275-305 *in* Soil microbial ecology: application in agricultural and environmental management (Metting, F.B., ed.). New York, USA: Marcel Dekker.

Barber, D.A., Bowen, G.D., and Rovira, A.D. 1976. Effects of microorganisms on absorption and distribution of phosphate in barley. Australian Journal of Plant Physiology 3: 801-808.

Bolton, H., Frederickson, J.K., and Elliott, L.F. 1993. Microbial ecology of the rhizosphere. Pages 27-63 *in* Soil microbial ecology: application in agricultural and environmental management (Metting, F.B., ed.). New York, USA: Marcel Dekker.

Bowen, G.D. 1983. Microbial determinants of plant nutrient uptake. Pages 693-710 *in* Soils: an Australian viewpoint (Division of Soils, CSIRO, Melbourne, Australia). London, UK: Academic Press.

Bowen, G.D., and Rovira A.D. 1991. The rhizosphere: the hidden half of the hidden half. Pages 641-670 *in* Plant roots: the hidden half (Waisel, Y., Eshel, A., and Kafkafi, U., eds.). New York, USA: Marcel Dekker.

Cohn, E., and Spiegel, Y. 1991. Root-nematode interactions. Pages 789-805 *in* Plant roots: the hidden half (Waisel, Y., Eshel, A., and Kafkafi, U., eds.). New York, USA: Marcel Dekker.

Cooper, R. 1959. Bacterial fertilizers in the Soviet Union. Soils and Fertilizers 22: 327-333.

Dalai, R.C. 1977. Soil organic phosphorus. Advances in Agronomy 29: 83-117.

Darrah, P.R. 1993 . The rhizosphere and plant nutrition: a quantitative approach. Pages 3-22 *in* Plant nutrition — from genetic engineering to field practice (Barrow, N.J. ed.). Dordrecht, The Netherlands: Kluwer Academic.

Dickman, M.B. 1993. Molecular biology of plant-parasite relations. Pages 177-202 *in* Soil microbial ecology: application in agricultural and environmental management (Metting, F.B., ed.). New York, USA: Marcel Dekker.

Dinkelaker, B., and Marschner, H. 1992. *In vivo* demonstration of acid phosphatase activity in the rhizosphere of soil-grown plants. Plant and Soil 144: 199-205.

Duff; R.B., Webley, D.M., and Scott, R.O. 1963. Solubilization of minerals and related materials by 2-ketogluconic acid producing bacteria. Soil Science 95:105-114.

Gardner, W.K., Barber, D.A., and Parbery, D.G. 1982a. Effects of microorganisms on the formation and activity of proteoid roots of *Lupinus albus* L. Australian Journal of Botany 30: 303-309.

Gardner, W.K., Parbery, D.G., and Barber, D.A. 1982b. The acquisition of phosphorus by *Lupinus albus* L. I. Some characteristics of the soil/root interface. Plant and Soil 68: 19-32.

Gardner, W.K., Parbery, D.G., and Barber, D.A. 1982c. The acquisition of phosphorus by *Lupinus albus* L. II. The effect of varying phosphorus supply and soil type on some characteristics of the soil/root interface. Plant and Soil 68: 33-41.

Gardner, W.K., Barber, D.A., and Parbery, D.G. 1983. The acquisition of phosphorus by *Lupinus albus* L. III. The probable mechanism by which phosphorus movement in the soil/ root interface is enhanced. Plant and Soil 70: 107-124.

Gerretsen, F.C. 1948. The influence of microorganisms on the phosphate intake by the plant. Plant and Soil 1: 51-81.

Gerson, U. 1991. Arthropod root pests. Pages 807-822 *in* Plant roots: the hidden half (Waisel, Y., Eshel, A., and Kafkafi, U., eds.). New York, USA: Marcel Dekker.

Grierson, P.F. 1992. Organic acids in the rhizosphere of *Banksia integrifolia* L.f. Plant and Soil 144: 259-265.

Hayman, D.S. 1975. The occurrence of mycorrhiza in crops as affected by soil fertility. Pages 495-510 in Endomycorrhizas (Sanders, F.E., Mosse, B., and Tinker, P.B., eds.). London, UK: Academic Press.

Helal, H.M., and Dressier, A. 1989. Mobilization and turnover of soil phosphorus in the rhizosphere. Zeitschrift fur Pflanzenernahrung und Bodenkunde 152: 175-180.

Helal, H.M., and Sauerbeck, D. 1984. Influence of plant roots on carbon and phosphorus metabolism in soil. Plant and Soil 76: 175-182.

Helal, H.M., and Sauerbeck, D. 1991. Soil and root phosphatase activity and the utilization of inositol phosphates as dependent on phosphorus supply. Pages 93-97 *in* Plant roots and their environment: proceedings of an International Society of Root Research symposium, 21-26 Aug 1988, Uppsala, Sweden (McMichael, B.L., and Persson, H., eds.). Amsterdam, The Netherlands: Elsevier.

Jungk, A., Seeling, B., and Gerke, J. 1993. Mobilization of different phosphate fractions in the rhizosphere. Pages 95-98 *in* Plant nutrition — from genetic engineering to field practice (Barrow, N.J., ed.). Dordrecht, The Netherlands: Kluwer Academic.

Kalpage, F.S.C.P. 1979. Efficiency of phosphate utilization. Pages 60-73 *in* Soil microbiology and plant nutrition: proceedings of Symposium Somiplan, August 1976, Kuala Lumpur, Malaysia (Broughton, W.J., John, C.K., Rajarao, J.C., and Lim, B., eds.). Kuala Lumpur, Malaysia: Penerbit Universiti Malaya.

Kapulnik, Y. 1991. Plant-growth-promoting rhizobacteria. Pages 717-729 *in* Plant roots: the hidden half (Waisel, Y., Eshel, A., and Kafkafi, U., eds.). New York, USA: Marcel Dekker.

Kapulnik, Y., Gafny, R., and Okon, Y. 1983. Effect of *Azospirillum* spp. inoculation on root development and NO_3-uptake in wheat *(Trifolium aestivum* cv. Miriam) in hydroponic systems. Canadian Journal of Botany 63: 627-631.

Kapulnik, Y., Okon, Y., and Henis, Y. 1985. Changes in root morphology of wheat caused by *Azospirillum* inoculation. Canadian Journal of Microbiology 31: 881-887.

Katan, J. 1991. Interactions of roots with soil-borne pathogens. Pages 823-836 *in* Plant roots: the hidden half (Waisel, Y., Eshel, A., and Kafkafi, U., eds.). New York, USA: Marcel Dekker.

Katznelson, H., and Bose, B. 1959. Metabolic activity and phosphate dissolving capability of bacterial isolates from wheat roots, rhizosphere and non-rhizosphere soil. Canadian Journal of Microbiology 5: 79-85.

Kloepper, J.W. 1993. Plant growth-promoting rhizobacteria as biological control agents. Pages 255-274 *in* Soil microbial ecology: application in agricultural and environmental management (Metting, F.B., ed.). New York, USA: Marcel Dekker.

Krikun, J. 1991. Mycorrhizae in agricultural crops. Pages 767-786 *in* Plant roots: the hidden half (Waisel, Y., Eshel, A., and Kafkafi, U., eds.). New York, USA: Marcel Dekker, Inc.

Kunishi, H.M., and Bandel, V.A. 1991. Microbe enhanced P uptake by corn under no till and conventional till. Page 368 *in* The rhizosphere and plant growth: papers presented at a symposium, 8-11 May, Beltsville Agricultural Research Center, Beltsville, Maryland, USA (Keister, D.L., and Cregan, P.B., eds.). Dordrecht, The Netherlands: Kluwer Academic.

Lamont, B.B. 1993. Why are hairy root clusters so abundant in the most nutrient impoverished soils of Australia. Pages 309-312 *in* Plant nutrition—from genetic engineering to field practice (Barrow, NJ., ed.). Dordrecht, The Netherlands: Kluwer Academic.

Lifshitz, R., Kloepper, J.W., Kozlowski, M., Simonson, C., Carlson, J., Tipping, E.M., and Zaleska, I. 1987. Growth promotion of canola (rapeseed) seedling by a strain of *Pseudomonas putida* under gnotobiotic conditions. Canadian Journal of Microbiology 33: 390-395.

Lin, W., Okon, Y., and Hardy, R.W. 1983. Enhanced mineral uptake by *Zea mays* and *Sorghum bicolor* roots inoculated with *Azospirillum brazilense.* Applied and Environmental Microbiology 45: 1775-1779.

Malajczuk, N., and Bowen, G.D. 1974. Proteoid roots are microbially induced. Nature (London) 251: 316-317.

McLachlan, K.D. 1980. Acid phosphatase activity of intact roots and phosphorus nutrition in plants. I. Assay conditions and phosphatase activity. Australian Journal of Agricultural Research 31: 429-440.

Moghimi, A., and Tate, M.E. 1978. Does 2-ketogluconic acid chelate calcium in the pH range 2.4 to 6.4? Soil Biology and Biochemistry 10: 289-292.

Moghimi, A., Lewis, D.G., and Oades, J.M. 1978a. Characterization of rhizosphere products especially 2-ketogluconic acid. Soil Biology and Biochemistry 10: 283-287.

Moghimi, A., Tate, M.E., and Oades, J.M. 1978b. Release of phosphate from calcium phosphates by rhizosphere products. Soil Biology and Biochemistry 10: 277-281.

Nye, P.H., and Tinker, P.B. 1977. Solute movement in the soil-root system. Oxford, UK: Blackwell. 342 pp.

O'Dell, T.E., Castellano, MA., and Trappe, J.M. 1993. Biology and application of ectomycorrhizal fungi. Pages 379-416 *in* Soil microbial ecology: application in agricultural and environmental management (Metting, F.B., ed.). New York, USA: Marcel Dekker.

Smith, S.E., Robson, A.D., and Abbott, L.K. 1992. The involvement of mycorrhizas in assessment of genetically dependent efficiency of nutrient uptake and use. Plant and Soil 146: 169-179.

Tarafdar, J.C., and Claassen, N. 1988. Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatase produced by plant roots and microorganisms. Biology and Fertility of Soils 5: 308-312.

Tarafdar, J.C., and Jungk, A. 1987. Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. Biology and Fertility of Soils 3: 199-204.

Tinker, P.B. 1984. The role of microorganisms in mediating and facilitating the uptake of plant nutrients from soil. Plant and Soil 76: 77-91.

Wilcox, H.E. 1991. Mycorrhizae. Pages 731-765 *in* Plant roots: the hidden half (Waisel, Y., Eshel, A., and Kafkafi, U., eds.). New York, USA: Marcel Dekker.

Genetic control and manipulation of phosphorus uptake mechanisms

F W Smith[1]

Abstract

Anions are taken up by plants roots through high affinity H^+lanion cotransporters. The rates of uptake of such anions as phosphate and sulfate are subject to feedback regulation. This provides for considerable increase in the capacity of plants under nutrient stress to take up nutrients, should they become available from the soil solution. Elucidation of these regulatory mechanisms at the genetic and molecular level is being facilitated by recent advances in the cloning of genes involved in ion transport. Genes encoding potassium, nitrate, and sulfate transporters in plants have been cloned and studies on the regulation of expression of these genes are under way. There have been no reports of the cloning of a gene encoding a plant phosphate transporter to date. Through these advances, manipulation of the regulatory mechanisms controlling ion transport will be feasible, but considerable research is required before there is sufficient information to determine whether the widespread application of these technologies to crop plants is desirable.

Introduction

It is now generally accepted that the uptake of nutrient anions into the cells of plant roots is mediated via ion-specific transport proteins. Specific transport proteins appear to exist for each of the major nutrient anions. However, all share a common process for deriving the necessary energy for uptake, against large concentration gradients, of negatively charged molecules into the highly negatively charged environment on the inner surface of the plasmalemma. This process, proton

1. Division of Tropical Crops and Pastures, CSIRO. Cunningham Laboratory, 306 Carmody Rd, St. Lucia, Queensland 4067, Australia.

Smith, F.W. 1995. Genetic control and manipulation of phosphorus uptake mechanisms. Pages 87-96 *in* Genetic manipulation of crop plants to enhance integrated nutrient management in cropping systems—1. Phosphorus: proceedings of an FAO/ICRISAT Expert Consultancy Workshop. 15-18 Mar 1994, ICRISAT Asia Center. India (Johansen, C., Lee, K.K., Sharma, K.K., Subbarao, G.V., and Kueneman, E.A., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

pumping, involves the hydrolysis of ATP by a H^+ ATPase. The diffusion of protons back into the root cells results in a loss of free energy, which can be coupled to the uptake of anions by ion-specific transport proteins in the plasmalemma.

In recent times, physiologists have enlisted the assistance of molecular geneticists to answer some of the vexed questions concerning the exact nature of nutrient transport proteins and, importantly, unravel the complex regulatory mechanisms associated with their synthesis and operation. Basic research of this nature inevitably leads to thoughts about how these processes might be manipulated to improve crop production and hence the convening of such workshops as this. Although it is very early days in this area of science, this paper attempts to provide a brief summary of recent advances. When considering phosphate transport, it is instructive to also consider sulfate transport as there are both similarities and important differences in the regulation of the uptake of these two anions. The paper is concluded by raising some of the implications that may result from attempts to manipulate rates of phosphate uptake into crop plants by intervening in genetic control processes.

Physiological evidence

The concentration of phosphate and sulfate in the soil solution of all but very heavily fertilized agricultural soils is in the micromolar or submicromolar range. Within this range, anions are taken up by high affinity H^+/anion cotransporters. It is transporters of this type that are of interest to this workshop.

In studies with the tropical pasture legume *Macroptilium atropurpureum* by Clarkson et al. (1983), the capacity of plant roots to take up sulfate increased rapidly when plants were deprived of sulfate. Plants adequately supplied with sulfate had potential sulfate uptake rates of 200 nmol h^{-1} g^{-1} root fresh weight. The potential sulfate uptake rate increased six-fold after 24 hours' deprivation of an external sulfur supply and nine-fold after sulfate was withheld for 48 hours. Upon restoration of the sulfur supply, the potential sulfate uptake rate declined rapidly, returning within 8 hours to levels approaching those in plants that had been adequately supplied with sulfur throughout, and within 24 hours to the same level. Similar results have been observed with other plant species, cultured cells, and isolated plant vesicles (Smith 1975, Jensen and Konig 1982, Clarkson and Saker 1990, Rennenberg et al. 1989, Clarkson et al. 1992, Hawkesford et al. 1993). Such experiments are interpreted as indicating that the sulfate transport function is repressed in plants adequately supplied with sulfate and derepressed when the external sulfate supply becomes limiting. By manipulating the growth rate of plants by shading, Clarkson et al. (1983) demonstrated a close positive relationship between plant growth rate and the derepression of sulfate uptake capacity. Further, split-root experiments in which part of the root system was deprived of an external sulfate supply showed that the remainder of the root system compensated by

increasing its rate of sulfate uptake. These experiments indicate, therefore, a very close coupling between the factors that control the rate of uptake of sulfate and other plant growth processes. This must be borne in mind when considering the implications of intervention in the control mechanisms that regulate nutrient transport.

Similar deprivation experiments have been done with phosphate (Clarkson and Scattergood 1982, Lefebvre and Glass 1982, Cogliatti and Clarkson 1983). Again, phosphate uptake capacity increased when the external phosphate supply was limiting and returned to that of adequately supplied plants when the phosphorus supply was restored. However, the magnitude of the responses and the time courses are quite different to those for sulfur. In most experiments, phosphate deprivation resulted in only two- to four-fold increases in phosphate uptake capacity. This increase was usually achieved 1 to 3 days after removal of the external phosphate, depending upon the internal phosphorus status of the plants when the external phosphate supply was withdrawn. Upon resumption of external phosphate supply, the phosphate uptake capacity took approximately 3 days to return to that of plants that had remained on an adequate phosphate supply throughout. During this period, the higher uptake rates resulted in accumulation of high concentrations of phosphate in previously stressed plants and, in some cases, phosphate toxicity. Thus, the phosphate uptake system is not as responsive as the sulfate uptake system and appears to be not as tightly controlled.

The level of control

The above observations are indicative of feedback control of the sulfate and phosphate uptake systems. This raises questions about the nature of the controlling molecules and the level at which control is exerted. In this regard, we need to distinguish between short-term and long-term modulation of ion uptake. In the short-term, changes in the activity of the transporters resulting from allosteric regulation may be important. In feedback systems, this is often brought about by conformational changes in the protein associated with the binding of a ligand. Allosteric regulation takes place within very short time frames and may be likened to fine tuning the rate of ion uptake.

Glass (1976, 1983) demonstrated how potassium uptake may be allosterically regulated by the intracellular potassium concentration. The case of phosphate uptake, however, is an enigma. Using NMR spectroscopy to differentiate between cytoplasmic and vacuolar pools, Lee and Radcliffe (1983) showed that the concentration of inorganic phosphate in the cytoplasm remained relatively constant when the external phosphate supply was withdrawn. Over this period, the total phosphorus status of the roots declined and their phosphate uptake capacity increased. In these experiments, it was the vacuolar phosphate that declined. Since the vacuolar pool is not in direct contact with the plasmalemma, and in fact buffers

the cytoplasmic pool against changes in phosphate concentration, it is difficult to visualize how it might act to control the rate of phosphate uptake by allosteric regulation. In reviewing these NMR data, Clarkson and Saker (1990) pointed out that NMR spectroscopy measures the average phosphate concentration in the cytoplasm of all cells in the tissue. They argued that such cells as those near the root surface may play a much greater role in regulating phosphate uptake than other cells through subtle changes in their cytoplasmic phosphate concentrations. Such changes may be obscured by the NMR signal from the whole cell mass. They also pointed out that the cytoplasm is not homogeneous; it contains organelles that cause localized concentrations of phosphate within the cytoplasm.

Over longer time frames, the number of transporters in the plasmalemma may control the rate of phosphate or sulfate uptake. This number represents the balance between the rate of degradation of transporter molecules and the rate of synthesis of new transporters. Control at this level provides opportunities for geneticists and molecular biologists to intervene in these uptake processes by manipulating the expression of genes involved in anion transport.

Using inhibitors of protein synthesis (cycloheximide, azetidine carboxylic acid, p-fluorophenylalanine, and puromycin), Clarkson et al. (1992) demonstrated that protein synthesis was necessary for the derepression of the sulfate uptake system in barley roots when the external sulfate supply was withdrawn. Their data led them to conclude that the sulfate transporter turns over rapidly with a half-life of approximately 2.5 hours. The effects on phosphate influx in these roots were much less and took longer to be exhibited. They concluded that the turnover of the phosphate transporter was much slower than that of the sulfate transporter. Using a different system, Smith and Jackson (1987) also showed a requirement for protein synthesis during derepression of phosphate transport. They found that the amino acid analogs azetidine carboxylic acid and p-fluorophenylalanine inhibited the increase in phosphate transport capacity caused by pretreatment of maize roots with nitrogen.

Involvement of protein synthesis in derepression of sulfate and phosphate transport and the differences in turnover rates of the proteins involved provide an explanation for the physiological differences between the two systems highlighted earlier in this paper. Rapid changes in the sulfate uptake capacity can be attributed to changes in the rates of synthesis of the transporters or of some protein that regulates their synthesis. The relatively slower responses of the phosphate transport system may be due to the slower turnover rates of the phosphate transporter or of some regulatory protein. There are some limited data that suggest that control of the sulfate transport system may be exerted at

the translational level rather than at the transcriptional level (Rennenberg et al. 1989).

The nature of membrane transporters

Regrettably, we are still at the stage of having to infer the structure of membrane transport proteins responsible for sulfate and phosphate uptake into plant roots. The situation is improving rapidly, however, with the availability of sequences of clones for a high-affinity potassium transporter (Sentenac et al. 1992) and a low-affinity nitrate transporter (Tsay et al. 1993) that are expressed in *Arabadopsis* roots. Sequences of genes from filamentous fungi encoding for high-affinity sulfate (Ketter et al. 1991) and phosphate (Mann et al. 1989) transporters are also available. A gene from yeast that, among other functions, restores high-affinity phosphate transport in yeast PH084 mutants has also been cloned (Bun-ya et al. 1991) and my laboratory has very recently cloned and sequenced a high-affinity sulfate transporter from yeast.

The low-affinity nitrate transporter from *Arabadopsis* is 590 amino acids in length and the yeast PH084 phosphate transporter has 596 amino acids. The other transporters referred to above are larger, ranging from 781 to 859 amino acids. With the exception of the *Arabadopsis* potassium transporter, all of these sequences have 12 hydrophobic regions, which form alpha-helices that span the plasmalemma. The potassium transporter has only six membrane-spanning domains and appears to belong to a different class of proteins known as the Shaker channels (Sussman 1992). Between the hydrophobic membrane-spanning regions, loops protrude into the aqueous external and internal environments. Some of these loops, no doubt, contain domains associated with recognition of specific ions and allosteric regulation. Readers are referred to the interesting review on sequence comparisons and analyses of membrane transport proteins by Griffith et al. (1992) and the paper by Clarkson and Hawkesford (1993) for further information on the nature of membrane transporters. Smith et al. (1993) have outlined some approaches to cloning anion transporters.

Genetic controls

Upstream of the coding region of structural genes on the chromosome are promoter regions that regulate the expression of the gene. These may be likened to 'switches' that determine whether the structural gene will be transcribed or not. These regions contain specific base sequences that interact with DNA binding proteins and other factors that control transcription. Depending upon the type of promoter, binding may either block transcription of the downstream coding region or enable its transcription.

In the long term, intervention in the control of these genetic switches is one of the most attractive options for attempting to experimentally manipulate ion

transport in plant roots. However, a full understanding of the nature of the regulatory circuits involved is an essential prerequisite to intervening in their genetic control. These same switches also control developmental processes in the plant and, as noted earlier, nutrient transport is closely coupled to other plant growth processes. This is facilitated by coordinated regulatory systems in which common transcriptional factors influence the expression of a number of genes. For example, phosphorus deprivation not only derepresses phosphate transport but it also promotes excretion of acid phosphatases by tomato cells (Goldstein et al. 1988a, 1988b, 1989). The uptake of sulfate by yeasts and the enzymes involved in its assimilation are coordinately controlled (Thomas et al. 1992) and, in some organisms, there is good evidence for coordinated regulation of aspects of the uptake of nitrate, its reduction, and subsequent metabolism (Fu and Marzluf 1990, Clarkson and Luttge 1991, Crawford and Arst 1993).

The regulatory circuits involved in coordinated control of gene expression are complex and, to date, poorly developed for plants. To illustrate this complexity and their operation, the sulfur regulatory circuit in the filamentous fungus *Neurospora crassa* will be used. This circuit has been researched by Marzluf and colleagues for some years (Marzluf 1993).

Neurospora crassa can use a variety of sulfur sources including sulfate and a number of aromatic sulfates. These aromatic sulfates must be broken down by catabolic enzymes before the sulfur that they contain can enter the assimilatory pathway. The synthesis of these catabolic enzymes and two sulfate permease (transporter) species is under coordinated regulation and only occurs when sulfur is limiting. Figure 1 (from Marzluf 1993) illustrates this regulatory circuit using a sulfate permease (coded by *cys-14)* and a catabolic enzyme, aryl sulfatase (coded by *ars),* as examples of this entire family of proteins.

Expression of these proteins is controlled by three regulatory genes, namely, *scon1, scon2,* and *cys-3.* The *cys-3* gene is turned off when the cellular sulfur supply is adequate and is expressed only when cellular sulfur is limiting. The product of the *cys-3* gene acts in a positive manner, binding to three sites in the promoter region of *cys-14* (the sulfate permease) and to sites in the *ars* region (the aryl sulfatase), turning on transcription of these genes. The *cys-3* product also binds to a single duplex site upstream of *cys-3* itself, thereby enhancing its own transcription through autogenous regulation. The product of the *sconl* and *scon2* genes acts in a negative manner on *cys-3,* turning the expression of *cys-3* off when the cellular sulfur supply is adequate. Thus the regulatory gene *cys-3* is itself highly regulated negatively by the products of the *sconl* and *sconl* genes and positively by autogenous regulation by its own gene product.

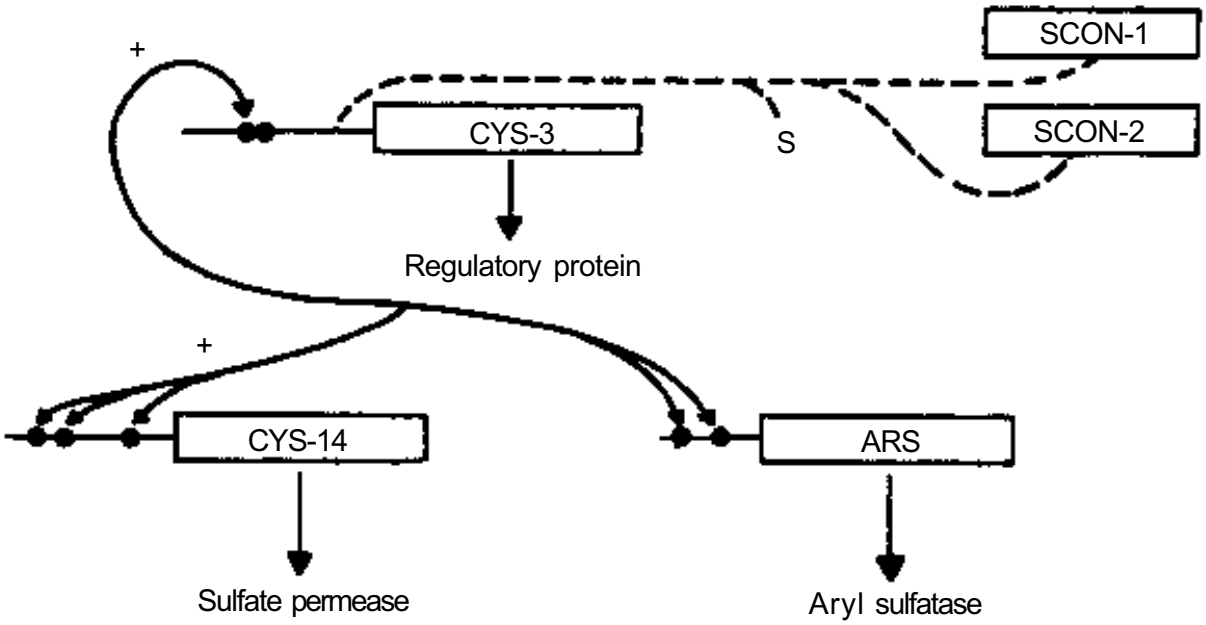


Figure 1. The sulfur regulatory circuit in *Neurospora crassa* (from Marzluf 1993). Reproduced, with permission, from the Annual Review of Microbiology, vol. 47, © 1993, by Annual Reviews Inc.

Genetic manipulation of phosphorus uptake mechanisms

Technologies are becoming available that could permit over- or under-expression of the P uptake system. We need to consider what the implications of this might be and under what circumstances it might be used in solving agricultural and environmental problems.

It is clear that plants use only a fraction of the potential of the root system for P uptake. Further, they already possess the facility to markedly increase P uptake capacity when P becomes limiting, It would seem therefore that neither the number of transporters on the plasmalemma nor their activity is the most limiting factor in the P nutrition of crop plants. Model studies suggest that the rate of diffusion of P to the root surface is often the primary constraint. One of the major limitations to diffusion of P in soils of the semi-arid tropics is water deficit. For considerable periods during the growing season, crops are unable to take up P because the surface layers of soil that contain most of the P are dry. Plants therefore need to accumulate P rapidly in periods following rain. Perhaps this suggests one circumstance under which over-expression of the P uptake system might be useful.

Down-regulation of uptake systems is a simpler technology than over-expression. There are situations in which, during specific developmental stages in some crops, it might seem attractive to turn off nitrate uptake, for example. I am unaware of any similar circumstances with P uptake, but the workshop may wish to consider this further.

What might be the implications of over-expressing the P uptake system on the biology of the crop plant? As indicated earlier, considerable caution is necessary

because of the close interaction among nutrient transport, growth, and developmental processes. This new era of plant nutrition offers the tools to address some physiological problems that have remained intractable for a long time. A considerable input into basic research is needed. This will lead to the insertion of constructs in such model systems as *Arabadopsis* to test the interactions that will undoubtedly arise from intervention in the control of nutrient transport processes. These are early days and such work is a prerequisite to wide-scale application of these technologies to agricultural plants.

One obvious effect of over-expressing phosphate uptake will be to increase the P concentration in plant tissues. In many plants, P concentrations in excess of 0.6% of the dry weight are toxic to the plant. It may be that, in order for over-expression of P uptake to be effective, plants will also require an internal nontoxic pool of storage phosphorus that they can mobilize for growth when external P is limiting.

One aspect of plant P nutrition that may not have been adequately addressed in the background paper for this workshop relates to the efficiency of utilization of P within the plant. Many plants that use P efficiently have low concentrations of P in their tissues. One of the primary attributes of the P-efficient tropical pasture legume *Stylosanthes hamata* is its ability to rapidly remobilize its internal P resources to maximize its use of P for plant growth (Smith and Jackson 1990). Perhaps this aspect of P nutrition warrants some consideration by the workshop participants.

References

Bun-ya, M., Nishimura, M., Harashima, S., and Oshima, Y. 1991. The PH084 gene of *Saccharomyces cerevisiae* encodes an inorganic phosphate transporter. Molecular and Cellular Biology 11: 3229-3238.

Clarkson, D.T., and Hawkesford, M J . 1993. Molecular biological approaches to plant nutrition. Pages 23-33 *in* Plant nutrition—from genetic engineering to field practice. (Barrow, N.J., ed.). Dordrecht, The Netherlands: Kluwer Academic.

Clarkson, D.T., and Liittge, U. 1991. Mineral nutrition: inducible and repressible nutrient transport systems. Progress in Botany 52: 61-83.

Clarkson, D.T., and Saker, L.R. 1990. The regulation of ion transport in roots: a role for phloem transport? Pages 1200-1206 *in* Proceedings of the International Congress of Plant Physiology, Volume 2, 15-20 Feb 1988, New Delhi, India (Sinha, S.K., Sane, P.V., Bhargava, S.C., and Agrawal, P.K., eds.). New Delhi, India: Society of Plant Physiology and Biochemistry.

Clarkson, D.T., and Scattergood, C.B. 1982. Growth and phosphate transport in barley and tomato plants during development of, and recovery from, phosphate stress. Journal of Experimental Botany 33: 865-875.

Clarkson, D.T., Smith, F.W., and Vanden Berg, P.J. 1983. Regulation of sulfate transport in a tropical legume, *Macroptilium atropurpureum,* cv Siratro. Journal of Experimental Botany 34: 1463-1483.

Clarkson, D.T., Hawkesford, M.J., Davidian J.C., and Grignon, C. 1992. Contrasting responses of sulfate and phosphate transport in barley *(Hordeum vulgare* L.) roots to protein-modifying reagents and inhibition of protein synthesis. Planta 187: 306-314.

Cogliatti, D.H., and Clarkson, D.T. 1983. Physiological changes in potato plants during development of, and recovery from phosphate stress. Physiologia Plantarum 58: 287-294.

Crawford, N.G., and Arst, H.N. 1993. The molecular genetics of nitrate assimilation in fungi and plants. Annual Review of Genetics 27: 115-146.

Fu, Y-H., and Marzluf, G.A. 1990. *nit-2,* the major nitrogen regulatory gene of *Neurospora crassa,* encodes a protein with a putative zinc finger DNA-binding domain. Molecular and Cellular Biology 10: 1056-1065.

Glass, A.D.M. 1976. Regulation of potassium absorption in barley roots. An allosteric model. Plant Physiology 58: 33-37.

Glass, A.D.M. 1983. Regulation of ion transport. Annual Review of Plant Physiology 34: 311-326.

Goldstein, A.H., Baertlein, D.A., and McDaniel, R.G. 1988a. Phosphate starvation inducible metabolism in *Lycopersicon esculentum.* I. Excretion of acid phosphatase by tomato plants and suspension cultured cells. Plant Physiology 87: 711-715.

Goldstein, A.H., Danon, A., Baertlein, D.A., and McDaniel, R.G. 1988b. Phosphate starvation inducible metabolism *in.Lycopersicon esculentum.* II. Characterisation of the phosphate starvation inducible-excreted acid phosphatase. Plant Physiology 87: 716-720.

Goldstein, A.H., Mayfield, S.P., Danon, A., and Tibbot, B.K. 1989. Phosphate starvation inducible metabolism in *Lycopersicon esculentum.* III. Changes in protein secretion under nutrient stress. Plant Physiology 91: 175-182.

Griffith, J.K., Baker, M.E., Rouch, D.A., Page, M.G.P., Skurray, R.A., Paulsen, I.T., Chater, K.F., Baldwin, S.A., and Henderson, P.J.F. 1992. Membrane transport proteins: implications of sequence comparisons. Current Opinion in Cell Biology 4: 684-695.

Hawkesford, M J., Davidian, J-C., and Grignon, C. 1993. Sulfate/proton cotransport in plasma-membrane vesicles isolated from roots of *Brassica napus* L.: increased transport in membranes isolated from sulfur-starved plants. Planta 190: 297-304.

Jensen, P., and Konig, T. 1982. Development of regulation mechanisms for SO_4^{2}-influx in spring wheat. Physiologia Plantarum 55: 459-464.

Ketter, J.S., Jarai, G., Fu, Y-H., and Marzluf, G.A. 1991. Nucleotide sequence, messenger-RNA stability, and DNA recognition elements of *cys-14,* the structural gene for sulfate permease II in *Neurospora crassa.* Biochemistry 30: 1780-1787.

Lee, R.B., and Radcliffe, R.G. 1983. Phosphorus nutrition and the intracellular distribution of inorganic phosphate in pea root tips: a quantitative study using ^{31}P-NMR. Journal of Experimental Botany 34: 1222-1224.

Lefebvre, D.E., and Glass, A.D.M. 1982. Regulation of phosphate influx in barley roots: effects of phosphate deprivation and reduction of influx with provision of orthophosphate. Physiologia Plantarum 54: 199-206.

Mann, B.J., Bowman, B.J., Grotelueschen J., and Metzenberg, R.L. 1989. Nucleotide sequence of *pho-4*$^{+}$,encoding a phosphate-repressible phosphate permease of *Neurospora crassa.* Gene 83: 281-289.

Marzluf, G.A. 1993. Regulation of sulphur and nitrogen metabolism in filamentous fungi. Annual Review of Microbiology 47: 31-55.

Rennenberg, H., Kemper, O., and Theone, B. 1989. Recovery of sulfate transport in heterotrophic tobacco cells from inhibition by reduced glutathione. Physiologia Plantarum 76: 271-276.

Sentenac, H., Bonneaud, N., Minet, M., Lacroute, F., Salmon, J-M, Gaymard, F., and Grignon, C. 1992. Cloning and expression in yeast of a plant potassium ion transport system. Science 256: 663-665.

Smith, F.W., and Jackson, W.A. 1987. Nitrogen enhancement of phosphate transport in roots of *Zea mays* L. II. Kinetic and inhibitor studies. Plant Physiology 84:1319-1324.

Smith, F.W., and Jackson, W.A. 1990. Internal phosphorus flows during development of phosphorus stress in *Stylosanthes hamata.* Australian Journal of Plant Physiology 17: 451-464.

Smith, F.W., Hawkesford, M.J., Prosser, I.M., and Clarkson, D.T. 1993. Approaches to cloning genes encoding for nutrient transportation in plants. Pages 151-154 *in* Plant nutrition—from genetic engineering to field practice (Barrow, N.J., ed.). Dordrecht, The Netherlands: Kluwer Academic.

Smith, I.K. 1975. Sulfate transport in cultured tobacco cells. Plant Physiology 55: 303-307.

Sussman, M.R. 1992. Shaking *Arabadopsis thaliana.* Science 256: 619.

Thomas, D., Jacquemin, I., and Surdin-Kerjan, Y.H. 1992. MET4, a leucine zipper protein, and a centromere-binding factor are both required for transcriptional activation of sulfur metabolism in *Saccharomyces cerevisiae.* Molecular and Cellular Biology 12: 1719—1727.

Tsay, Y-F, Schroeder, J.I., Feldmann, K.A., and Crawford N.M. 1993. The herbicide sensitivity gene CHLI *of Arabadopsis* encodes a nitrate-inducible nitrate transporter. Cell 72: 705-712.

Root exudate modification of rhizosphere nutrient availability and nutrient uptake by roots

R M Welch[1]

Abstract

Different mechanisms of root-cell plasma membrane transport developed in wild plant species as a result of environmental selection pressure during evolution that occurred in their native soil environments. These mechanisms regulate mineral acquisition from the rhizosphere by higher plants. This review summarizes the evidence that links iron deficiency stress responses to the dissolution, mobilization, and uptake of not only phosphorus but also of many other mineral nutrients including calcium, magnesium, iron, zinc, copper, manganese, and nickel. Finally, it ties these mechanisms to a plant hormone, ethylene, suggesting that ethylene evolution by roots may be linked to nutrient efficiency in higher plants.

Introduction

The excellent background paper prepared by our hosts (Johansen et al., these proceedings) provides a brief but thorough review of numerous ideas and approaches currently employed to find long-term solutions to phosphorus (P) deficiency problems that reduce yields of many staple food crops across the world, especially in developing countries. I will, therefore, briefly present some novel and controversial ideas that should, hopefully, stimulate additional thinking concerning the basic root mechanisms that modify the rhizosphere in ways that enhance P dissolution, mobilization, and uptake by roots. I hope these ideas will simulate new research effort that will ultimately fulfill the important charge given to us by our workshop organizers (namely, to find sustainable means to enhance P availability and efficiency in staple food crops grown on soils with low P reserves without major inputs of P fertilizers or other soil amendments, and in a way that is integrated with the management of other nutrients and is not environmentally damaging).

1. US Department of Agriculture, Agriculture Research Service, US Plant, Soil, and Nutrition Laboratory, Cornell University, Tower Road, Ithaca, New York, 14853-2901, USA.

Welch, R.M. 1995. Root exudate modification of rhizosphere nutrient availability and nutrient uptake by roots. Pages 97-105 *in in* Genetic manipulation of crop plants to enhance integrated nutrient management in cropping systems—1. Phosphorus: proceedings of an FAO/ICRISAT Expert Consultancy Workshop. 15-18 Mar 1994, ICRISAT Asia Center, India (Johansen, C., Lee, K.K., Sharma, K.K., Subbarao, G.V., and Kueneman, E.A., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

The goal of genetically manipulating crop plants to enhance integrated nutrient management in cropping systems requires a holistic view of plant nutrition. Plants did not evolve mechanisms of nutrient acquisition for just one nutrient, but responded to their need to absorb sufficient, but nontoxic, amounts of all the essential minerals from a complex and dynamic soil environment. During evolution, plants developing within different edaphic environments encountered differences in soil-weathering patterns, parent materials, minerals, micro-organisms, competing plant species, and chemical processes. These disparate soil-forming factors exerted distinctive selection pressures on plant development. Consequently, a variety of mineral acquisition mechanisms developed in root-cells of different plant species to mobilize and absorb adequate, but nontoxic, amounts of mineral nutrients from greatly different soil types. These strategies included increased root efflux of H^+ ions, organic acids, amino acids, soluble reductants, and mucilages, along with root hair proliferation, microorganism-root synergisms, changes in root morphology, and enhanced root-cell membrane transport processes.

At least three strategies are known to have evolved in higher plants for iron (Fe) acquisition by plant roots, including one for dicotyledons (dicots) and nongrass monocotyledons (monocots), associated with redox and pH changes at the root soil interface (Strategy I); another for grasses, related to root phytosiderophore efflux and influx (Strategy II); and a third, correlated to siderophore release by rhizosphere microorganisms, which affects iron availability in a wide range of monocot and dicot species (Strategy III)(Welch 1994). Importantly, each of these Fe deficiency stress responses also affects P dissolution, mobilization, and availability. For this reason, they are the major focus of the forthcoming discussions.

Soil-phosphorus forms and pools

Knowledge of the major forms of soil P, the dynamics of soil P pools, and the rate-limiting step in P mobilization to roots is central to our understanding of the strategies that evolved for P acquisition by plants (Barber 1984). Total P in soils can be divided into four general pools: (1) soluble P species and soluble compounds in soil solution, (2) P adsorbed on inorganic soil constituent surfaces (e.g., anion exchange sites), (3) crystalline and amorphous P minerals, and (4) organic P compounds incorporated into soil organic matter and soil organisms. Most of the total P in soils is not soluble or readily available for root uptake; rather, it is associated with nonlabile pools that slowly release P to labile pools for mobilization to root surfaces for uptake.

The soluble pool of P contains the phosphate readily available for root absorption, but this pool is very small relative to that for other macronutrient ions, averaging only 0.06 mg P L^{-1} (about 2 µM P) for a wide range of soils in the

United States. Within the pH range of most soils (4.0-8.5), the inorganic ionic species of P in soil solution are dominated by the phosphate ions, H_2PO_4- and $HP0_4^{2-}$. At pH 7.2, 50% of the soluble inorganic P will be in the H_2PO_4- form. Plant root cells absorb P from soil solution primarily as the H_2PO_4- ion.

The exchange of phosphate between solid forms and soil solution is based on the rate of reactions involved. This kinetic approach of labile and nonlabile P forms is useful in understanding the phosphate supply to roots (Barber 1984). As phosphate is removed from soil solution via root uptake, the labile pools of phosphate are depleted rapidly. This disturbance in equilibrium between nonlabile and labile P pools results in phosphate moving very slowly from nonlabile to labile forms. In soils having large amounts of aluminum (Al) and Fe oxides (i.e., many acid soils), relatively little total P may move to labile forms as P is adsorbed, as compared to that in soils (e.g., calcareous soils) with higher amounts of P in other types of nonlabile forms (e.g., calcium phosphates).

Soil P minerals are mainly those of calcium (Ca), Al, and Fe. Generally, soils above pH 7 are dominated by Ca phosphates whereas in many acid soils, Fe and Al phosphates predominate (Barber 1984). Less weathered soils (e.g., Mollisols) are higher in Ca phosphates whereas highly weathered soils (e.g., Oxisols) contain larger amounts of occluded phosphates, especially Fe phosphates (Chang and Jackson 1958). Alfisols and Vertisols are major soil types in the semi-arid tropics. In some acid Alfisols, the majority of the P is in Fe phosphate forms, while in alkaline Vertisols, proportionately more P is in Ca phosphate forms (Ae et al. 1990).

Most plant species evolved on soils with low amounts of labile P pools. Plants that evolved on soils dominated by Fe and Al forms of P were presented with different selection pressures from those developed on alkaline, calcareous soils where Ca forms of P predominate. Presumably, each species develops heritable rhizosphere strategies to access nonlabile P pools in order to successfully compete and survive. What types of root mechanisms evolved in plants developed within these two distinctive soil environments? In other words, what strategies were developed primarily by plants evolved on soils dominated by Al and Fe oxides, hydroxides, and phosphates as compared to those strategies acquired primarily by plants native to neutral or basic calcareous soils dominated by Ca phosphates with relatively less Fe and Al phosphate forms? The answer to this question is complex, but some basic differences in strategies have been proposed for Fe acquisition by plant roots that also relate to P availability.

Prospective on root exudates

As stated in the background paper (Johansen et al., these proceedings), genetic manipulation of root rhizosphere modification processes, and of their associated microorganisms, is seen as one of the key research targets in the search for more

efficient use of P by plants. While root exudates (including H+, sugars, amino acids, organic acids, reductants, and mucilages) are important contributors to rhizosphere modifications and P availability, they also appear to be general adaptations to rhizosphere stress, and thus are not solely related to P-deficiency resistance strategies.

Why are increased rates of root exudation associated with a wide variety of rhizosphere stress conditions including deficiencies of P, Fe, zinc (Zn), potassium (K), and Al toxicity (Marschner 1986)? The answer is not known with any certainty. Possibly, release of root exudates by root cells is an integral part of a more basic, yet unrecognized, stress response mechanism operating in nutrient acquisition by roots. This process could be directly related to nutrient absorption mechanisms across the plasma membrane of root cells and/or indirectly to the dissolution and mobilization of many nutrients to root-cell surface.

Such a basic root cell membrane mechanism is known for H^+ ion efflux (the H+-translocating ATPase responsible for driving all cation absorption processes across the plasmalemma) as discussed by Johansen et al. (these proceedings). This fundamental membrane process (i.e., the H^+ efflux mechanism) is also known to greatly affect P dissolution, mobilization, and absorption by roots.

Are there other basic membrane mechanisms controlling root exudation rates and P acquisition by roots? Yes, some are known that could greatly influence P availability. For example, unknown mechanisms control organic acids, reductants, and nonprotein amino acids (the phytosiderophores) released by roots and they have profound effects on the ability of roots to absorb many divalent cations including Fe^{2+}, Mn^{2+}, Zn^2+, Cu^{2+}, and Ni^{2+}. Furthermore, root exudates also greatly affect P dissolution, mobilization, and uptake because they contribute to the control of free Al, Fe, and other transition metal ion activities in soil solution that can reduce P solubility.

Basic root-cell membrane mechanisms that highly regulate the efflux and influx of these important rhizosphere substances are currently being researched in many laboratories globally. Ironically, they are being studied because of their implications in Fe-deficiency stress resistance, and not for their possible importance to increasing P availability to roots. This aspect of their importance to plant nutrition has not been widely considered. In the following section, I focus on the possible importance of these putative Fe uptake mechanisms to P dissolution, mobilization, and availability to plants.

Relevance of root-cell plasmalemma reductases to phosphorus availability

Marschner and his colleagues developed the concept of Strategy I for the mobilization and uptake of Fe by dicots and nongrass monocots (Marschner et al. 1986, 1989; Romheld and Marschner 1986). The key to understanding the regulation of Fe uptake by nongrass species was the realization that redox changes in the rhizosphere controlled Fe uptake by these species. The discovery of a highly regulated and inducible root-cell plasma membrane enzyme system, the Fe(III)-chelate reductase system (also termed "Turbo' reductase or inducible reductase) in dicots, led to the development of the Strategy I concept. Interestingly, recent research has shown that this reductase system is induced not only by Fe deficiency stress conditions but also by copper (Cu) and, possibly, by Zn and manganese (Mn) deficiency stress conditions; it is not solely tied to the regulation of iron nutrition (Welch 1994). Currently, much research is being directed at delineating the genes responsible for the induction of this reductase under micronutrient metal-deficiency stress conditions.

What properties of this micronutrient-metal-chelate reductase system relate to P availability? The induction of this reductase at the exterior surface of root-cell plasma membranes, in response to micronutrient metal deficiency stress, results in several beneficial reactions that directly affect P dissolution, mobilization, and absorption. Firstly, the activity of this reductase system is associated with increased activity of the H^+-translocating ATPase leading to root-H^+-ion efflux and a lowering of the rhizosphere pH, a prerequisite for P dissolution and mobility in calcareous soils. In neutral or alkaline soils, a decrease in pH within the rhizosphere would result in more phosphate dissolution and absorption because increased H^+ ion activity in the rhizosphere would dissolve more P from nonlabile pools. This decrease in pH would also result in a shift from the more strongly adsorbed HPO_4^{2-} species to the more soluble and absorbable $H_2PO_4^-$ species. Secondly, increased activity of this reductase system results in the indirect release of reductants by root cells and increased dissolution of Fe(III)-nonlabile pools, another important positive factor in P dissolution, mobilization, and uptake from important nonlabile amorphous Fe-P pools in many neutral and alkaline soils. Thirdly, regulation of inducible reductase activity may control the uptake of divalent cations by root-cells, including Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, and Ni^{2+}, via alterations in the gating of root-cell plasma membrane divalent cation channels (Welch et. al. 1993). Increased uptake of these divalent cations would lower divalent cation activities in the rhizosphere. This, in turn, would lead to increased P solubility and uptake by roots under P deficiency. Fourthly, the activity of the inducible reductase system is positively correlated with the proliferation of root hairs, another advantage in terms of P uptake.

In summary, the activity of the inducible reductase system may have profound effects on P dissolution, mobilization, and uptake by roots. We should consider this possibility seriously in our deliberations on finding genetically controlled ways of increasing P availability to nongrass plant species.

Relevance of the phytometallophore system of grasses to phosphorus availability

In contrast to roots of dicots and nongrass monocots, grasses do not increase H^+ ion efflux in response to Fe deficiency. Rather, grasses have developed another mechanism to solubilize Fe from insoluble soil pools. Marschner and his colleagues distinguish this mechanism as a second process (Strategy II) for controlling Fe uptake by Fe-deficiency stressed grasses—the biosynthesis, efflux, and absorption by roots of a novel group of nonprotein amino acids, the phytosiderophores. Importantly, this strategy is highly regulated by grasses and is inheritable. In a recent review (Welch 1994), I suggested the term 'phytometallophores' for this class of root-synthesized compounds because they are not associated with Fe dissolution, mobilization, and uptake alone, but form very stable chelates with many transition metal cations in soil solution including Cu(II), Mn(II), Zn(II), Co(II), and Ni(II) and all of these metal chelates can be absorbed by root-cells of grasses.

Grass roots, encountering Fe and/or Zn deficiency stress conditions in the rhizosphere, respond by increasing their release of phytometallophores into the soil solution and not by increased H^+ion excretion, as do dicots and other nongrass monocots. In many acid soils, the nonlabile P pool is predominately associated with Fe and Al phosphate forms, as discussed previously. Increased concentrations of rhizosphere phytometallophores would result in increased rates of dissolution of both Fe and Al from their nonlabile P pools. Concomitant increases in soil solution P levels would also be associated with this process. The phytometallophores bind not only Fe and Al ions, but also other transition metal cations that could form insoluble precipitates with the phosphate released during dissolution of insoluble P pools if these metal ions were free in solution and not bound to phytometallophores. Therefore, the release of phytometallophore compounds by grass roots should increase the pool of soluble phosphate ions available to them. Interestingly, because Strategy II is not linked to H^+ efflux by roots, as in Strategy I species, the serious problem of Al toxicity in many acid soil environments would not be exacerbated by roots employing the Strategy II system. Summarizing, the operation of Strategy II in grass species should make more P in nonlabile soil pools available to grass roots in many acid soil environments dominated by Fe and Al phosphate forms.

A role for rhizosphere-stress-induced ethylene in controlling phosphorus availability?

Evidence exists in the literature that the stress-induced release of ethylene from the root is intimately involved in the regulation of the inducible reductase system in root-cell plasma membranes of Strategy I species (personal communications from Francisco J Romera, University of Cordoba, Spain; see also Romera and Alcantara 1993). Supplying Strategy I species with promoters of ethylene action (e.g., ethephon or 1-aminocyclopropane-l-carboxylate) in their growth media greatly stimulates root-cell plasma membrane Fe(II)-chelate reductase activity even when the plants are adequately supplied with Fe. Conversely, supplying low levels of inhibitors of ethylene action (i.e., Co^{2+}, Ag^{+}, aminoethoxyvinylglycine, and aminooxyacetic acid) to Fe-deficient roots prevented the induction of the root-cell Fe(III)-chelate reductase. Stimulation of the reductase by promoters of ethylene action resulted in the accumulation of Fe, Mn, Zn, and magnesium (Mg) in roots and shoots just as found with Fe-deficient conditions previously described. Thus, ethylene plays some, as yet unknown, role in stimulating Fe(III)-chelate reductase activity in Strategy I plants.

Curiously, a close parallel exists between ethylene biosynthesis and the biosynthesis of an important phytometallophore, nicotianamine, in plants. Nicotianamine is responsible for the intracellular and intercellular movement of Fe and possibly other transition metal ions. Furthermore, nicotianamine is the biosynthetic precursor of all known higher plant phytometallophores (e.g., mugineic acid and avenic acid). It is the only known phytometallophore that has a higher affinity for Fe(II) than for Fe(III) and may play a role in regulating the activity of the inducible reductase system in Strategy I species and in phytometallophore synthesis in Strategy II species. Ethylene is synthesized from methionine, via S-adenosylmethionine (SAM) and 1-aminocyclopropane-l-carboxylic acid (ACC) (Yang and Hoffman 1984), while nicotianamine is synthesized via the same precursors (methionine and SAM), but excludes the final biosynthetic step involving ACC and ACC synthase in ethylene synthesis (Shojima et. al. 1990). Both increased ethylene levels and the lack of nicotianamine biosynthesis result in increased activity of the inducible reductase in roots of Strategy I plant species. For example, the *Chloronerva* tomato mutant, which cannot synthesize nicotianamine, always exhibits stimulated root Fe(III)-reductase activity and high rates of divalent cation accumulation (Scholz et. al. 1988). Furthermore, both ethylene and nicotianamine affect root development and root hair formation as well as the mineral nutrient cation uptake by plants. These close relationships between ethylene and nicotianamine biosynthetic pathways, and their ability to influence root reductase activity, phytometallophore synthesis, root morphology, and nutrient status, suggest that these compounds may be tightly

linked in controlling nutrient stress resistance processes in roots. In the future, ethylene production in higher plants may be shown to interact with nicotianamine biosynthesis and, thus, play a vital role in regulating cation transport processes.

As previously discussed, the activity of both the inducible reductase system and phytornetallophore synthesis may play a critical role in P availability to plants. If additional research confirms these speculations, then techniques could be developed to screen plants for their ability to increase either inducible reductase activity or their phytornetallophore synthesis under P-deficiency stress conditions. Potentially, determining ethylene production by P-deficient roots might be used as a rapid method to identify P-efficient genotypes of important crop species.

Conclusions

This review has attempted to present some unique ideas concerning the basic processes developed by plants to acquire P from nonlabile P soil pools. In doing so, I have pointed out three processes that are linked to basic membrane transport processes in root-cells, namely, the H^+-translocating ATPase, the inducible reductase system in dicots and nongrass monocots, and the phytornetallophore system that is responsible for the synthesis, efflux, and influx of these natural metal chelates by grass roots. Much more needs to be learned about the roles these systems play in mineral acquisition by plant roots and the role of plant hormones in regulating their activity. Furthermore, research directed at finding the genes responsible for their synthesis and regulation should be increased. Studying these basic systems could lead not only to an understanding of P acquisition by plant roots but also to a holistic understanding of the basic mechanisms controlling mineral uptake by all higher plants.

References

Ae, N., Arihara, J., Okada, K., Yoshihara, T., and Johansen, C. 1990. Phosphorus uptake by pigeon pea and its role in cropping systems of the Indian subcontinent. Science 248: 477-480.

Barber, S.A., 1984. Phosphorus. Pages 201-228 *in* Soil nutrient bioavailability. New York, USA: John Wiley.

Chang, S.C., and Jackson, M.L. 1958. Soil phosphorus fractions in some representative soils. Journal of Soil Science 9: 109-119.

Marschner, H. 1986. Mineral nutrition of higher plants. London, UK: Academic Press. 674 pp.

Marschner, H., Romheld, V., and Kissel, M. 1986. Different strategies in higher plants in mobilization and uptake of iron. Journal of Plant Nutrition 9: 695-697.

Marschner, H., Treeby, M., and Romheld, V. 1989. Role of root-induced changes in the rhizosphere for iron acquisition in higher plants. Zeitschrift fur Pflanzenernahrung und Bodenkunde 152: 197-204.

Romera, F.J., and Alcantara, E. 1993. Ethylene involvement in the regulation of iron deficiency responses in cucumber. Plant Physiology 102 (Supplement): 63.

Romheld, V., and Marschner, H. 1986. Mobilization of iron in the rhizosphere of different plant species. Advances in Plant Nutrition 2: 155-204.

Scholz, G., Becker, R., Stephan, U.W., Rudolph, A., and Pich, A. 1988. The regulation of iron uptake and possible functions of nicotianamine in higher plants. Biochemie und Physiologie der Pflanzen 183: 257-269.

Shojima, S., Nishizawa, N.K., Fushiya, S., Nozoe, S., Irifune, T., and More, S. 1990. Biosynthesis of phytosiderophores. *In vitro* biosynthesis of 2'-deoxymugineic acid from L-methionine and nicotianamine. Plant Physiology 93: 1497-1503.

Welch, R.M. 1994. Micronutrient nutrition of plants. CRC Critical Reviews in Plant Science 12: (In press).

Welch, R.M., Norvell, W.A., Schaefer, S.C., Shaff, J.E., and Kochian, L.V. 1993. Induction of iron(III) and copper(II) reduction in pea *(Pisum sativum* L.) roots by Fe and Cu status: does the root-cell plasmalemma Fe(III)-chelate reductase perform a general role in regulating cation uptake? Planta 190: 555-561.

Yang, S.F., and Hoffman, N.E. 1984. Ethylene biosynthesis and its regulation in higher plants. Annual Review of Plant Physiology 35: 155-189.

Rhizosphere pH effects on phosphorus nutrition

H Marschner[1]

Abstract

Depending on plant and soil factors, rhizosphere pH may differ considerably from bulk soil pH. Of the plant factors, imbalance in cation-anion uptake ratio and excretion of organic acids are of major importance for root-induced changes in rhizosphere pH. At high bulk soil pH, rhizosphere acidification can enhance utilization of sparingly soluble calcium phosphates (e.g., rock phosphate) whereas in acid soils, root-induced pH increase in the rhizosphere may increase phosphorus (P) acquisition by enhanced desorption from the solid phase. Rhizosphere acidification due to enhanced net excretion of H^+ or of organic acids is also a common root response to P deficiency. Excretion of organic acids has several advantages for the P nutrition of plants, as it enhances P mobilization irrespective of the bulk soil pH, and also contributes to detoxification of aluminum. Selection of genotypes that respond to P deficiency by enhanced excretion of organic acids is considered a particularly promising approach for adaptation of crop plants to low-P soils.

Introduction

Rhizosphere pH may differ from that of the bulk soil by up to two units, depending on plant and soil factors. Of the plant factors, the most important ones responsible for root-induced changes in rhizosphere pH are imbalance in cation-anion uptake ratio and corresponding differences in net excretion of H^+ and HCO_3- (or OH-); excretion of organic acids and, indirectly, microbial acid production from root release of organic carbon; and enhanced CO_2 production. As a rule, the most common of these plant factors is imbalance in cation-anion uptake ratio.

The driving force behind uptake of cations and anions into roots is a plasma membrane-bound H^+-pumping ATPase, maintaining a high cytosolic pH and

1. Institut fur Pflanzenernahrung, Universitat Hohenheim, Fruwirthstrasse 20, 70593, Stuttgart 70, Germany.

Marschner, H. 1995. Rhizosphere pH effects on phosphorus nutrition. Pages 107-115 *in* Genetic manipulation of crop plants to enhance integrated nutrient management in cropping systems—1. Phosphorus: proceedings of an FAO/ICRISAT Expert Consultancy Workshop, 15-18 Mar 1994, ICRISAT Asia Center. India (Johansen, C., Lee, K.K.. Sharma. K.K., Subbarao, G.V., and Kueneman, E.A., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

thereby creating differences in pH and electropotential between cytosol and the cell wall compartment (apoplasm). Along this gradient, cations are transported as uniport, or countertransport, and anions as a proton-anion cotransport. Accordingly, high uptake rates of cations compared to those of anions lead to enhanced net excretion of protons (rhizosphere acidification) whereas high uptake rates of anions compared to those of cations lead to H^+ consumption (and rhizosphere pH increase). The functioning of the model implies that with decrease in external pH, cation uptake decreases and anion uptake increases. This is also true for phosphate where this enhancement effect of lower pH values is accentuated by the shift in the ionic species from HPO_4^{2-} to H_2PO_4-. The monovalent form $H_2PO_4^-$ is taken up at much higher rates than the divalent anion HPO_4^{2-}. In the pH range of 8.5 to 5.6 there is, therefore, a striking positive correlation between the proportion of H_2PO_4- in the external solution and the uptake rate of phosphate (Hendrix 1967, Li and Barber 1991).

Form of nitrogen supply

The form of nitrogen (N) supply (NH_4^+, NO_3-, and N_2 fixation) plays a key role in the cation-anion relationships and thus rhizosphere pH (Romheld 1986). About 70% of the cations and anions taken up by plants are represented by either NH_4+or NO_3-. Accordingly, and in contrast to NO_3^--fed plants, NH_4^+-fed plants are characterized by a high cation-anion uptake ratio, rhizosphere acidification, and enhanced phosphorus (P) uptake when grown in neutral or alkaline soils (Table 1). This difference in rhizosphere acidification and P uptake is particularly marked when such nitrification inhibitors as N-Serve are used.

Differences in rhizosphere pH brought about by the form of N supply are caused not only by different uptake rates but also by the assimilation of N in the plants, which either produces or consumes H^+ or OH^- (Raven 1986). Assimilation

Table 1. Effect of nitrogen fertilizer form on bulk soil pH, rhizosphere pH, and phosphorus contents in the shoots of *Phaseolus vulgaris* L. plants supplied with water soluble [(P_{sol})] or rock [(P_{rock})] phosphate.

	pH (microelectrode)		P content (mg g^{-1} shoot dry mass)	
N-form	Bulk soil	Rhizosphere soil	"(sol)	P(rock)
-N	6.1	6.2	3.68	1.35
NO3-	6.6	6.6	3.00	1.00
NH_4+	5.7	5.6	4.30	2.00
NH_4^+ + N-Serve	6.6	4.5	5.58	2.34

Source: Thomson et al. (1993).

of NH_4+ produces H^+ (3 NH_4+ -► 4H^+) and assimilation of NO_3-(nitrate reduction) consumes H^+ (3 NO_3- -► 2 OH^-). The prevailing sites of nitrate reduction (roots or shoots) differ among plant species, and the degree of pH increase in the rhizosphere of NO_3^--fed plants will differ accordingly. Typical differences in rhizosphere pH, of soybean for example, even among cultivars (6.8 compared to 5.6; Romheld and Marschner 1984) may be related to differences in the sites of nitrate reduction.

Legumes that depend on biological N_2 fixation are characterized by a cation-anion ratio of 1 and, thus, enhanced net excretion of H+, although in lower amounts as compared to NH_4+-fed plants (1,5 N_2 fix -► 1 H+; Raven 1986). For different legume species, a molar ratio of 0.5 (mM N_2 fixed/mM H^+ released) was calculated (de Swart and van Diest 1987). The capacity of plants to utilize P from rock phosphate, or from other sparingly soluble calcium (Ca) phosphates in neutral and alkaline soils, is therefore higher in N_2-fixing plants than in NO_3--fed plants (Table 2). On severely P-deficient soils, utilization of rock phosphate as a P source for legumes can be low when nodulation is limited by P deficiency. Thus, a starter supply of soluble P can enhance nodulation, N_2 fixation, and rhizosphere acidification and thereby utilization of rock phosphate (de Swart and van Diest 1987). Rhizosphere acidification and corresponding P mobilization have to be considered also in mechanistic simulation models that predict P uptake by legumes from calcareous soils (Table 3). In maize predicted and observed P uptake agreed

Table 2. Effect of nitrogen source on net release of H+ and OH(HCO_3-) by roots of alfalfa plants, phosphorus uptake from rock phosphate, and plant dry mass.

N-source	Acidity/alkalinity generated (meq g^{-1} dry mass)		Substrate pH(H_2O)	P uptake (mg pot^{-1})	Dry mass (g pot^{1})
NO_3-N	-	0.8	7.3	23	18.8
N_2fix	1.4	-	5.3	49	26.9

Source: Aguilar and van Diest (1981).

Table 3. Relationship between bulk soil pH and observed and predicted (mechanistic model) uptake of phosphorus in maize and alfalfa.

Bulk soil pH	P uptake (umol pot^{-1})			
	Maize		Alfalfa	
	Observed	Predicted	Observed	Predicted
8.3	23	21	157	26
7.6	212	208	240	133
7.3	381	366	265	201
5.8	718	746	431	386

Source: Li and Barber (1991).

over the pH range of 8.3 to 5.8, whereas in alfalfa observed uptake was much higher than predicted uptake, particularly at high bulk soil pH. This underestimation of P uptake in alfalfa and other legume species was due to rhizosphere acidification by 0.39 to 0.77 units and a corresponding increase in P availability (solubilization and shift in favor of H_2PO_4-) by 21 to 242% (Li and Barber 1991).

Striking differences in rhizosphere pH exist among plant species supplied with NO_3- and growing in the same soil. Typically, such plant species as chickpea, white mustard, and buckwheat have a very low rhizosphere pH compared, for example, to that of wheat, sorghum, or maize (Marschner and Romheld 1983). These differences mainly reflect differences in the cation-anion uptake ratio (Table 4). Accordingly, utilization of sparingly soluble Ca phosphates is low, for example, in maize compared to buckwheat. At least some of these plant species with marked high cation-anion uptake ratios and rhizosphere acidification are characterized by high uptake rates of Ca, precipitation of Ca carbonate within root cells, and calcification of the roots (Jaillard 1985).

With a decrease in bulk soil pH, rhizosphere acidification, either inherent or induced by NH_4^+-N or N_2 fixation, becomes less important for P mobilization. At a bulk soil pH below about 5.5, further rhizosphere acidification by enhanced net excretion of H+ has either no effect on P acquisition or it may even depress it directly via enhancement of P sorption on sesquioxide surfaces and indirectly via inhibition of root growth induced by aluminum (Al) toxicity. Accordingly, on acid soils, nitrate supply increases not only rhizosphere pH but also plant uptake of P, presumably by exchange with HCO_3- for phosphate adsorbed to sesquioxides (Gahoonia et al. 1992). For various pasture grasses grown in P-deficient soils, depletion of P in the rhizosphere and an increase in rhizosphere pH have been found to be closely associated (Armstrong and Helyar 1992).

Table 4. Dry matter yield and cation-anion uptake pattern in NO_3-fed maize and buckwheat supplied with triple superphosphate (TPS) and rock phosphate (RP).

Plant species	P source	Dry mass (g plot^{-1})	Meq g^{-1} dry mass		C-A	Final soil pH
			Cations(c)	Anions(A)		
Maize	TPS	30.0	943	979	-36	6.4
	RP	2.6	2350	2760	-410	6.4
Buck-wheat	TPS	31	2058	1213	+845	4.9
	RP	27	2467	1488	+979	5.1

Source: Bekele et al. (1983).

Variation in rhizosphere pH

Average values of rhizosphere pH, however, can be misleading and may result in erroneous conclusions being reached concerning nutrient mobilization and immobilization in the rhizosphere. For example, within the root system of an individual plant, pH differences exceeding two units may sometimes occur between the primary and lateral roots, or along the root axis (Marschner and Romheld 1983). In a given plant species, the extent of root-induced changes in rhizosphere pH also strongly depend on soil-water content and bulk soil pH. High soil-water content facilitates diffusion of NH_4^+ more than that of NO_3- and thus favors rhizosphere acidification (Gijsman 1991). For a given pressure of CO_2 in the pH range of 5 to 6, both H^+ and HCO_3- concentrations are low in the soil solution and thus root-induced changes in pH are maximal in both pH at the rhizoplane and extent of the pH changes into the rhizosphere (Nye 1986) (Table 5).

Table 5. Relationship between bulk soil pH and extent of rhizosphere acidification in groundnut grown in different soils.

Bulk soil pH	Rhizoplane pH	A pH	Extension of rhizosphere acidification (mm)
7.6	6.40	1.20	1.8
6.3	4.75	1.55	2.2
5.5	4.10	1.40	2.8
5.0	4.25	0.75	1.3

Source: Schaller (1987).

Although the extension of the root-induced changes in rhizosphere pH may not exceed the root hair cylinder, these pH changes may be of crucial importance for P acquisition. Since usually less than 20% of the top soil is explored by roots for P during a growing season, the rate of replenishment within the root hair cylinder needs to be very high (about 10-20 times a day) to meet the P requirement of plants. In nonmycorrhizal plants with coarse root systems, high influx rates of P per unit root length are required (Fdhse et al. 1991), and root-induced pH changes in the rhizosphere are likely to be particularly important for efficiency in P uptake.

Root-induced changes in rhizosphere pH are also related to the nutritional status of plants. Examples are rhizosphere acidification in cotton and other dicotyledons under zinc (Zn) deficiency (Cakmak and Marschner 1990) and in nongraminaceous species under iron (Fe) deficiency (Romheld 1987). In both instances, the increase in net release of H^+ is closely related to increase in cation-anion uptake ratio. Under Fe deficiency, this acidification also takes place in NO_3^- -fed plants and is confined to root apical zones, where the actual rates of H+ excretion are nearly eight times higher than those in NH4+-fed plants.

Rhizosphere acidification and P deficiency

Rhizosphere acidification is also a widespread root response to P deficiency in many dicotyledons. Depending on the plant species, this rhizosphere acidification is brought about by either enhanced net excretion of H^+ or of organic acids. An example of enhanced net excretion of H+ in P-deficient plants is shown for tomato in Table 6. In contrast, in many such other species as rape (Hoffland et al. 1989, Hoffland 1992) and many legume species (Ohwaki and Hirata 1992), rhizosphere acidification is caused mainly by excretion of organic acids, malic and citric acids in particular, or of piscidic acid in pigeonpea (Ae et al. 1990). As a common feature under P deficiency, synthesis of organic acids is enhanced in plants and, for stabilization of the internal pH, either the H^+-cation countertransport is enhanced (e.g., in tomato) or the organic acid anions are excreted via a H^+-anion cotransport (e.g., in white lupin). Rhizosphere acidification by excretion of organic acids has several advantages to the plants compared to acidification by H+ secretion, particularly for P acquisition. Citric acid, for example, is highly effective in complexing not only Ca but also Fe and Al, and thus mobilizes sparingly soluble inorganic P compounds in both calcareous (Dinkelaker et al. 1989) and acid soils (Gardner et al. 1982) and liberates P complexed by soil organic matter (Gerke 1992). On the other hand, piscidic acid is a strong chelator of ferric iron but not of Ca, which explains the higher efficiency of pigeonpea in P acquisition from Alfisols compared to that from Vertisols (Ae et al. 1990). The widespread excretion of organic acids in legume species in response to P deficiency is of particular importance for adaptation to acid mineral soils, not only for P acquisition but also for detoxification of Al, which is solubilized in response to rhizosphere acidification during N_2 fixation.

In many instances, P-deficiency-induced enhancement of organic acid excretion is confined to certain root zones, for example, subapical root zones in rape (Hoffland 1992), the proteoid root zones in such annual legumes as white lupin (Dinkelaker et al. 1989), and the zone around the cluster-root in such tree species as *Banksia* spp. (Grierson 1992). Such localized excretion restricts microbial degrada-

Table 6. Effect of phosphorus nutritional status of NO_3-fed tomato plants on net release of H^+ and OH^-, and on cation-anion uptake ratio.

	Uptake ratio at day 7 (ueq g^{-1} root fresh mass day^{-1})		
P supply	Cation(C)	Anion(A)	C-A
+ P	354	453	-99
- P	213	160	+55

Source: Heuwinkel et al. (1992).

Table 7. Cation-anion uptake ratio, rhizosphere acidification (proteoid root zone), and citrate excretion in nitrate-fed white lupin plants grown in a phosphorus-deficient calcareous soil of pH 7.5.

Total content (meq 100 g^{-1} dry mass)			Proteoid root zone	Citrate excretion (meq 100 g^{-1} dry mass)
Cation(C)	Anion(A)	C-A	pH	
159	115	+44	4.8	356

Source: Dinkelaker et al. (1989).

tion of exudates and simultaneously allows an intensive extraction of a limited soil volume. In white lupin grown in a calcareous (23% $CaCO_3$) P-deficient soil, nearly 90% of the total acidity excreted could be attributed to citric acid in the proteoid root zones (Table 7). This amount of citric acid released accounted for 1 g $plant^{-1}$, or 23% of the net photosynthesis, after 13 weeks of growth. When grown in mixed culture in a P-deficient soil supplied with rock phosphate, wheat can profit from the P mobilized in the rhizosphere of the white lupin (Horst and Waschkies 1987).

In conclusion, temporal and localized rhizosphere acidification by excretion of organic acids into the rhizosphere of soil-grown plants might be more important and abundant in higher plants than indicated at present from studies in nonsterile nutrient solutions.

References

Ae, N., Arihara, J., Okada, K., Yoshihara, T., and Johansen, C. 1990. Phosphorus uptake by pigeon pea and its role in cropping systems of the Indian subcontinent. Science 248: 477-480.

Aguilar, S.A., and van Diest, A. 1981. Rock-phosphate mobilization induced by the alkaline uptake pattern of legumes utilizing symbiotically fixed nitrogen. Plant and Soil 61: 27-42.

Armstrong, R.D., and Helyar, K.R. 1992. Changes in soil phosphate fractions in the rhizosphere of semi-arid pasture grasses. Australian Journal of Soil Research 30: 131-143.

Bekele, T., Cino, B.J., Ehlert, P.A.I., van der Mass, A.A., and van Diest, A. 1983. An evaluation of plant-borne factors promoting the solubilization of alkaline rock phosphates. Plant and Soil 75: 361-378.

Cakmak, I., and Marschner, H. 1990. Decrease in nitrate uptake and increase in proton release in zinc deficient cotton, sunflower and buckwheat plants. Plant and Soil 129: 261—268.

de Swart, P.-H., and van Diest, A. 1987. The rock phosphate solubilizing capacity of *Pueraria javanica* as affected by soil pH, superphosphate priming effect and symbiotic N_2 fixation. Plant and Soil 100: 135-147.

Dinkelaker, B., Romheld, V., and Marschner, H. 1989. Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (*Lupinus albus* L.). Plant, Cell and Environment 12: 285-292.

Fohse, D., Claassen, N., and Jungk, A. 1991. Phosphorus efficiency of plants. II. Significance of root hairs and cation-anion balance for phosphorus influx in seven plant species. Plant and Soil 132: 261-272.

Gahoonia, T.S., Claassen, N., and Jungk, A. 1992. Mobilization of phosphate in different soils by ryegrass supplied with ammonium or nitrate. Plant and Soil 140: 241-248.

Gardner, W.K., Parbery, D.G., and Barber, D.A. 1982. The acquisition of phosphorus by *Lupinus albus* L. II. The effect of varying phosphorus supply and soil type on some characteristics of the soil/root interface. Plant and Soil 68: 33-41.

Gerke, J. 1992. Phosphate, aluminum and iron in the soil solution of three different soils in relation to varying concentrations of citric acid. Zeitschrift fur Pflanzenernahrung und Bodenkunde 155: 339-343.

Gijsman, A J. 1991. Soil water content as a key factor determining the source of nitrogen (NH_4^+ or NO_3^-) adsorbed by Douglas-fir *(Pseudotsuga menziesii)* along its roots. Canadian Journal of Forest Research 21: 616-625.

Grierson, P.F. 1992. Organic acids in the rhizosphere of *Banksia integrifolia* L. Plant and Soil 144: 259-265.

Hendrix, J.E. 1967. The effect of pH on the uptake and accumulation of phosphate and sulfate ions by bean plants. American Journal of Botany 54: 560-564.

Heuwinkel, H., Dirkby, E.A., Le Bot, J., and Marschner, H. 1992. Phosphorus deficiency enhances molybdenum uptake by tomato plants. Journal of Plant Nutrition 15: 549-568.

Hoffland, E. 1992. Quantitative evaluation of the role of organic acid exudation in the mobilization of rock phosphate by rape. Plant and Soil 140: 279-289.

Hoffland, E., Findenegg, G.R., and Nelemans, J.A. 1989. Solubilization of rock phosphate by rape. II. Local root exudation of organic acids as a response to P-starvation. Plant and Soil 113: 161-165.

Horst, W.J., and Waschkies, Ch. 1987. [Phosphatversorgung von Sommerweizen *(Triticum aestivum* L.) in Mischkultur mit WeiBer Lupine *(Lupinus albus* L.)]. Zeitschrift fur Pflanzenernahrung und Bodenkunde 150: 1-8.

Jaillard, B. 1985. [Activite racinaire et rhizostructures en milieu carbonat]. Pedologie 35: 297-313.

Li, Y., and Barber, S.A. 1991. Calculating changes of legume rhizosphere soil pH and soil solution phosphorus from phosphorus uptake. Communications in Soil Science and Plant Analysis 22: 955-973.

Marschner, H., and Romheld, V. 1983. *In vivo* measurement of root-induced pH changes at the soil-root interface: effect of plant species and nitrogen source. Zeitschrift fur Pflanzenphysiologie 111: 241-251.

Nye, P.H. 1986. Acid-base changes in the rhizosphere. Advances in Plant Nutrition 2: 129-153.

Ohwaki, Y., and Hirata, H. 1992. Differences in carboxylic acid exudation among P-starved leguminous crops in relation to carboxylic acid contents in plant tissues and phospholipid level in roots. Soil Science and Plant Nutrition 38: 235-243.

Raven, J.A. 1986. Biochemical disposal of excess H^+ in growing plants? New Phytologist 104: 175-206.

Romheld, V. 1986. [pH-Veranderungen in der Rhizosphare verschiedener Kulturpflanzenarten in Abhangigkeit vom Nahrstoffangebot]. Kali-Briefe 18: 13—30.

Romheld, V. 1987. Different strategies for iron acquisition in higher plants. Physiologia Plantarum 70: 231-234.

Romheld, V., and Marschner, H. 1984 Plant-induced pH changes in the rhizosphere of 'Fe-efficient' and 'Fe-inefficient' soybean and corn cultivars. Journal of Plant Nutrition 7: 623-630.

Schaller, G. 1987. pH changes in the rhizosphere in relation to the pH-buffering of soils. Plant and Soil 97: 439-444.

Thomson, C.J., Marschner, H., and Romheld, V. 1993. Effect of nitrogen fertilizer form on pH of the bulk soil and rhizosphere, and on the growth, phosphorus, and micronutrient uptake of bean. Journal of Plant Nutrition 16: 493-506.

Effects of specific compounds exuded from roots on phosphorus nutrition

N Ae[1], T Otani[1], and J Arihara[2]

Abstract

Pigeonpea is a major grain legume crop grown widely in the semi-arid tropics. The response of pigeonpea to phosphorus (P) fertilizer is generally low even in low-P Alfisols, where a major fraction of inorganic P is in the iron-bound form (Fe-P). The ability of pigeonpea to take up P from an iron-bound form has been attributed to piscidic acid and its derivatives released in root exudates. It was hypothesized that these substances chelate with Fe^{3+} and release P from Fe-P. So far, we have not been able to identify these substances in root exudates of other crops. Piscidic acid release in root exudates and the associate P uptake from Fe-P therefore appear to be unique to pigeonpea. Upland rice has the ability to take up P from Fe-P, but not through chelating compounds in the root exudates; a different mechanism appears to be operating. Evidence is presented indicating genotypic variation in upland rice in this respect.

Introduction

Sustainability has become a major issue of global concern during this decade as many technologies adopted to date, including the intensive use of chemical inputs in agriculture, show adverse impact on the environment. Crop production practices that incorporate a reduced use of fertilizers while maintaining and/or increasing yields are likely to enhance sustainability as these would lead to reduced pollution of ground water besides reducing the demand on nonrenewable natural resources. Our research has shown that several crop species possess unique mechanisms of survival in soils of low fertility. We believe that a greater understanding of these mechanisms would enable us to exploit the potential benefits from these crops and to design sustainable crop and land management practices.

1. Soil Biochemistry, National Institute of Agro-Environmental Sciences, Kannondai, Tsukuba, Ibaraki 305, Japan.
2. Hokkaido National Agricultural Experiment Station, 1 Hitsujigaoka, Toyohira-ku, Sapporo 062, Japan.

Ae, N., Otani, T., and Arihara, J. 1995. Effects of specific compounds exuded from roots on phosphorus nutrition. Pages 117-128 *in* Genetic manipulation of crop plants to enhance integrated nutrient management in cropping systems—1. Phosphorus: proceedings of an FAO/ICRISAT Expert Consultancy Workshop, 15-18 Mar 1994, ICRISAT Asia Center, India (Johansen, C., Lee, K.K., Sharma, K.K., Subbarao, G.V., and Kueneman, E.A., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Pigeonpea *[Cajanus cajan* (L.) Millsp.] has been widely grown as an intercrop with such cereals as sorghum *[Sorghum bicolor* (L.) Moench], pearl millet *[Pennisetum glaucum* (L.) R.Br.], and maize *(Zea mays* L.) on the Indian subcontinent since ancient times. Many field experiments have shown that the response of pigeonpea to phosphorus (P) is low, as compared with that of other crops, even when grown on soils with low P availability. Possible reasons for this include (1) root penetration to deeper soil layers, (2) mycorrhizal associations, and (3) exudation of specific compounds from roots, which may solubilize sparingly soluble P in the soil. In this paper, we examine the mechanisms by which pigeonpea extracts P from soils of low P availability.

In another recent study, which involved screening of several crops for their ability to solubilize sparingly available P in the soil, we identified upland rice *(Oryza sativa* L.) as another crop having unique mechanisms of P uptake, especially in relation to utilization of iron-bound P (Fe-P), which is normally unavailable to plants. Root distribution in upland rice is in no way superior to that in other crops like sorghum, which does not show any particular ability to utilize Fe-P. The P uptake coefficient (Km) of rice is also similar to that of other crops, including sorghum. Further, the root exudates collected during the rice-growing season do not show any active solubilizing factors when compared with those of pigeonpea. Our study, albeit incomplete, suggests that the mechanisms of P uptake by upland rice should be further explored.

Phosphorus response of pigeonpea

In the semi-arid tropics, Alfisols and Vertisols are major soil types. As part of the comparative assessment of the responses of pigeonpea and sorghum to P application, experiments were conducted at ICRISAT Asia Center in carefully chosen Alfisol and Vertisol fields with low P fertility (Ae et al. 1991b). Electrical conductivity, pH, and P status of the soils used are shown in Table 1. In Alfisols, most of the P is associated with iron (Fe-P); in Vertisols, although Fe-P is the largest fraction, there is also a large fraction of calcium-bound P (Ca-P). The Vertisol is not as weathered as the Alfisol, and available P (EDTA-Olsen, Bray 2, and Ca-lactate methods) in the Vertisol is much higher than that in the Alfisol. Nitrogen (N) at 120 kg N ha^{-1} was applied to sorghum but not to pigeonpea. In the absence of fertilizer P application, sorghum exhibited much greater dry matter production and P uptake in the Vertisol than in the Alfisol. However, the reverse was true for pigeonpea (Table 2).

In the field experiment, the rooting zone of these crops was not limited. Therefore, pigeonpea, with its deeper rooting habit (Sheldrake and Narayanan 1979), could absorb more available P, which lies deeper, than sorghum could. To eliminate the effect of rooting habit of these crops, a pot experiment with restricted rooting volume was conducted (Ae et al 1991a; Table 3). Without P

Table 1. Some chemical characteristics of soil from the experimental plots in an Alfisol and a Vertisol, ICRISAT Asia Center, 1986.

Parameter	Alfisol	Vertisol
pH (H_2O)	6.9	8.3
EC (mS cm^{-1})	0.05	0.16
	1340	7380
Total P(mg kg^{-1})	122	153
Inorganic P (mg kg^{-1})		
Ca-P	4	58
Al-P	5	20
Fe-P	48	55
Available P (mg kg^{-1})		
Olsen	3.5	1.5
Truog	6.5	49.2
Bray P2	1.5	18.1

Source: Ae et al. (1991a).

Table 2. Response of grain yield and P uptake to phosphorus fertilizer application in an Alfisol and a Vertisol fields, ICRISAT Asia Center, rainy season, 1987.

Crop	Soil	P fertilizer application (kg ha^{-1}) 0	9	18	Standard error
		P uptake at the flowering stage (kg ha^{-1})			
Sorghum	Alfisol	2.00	3.43	7.38	±1.19
	Vertisol	6.21	6.61	9.35	±2.23
Pigeonpea	Alfisol	3.18	3.73	6.91	±1.28
	Vertisol	2.46	2.50	4.04	±0.69
		Seed yield at harvest (kg ha^{-1})			
Sorghum	Alfisol	87	673	2101	±725
	Vertisol	3043	3364	3853	±570
Pigeonpea	Alfisol	929	727	1113	±393
	Vertisol	248	457	674	±158

Source: Ae et al. (1991b).

Table 3. Shoot phosphorus contents (mg pot^{-1}) of several crop species at the grain-filling stages after growth in potted Alfisol and Vertisol without phosphorus fertilizer.

Soil	Pigeonpea	Sorghum	Soybean	Pearl millet	Maize
Alfisol	5.72	0.59	1.40	0.64	0.51
Vertisol	2.34	3.91	6.53	5.38	6.13
Standard Error	±0.82	±0.39	±0.20	±0.34	±0.25

Source: Ae et al. (1991a).

addition, growth and P uptake of sorghum, pearl millet, and maize were severely limited on the Alfisol and these crops died as a result of P deficiency within one month after sowing. In contrast, pigeonpea survived. The similar results from both field and pot trials suggest that the better P acquisition by pigeonpea in Alfisol is not caused by a better root distribution when compared to that in the other species. These results also suggest that pigeonpea may have an ability to utilize Fe-P, the dominant form of P in the Alfisol.

Utilization of Fe-P in pigeonpea

To test the ability of pigeonpea to utilize Fe-P, a pot experiment was conducted to compare calcium phosphate ($CaHPO_4$), aluminum phosphate ($AlPO_4$), and iron phosphate ($FePO_4$) as P sources (Ae et al. 1991b). Solubilities of these P sources were 44 mg P kg^{-1} for $CaHPO_4$, 5.1 mg P kg^{-1} for $AlPO_4$, and 2.9 mg P kg^{-1} for $FePO_4$ at pH 7.0 in sand-vermiculite medium. A complete nutrient solution but excluding P was applied to the sand-vermiculite medium. Figure 1 shows P uptake from these sources by several crop species, which were harvested before the flowering stage. Pigeonpea can take up 2.5 to 7.0 times more P from $FePO_4$ than the other crops can at 80 mg P kg^{-1}. This result indicates a unique ability of pigeonpea to solubilize Fe-P.

Mycorrhizal association

It is known that pigeonpea has a strong association with vesicular-arbuscular mycorrhizae (VAM) (Manjunath and Bagyaraj 1984). Ae et al. (1991b) reported experiments showing that the enhanced ability of pigeonpea to extract P from Alfisol, compared to that of sorghum, could not be attributed to VAM accessing Fe-P. In Alfisol, sorghum failed to survive with or without VAM inoculation. This study indicated that VAM acts not by dissolving such relatively unavailable forms of P as Fe-P, but by allowing more efficient uptake of P that is already in a soluble form. This mode of action of VAM has been previously described (Mosse 1981). Therefore, the ability to solubilize Fe-P in Alfisols appears to be an inherent characteristic of pigeonpea.

Root exudates

Marschner (1986) has summarized the role of root exudates in dissolving the unavailable soil P. Gardner et al. (1983) proposed that citric acid exuded from lupin *(Lupinus albus* L.) roots acts to mobilize Fe-P for P uptake. It is in this connection that we examined the role of root exudates in pigeonpea.

Root exudates were collected from pigeonpea grown in a low-P sand culture experiment and separated into three fractions by ion-exchange resins (Ae et al.

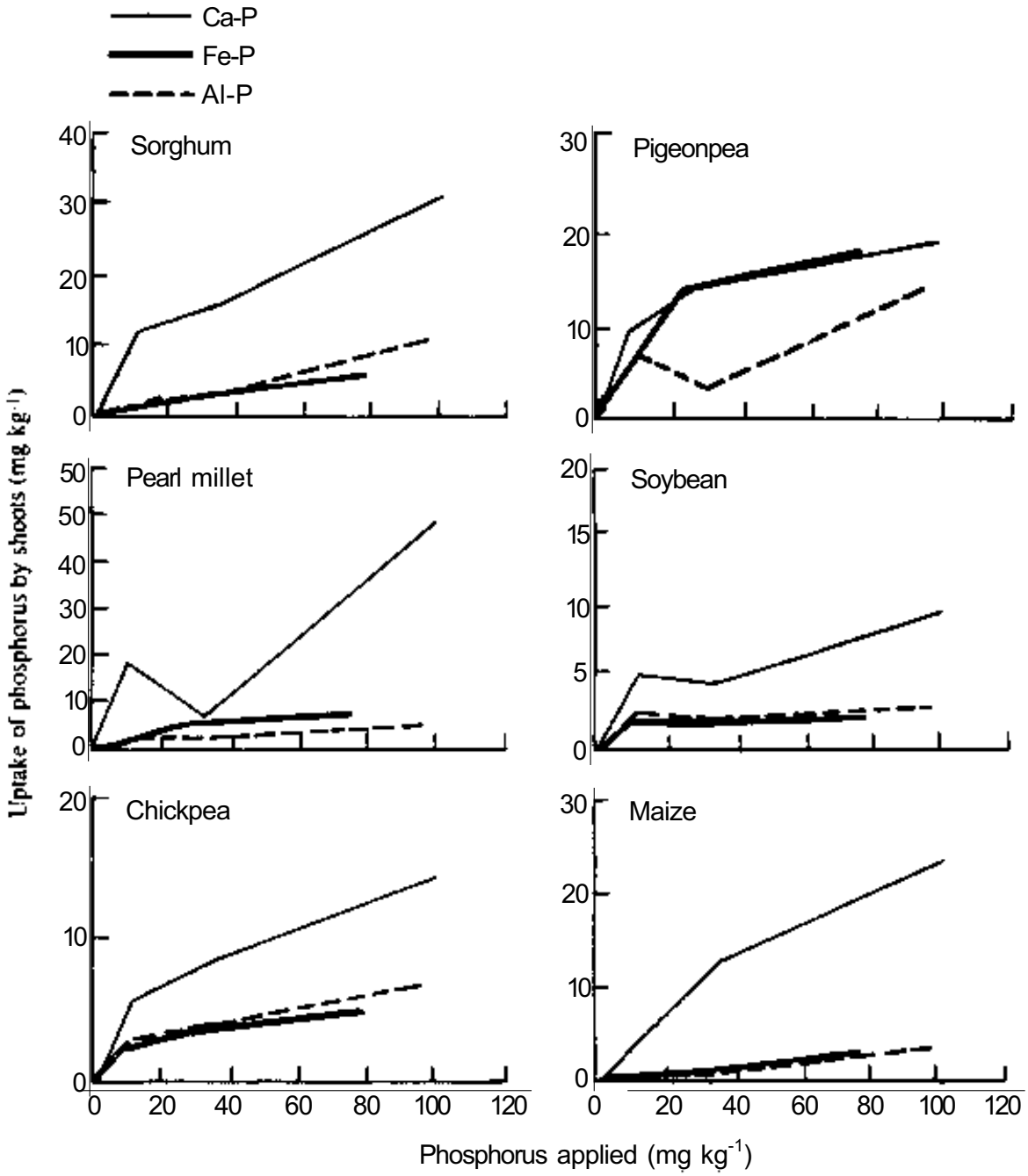


Figure 1. Effect of phosphorus applied through different sources ($CaHPO_4$ = Ca-P, $AlPO_4$ = Al-P, and $FePO_4$ = Fe-P) on its uptake by shoots of six crop species at the pre-flowering stage in sand culture (from Ae et al. 1991b).

1991b). The acid fraction was twice as efficient as the cationic fraction in solubilizing $FePO_4$ whereas the neutral fraction was inactive. The acid fraction from 2-month-old plants was collected and major organic acids in each crop tested were analyzed (Table 4). Citric acid was found to be the major organic acid, the content of which was the highest in soybean *(Glycine max* L.), followed by pigeonpea and then sorghum. The amount of citric acid produced by pigeonpea

Table 4. Major organic acids in root exudates from sorghum, soybean, and pigeonpea.

Crop	Organic acid (mg g^{-1} dry root)			
	Malonate	Succinate	Citrate	Malate
Sorghum	Trace	Trace	0.045	0.008
Pigeonpea	Trace	0.025	0.101	0.047
Soybean	0.324	0.046	0.481	0.078

Source: Ae et al. (1991a).

does not therefore explain the unique P absorption by pigeonpea from Fe-P sources as compared to that by soybean and sorghum.

In gas chromatograms (GC) of the acid fraction of root exudates from soybean, sorghum, and pigeonpea, there were peaks peculiar to pigeonpea at a retention time of 23-24 min. Subsequent GC-mass spectrometry and nuclear magnetic resonance (NMR) analysis identified molecular weight and chemical structure of the compounds associated with these peaks (Ae et al. 1990,1991b, 1993). These were (p-hydroxy benzyl) tartaric acid and (p-methoxy benzyl) tartaric acid. The former compound is named piscidic acid and is one of the chemical constituents of hypnotic and narcotic drugs that have long been extracted from the bark of Jamaica dogwood tree *(Piscidia erythrina* L.) (Freer and Clover 1901, Bridge et al. 1948). However, these substances have not been previously considered in relation to the P-acquisition ability of roots.

Piscidic acid from *Narcissus poeticum* bulbs (Smeby et al. 1954) and some derivatives of fukiic acid from *Petasites japonicus* (Sakamura et al. 1973) were also synthesized to investigate the chelating ability between Fe^{3+} and such reactive entities as phenolic, alcoholic, and carboxylic groups in piscidic acid (Ae et al. 1990,1991b, 1993; Table 5). The absolute configuration of fukiic acid is the same as that of piscidic acid. Piscidic acid and dimethyl fukiic acid have similar P-releasing ability while the trimethyl fukiic acids have lower P-releasing ability. This shows that the alcoholic and carboxylic acid groups the tartaric portion of piscidic acid are involved in chelation of Fe^{3+}, and that the phenolic -OH group is not involved in chelation.

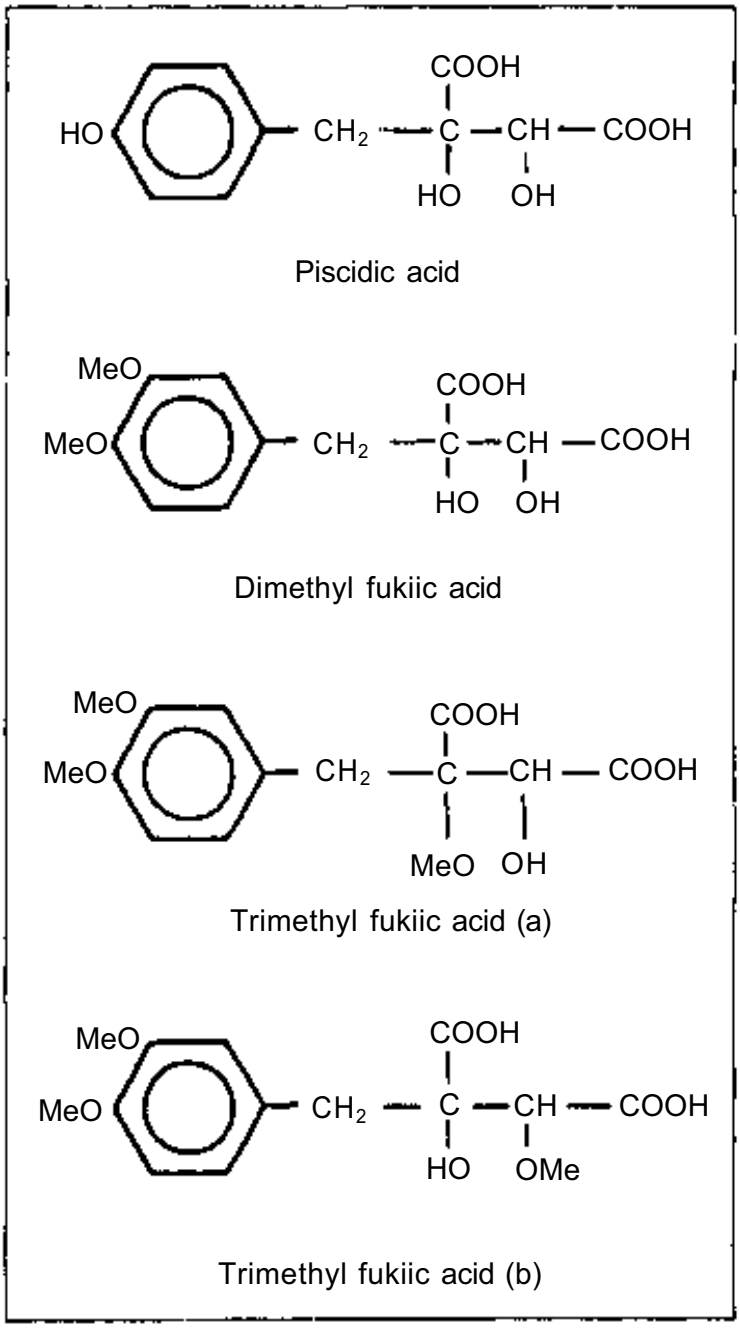
Postulated mechanisms of P uptake by pigeonpea

It is proposed that pigeonpea exudes piscidic acid to take up P from Fe-P in such iron-rich soils as Alfisols. It is necessary, however, that piscidic acid-Fe^{3+} complexes be excluded from the rhizosphere because of the possibility of excess Fe uptake into roots, which could result in precipitation of P in plant cells. Mineral element concentrations of several crop species (pigeonpea, sorghum, soybean, pearl

Table 5. Effects of piscidic acid and its derivatives on phosphorus release from ferric phosphate ($FePO_4$).

Chemical	Released P ug mL^{-1})
Control (water)	1.48
Piscidic acid	4.37
Dimethyl fukiic acid	4.44
Trimethyl fukiic acid (a)	3.27
Trimethyl fukiic acid (b)	3.23
Standard Error	±0.40

Source: Ae et al. (1991b).



millet, and maize), which were grown on the Alfisol at various P levels, were measured (Ae et al. 1991b, 1993). The Fe/P ratio, which indicates the degree of exclusion of P in relation to P uptake, is the lowest for pigeonpea (Table 6). The results suggest that the phosphate ions solubilized by the chelating agents at the root surface are taken up and that the piscidic acid-Fe^{3+} complex is excluded from the rhizosphere (Ae et al. 1991b, 1993).

Table 6. Concentrations (mean ± standard error) of phosphorus (P) and iron (Fe), and Fe:P ratio, in shoots of crop species at the grain-filling stage grown on Alfisol of low P status.

Crop	P(%)	Fe(mg kg^{-1})	Fe:P
Pigeonpea	0.27 ± 0.03	259 ± 15	0.101 ±0.01
Sorghum	0.14 ±0.02	168 ± 17	0.129 ±0.02
Soybean	0.19 ±0.03	410 ±123	0.292 ±0.15
Pearl millet	0.12 ±0.03	167 ± 23	0.159 ±0.03
Maize	0.13 ±0.02	274± 65	0.235 ± 0.06

Source: Ae et al. (1991b).

Is the P uptake mechanism in pigeonpea applicable to other crops?

Over the previous half century, high doses of fertilizers have been applied to arable lands to increase agricultural production in several countries, including Japan. Consequently, ground and surface waters are becoming increasingly polluted with such leached nutrients as nitrogen and P from fertilizers. Indeed, the accumulated P level in some farmers' fields in Japan is far above the optimum P fertility status necessary to support crop growth. In order to improve the sustainability of cropping systems and reduce the environmental degradation caused by land and crop management practices, a search for soil ameliorative crops including those that can take up accumulated nutrients is of paramount importance.

With this in mind, a pot experiment aimed at identifying crops that can remove Fe- or Al-bound P more efficiently was conducted at the National Institute of Agro-Environmental Sciences (NIAES), Japan. The soil used for this experiment was collected from Kawatabi, Miyagi Prefecture. The chemical analysis (Table 7) indicated that the soil is characterized by low pH, high Al^{3+} concentration, low P availability, and high contents of organic P associated with Al^{3+} and Fe^{3+}. Upland rice (cv Toyohatamochi) proved to be much superior to the other crops in dry matter production and P uptake (Figure 2). Upland rice not only showed a higher tolerance to Al^{3+} toxicity, but also a greater ability to remove accumulated P from the soil. Another pot experiment, without P application, was conducted with rice genotypes collected from different parts of the world, after adjusting the soil pH to 6.5 (by adding of $CaCO^3$, Al^{3+} concentration extracted by IM KCl was 1.55 mmol

Table 7. Phosphorus (P) status of an Andosol at Kawatabil with which pot experiments were conducted (National Institute of Agro-Environmental Sciences, Japan.)

Parameter	Value
pH (H_2O)	5.1
Al(KCl)(mmol kg^{-1})	13.5
Total P(mg kg^{-1})	752
Inorganic P (mg kg^{-1})	
Ca-P	0
Al-P	36
Fe-P	23
Organic P(mg kg^{-1})	
Ca-P	1
Al-P	405
Fe-P	220
Available P (mg kg^{-1})	
Olsen	12
Truog	0.0
Bray p2	7.8

kg^{-1}) to eliminate the effect of low pH on their growth. Some rice varieties originating from Japan and Brazil grew better, especially Nohrinmochi 4 from Japan (Figure 3). On the other hand, genotypes from the International Rice Research Institute (IRRI), the Philippines, and India did not show much ability to

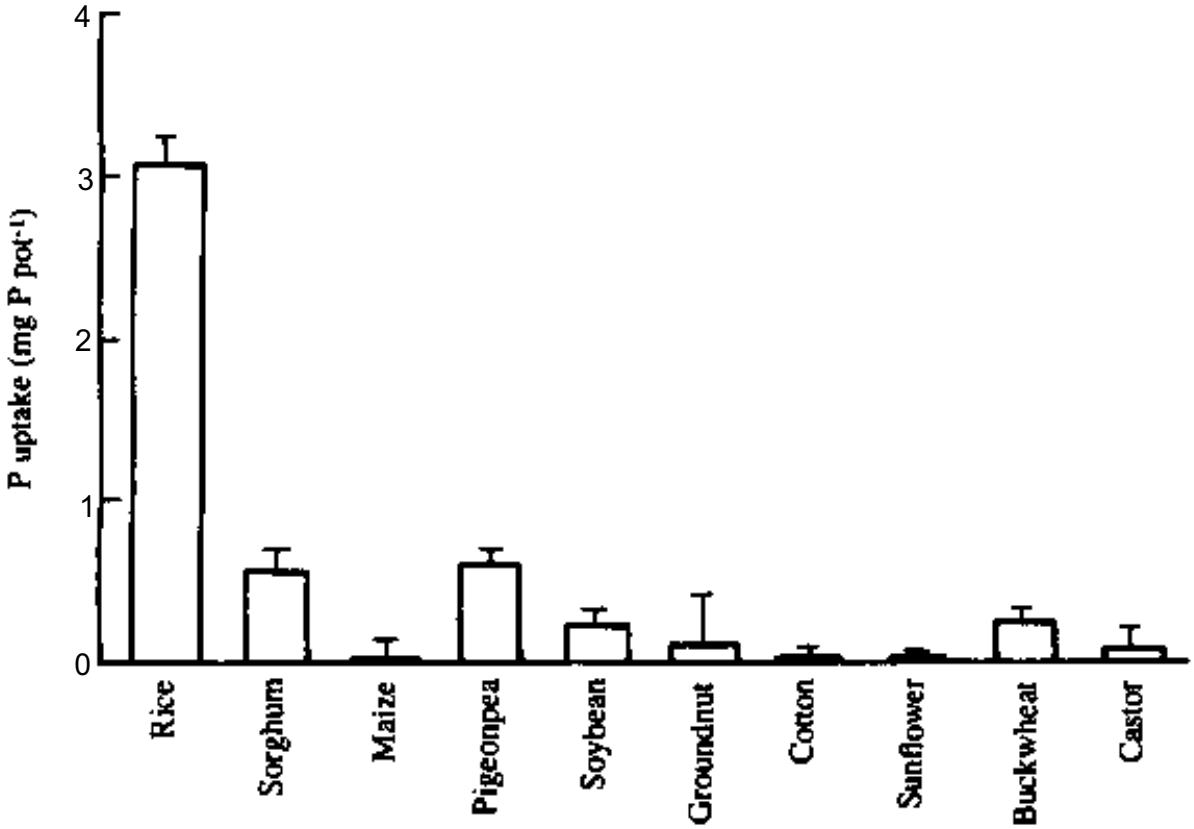


Figure 2. Uptake of phosphorus (P) by ten crop species grown without P application on an Andosol with low pH and low P-availability. (Standard errors are indicated.)

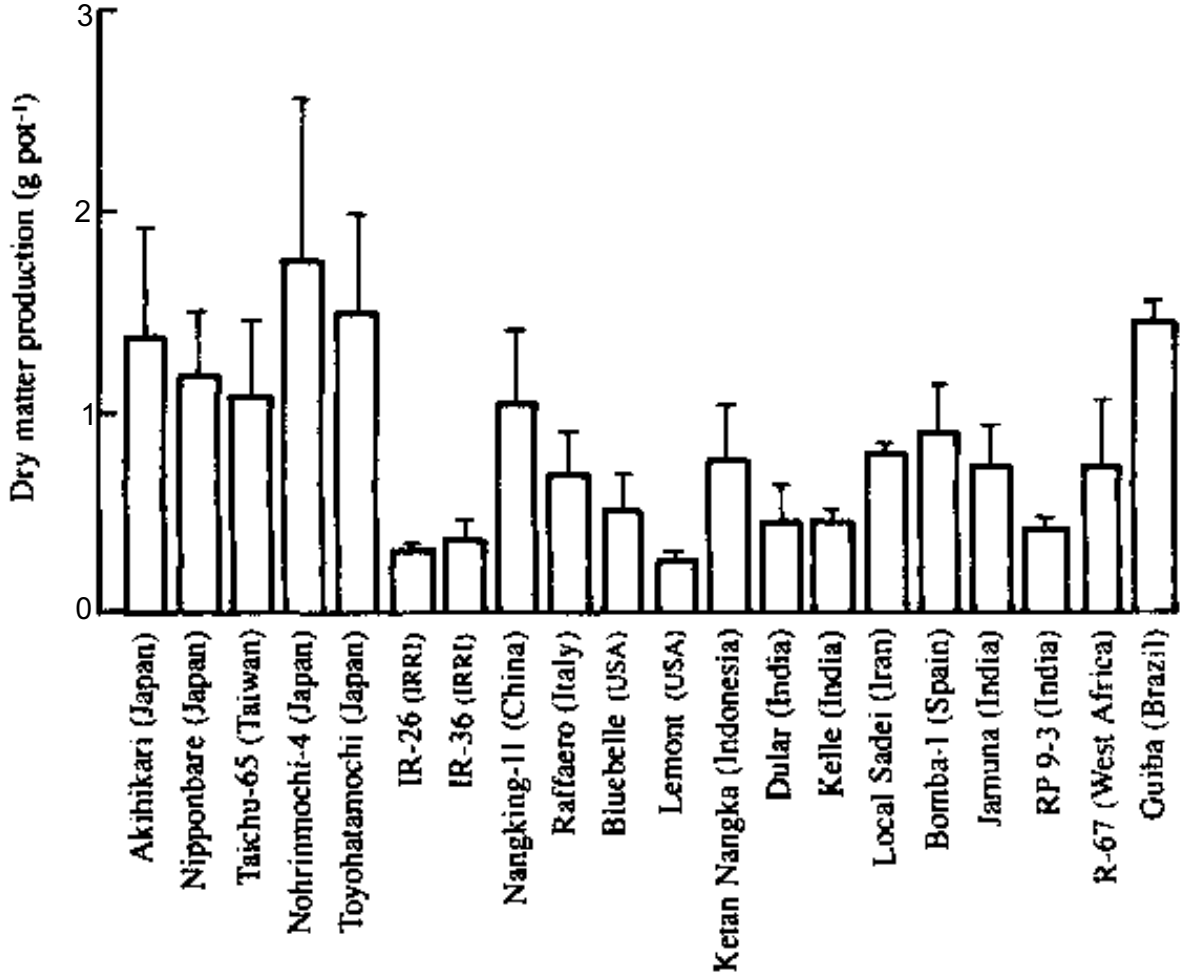


Figure 3. Dry matter production in 20 genotypes of upland rice grown without phosphorus (P) application on an Andosol with low P-availability. The Andosol was adjusted to pH 6.5 with $CaCO_3$. Standard errors are indicated.

grow and take up P from the low P-fertility Andosol. These findings suggest evolutionary adaptation by upland rice to acid soils of low-P availability, as evidenced by the better performance of genotypes from the high P-fixing soils of Japan and Brazil.

We confirmed this superior ability of upland rice to use $FePO_4$ as a P source in vermiculite pot culture (data not shown). The results therefore suggest that the greater P absorption ability of upland rice is related to neither rooting pattern nor VAM association. The results then raise the question whether a special mechanism to utilize P in Fe-P exists in upland rice, similar to that in pigeonpea. Sorghum, pigeonpea, rice, and soybean were grown in water culture. Seedlings were grown in Arnon's medium for two weeks before transferring them to the modified Arnon's medium with very low P concentration (2 mg P L^{-1}). Root exudates were collected every week after the transfer to the low-P medium, and the capacity of exudates to solubilize $FePO_4$ was determined. Only root exudates from pigeonpea enhanced the solubility of $FePO_4$ while those from the other crops had little effect (Table 8).

Table 8. Phosphorus (P) released from $FePO_4$ by root exudates of several crops grown in water culture with low P concentration.

	Released P (ug mL^{-1})[1]			
Crop	1W[2]	2W	3W	4W
Rice	2.51	1.42	n.d.[3]	n.d.
Sorghum	2.05	n.d.	2.48	n.d.
Pigeonpea	n.d.	n.d.	8.51	4.95
Soybean	n.d.	n.d.	n.d.	n.d.

1. On a dry root basis.
2. Weeks after exposure to the low-P medium.
3. Not detected.

In pigeonpea, we have not yet tackled the question of how, and how much, effective chelating agents are exuded from the root surfaces. In upland rice, however, we found significant genotypic variation in absorption of normally unavailable P from soils of low P status. Since we have not yet been able to find any evidence of a chelating function of root exudates in rice, it is necessary for us to explore other possible mechanisms to elucidate its P-solubilizing effect.

Conclusion

The fact that pigeonpea performed better on the Alfisol than the other crops (maize, sorghum, soybean, and pearl millet) suggests that pigeonpea is able to access the large Fe-P fraction in the Alfisol. Generally, Fe-P is more insoluble than Ca-P or Al-P. Gardner et al. (1983) and Mullette et al. (1974) proposed that citric and oxalic acids exuded from *Lupinus albus* and *Eucalyptus gummifera* chelated with Al^{+3} and/or Fe^{3}+ to release P in soils of low fertility. However, pigeonpea exuded much less citric acid than did soybean, which does not show the capacity to solubilize Fe-P. Another organic acid, which is unique to pigeonpea, was then identified. Piscidic acid and its derivatives were detected and these chemical compounds seem to be involved in chelation with Fe^{3+}. In another study, crops were screened for their ability to take up P from an acid Andosol with low available P. Upland rice was found to be a promising candidate crop having unique P uptake ability. Since the precise mechanisms involved are however as yet unknown, including the chelating function of rice root exudates, a thorough study on these aspects is necessary.

References

Ae, N., Arihara, J., Okada, K., Yoshihara, T., and Johansen, C. 1990. Phosphorus uptake by pigeon pea and its role in cropping systems of the Indian subcontinent. Science 248: 477-480.

Ae, N., Arihara, J., and Okada, K. 1991a. Phosphorus response of chickpea and evaluation of phosphorus availability in Indian Alfisols and Vertisols. Pages 33-41 *in* Phosphorus

nutrition of grain legumes in the semi-arid tropics (Johansen, C., Lee, K.K., and Sahrawat, K.L., eds.). Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Ae, N., Arihara, J., and Okada, K. 1991b. Phosphorus uptake mechanisms of pigeonpea grown in Alfisols and Vertisols. Pages 91-98 *in* Phosphorus nutrition of grain legumes in the semi-arid tropics (Johansen, C., Lee, K.K., and Sahrawat, K.L., eds.). Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Ae, N., Arihara, J., Okada, K., Yoshihara, T., Otani, T., and Johansen, C. 1993. The role of piscidic acid secreted by pigeonpea roots grown in an Alfisol with low P-fertility. Pages 270-288 *in* Genetic aspects of plant mineral nutrition (Randall, P. J., Delhaize, E., Richards, R. A., and Munns, R., eds.). Dordrecht, The Netherlands: Kluwer Academic.

Bridge, W., Coleman F., and Robertson, A. 1948. Constituents of 'Cortex pisciae erythrinae'. Part 1. The structure of piscidic acid. Journal of Chemical Society 1948: 257-260.

Freer, P.C., and Clover, A.M. 1901. On the constituents of Jamaica dogwood. American Chemical Journal 25: 390-413.

Gardner, W.K., Barber, D.A., and Parbery, D.G. 1983. The acquisition of phosphorus by *Lupinus albus* L. III. The probable mechanism by which phosphorus movement in the soil/ root interface is enhanced. Plant and Soil 70: 107-124.

Manjunath, A., and Bagyaraj, D.J. 1984. Response of pigeonpea and cowpea to phosphates and dual inoculation with vesicular-arbuscular mycorrhiza and *Rhizobium.* Tropical Agriculture (Trinidad) 61: 48-52.

Marschner, H. 1986. Mineral nutrition of higher plants. London, UK: Academic Press. 456 pp.

Mosse, B. 1981. Vesicular-arbuscular mycorrhiza research for tropical agriculture. Research Bulletin, Hawaii Institute of Tropical Agriculture and Human Resources, University of Hawaii. Honolulu, Hawaii, USA: University of Hawaii. 82 pp.

Mullette, K.J., Hannon, N.J., and Elliott, A.G.L. 1974. Insoluble phosphorus usage by *Eucalyptus.* Plant and Soil 41: 199-205.

Sakamura, S., Yoshihara, T., and Toyoda, K. 1973. The constituents of *Petasites japonicus:* Structure of fukiic acid and fukinolic acid. Agricultural and Biological Chemistry 37: 1915-1921.

Sheldrake, A.R., and Narayanan, A. 1979. Growth, development and nutrient uptake in pigeonpeas *(Cajanus cajan).* Journal of Agricultural Science, Cambridge 92: 513-526.

Smeby, R.R., Zbinovsky, V., and Burris R.H. 1954. The organic acids of *Narcissius poeticum.* American Chemical Society 76: 6127-6130.

A strategy for cloning the genes in the synthetic pathway of mugineic acid-family phytosiderophores

S Mori[1]

Abstract

Genes involved in the biosynthetic pathway of mugineic acid-family phytosiderophores were cloned. Initially, the genes for nicotinamine synthase and nicotinamine aminotransferase were confirmed to be induced by iron (Fe) deficiency and were partially purified. The partial amino acid sequences of the 'd' peptide were determined on 2D-PAGE, which appeared to be specific to Fe-deficient barley roots. Finally, seven Fe-deficiency specific clones were selected by "differential screening' of a cDNA library constructed from Fe-deficient barley roots and three DNA clones (Idsl, Ids2, *and* Ids3) *were sequenced from amongst these. Strategies to clone the genes essential for the synthesis of phytosiderophores are discussed.*

Introduction

The biosynthetic pathways of mugineic acid-family phytosiderophores (MAs) have been discussed both *in vivo* (Mori and Nishizawa 1987, Kawai et al. 1988, Mori and Nishizawa 1989, Mori et al. 1990, Ma and Nomoto 1993) and *in vitro* (Shojima et al. 1989,1990). The overall plausible synthetic pathway of MAs is shown in Figure 1. 2-deoxymugineic acid (DMA), which is formed from methionine (Met), is a precursor of such other MAs as mugineic acid (MA), 3-epihydroxymugineic acid (epiHMA), 3-hydroxymugineic acid (HMA), and avenic acid (AVA).

The identification of Met as a precursor of MAs (Mori and Nishizawa 1987) has raised the possibility of producing transgenic cultivars by introducing one of the genes of MAs biosynthesis into cultivars susceptible to Fe deficiency. Interestingly,

1. Laboratory of Plant Nutrition and Fertilizer, Faculty of Agriculture, The University of Tokyo, 1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan.

Mori, S. 1995. A strategy for cloning the genes in the synthetic pathway of mugineic acid-family phytosiderophores. Pages 129-144 *in* Genetic manipulation of crop plants to enhance integrated nutrient management in cropping systems—1. Phosphorus: proceedings of an FAO/ICRISAT Expert Consultancy Workshop, 15-18 Mar 1994,ICRISAT Asia Center, India (Johansen, C., Lee, K.K., Sharma, K.K., Subbarao, G.V., and Kueneman, E.A., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

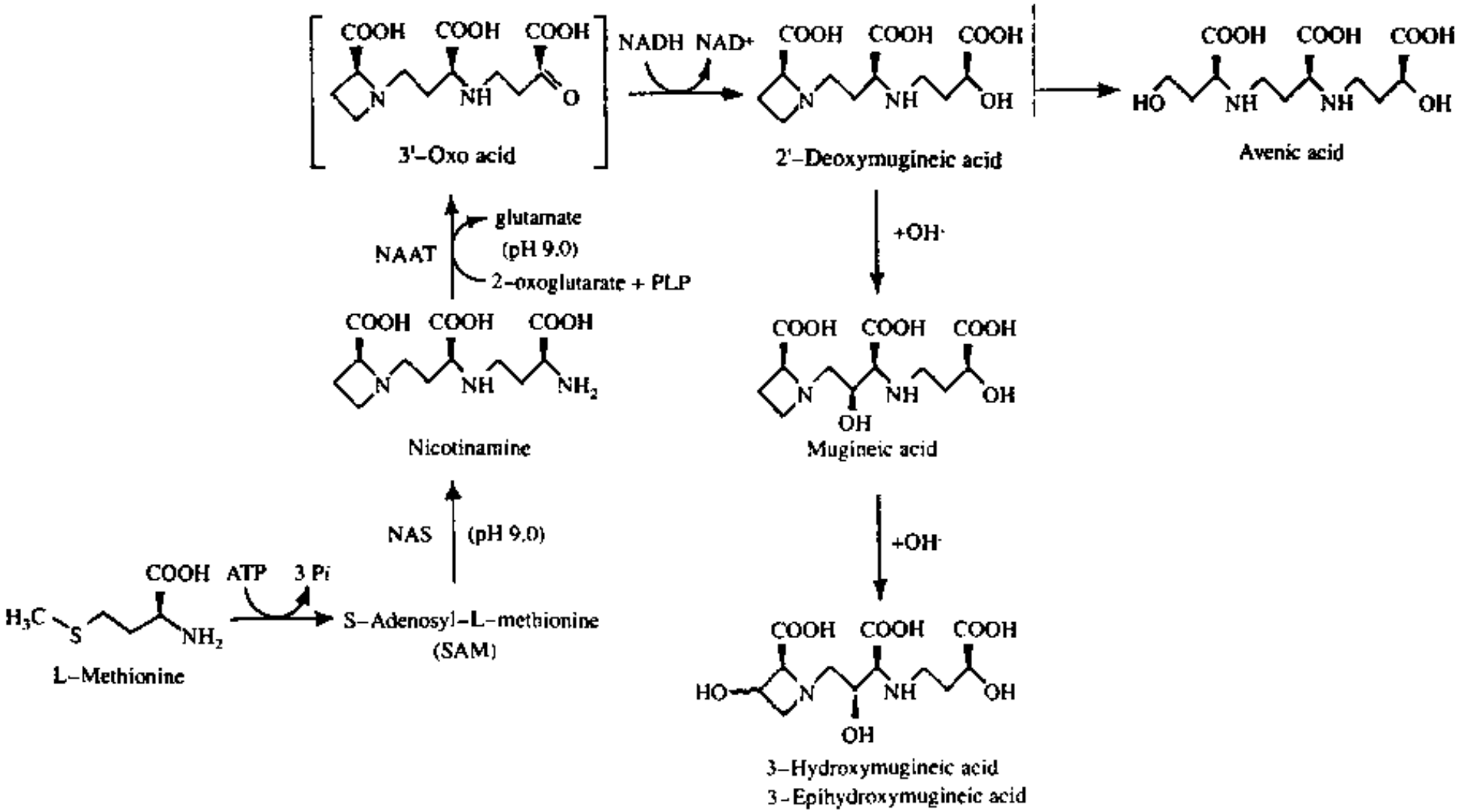


Figure 1. Biosynthetic pathway of mugineic acid-family phytosiderophores (MAs). MAs is composed of 2'-deoxymugineic acid, mugineic acid, 3-hydroxymugineic acid, 3-epihydroxymugineic acid, and avenic acid. NAS = nicotianamine synthase. NAAT = nicotianamine aminotransferase.

amongst the many graminaceous plants, the degree of tolerance to Fe deficiency correlates with the capacity to produce and secrete MAs into the rhizosphere under iron (Fe)-deficient conditions in the order barley > rye > wheat, oat > sorghum, maize > rice (Romheld and Marschner 1990, Singh et al. 1993). Thus, introducing one of the key enzymatic genes such as nicotinamine synthase (NAS) or nicotinamine aminotransferase (NAAT) or other subsequent hydroxylation enzymes for MAs synthesis from DMA could play an important role in developing transgenic plants (especially monocotyledons) tolerant to Fe deficiency (see Figure 1).

Although dicotyledons (dicots) have also been shown to possess NAS activity (Shojima et al. 1989, Higuchi et al. 1994), they apparently lack NAAT activity and transporter protein in the plasma membrane of root cells (Romheld and Marschner 1986). Hence, by introducing NAAT genes from cereals into dicots, it could be possible to develop dicots with ability to produce DMA. However, such transgenic dicots may prove susceptible to some degree to Fe deficiency, since they do not possess the necessary transporters in their root plasma membranes. Nevertheless, there may be a favorable influence in increasing the soluble iron (Fe) in the rhizosphere in the form of Fe^{3+}-DMA due to DMA secretion from the roots of the transgenic plants.

To begin applying this concept, it is suggested that rice should be the first target crop for genetic manipulation of MAs synthesis since it is the crop most susceptible to Fe deficiency amongst graminaceous crops (Mori et al. 1991).

In our attempts to isolate the genes that are specifically expressed in the roots of Fe-deficient barley by differential hybridization, we hope that some of these expressed genes could be involved in MAs synthesis.

Methodology

Plant culture conditions

Seeds of barley *(Hordeum vulgare* L. cv Ehimehadaka no.1) were germinated and transplanted to water culture as previously described (Okumura et al. 1992). Fe at 0.05 mg L^{-1} was supplied from a 1% Fe-citrate stock solution when required. The nutrient concentration was gradually increased from half-strength to reach full strength at pH 5.5 when the fourth leaf had emerged. The Fe deficiency treatment was then begun using deionized-redistilled water without controlling the pH. The culture solutions were renewed every 5 days. One month after the Fe-deficiency treatment, when severe chlorosis appeared, 1-cm (for RNA preparation) or 5-cm (for enzyme purification) lengths of the tips of the newly growing roots were harvested and put in liquid nitrogen in 50-µL plastic tubes (Corning Co.) and stored at -80°C prior to use. Root tips from control plants, cultured maintaining the pH at 5.5, were also collected and stored similarly. A chlorophyll-meter (Spad-502, Minolta, Japan) was used to quantify the chlorophyll content in the new leaf. For

Northern and genomic Southern hybridization or for enzyme assays of NAS and NAAT, barley leaves from Fe-deficient and Fe-sufficient (control) plants were also harvested and stored as in the case of the root tips.

Enzyme assay of nicotinamine synthase and nicotinamine aminotransferase

The root tips (100 g) were homogenized with 250 mL of extraction buffer (0.2M Tris, 1 mM EDTA, 5% (v/v) glycerol, 10 mM DTT, 50 u*M* Leupeptin, 5% (v/v) PVP, pH 8.0), passed through four layers of gauze, and centrifuged at 5000g for 20 min. The supernatant was applied to a TSK gel Butyl-Toyopearl 650 M (Fractogel TSK Butyl-650M, Merck) column (1 x 11 cm) equilibrated with the starting buffer for the hydrophobic chromatography (50 mM Tris, 1 mM EDTA, 3 mM DTT, 0.4 M ammonium sulfate, pH 8.0). NAAT activity was recovered in the first nonabsorbable fractions and NAS activity was recovered in the eluates with water containing 1% glycerol.

Nicotinamine synthase. Nicotinamine synthase activity was assayed as reported by Higuchi et al. (1994). Enzyme solutions were equilibrated with reaction buffer (50 mM Tris, 1 mM EDTA, 3 mM DTT, pH 8.7) concentrated by gel filtration or ultrafiltration using Ultrafree C3LGC NMWL10000 or Ultrafree-PC (LGC) NMWL10000 (Millipore Co.) or Column PD-10 (Pharmacia LKB). [^{14}C]SAM was added to the enzyme solution at the final concentration of 20 uM. After 1 h incubation at 25°C, the enzyme reaction was stopped by adding ethanol at a final concentration of 50% (v/v). For the qualitative analysis, radioactivity of [^{14}C]NA was detected by a radio analyzer RLC-700 (Aloka) after separation with HPLC. For the quantitative analysis, 10 uL of reaction mixtures and authentic [^{14}C]NA were spotted on a silica gel TLC plate LK6 (Whatman), and the plates were developed with phenol:butanol:formate:water at 12:3:2:3, v/v. Radioactivity of [^{14}C]NA was detected by an image analyzer BAS2000 (Fuji film). The protein content was estimated using a protein assay kit with Standard I (Bio Rad).

Nicotinamine aminotransferase. Nicotinamine aminotransferase activity was assayed by the method of Ohata et al. (1993) with minor modifications: 2-oxoglutarate and NA were added to 50 uL of enzyme preparation containing 0.1 or 0.2 mg proteins, and the mixture was kept at 25°C for 30 min or 1 h. Final concentration of 2-oxoglutarate was 5 mM, and of NA 122 uM. The reaction was stopped by cooling the mixture at 4°C and the enzyme separated from the mixture by using ultrafree (molecular cutoff 30000). Then, 4 uL of 0.25M $NaBH_4$ was added to the protein-free mixture to reduce 3'-oxo acid to DMA (see Figure 1) and the mixture kept at room temperature for 5 min. Excess reductant ($NaBH_4$)was consumed by adding 2 uL of 0.25M 2-oxoglutarate to the mixture, and the mixture

was stored at -20°C. The final mixture was about 60 μL, and 30 μL of this mixture was analyzed by HPLC for the product DMA.

Identification and partial determination of amino acids sequence of 'd' spot

For the extraction of protein, the roots were homogenized in liquid nitrogen with a mortar and pestle, and resuspended in a cold solution of 10% trichloroacetic acid (TCA) in acetone with 0.1% β-mercaptoethanol. Proteins were allowed to precipitate for 45 min at -20 °C, centrifuged at 16000g for 15 min, and the pellet was dried under reduced pressure. The pellet was dissolved in 50 μL in a sample buffer (1 M thiourea, 5 M urea, 2% Triton-X100, 0.2% β-mercaptoethanol) at 50 u*L* per mg of pellet, centrifuged at 16000g for 5 min, and the supernatant used for 2D-PAGE. 2D-PAGE was performed following the method of O'Farrel (1975), with some modifications. To determine the pH in the gel, 2D-IEF marker 'DAIICHI' (Daiichi Pure Chemicals, Tokyo) was used. After 2D-PAGE, the 36 kDa peptide was cut out and collected from 400 gel sheets. The peptides were electroeluted out of the gel at 200V for 2.5 h, dialyzed against distilled water for 27 h at 4 °C, and dried under reduced pressure. Unfortunately, after direct sequencing of the 'd' peptide by the automated Edman degradation on a gas-phase sequencer (Model 477A Protein Sequencer and 120A PTH Analyzer; Applied Biosystem Japan, Tokyo), its N-terminal amino acid was blocked. To determine internal amino acid sequence of the 36 kDa peptide, enzymatic digestion with V8 protease (Sigma) (Cleveland et al. 1977) or lysylendopeptidase (Kawasaki et al. 1990) was performed. The digested peptides were separated by SDS-PAGE, transferred to a PVDF membrane, and the amino acid sequences of the membrane bands were analyzed.

Isolation of cDNA specifically expressed in Fe-deficient barley roots

Total RNA from Fe-deficient barley root tips was extracted by the revised method of Logman et al. (1987). Poly(A+)RNA was purified by Oligotex TM-dT30 (Daiichikagakuyakuhin Co. Japan), and the cDNA library of λ ZAPII was constructed following the manufacturer's protocol (Stratagene). This library was screened by differential plaque hybridization (see Figure 2) between the cDNA probes of (-)Fe and (+)Fe. ^{32}P-cDNA probes were labeled from [α-^{32}P]dCTP following the method of Gasser et al. (1989). After the third screening, seven independent clones were selected and DNA sequences of *Idsl, Ids2, Ids3,* and *Ids4* were determined. Then, using a commercial genomic library (λ EMBL-III), the genomic DNA of barley was sequenced for *Idsl, Idsl,* and *Ids3.* Using the probe of *Ids* series, Northern hybridization was performed on the total RNA from the barley roots and shoots of (-)Fe or (+)Fe treatments.

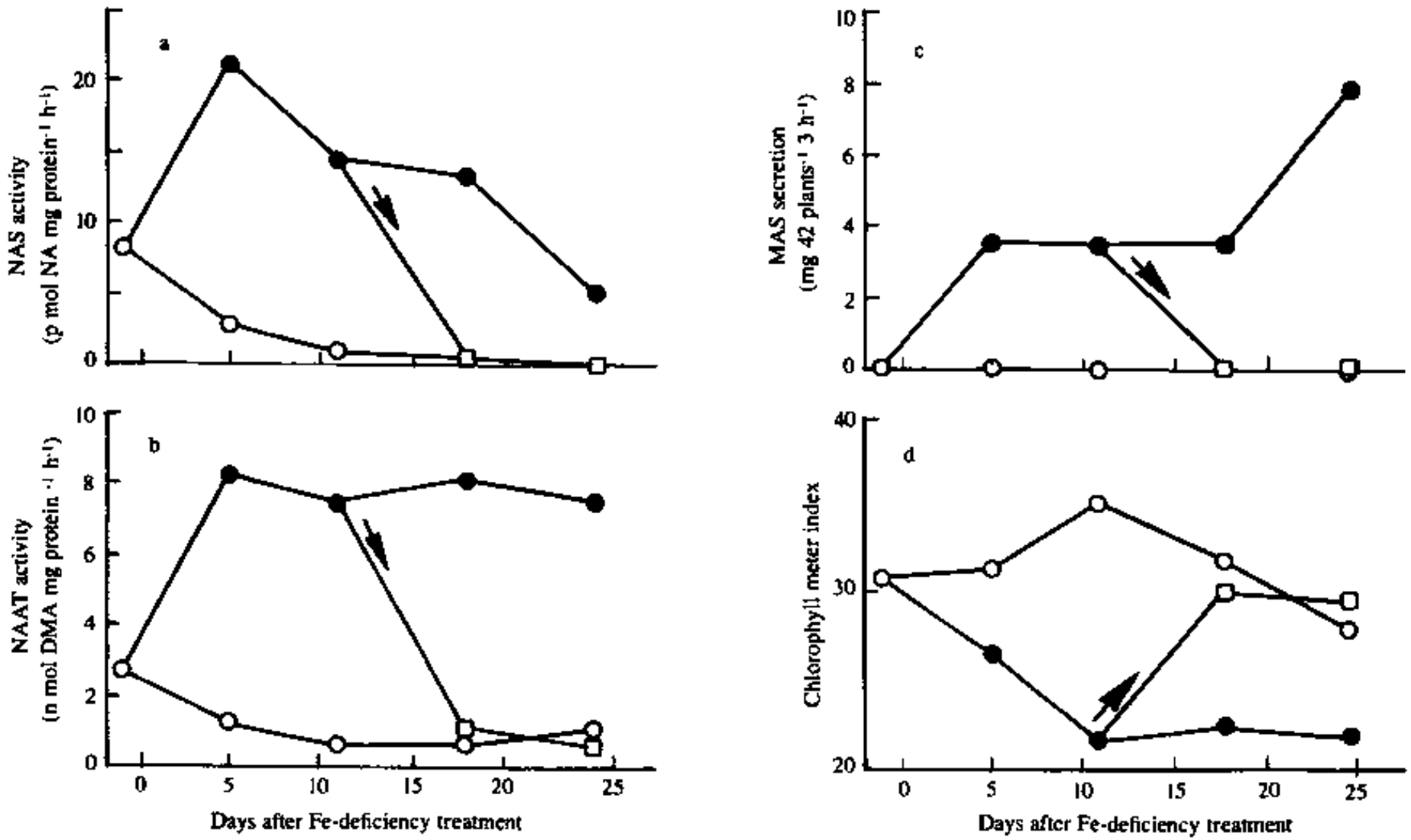


Figure 2. Regulation of NAS activity (a) and NAAT activity (b) first by Fe deficiency treatment and then Fe resupply, in comparison with MAs secretion (c) and chlorophyll deter index of the newest leaf (d). Arrows indicate the trends after Fe resupply. Open circles refer to the sufficient supply of Fe in the culture medium, closed circles refer to Fe-deficiency treatment of the culture medium, and the open square refers to the resupply of Fe.

Results and discussion

Induction of enzyme activities for NAS and NAAT by iron deficiency

Almost no MAs were secreted from the Fe-sufficient plant roots (Figure 2c). The amount of secreted MAs gradually increased until day 26 after beginning the Fe-deficiency treatment. The secretion declined to the control level within 7 days after resupplying Fe in the growth medium at day 18. Both NAS activity (Figure 2a) and NAAT activity (Figure 2b) showed profiles similar to those of MAs secretions. The induced enzyme activities of Fe-deficient plants decreased to the control level within 7 days after Fe resupply (Figures 2a and 2b). On the other hand, chlorophyll contents gradually reduced to reach a minimal level 12 days after the onset of Fe-deficiency treatment (Figure 2d); these changes were slower than those recorded for MAs secretion or enzyme activities.

A unique secretion pattern of MAs in the circadian rhythm has been extensively studied by Takagi and co-workers (Takagi 1993). We investigated changes in enzyme activities over a 24-h period to determine whether these phenomena were preceded by changes in these enzyme activities. The enzyme activities were not constant but the changes were not so extreme (Figure 3b) as to affect the MAs secretion pattern during a day (Figure 3a).

Characteristics of NAS and NAAT

The optimum pH of NAS was 9.0 (Figure 4b) and its molecular weight, determined by gel filtration, was about 45 kDa (Figure 4a). The optimum pH of NAAT was 8.5-9.0 (Figure 5b) and its molecular weight about 100 kDa (Figure 5a). But, to date, NAAT has been shown to be an isozyme of at least three different molecular weights (unpublished data). The exact characteristics of NAATs are now under investigation.

Partial amino acid sequences of 'd' spot

Several peptides induced by Fe deficiency in barley roots were observed on two-dimensional electrophoresis (2D-PAGE) (Mori et al. 1988, Suzuki et al., in press). Among them, a 36-kDa peptide was found to be induced most strongly by Fe deficiency. The induction of the 36-kDa peptides seemed to be specific to Fe deficiency, since it was not induced by the other types of nutrient stress (Suzuki et al., in press). The partial amino acid sequences of the resultant peptides digested by V8 were LYSFDTATS(X)W, AFVLDT, and VTINQ. However, these peptides were not suitable to design probe mixtures for screening genes from the cDNA library prepared from Fe-deficient barley roots (Okumura et al. 1991) because the component amino acids had highly degenerated codon usages. The 36-kDa peptide was then digested with lysylendopeptidase, and the sequence of DHTEL-

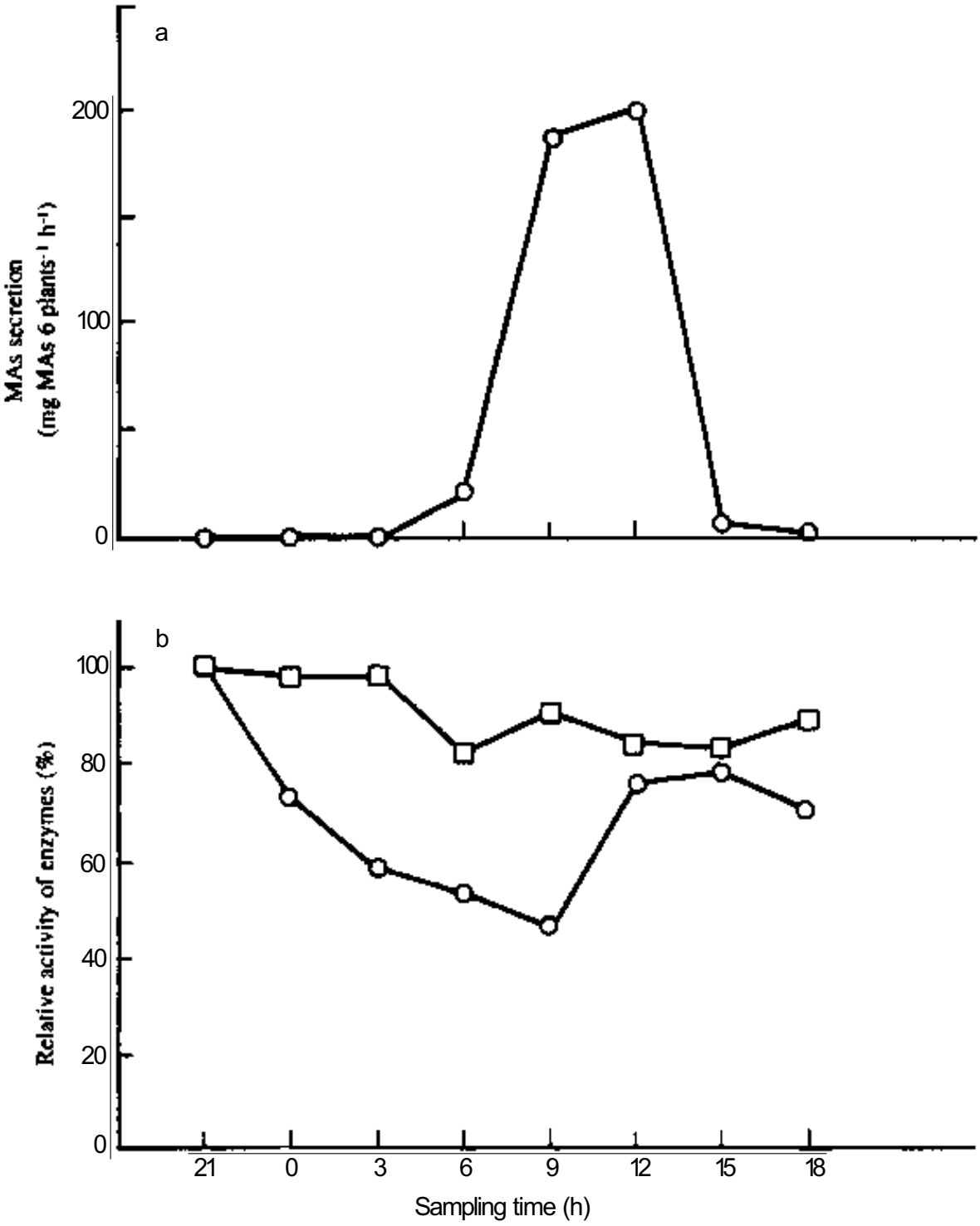


Figure 3. Diurnal changes in MAs secretion (a), NAS activity (b; circle), and NAAT activity (b; square).

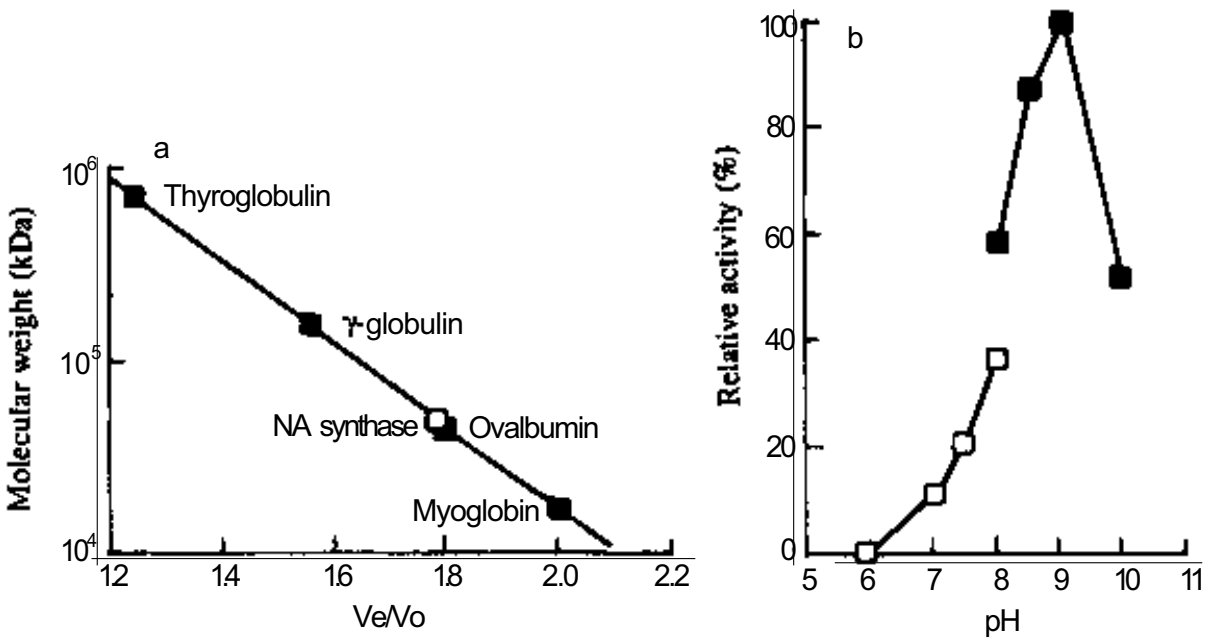


Figure 4. Molecular weight of NA synthase (a) and its pH dependency (b).

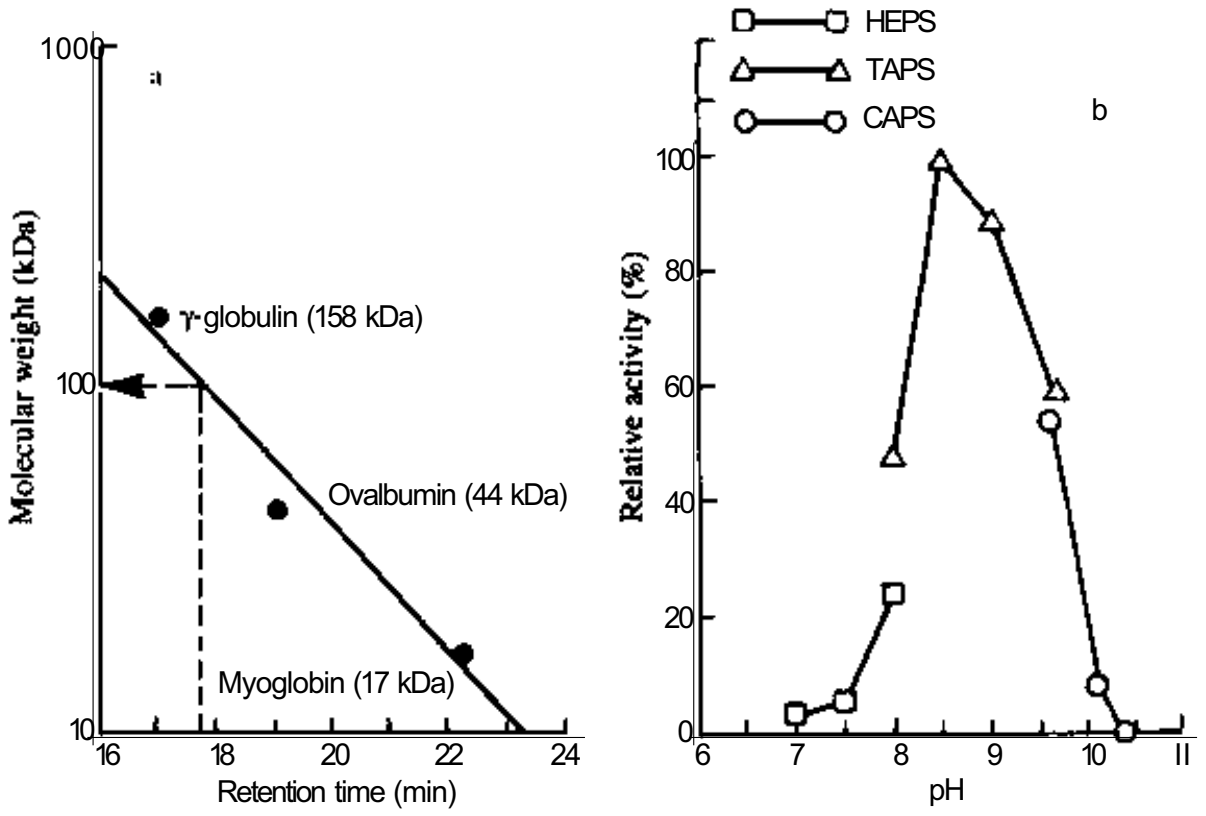


Figure 5. Molecular weight of NAAT (a) and its pH dependency (b).

NELYSFDTATS(X)W(X)L was obtained. The latter half of this entire sequence was the same as that determined by V8 protease digestion.

Amino acid homology of the deduced gene products of *Ids* series

Idsl. When *Idsl* was searched for on the DNA database, it was revealed as a plant-MT (metallothionein) (Figure 6). After the discovery of pea (*Pisum sativum*)-MT by Evance et al. (1990), the plant-MT was found in *Mimulus guttatus* (de Miranda et al. 1990), *Arabidopsis* (Takahashi et al. 1991), barley (Okumura et al. 1991, 1992, our data), maize (de Framond 1991), soybean (Kawashima et al. 1991), and, recently, in wheat (Snowden and Gardner 1993). When the genomic DNA of *Idsl* was cloned and sequenced, *Idsl* had a sequence of 5'-TGCACACC-3', which is similar to the animal Metal Responsive Elements (MREs; 5'-TGCRCNCX-3', R = A or G, X = C or G) (Palmiter 1987) between TATAA and translation starting point (ATG) (unpublished). The gene had one intron inserted in a similar position as described in maize (de Framond et al. 1991). Although the true function of plant-MT is still unclear, it seems that it has some relation to the metabolism of such minerals as copper (pea, *Mimulus guttatus),* zinc (*Arabidopsis,* soybean), Fe (barley), and aluminum (wheat). Since *Idsl* is expressed highly specifically in the roots of Fe-deficient barley, the 5'-flanking region of the gene will also have the 'root-specific cis element(s)' not identified so far in other plant species.

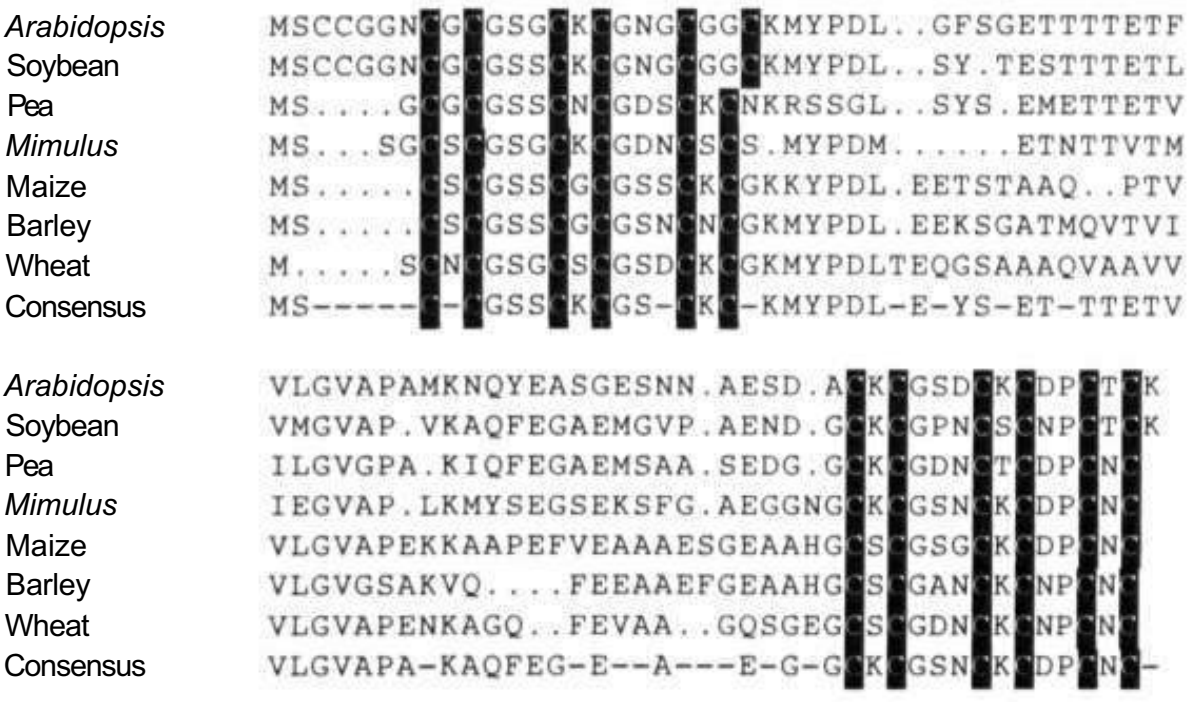


Figure 6. Homology of barley *(Idsl)* with other plant-metallothionein. The reference of each gene is written in the text.

Ids2. The deduced amino acid sequence of *Ids2* was highly homologous to the plant 2-oxoglutarate-dependent dioxygenases, which need 2-oxoglutarate, Fe^{2+}, and ascorbate as cofactors (Figure 7). The DNA of *Ids2* had three introns, and the total open reading frame (ORF) was 338 amino acids (Okumura et al., 1994). The 5'-flanking region had several specific elements: 5'-TGCACCC-3'(MREs), TCTTTT and AAAAGA [upstream activating sequences of CUP1 (yeast-MT) gene of *Saccharomyces cerevisiae]* (Butt et al. 1984), TGACG (root specific sequence ? of CaMV35S promoter; Lam et al. 1989) and CTCTTC (nodule specific sequence

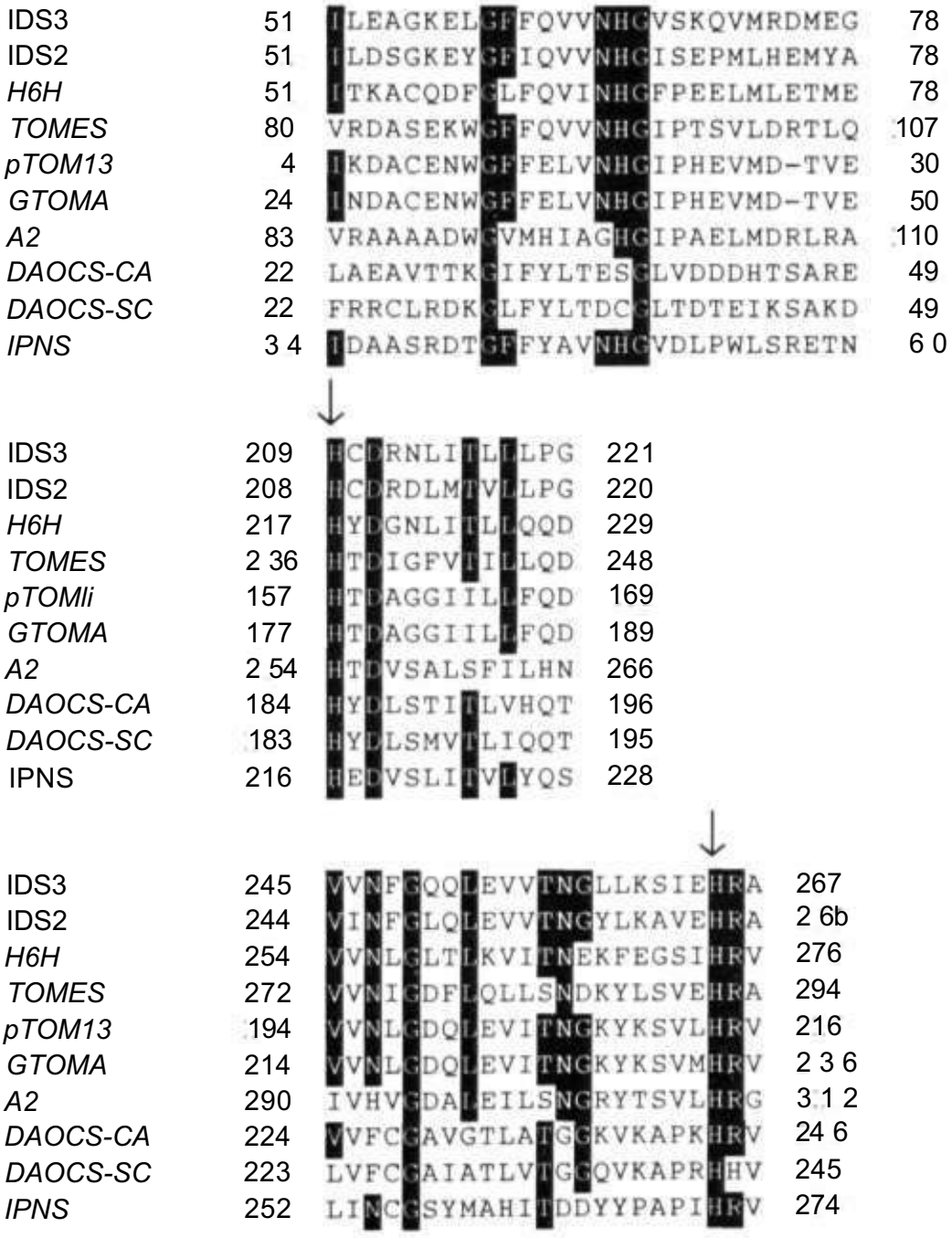


Figure 7. Homology of *Ids2* and *Ids3* with other plant dioxygenases. References of other genes are listed in Nakanishi et al. (1993).

motif in the regulatory region of leghemoglobin genes (Bogsz et al. 1990, Sandal et al. 1987). Northern hybridization studies showed that *Ids1* was specifically expressed under the conditions of Fe deficiency in the roots of barley, just like *Ids1.* However, the substrate of this dioxygenase is still unknown.

Ids3. The deduced amino acid sequence of *Ids3* was also highly homologous to plant dioxygenases (Figure 5) and had 50% homology with that of *Ids1.* The genomic DNA of *Ids3* had three introns in the same positions as *Ids2* (or H6H; see Figure 7) and the total ORF was 339 amino acids. *Ids3* was expressed specifically in the roots tips of Fe-deficient barley. The expression was more strictly regulated by Fe than in the case of *Ids2* or *Ids1* [Okumura et al. 1992 and 1994; Nakanishi et al. 1993]. When an antisense-RNA labeled with digoxigenin-antibody-gold was used to look for the site of the expression of *Ids3* in the root of Fe-deficient barley, the luminescence was detected in the stele (Sakaguchi et al. 1993). This suggests that the gene has organ(stele)-specific regulatory element(s) in the promoter region, as in the case of hyoscyamine-6p-hydroxylase gene, which was also reported as a dioxygenase (see H6H in Figure 7) expressed in the pericycle of the roots of *Hyoscyamus niger* (Hashimoto et al. 1991). By using the Fe-deficient roots of 'wheat (Chinese Spring)-barley (Betzes) chromosome addition lines (Add-1, Add-2, Add-3, Add-4, Add-6 and Add-7)', Northern hybridization was carried out and the results showed that *Ids3* was expressed in the roots of Betzes barley and in Add-4 as well, while Chinese Spring wheat and the other addition series were negative in its expression. So far, only Add-4 has been speculated to have the gene of MA synthase (Mori and Nishizawa 1989), which hydroxylates DMA to MA (Figure 1). Therefore, *Ids3* is a possible candidate for the MA synthase gene (Nakanishi et al. unpublished data).

Both NAS and NAAT have been only partially purified and are not yet pure enough to determine partial amino acid sequences for the synthesis of DNA probes for cloning those enzymatic genes. The limiting problem has been the difficulty in collecting sufficient amounts of material from root tips of barley. But there is no other effective way to access these genes. Even though we may succeed in isolating and determining the amino acid sequences of some unknown genes specifically expressed in the roots of Fe-deficient barley, we must know at least the partial amino acid sequences of NAS or NAAT if we want to identify the unknown gene products as either NAS or NAAT. Therefore, the purification of NAS or NAAT up to partial amino acids sequencing has a high priority.

According to the protein homology search in the NBRF-PIR database and in the EMBL-SWISS-PROTT database (DNASIS; Hitachi Software, Japan), no homologous protein was found with partial amino acid sequences of 'd' spot. Though the function of the peptide is not yet known, it is considered to have an important role in Fe-deficiency responses since it is strongly, reproducibly, and specifically

induced by Fe-deficiency. Hence, this peptide was not observed in the roots of other nutrient stresses (Suzuki et al., in press). This will be the first peptide whose partial amino acid has been sequenced under 'nutrient deficiency' stress. Gene cloning of this peptide is now under way.

We have sequenced the complete genomic DNA sequences of *Idsl, Ids2,* and *Ids3* among the seven clones. It is highly probable that *Ids3* is the gene of MA synthase. To determine the function of *Ids3* protein, *Ids3* gene was introduced and synthesized in *Escherichia coli,* purified, and the enzyme activity was assayed using Fe^{2+} and ascorbate as cofactors under 2-oxoglutarate dependent conditions. However, this study did not provide successful results. On the other hand, we speculate that *Ids2* may be the gene of synthase of epiHMA from MA, because *Ids2* has high homology with *Jds3;* also, it seems to be a dioxygenase gene, one of the hydroxylases like MA synthase. A cDNA of *Ids4* was also determined (DDBJ/EMBL/Genbank DNA database accession number D 14161; unpublished), but it had no homology with any genes. The residual three clones are now being sequenced. We hope to confirm the existence of NAS or NAAT amongst *Ids4, Ids5, Ids6,* and *Ids7* clones.

References

Bogsz, D., Llewellyn, D.J., Crag, S., Dennis, E.S., Appleby, C.A., and Peacock, W.J. 1990. Nonlegume haemoglobin genes, retain organ-specific expression in heterologous transgenic plants. The Plant Cell 2: 633-641.

Butt, T.R., Steinberg, E.J., Gorman, J.A., Clark, P., Hamer, D., Rosenberg, M., and Crooke, S.T. 1984. Copper metallothionein of yeast, structure of the gene, and regulation of expression. Proceedings of National Academy of Sciences (USA) 81: 3332-3336.

Cleveland, D.W., Fisher, S.G., Kirschner, M.W., and Laemmli, U.K. 1977. Peptide mapping by limited proteolusis in sodium dodesyl sulfate and analysis by gel electrophoresis. Journal of Biological Chemistry 252: 1102-1106.

de Framond, A J. 1991. Metallothionein-like gene from maize *(Zea mays).* F E B S Letters 290: 103-106.

de Miranda, J.R., Thomas, M.A., Thurman, D.A., and Tomset, A.B. 1990. Metallothionein genes from the flowering plant *Mimulus guttatus.* F E B S Letters 260: 227-280.

Evance, I.M., Gatehouse, L.N., Gatehouse, J.A., Robinson, N.J., and Croy, R . R . D . 1990. A gene from pea *(Pisum sativum* L.) with homology to metallothionein genes. F E B S Letters 262: 29-32.

Gasser, C.S., Budelier, K.A., Smith, A.G., Shah D.M., and Fraley R.T. 1989. Isolation of tissue specific cDNAs from tomato pistils. The Plant Cell 1: 15-24.

Hashimoto, T., Hayashi, A., Amano, Y., Kohno, J., Iwanari, H, Usuda, S., and Yamada, Y. 1991. Hyoscyamine 6B-hydroxylase, and enzyme involved in tropane alkaloid

biosynthesis, is localized at the pericycle of the root. Journal of Biological Chemistry 226: 4648-4653.

Higuchi, K., Kanazawa, K., Nishizawa, N.K., Chino, M., and Mori, S. 1994. Purification and characterization of nicotinamine synthase from Fe deficient barley roots. Plant and Soil 165: 173-179.

Kawasaki, H., Emori, Y., and Suzuki, K. 1990. Production and separation of peptides from proteins stained with Coomassie Brilliant Blue R-250 after separation by SDS polyacrylamide gel electrophoresis. Analytical Biochemistry 191: 332-336.

Kawai, S., Takagi, S., and Sato, Y. 1988. Mugineic acid-family phytosiderophores in root-secretions of barley, corn and sorghum varieties. Journal of Plant Nutrition 11: 633-642.

Kawashima, I., Inokuchi, Y., Chino, M., Kimura, M., and Shimizu, N. 1991. Isolation of a gene for a metallothionein-like protein from soybean. Plant Cell Physiology 32: 913-916.

Lam, E., Benfay, P.N., Gilmartin, P.M., Fang, R.-X., and Chua N-H. 1989. Site specific mutations alter *in vitro* 24 factor binding and change promoter expression pattern in transgenic plants. Proceedings of the National Academy of Sciences (USA) 86: 7890-7894.

Logman, J., Schell, J., and Willmitzer, L. 1987. Improved method for the isolation of RNA from plant tissues. Analytical Biochemistry 163: 16-20.

Ma, J.F., and Nomoto, K. 1993. Two related biosynthetic pathways of mugineic acids in gramineous plants. Plant Physiology 102: 373-378.

Mori, S., and Nishizawa, N. 1987. Methionine as a dominant precursor of phytosiderophores in Gramineae plants. Plant Cell Physiology 28: 1081-1092.

Mori, S., and Nishizawa, N. 1989. Identification of barley chromosome no.4, possible encoder of genes of mugineic acid synthetase from 2'-deoxymugineic acid using wheat-barley addition line. Plant and Cell Physiology 30: 1057-1061.

Mori, S., Hachisuka, M., Kawai, S., Takagi, S., and Nishiwaza, N.K. 1988. Peptides related to phytosiderophores secretion by Fe-deficient barley roots. Journal of Plant Nutrition 11:653-662.

Mori, S., Nishizawa, N.K., and Fujigaki, J. 1990. Identification of rye chromosome 5R as a carrier of the genes for mugineic acid synthetase and hydroxymugineic acid synthetase using wheat-rye addition lines. Japanese Journal of Genetics 65: 343-352.

Mori, S., Nishizawa, N., Hayashi, H., Chino, M., Yoshimura, E., and Ishihara, J. 1991. Why are young rice plants highly susceptible to iron deficiency? Plant and Soil 130: 143-156.

Nakanishi, H., Okumura, N., Umehara, Y, Nishizawa, N.K., Chino, M., and Mori, S. 1993. Expression of a gene specific for iron deficiency *(Ids3)* in the roots of *Hordeum vulgare.* Plant and Cell Physiology 34: 401-410.

O'Farrel, P.H. 1975. High resolution two-dimensional electrophoresis of proteins. Journal of Biological Chemistry 250: 4007-4021.

Ohata, T., Kanazawa, K., Mihashi, S., Nishizawa, N.K., Fushiya, S., Nozoe, S., Chino, M., and Mori, S. 1993. Biosynthetic pathway of phytosiderophores in iron-deficient graminaceous plants; establishment of an assay system for the detection of nicotianamine aminotransferase activity. Soil Science and Plant Nutrition 39: 745-749.

Okumura, N., Nishizawa, N.K., Umehara Y., and Mori, S. 1991. An iron deficiency specific cDNA from barley roots having two homologous cysteine-rich MT domains. Plant Molecular Biology 17: 531-533.

Okumura, N., Nishizawa, N.K., Umehara, Y., Ohata, T., and Mori S. 1992. Iron deficiency specific cDNA *(Idsl)* with two homologous cysteine rich domains from the roots of barley. Journal of Plant Nutrition 15: 2157-2172.

Okumura, N., Nishizawa, N.K., Umehara Y., Ohata T., Nakanishi H., Yamaguchi T., Chino, M., and Mori, S. 1994. A dioxygenase gene *{Ids2)* expressed under iron deficiency conditions in the roots *of Hordeum vulgare.* Plant Molecular Biology 25: 705-719.

Palmiter, R.D. 1987. Molecular biology of metallothionein gene expression. Pages 63-83 *in* Metallothionein II (Kaji, J.H.R., and Kojima, Y., eds.). Basel, Switzerland: Birkhauser Verlag.

Rom held, V., and Marschner, H. 1986. Evidence for a specific uptake system for iron phytosiderophore in roots of grasses. Plant Physiology 80: 175-180.

Rom held, V., and Marschner, H. 1990. Genotypical differences among graminaceous species in release of phytosiderophores and uptake of iron phytosiderophores. Plant and Soil 123: 585-592.

Sakaguchi, T., Nishizawa, N.K., Okumura, H., Nakanishi, H., Umehara, M., Chino, M., and Mori, S. 1993. Localization *of Ids1, Ids2* and *Ids3-mRNA* in barley roots revealed by *in situ* hybridization. Page 87 *in* Abstracts of the Papers of the 7th International Symposium on Iron Nutrition and Interactions in Plants, Zaragoza, Spain.

Sandal, N.N., Bojsen, K., and Marker, K.A. 1987. A small family of nodule-specific genes from soybean. Nucleic Acids Research 15: 1507-1519.

Shojima, S., Nishizawa, N.K., Fushiya, S., Nozoe, S., Kumashiro, T., Nagata T., Ohata, T., and Mori, S. 1989. Biosynthesis of nicotianamine in the suspension-cultured cells of tobacco (*Nicotiana megalosiphon).* Biological Metals 2: 142-145.

Shojima, S., Nishizawa, N.K., Fushiya, S., Iriftine T., and Mori, S. 1990. Biosynthesis of phytosiderophores. *In vitro* biosynthesis of 2'-deoxymugineic acid from L-methionine and nicotianamine. Plant Physiology 93: 1497-1503.

Singh, K., Chino, M., Nishizawa, N.K., Ohata, T., and Mori, S. 1993. Genotypic variation among Indian graminaceous species with respect to phytosiderophore secretion. Pages 335-339 *in* Genetic aspects of plant mineral nutrition (Randall, R.J., Delhaize, E., Richards, R.A., and Munns, R., eds.). Dordrecht, The Netherlands: Kluwer Academic.

Snowden, K.C., and Gardner, R.C. 1993. Five genes induced by aluminum in wheat *(Triticum aestivum* L.) roots. Plant Physiology 103: 855-861.

Suzuki, K., Hirano, H., Yamaguchi, H., Iriftine, T., Nishizawa, N.K., Chino, M., and Mori, S. 1994. Partial amino acid sequences of a peptide induced by Fe deficiency in barley roots. Plant and Soil (in press).

Takagi, S. 1993. Production of phytosiderophores. Pages 113-131 *in* Iron chelation in plants and soil microorganisms (Barton, L., and Hemming, H., eds.). New York, USA: Academic Press.

Takahashi, K., Kawashima, I., and Chino, M. 1991. Cloning of MT-like gene in *Arabidopsis.* Page 263 *in* Proceedings of the Annual Meeting and 31st Symposium, The Japanese Society of Plant Physiologists, Okayama, Japan.

Genetic control and manipulation of root exudates

E Delhalze[1]

Abstract

Organic acids and acid-phosphatases exuded by roots have been implicated in the phosphorus (P) nutrition of plants. The mechanism used by plants to excrete organic acids is not known but may involve anion channels. These putative anion-channels are a potential site for genetic manipulation to enhance the ability of plants to acquire P. Acid-phosphatases are enzymes that cleave off esterified phosphate from organic compounds and represent a different class of excreted substances involved in P nutrition. In this review, the mechanisms of excretion and potential for genetic manipulation of organic acids and acid-phosphatases are discussed.

Introduction

Plants exude a wide range of compounds that include amino acids, sugars, organic acids, polysaccharides, and proteins. These compounds may influence the mineral nutrition of a plant directly by solubilizing or chelating nutrients or indirectly by influencing microbial growth in the rhizosphere. There are several factors to consider when deciding whether specific exudates from roots can be manipulated by genetic means to improve efficiency of nutrient use. These factors include (1) the biosynthetic pathway used to synthesize the compound, (2) the mechanism used to transport the compound across the plasma membrane to the external solution, and (3) regulation of synthesis or transport (or both) of the compound by the nutrient status of the plant. In this review, I consider these in relation to two types of root exudates that are likely to be directly involved in the phosphorus (P) nutrition of plants: organic acids and acid-phosphatases.

1. CSIRO Division of Plant Industry, G P 0 Box 1600, Canberra. ACT 2601, Australia.

Delhaize, E. 1995. Genetic control and manipulation of root exudates. Pages 145-152 *in* Genetic manipulation of crop plants to enhance integrated nutrient management in cropping systems—1. Phosphorus: proceedings of an FAO/ICRISAT Expert Consultancy Workshop, 15-18 Mar 1994, ICRISAT Asia Center. India (Johansen, C, Lee. K.K., Sharma, K.K., Subbarao, G.V., and Kueneman, E.A.. eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Organic acids

Citric and malic acids excreted by roots have been implicated in acquisition of P from some soils by aiding the solubilization of phosphate-containing compounds (Hoffland 1992). All plants are capable of synthesizing organic acids of the tricarboxylic acid cycle but only a limited number of species have been reported to excrete organic acids as a means of increasing P availability (Dinkelaker et al. 1989, Gardner et al. 1983, Grierson 1992, Hoffland et al. 1992, Lipton et al. 1987). To date, the mechanisms used by roots to excrete organic acids are not known. For both malate and citrate, there is a large concentration gradient from within root cells to the external medium and coupled to this is a negative membrane potential. At the pH of the cytoplasm (approximately 7), most of the malate will exist as divalent anions while most of the citrate will exist as trivalent anions (organic-acid anions). Therefore, one way that these organic-acid anions could be released from root cells is through anion channels, which would allow the passive movement of these anions from the cytoplasm to the apoplasm. Channels are proteins that span membranes and facilitate the transport of compounds down their electrochemical gradient. To maintain charge balance in the cell, either cations must also be transported across the membrane or other anions must be taken up from the external solution. In the case of excretion of organic-acid anions induced by P deficiency, it is likely that protons are also excreted since the rhizosphere is acidified (Dinkelaker et al. 1989, Hoffland et al. 1992). Protons are probably transported by an H^+-ATPase of the plasma membrane as a consequence of the cytoplasm becoming acidified with the loss of organic-acid anions.

Organic acid excretion in some plant species is stimulated by P deficiency (Gardner et al. 1983, Hoffland 1992, Lipton et al. 1987). The molecular events leading from the sensing of P deficiency by the plant to efflux of malate or citrate from roots are not known but may involve synthesis of specific anion channels or the opening of pre-existing channels. In wheat, Al^{3+} stimulates the efflux of malate from root tips (Delhaize et al. 1993) and recent work indicates that Al^{3+} opens a malate-permeable channel present in the plasma membrane of root cells, allowing malate to flow down its electrochemical gradient (Ryan, PR, et al., CSIRO, personal communication). The Al-stimulated efflux of malate was found to be a direct effect of Al and not due to Al-induced P deficiency.

Several malate-permeable channels of plants have been described including channels in the tonoplast of leaf cells (Iwasaka et al. 1992). To date, a malate-permeable channel in root plasma membranes has not been identified. Ouyang et al. (1991) suggested that nodulin-26, a protein of the peribacteroid membrane of soybean nodules, is a malate transporter. The peribacteroid membrane is derived from the plant plasma membrane and encapsulates the bacterial nodule. The gene for nodulin-26 has been cloned (Miao et al. 1992) and is encoded by the plant but

its transcription is triggered by the invading *Rhizobium.* There is indirect evidence to suggest that nodulin-26 transports organic-acid anions across the peribacteroid membrane: phosphorylation of nodulin-26 coincides with malate transport across the peribacteroid membrane and transport ceases when the protein is de-phosphorylated (Ouyang et al. 1991). In addition, from the sequence of nodulin-26, it is clear that the gene encodes a membrane protein with features of a channel (Miao et al. 1992).

Under P deficiency, some lupin species produce specialized roots (proteoid roots) that excrete large amounts of citric acid (Gardner et al. 1983). The processes leading to production of these roots are not known but probably involve low internal phosphate concentrations triggering a developmental pathway in certain root cells. Little is known of the biochemistry of proteoid roots but they are likely to possess a very active biosynthetic pathway for organic acids as well as abundant transporters for citrate. This contrasts with rape, where roots excrete organic acids from pre-existing roots when P-deficient (Hoffland et al. 1992). While all plants are able to synthesize organic acids, species that excrete large quantities might possess an enhanced biosynthetic pathway for organic acids.

Phosphoenolpyruvate carboxylase has been suggested as a key enzyme in plant cells that accumulate large quantities of organic acids (Latzko and Kelly 1979). Phosphoenolpyruvate carboxylase catalyzes the formation of oxaloacetate from phosphoenolpyruvate and HCO_3^-. Oxaloacetate is then converted to malate by malate dehydrogenase. The activity of this enzyme might be enhanced in tissues that excrete organic acids for extended periods. Genes for phosphoenolpyruvate carboxylases have been cloned from many species and from a variety of plant organs (Toh et al. 1994).

Phosphatases

Acid phosphatases are enzymes that cleave esterified-phosphate groups off a variety of organic substrates. In many plant species, the activity of these enzymes in various plant parts is increased with the onset of P deficiency (Bieleski 1973) and in some species, acid phosphatases are excreted (Duff et al. 1991b, Goldstein et al. 1988, Ueki and Sato 1971), making a discussion of these enzymes relevant to this review.

Excreted acid phosphatases may enhance the availability of P from organic compounds in the rhizosphere. Phosphate is a potent inhibitor of an acid phosphatase excreted by *Brassica nigra* cells in culture, which suggests that the enzyme functions under P-starved conditions but is inactivated when phosphate becomes abundant (Duff et al. 1991a). Different isoenzymes of excreted acid-phosphatases may have different substrate specificities and under some conditions where a particular organic-P compound predominates in the soil, a specific acid-phosphatase may be advantageous to the plant. In many soils, the major organic-P

fraction is inositol hexaphosphate, which is also known as phytate (Dalal 1977). This organic-P fraction is potentially a valuable source of P for plants but it is uncertain whether plants are able to access it directly through their acid-phosphatases (Richardson 1994). Although the acid-phosphatases excreted by plants generally have a broad substrate specificity, they show poor activity towards inositol hexaphosphate and it is generally assumed that the poor solubility of inositol hexaphosphate in soil may also limit its potential availability. A recent report showed that clover *(Trifolium subterraneum)* was unable to use inositol hexaphosphate as a P source even though it excreted acid-phosphatases (Barret-Lennard et al. 1993). Although plants have phytases (phosphatases specific for inositol hexaphosphate), these are restricted to seeds (Gabard and Jones 1986). By contrast, microorganisms excrete phytases that are specific for inositol hexaphosphate (Shimizu 1992, van Hartingsveldt et al. 1993) and plants probably access the P from inositol hexaphosphate indirectly through soil microorganisms.

Several acid phosphatases excreted from plants have been purified to homogeneity (Duff et al. 1991a, Goldstein et al. 1988, LeBanksky et al. 1992). The increase in amount of acid-phosphatase excreted by *Brassica nigra* cell cultures under P deficiency was shown to be due to enhanced synthesis of the protein (Duff et al. 1991b). This result implies that transcription of the gene encoding the excreted acid-phosphatase is controlled by the P status of the plant. As noted above for organic acid excretion, the molecular events that allow a plant to sense that it is P deficient and to activate specific genes are not known. This contrasts with the situation in certain microorganisms where the molecular events leading from perception of P-deficiency to activation of specific genes have been well studied. More than 20 genes have been identified in *Escherichia coli* to be involved in regulating the uptake of phosphate from the external medium (Torriani-Gorini et al. 1987).

Proteins that are secreted out of plant cells follow a 'default pathway', which starts with the protein being sequestered into the endoplasmic reticulum or incorporated into endoplasmic reticulum membranes (Chrispeels 1991). Unlike specific transporters that are required for such small molecules as organic acids, the machinery for secreting proteins is common to all plants.

Prospects for genetically manipulating plant excretions

I limit this discussion to the possibility of manipulating excretions by genetically engineering plants. This assumes that the methods for transferring genes to specific plant species have been developed. Traditional plant breeding will clearly be useful for transferring genes within a species but will be limited when the variation of a particular attribute within that species is insufficient. One needs to identify the genes that must be transferred to the recipient plant for it to acquire the desired

phenotype. The simplest case would be where the desired character can be obtained by transferring a single gene. The procedure becomes more difficult where several genes need to be transferred as in the case of a biosynthetic pathway and associated transport genes, which together confer the desired phenotype. The isolation of the desired genes can also be a major obstacle if the genes have not already been cloned. The following discussion includes considerable speculation because, as noted above, the molecular basis of many of the processes involved is not fully understood.

Manipulation of organic acid excretion

If, under P deficiency, the efflux of organic acids from roots is controlled by channel activity at the membrane, then this could be a site to exploit. The rate of efflux could be controlled by the number of channels present in the membrane or by the time they remain open. A species that does not excrete organic acid or excretes low quantities of organic acid under P deficiency could be genetically modified to express an organic-acid anion channel in the appropriate region of the root. Efflux of organic-anions will probably result in co-incident efflux of protons by plasma membrane ATPases for the cell to maintain its internal pH and charge balance. Organic acids within the plant would need to be synthesized as they are depleted but this is likely to occur without the need to introduce additional genes. All plants synthesize organic acids and their synthesis within cells is regulated by internal concentrations.

To date, the gene for a channel involved in organic-acid anion transport across the root plasma membrane has not been cloned. The best candidate is the nodulin-26 gene discussed above, but while it has the necessary attributes of a channel, it has not yet been shown to transport organic-acid anions. At CSIRO, we are interested in cloning a gene coding for a malate-permeable channel from root tips of wheat. We believe that the activity of such a channel controls the rate of malate efflux across the root membrane and that Al tolerance is associated with this efflux. Although this putative channel appears to be specifically stimulated by Al, isolation of the gene could be relevant for P acquisition and similar genes may be involved in the P deficiency response of some species.

It is not only necessary that a gene be transferred to a new host but it must also be expressed in the recipient plant under the appropriate conditions and in the correct tissues. In the case of an organic-acid anion channel, it would be desirable that organic acid efflux occur only when the plant becomes P-deficient and that the gene be expressed specifically in roots. This requires that a suitable promoter be used such as one derived from a P responsive acid-phosphatase from roots (see below).

Manipulation of excretion of acid-phosphatases

Plants could be engineered to excrete high levels of acid-phosphatase to allow them to readily use organic-P substrates. The acid-phosphatase gene could be an endogenous gene that is expressed at a greater level than normal. Alternatively, the gene could be derived from a different plant or even from a different organism. For example, a phytase gene from *Aspergillus niger* has been engineered for expression in tobacco *(Nicotiana tabacum)* seeds to a level of about 1% of soluble protein, a concentration far in excess of the endogenous phytase (Pen et al. 1993). The phytase acts on inositol hexaphosphates present in the seed and releases the phosphate, which becomes available for enhancing the P nutrition of animals. Using a similar approach, a phytase gene could be expressed in roots and, with the appropriate signal sequence, be excreted. Recent work at Plant Industry CSIRO (A E Richardson and P A Hadobas, CSIRO, personal communication) has involved screening soil bacterial isolates for their ability to grow on inositol hexaphosphate as the sole source of C and P. From several hundred isolates obtained initially, a few isolates capable of liberating large amounts of inorganic phosphate from inositol hexaphosphate were identified. If a suitable phytase gene is cloned from these bacterial isolates, then it opens up the possibility of introducing the gene into plants.

In addition to understanding their role in P nutrition, the acquisition of acid-phosphatase genes from plants may also prove useful in providing suitable promoters for engineering other genes. As noted for the organic-acid anion channels, it is desirable that the introduced genes be activated specifically under P deficiency conditions and be expressed in roots. To date, cloning of a P-regulated acid phosphatase gene from plant roots has not been reported although, as indicated above, several acid phosphatases have been purified to homogeneity.

Concluding remarks

The two examples discussed of substances excreted by roots under P deficiency serve to illustrate some of the factors that need to be considered when attempting to manipulate exudates by genetic means. Our understanding of the molecular basis of P nutrition in plants is still in its infancy compared to the depth at which it is understood in some microorganisms. Understanding the molecular basis of P nutrition in plants will aid attempts at genetically engineering plants for increased P efficiency either by manipulation of exudates or by other means. In this respect, the small plant *Arabidopsis thaliana* can make a major contribution as a model system (Meyerowitz 1987). Analysis of *Arabidopsis* mutants with respect to P nutrition can provide information on gene function as well as being the starting point for gene isolation (Dean 1993). The search for genes that may be useful in improving the P nutrition of plants need not be restricted to plant genes; genes derived from

other organisms should also be assessed for their ability to improve the P nutrition of plants.

References

Barret-Lennard, E.G., Dracup, M., and Greenway, H. 1993. Role of extracellular phosphatases in the phosphorus-nutrition of clover. Journal of Experimental Botany 44: 1595-1600.

Bieleski, R.L. 1973. Phosphate pools, phosphate transport, and phosphate availability. Annual Review of Plant Physiology 24: 225-252.

Chrispeels, M.J. 1991. Sorting of proteins in the secretory system. Annual Review of Plant Physiology and Plant Molecular Biology 42: 21-53.

Dalai, R.C. 1977. Soil organic phosphorus. Advances in Agronomy 29: 85-117.

Dean, C. 1993. Advantages of *Arabidopsis* for cloning plant genes. Philosophical Transactions of the Royal Society of London. Series B 342: 189-195.

Delhaize, E., Randall, P.J., and Ryan, P.R. 1993. Aluminum tolerance in wheat *(Triticum aestivum* L.) II. Aluminum-stimulated excretion of malic acid from root apices. Plant Physiology 103: 695-702.

Dinkelaker, B., Rom held, V., and Marschner, H. 1989. Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin *(Lupinus albus* L.). Plant, Cell and Environment 12: 285-292.

Duff, S.M.G., Lefebvre, D.D., and Plaxton, W.C. 1991a. Purification, characterization, and subcellular localization of an acid phosphatase from black mustard cell-suspension cultures: comparison with phosphoenolpyruvate phosphatase. Archives of Biochemistry and Biophysics 286: 226-232.

Duff, S.M.G., Plaxton, W.C, and Lefebvre, D.D. 1991b. Phosphate-starvation response in plant cells: *De novo* synthesis and degradation of acid phosphatases. Proceedings of the National Academy of Sciences, USA 88: 9538-9542.

Gabard, K.A., and Jones, R.L. 1986. Localization of phytase and acid phosphatase isoenzymes in aleurone layers of barley. Physiologia Plantarum 67: 182-192.

Gardner, W.K., Barber, D.A., and Parbery, D.G. 1983. The acquisition of phosphorus by *Lupinus albus* L. III. The probable mechanism by which phosphorus movement in the soil/ root interface is enhanced. Plant and Soil 70: 107-124.

Goldstein, A.H., Danon, A., Baertlein, D.A., and McDaniel, R.G. 1988. Phosphate starvation inducible metabolism in *Lycopersicon esculentum* II. Characterization of the phosphate starvation inducible-excreted acid phosphatase. Plant Physiology 87: 716-720.

Grierson, P.F. 1992. Organic acids in the rhizosphere of *Banksia integrifolia* L.f. Plant and Soil 144: 259-265.

Hoffland, E. 1992. Quantitative evaluation of the role of organic acid exudation in the mobilization of rock phosphate by rape. Plant and Soil 140 : 279-289.

Hoffland, E., Van Den Boogaard, R., Nelemans, J., and Findenegg, G. 1992. Biosynthesis and root exudation of citric and malic acids in phosphate-starved rape plants. New Phytologist 122: 675-680.

Iwasaka, I., Arata, H., Kijima, H., and Nishimura, M. 1992. Two types of channels involved in the malate ion transport across the tonoplast of a crassulacean acid metabolism plant. Plant Physiology 98: 1494-1497.

Latzko, E., and Kelly, G.J. 1979. The many-faced function of phosphoenol-pyruvate carboxylase in C3 plants. Physiologie Vegetale 21: 805-815.

LeBanksky, B.R., McKnight, T.D., and Griffing, L.R. 1992. Purification and characterization of a secreted purple phosphatase from soybean suspension cultures. Plant Physiology 99: 391-395.

Lipton, D.S., Blanchar, R.W., and Blevins, D.G. 1987. Citrate, malate, and succinate concentration in exudates from P-sufficient and P-stressed *Medicago sativa* L. seedlings. Plant Physiology 85: 315-317.

Meyerowitz, E.M. 1987. *Arabidopsis thaliana.* Annual Review of Genetics 21: 93-111.

Miao, G.-H., Hong, Z., and Verma, D.P.S. 1992. Topology and phosphorylation of soybean nodulin-26, an intrinsic protein of the peribacteroid membrane. Journal of Cell Biology 118: 481-490.

Ouyang, L.-I., Whelan, J., Weaver, CD., Roberts, D.M., and Day, D.A. 1991. Protein phosphorylation stimulates the rate of malate uptake across the peribacteroid membrane of soybean nodules. F E B S Letters 293: 188-190.

Pen, J., Verwoerd, T.C., van Paridon, P.A., Beudeker, R.F., van den Elzen, P.J.M., Geerse, K., van der Klis, J.D., Versteegh, H.A J., van Ooyen, A.J.J., and Hoekema, A. 1993. Phytase-containing transgenic seeds as a novel feed additive for improved phosphorus utilization. Bio/Technology 11: 811-814.

Richardson, A.E. 1994. Soil microorganisms and phosphorus availability. Pages 50-62 *in* Soil biota—management in sustainable farming systems (Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R., and Grace, PR., eds.). Melbourne, Australia: CSIRO Editorial and Publishing Unit.

Shimizu, M. 1992. Purification and characterization of phytase from *Bacillus subtilis {natto)* N-77. Bioscience, Biotechnology, and Biochemistry 56: 1266-1269.

Toh, H., Kawamura, T., and Izui, K. 1994. Molecular evolution of phosphoenol pyruvate carboxylase. Plant Cell and Environment 17: 31-43.

Torriani-Gorini, A., Rothman, F.G., Silver, S., Wright, A., and Yagil, E. (eds.). 1987. Phosphate metabolism and cellular regulation in microorganisms. Washington, D.C., USA: American Society for Microbiology.

Ueki, K., and Sato, S. 1971. Effect of inorganic phosphate on the extracellular acid phosphatase activity of tobacco cells cultured *in vitro.* Physiologia Plantarum 24: 506-511.

van Hartingsveldt, W., Zeijl, C.M.J., Harteveld, G.M., Gouka, R.J., Suykerbuyk, M.E.G., Luiten, R.G.M., van Paridon, P.A., Selten, G.C.M., Veenstra, A.E., van Gorcom, R.F.M., and van den Hondel, C.A.M.J.J. 1993. Cloning, characterization and overexpression of the phytase-encoding gene *(phyA)* of *Aspergillus niger.* Gene 127: 87-94.

Preliminary draft proposal for a cooperative research program

Introduction

Phosphorus (P) is the next most limiting nutrient after nitrogen (N) for crop production in tropics. The World Phosphate Institute classified 65% of 500 soil samples collected from 42 countries in the tropics as acutely deficient in P and 27% as moderately deficient; only 8% of the samples were classified as not deficient (Koala et al. 1988). For example, in Peru, 70% of soils in the coastal region are low to medium in available P, and 80 to 90% of those in the 'Sierra' region are deficient. In Bolivia, 50% of the soils in the Altiplano region are low-P and 50% are medium-P. Similarly, in Montana and Santa Cruz areas, 60 to 80% of the soils are deficient in P. In parts of Africa, P deficiency is so severe that crop seedlings die as soon as they have exhausted the seed P reserves. Based on extensive surveys in India, it was concluded that 45.7% of the soils were low in available P, 49.7% were medium, and only 4.6% were considered as high in P (Tandon 1987). Application of processed mineral fertilizers or amendments cannot be relied upon to reasonably redress such nutrient deficiencies, for economic, logistic, and many other reasons.

Sustainability of agricultural production systems has become a major issue of global concern during this decade as most of the modern technology that is applied for improved crop production in developed countries is based on intensive use of chemical inputs. This can have an adverse impact on the environment. Thus, crop production practices that minimize the use of fertilizers while maintaining and/or increasing yields are likely to enhance sustainability. These would result in minimal pollution of water resources besides reducing the demand on non-renewable natural resources. It would thus be desirable for crop plants to access a greater proportion of the total soil nutrient pool than is otherwise available to them. For example, total soil P is often 100-fold more than the fraction of soil P normally available for uptake by crop plants (Al-Abbas and Barber 1964).

However, it is recognized that more effective mining of total soil nutrient reserves is only a medium-term solution and that long-term sustainability will require some compensation of nutrients removed from the system in agricultural produce or through other losses. In many tropical and subtropical areas, there are options to use less-processed fertilizers and amendments. An example is the use of unprocessed rock phosphate to supply P, which can be particularly effective in acid soils (Khasawneh and Doll 1978). However, it is desirable to identify plant species or genotypes that can best solubilize nutrients from sparingly soluble fertilizer

sources, and thus introduce available forms of nutrients into the nutrient cycle of the cropping systems at an earlier stage. Much evidence has accumulated that crop species differ in their ability to utilize rock phosphate (e.g., Khasawneh and Doll 1978, Flach et al. 1987, Hoffland et al. 1992).

There are three broad categories by which plants can increase their access to native or applied soil nutrients: (1) by increasing absorptive area, (2) by favorably modifying the absorption mechanisms to increase uptake from low ambient concentrations, and (3) by rhizosphere modification to increase nutrient availability. Options in the first category include increases in root surface area, either through root proliferation and/or root hair development, or through mycorrhizal associations, which allow capture of nutrients beyond the rhizosphere. With regard to the second category, plant species and genotypes have been shown to differ in their abilities to extract nutrients from low ambient concentrations, i.e., their threshold levels (e.g., Barber 1979). Thirdly, many possible mechanisms have been recorded by which root exudates, ranging from protons to complex organic molecules, influence nutrient availability and uptake either directly or through microbial inhabitants of the rhizosphere. A recently reported example in this category is the exudation of piscidic acid by pigeonpea roots, which facilitates release of P from normally plant-unavailable iron-bound soil P (Fe-P) (Ae et al. 1991).

Further, there have been recent advances in the understanding of genetic control of these various nutrient acquisition mechanisms (e.g., Bassam et al. 1990, Randall et al. 1993, Barrow 1993). This has opened up hitherto unrealized possibilities for favorable genetic manipulation of these mechanisms, either through standard plant breeding procedures or through new opportunities unfolding in the field of molecular biology. The latter suggests possibilities for identifying and transferring genes controlling nutrient ion uptake mechanisms and root exudation processes between genotypes and even species. Thus, opportunities have now arisen for applying advances in molecular biology to tackle problems of integrated nutrient management of agricultural production systems.

There are many possible candidate crops, nutrients, uptake mechanisms, and root exudates to consider. However, from the genetic improvement perspective, it is considered that root exudates that could mobilize such sparingly soluble soil P forms as Al-P, Fe-P, and Ca-P, and also permit better use of rock phosphates as P fertilizers, would offer greater scope for success in genetic manipulation. It is envisioned that, during the course of this proposed project, root exudates of a number of legumes would be evaluated for their ability to mobilize P from Fe-P, Al-P, and rock phosphates. The root exudate effects in mobilizing Fe-P and Al-P would be quantified at field level, so that the potential effects on the subsequent crop and the benefits to the cropping

systems as a whole could be envisioned. Efforts would be directed towards understanding the metabolic pathways of such specific root exudates as piscidic acid, malic acid, and citric acid with a view to developing means for transferring genes controlling their production and exudation to other crop species that lack this ability.

Most of the research attempted so far in this area has been component research done by separate laboratories with limited immediate research aims, even if a longer-term vision of practical contribution to integrated nutrient management (INM) was a guiding force. In view of the increasing problems related to INM, in cropping systems virtually throughout the world, and of this plethora of prospects for favorable genetic manipulation of nutrient acquisition mechanisms, time now seems opportune for a concerted, time-bound effort to test the hypothesis that genetic manipulation can significantly contribute to INM. Thus, a consortium approach involving a number of laboratories and institutes with expertise in various components of this research theme is proposed.

[References cited in this section are listed in the background paper (Johansen et al.) of these proceeding.]

C. Development Objective[1]

To enhance sustainable cropping systems in developing countries through improvement of acquisition and utilization of P from sparingly available sources. Major emphasis will be placed on improvement of grain legume/cereal cropping systems in soils with low P availability.

D. Immediate objective(s), outputs and activities

I. Roots component

1. Immediate Objective One (Root morphology). To determine the site of genetic control and to assess the extent to which root morphological characteristics are associated (determination of root-type function) with efficiency of P uptake in crop plants, using common bean *(Phaseolus vulgaris),* cowpea *(Vigna unguiculata),* and soybean *(Glycine max)* as examples.

1.1 Output One. A determination of which *Phaseolus* root morphology classes, if any, are related to P-uptake efficiency (dry matter per unit P applied) and the plasticity of this root morphology to changes in external P status.

1. Headings and numbering follow the format for UNDP project proposals.

Activity 1.1.1. A representative core sample (360 accessions) of the bean germplasm collection from the Centra Internacional de Agriculture Tropical (CIAT) will be assessed for variability in root morphology. Root imaging and cluster analysis techniques, done by CIAT and Pennsylvania State University teams, will be used to identify root morphology classes during Year 1 of the project (methods and classification scheme to be described). D Beck, CIAT; J Lynch, Pennsylvania State University[2]

Activity 1.1.2. Representatives of root types (20 genotypes total) will be grown under greenhouse conditions in an alumina-sand culture system at buffered levels of P at 0.5, 5, and 50 micromolar in solution. Plus and minus mycorrhizae treatments will be included. Measurements will be taken on root and shoot dry matter and root morphology classes, mycorrhizal infection, and P content of shoots and roots. D Beck, CIAT; J Lynch, Pennsylvania State University.

Activity 1.1.3. Field trials will be conducted in a highly P-fixing soil at three soil P levels with applied N (to eliminate N fixation /P interactions) using the 20 genotypes from Activity 1.1.2. Root systems will be excavated at 20 and 35 days after planting (DAP) for morphology evaluation, with concurrent analysis of root and shoot dry matter, P content, and VAM infection. Yield, biomass, and P content will be evaluated at harvest. D Beck, CIAT.

Activity 1.1.4. To verify the relationships identified above, reciprocal grafting experiments will be conducted. These experiments will provide information on interactions between the shoot genome and root genome in controlling root type. Experiments will be conducted in sand-alumina culture system at three P levels (0.5, 5, and 5 micromolar P) using four divergent genotypes. D Beck and J White, CIAT.

Activity 1.1.5. Verification of root morphology classes in the field using mini-rhizotrons (Perspex® tubes inserted into the soil in the root zone, with a video camera to observe root development under field conditions). D Beck, CIAT.

1.2 Output Two. The impact of previously determined root morphology classes of soybean on P uptake efficiency of soybean and determination of the plasticity of root morphology to external P status.

2. Indicates scientists/institutions where the work is intended to be implemented.

Activity 1.2.1. Assess the P uptake efficiency of soybeans representing root morphology classes at four soil P levels in field trials in Brazil. Root systems will be excavated at 35 DAP for morphology evaluation, with concurrent analysis of root and shoot dry matter, P content, and VAM infection. Yield, biomass, and P content will be evaluated at harvest. Brazilian researchers.

Activity 1.2.2. Image analysis of root morphological classes performed as in Activity 1.1.1 to update information on morphology classes and to relate it to information generated for *Phaseolus vulgaris.* D Beck, CIAT; Brazilian researchers.

1.3 Output Three. A determination of which cowpea *(Vigna)* root morphology classes, if any, are related to P-uptake efficiency (dry matter per unit P applied) and the plasticity of this root morphology to changes in external P status.

Activity 1.3.1. Published and unpublished data will be assessed for information on variation in root morphology in cowpea. W Payne, ICRISAT; IITA.

Activity 1.3.2. A representative core sample (200 accessions) of IITA's cowpea germplasm collection will be assessed for variability in root morphology. Root imaging and cluster analysis techniques, done by ICRISAT and Pensylvania State University teams, will be used to identify root morphology classes (methods and classification scheme to be described). W Payne, ICRISAT; J Lynch, Pennsylvania State University.

Activity 1.3.3. Field trials will be conducted in a highly P-deficient soil at three soil P levels using the 20 genotypes representative of different root morphology classes selected from Activity 1.3.2. Root systems will be excavated at 25 and 40 DAP for morphology evaluation, with concurrent analysis of root and shoot dry matter, P content, and VAM infection. Yield, biomass, P content, and N content will be evaluated at harvest. W Payne, ICRISAT.

Activity 1.3.4. Reciprocal grafting experiments will be conducted to verify the relationships identified above. These experiments will provide information on interactions between the shoot genome and root genome in controlling root type. Experiments will be conducted in large pots of the same soil used for Activity 1.3.3 at three P levels, using four divergent genotypes. W Payne, ICRISAT

Activity 1.3.5. Verification of root morphology classes in the field using mini-rhizotrons. W Payne, ICRISAT.

1.4 Output Four. Heritabilities of root morphological characteristics associated with P uptake determined in *Phaseolus vulgaris, Glycine max,* and *Vigna unguiculata.*

Activity 1.4.1. Crosses will be made among genotypes differing in root morphological characteristics that were associated with differences in P uptake efficiency. Appropriate progenies will be examined under controlled root environments and heritability analysis will be conducted by CIAT's bean breeding team for *Phaseolus,* by the Brazil team for *Glycine max,* and by IITA/ICRISAT for *Vigna.* D Beck, CIAT; Brazilian researchers; W Payne, ICRISAT; IITA.

Activity 1.4.2. Heritable root morphology traits associated with P uptake efficiency in *Phaseolus* recombinant inbred lines (PR90RILs) will be subjected to genetic analysis using randomly amplified polymorphic DNA (RAPD) markers (a wide range of markers exist for bean germplasm at CIAT). Loci representing effective root morphology will be identified and markers used in breeding programs to incorporate desirable traits in agronomically desirable germplasm. D Beck and S Beebe, CIAT.

1.5 Output Five. Associated physiological, morphological, and anatomical details related to phosphate uptake.

Activity 1.5.1. Identification of the sites of phosphate transporter activity in the different classes of roots of *Phasoelus* and *Glycine max. A* number of laboratories are currently attempting to clone genes that encode the phosphate transporter in plant roots. Once cloned, these will offer the tools necessary to identify those regions of the root systems most active in phosphate uptake. Antibodies will be used to detect P transporter proteins for *in situ* measurements of the location of P transporter proteins on different classes of roots of bean and soybean. F Smith, CSIRO.

Activity 1.5.2. Image analysis to identify possible associations between root morphological classes and the length, diameter, and frequency of root hairs. Bean, cowpea, and soybean plants grown in steady-state mist culture system at two P levels, with roots scanned by a video camera and subjected to computer analysis. J Caradus, AgResearch.

1.6 Output Six. Understand the interactive effects of soil moisture on P responses as related to root morphological differences in *Phaseolus* and *Vigna.*

Activity 1.6.1. Representatives of differnt root types (6 genotypes in total) will be grown under greenhouse conditions in an alumina-sand culture system at buffered levels of P at 0.5, 5, and 50 micromolar and under optimum and stress levels of available moisture for *Phaseolus,* Plus and minus mycorrhizae treatments will be included. Measurements will be taken on root and shoot dry matter and treatment effects on root morphology, mycorrhizal infection, and P content of shoots and roots. Cowpea will be evaluated in large pots, containing P-deficient soils, at three P levels. D Beck and J White, CIAT; W Payne, ICRISAT.

Activity 1.6.2. For *Phaseolus,* field trials will be conducted in a highly P-fixing soil at three soil P levels with applied N (to eliminate N fixation /P interactions) under conditions of adequate and stress levels of moisture (using a rainout shelter) with 10 representative genotypes from Activity 1.1.3. Root systems will be excavated at 20 and 35 DAP for morphology evaluation, with concurrent analysis of root and shoot dry matter, P content, and VAM infection. Yield, biomass, and P content will be evaluated at harvest. D Beck, J White, CIAT.

Activity 1.6.3. For *Vigna,* field trials will be conducted in a P-deficient soil at three soil P levels under adequate and stress levels of moisture (using a rainout shelter or overhead irrigation) with 10 representative genotypes from Activity 1.3.2. Root systems will be excavated at 25 and 40 DAP for morphology evaluation, with concurrent analysis of root and shoot dry matter, P content, and VAM infection. Yield, biomass, P content and N content will be evaluated at harvest. D Beck and J White, CIAT; W Payne, ICRISAT.

1.7 Output Seven. Determination of relationships between root morphology and P uptake in a model plant system *(Arabidopsis).*

Activity 1.7.1. Link with Pennsylvania State University laboratories investigating genetics of root morphology in *Arabidopsis,* with the aim of identifying genes responsible for pertinent characteristics. *Arabidopsis* mutants for root characteristics will be tested for performance at variable P levels. J Lynch, Pennsylvania State University.

II. Exudates component

2. Immediate Objective Two (Root modification of the rhizosphere: the role of exudates in P acquisition).

Background information. Root exudates play a critical role in P acquisition by plants. These exudates consist of high and low molecular weight compounds. In particular, amongst the low molecular weight compounds, organic acids have been shown to play an important role in P acquisition from sparingly soluble phosphate compounds. Organic acids, through chelation of metal ions and acidification of metal-phosphate complexes, solubilize the phosphate anion for uptake by plants. Additionally, the release of reductants from roots may also play an important role in solubilizing iron phosphates.

High molecular weight compounds include such enzymes as phosphatases, which can hydrolyze organic-phosphorus in its many different forms. Phosphatases that are exuded from plants typically are restricted to broad spectrum acid phosphatases, which show low to modest activities on many of the most abundant organic forms of P. However, microbial phosphatases present in the soil microflora can have many distinct enzymes that show high activity on these substrates.

In many cases, these high and low molecular weight compounds are exuded under P-deficiency stress. The stress response may be a key feature in controlling the production of these exudates.

There are substantial differences among species in the amounts and range of phosphorus-solubilizing compounds exuded by roots. Many important agricultural species are inefficient in acquiring P. One reason for this may be a limited ability to exude these compounds from their roots when faced with P stress.

Genetic manipulation of these traits and of their relative contributions to complex systems of integrated nutrient management, through traditional breeding and biotechnological methods, shows great promise for improving P acquisition by plants, thus alleviating one of the major constraints to sustainable crop production worldwide.

Piscidic acid. Following the work of Ae and his colleagues on pigeonpea there is good evidence to indicate a particular role for piscidic acid (PA) exuded from roots in the acquisition of P from iron and aluminum phosphate pools. In many soils, these pools form the major potential source of phosphate, but remain poorly available to most crop plants. The ability to expand the variety of crops that can grow on such soils would be a significant breakthrough. The role of PA in this ability needs to be assessed and quantified. Should it prove to be a causal agent in this trait, transfer of this ability to other crops can be considered.

2.1. Output One. A clear understanding of the mechanism of contribution of PA to P acquisition by pigeonpea on low-P soils. This will entail an understanding of its physical properties and metal stability constants, metabolism, genetic variation in its synthesis or excretion, and its quantification in the rhizosphere under phosphate deficiency stress.

Quantification of piscidic acid and its correlation with varietal differences in P acquisition will be established.

Activity 2.1.1. Assessments of pigeonpea accessions with sites of origin implicating differences in P acquisition abilities will be conducted under both sterile and field conditions. Piscidic acid exudation will be measured to determine the relationship between PA levels and developmental stage of the plant and, most importantly, to determine any correlation with P deficiency stress. A pre-screen of a set of pigeonpea genotypes grown under exceedingly low P conditions will be conducted to establish the extremes of variation that can be expected. A subset of these will then be evaluated for correlations between P acquisition ability and PA levels. C Johansen, ICRISAT; N Ae, National Institute of Agro-Environmental Sceinces (NIAES), Tsukuba, Japan.

Activity 2.1.2. Quantification of PA will be conducted on pigeonpea varieties grown under diverse conditions. Conditions that act as a key to trigger biosynthesis of PA will be determined for further studies on metabolic pathways of PA. N Ae, NIAES, Tsukuba, Japan; J Arihara, Hokkaido National Agricultural Experiment Station, Sapporo, Japan.

Activity 2.1.3. Metabolic pathways and enzymology of piscidic acid biosynthesis will be determined. N Ae, NIAES, Tsukuba, Japan.

Activity 2.1.4. Mechanisms regulating exudation of PA from cytoplasm to rhizosphere will be determined. Candidate enzymes or transporters will be purified and sequenced to enable the use of molecular genetic approaches. S Mori, University of Tokyo; J Arihara, Hokkaido National Agricultural Experiment Station, Sapporo, Japan.

Activity 2.1.5. Piscidic acid will be produced in substantial quantities to facilitate physicochemical and physiological studies of pigeonpea in acquiring P from Fe-P. N Ae, NIAES, Tsukuba, Japan.

Activity 2.1.6. The ability to take up iron-bound P in the soil will be confirmed using a radioisotope P value and a surface analysis of soil clay with XPS (ESCA). F W Smith, CSIRO; N Ae, NIAES and N Arihara, Hokkaido, Sapporo, Japan.

Activity 2.1.7. Initiate crosses between pigeonpea varieties showing extremes of P-acquisition abilities or PA production to see whether genetic

segregation will allow introgression of the trait by conventional breeding. C Johansen, ICRISAT.

Activity 2.1.8. Screen a wide variety of plant types, including *Arabidopsis,* and cereals grown under limiting P, for production of PA under soil conditions mimicking those found normally in the rhizosphere. N Ae, NIAES, Tsukuba, Japan..

Organic acid (malate/citrate) exudation

Organic acids have several potential roles in making P available for plant nutrition. The first is chelating the iron, aluminum, or calcium cations that form insoluble complexes with phosphate. The second is the direct effect of acidification, leading to increased solubility of the phosphate anion. Additionally, there is reason to suppose that a reducing function can be present in some organic acids that will stimulate changes in the redox state of the ferric phosphate complexes to allow their solubilization. Citrate and malate are the best characterized compounds in the category of organic acids, and their biochemistry and metabolism are well understood. Mechanisms for their excretion from roots are still obscure, however.

Citrate has been clearly implicated in P acquisition in lupins, whereas both malate and citrate are involved in rapeseed *(Brassica napus). Lupinus albus* and *Arabidopsis thaliana* will be used as model plant systems to further investigate organic acid secretion from roots. *Lupinus albus* exudes large quantities of citric acid under P-deficiency stress, making it a useful system to investigate the mechanisms of exudation. Genetic and biochemical analysis of mutants of *Arabidopsis* can be used to identify processes and genes important for P acquisition.

2.2 Output Two. Clarification of the relative roles of exuded organic acids and related compounds in P acquisition. Determination of the mechanisms of exudation and action, and identification of candidate processes that lend themselves to genetic manipulation.

Activity 2.2.1. Quantification of exudation of organic acids and other P-solubilizing compounds in *Lupinus albus.* Correlations with biochemical parameters will be sought, including activity of key enzymes of organic acid metabolism and the respiratory pathway. Carbon allocation to different root zones and calculation of carbon costs for P deficiency-enhanced root exudation will be determined. Anion channel inhibitors will be used to investigate mode of exudation. Studies will be made on the causal relationships between P deficiency-enhanced organic acid excretion and changes in root morphology in different lupin species.

Activity 2.2.1.1. Contingent on the above activity, genes encoding the candidate enzymes or transporters would be cloned and characterized using heterologous probes where available. H Marschner, University of Hohenheim, Germany, in collaboration with CSIRO Division of Plant Industry, Australia and USDA, ARS, Ithaca, USA.

Activity 2.2.2. Select mutants of *Arabidopsis* defective in P acquisition on a variety of P sources. These may include over-exuders, and those with qualitatively different exudate production, deregulated mechanisms, and hitherto unexpected mechanisms of P acquisition. Additionally, the mutants will be screened specifically for exuded organic acids, and correlations between these mutants and P acquisition will be made.

Categories of mutants that may arise are as follows.

- Exudation mutants (plus and minus)
- Phosphate transporters
- Organic acid synthesis mutants
- Root morphology mutants
- Redox mutants (e.g., membrane bound root reductases)
- Mutants in which any of the above process is deregulated.

Activity 2.2.2.1. Mutants will be extensively back-crossed to develop isogenic lines that will allow unambiguous characterization of the defects. E Delhaize and P Randall, CSIRO Division of Plant Industry.

Activity 2.2.3. Molecular and biochemical characterization of the defects in the *Arabidopsis* mutants that give rise to P utilization variants. E Delhaize, CSIRO; H Marschner, University of Hohenheim.

Genes expressed under phosphate-deficiency stress

All stresses thus far examined in plants are associated with responses in the plant that manifest themselves in changes in the patterns of gene expression. Many of these changes are caused by new mRNA transcription from promoters that respond to the stresses through signal transduction pathways of varying complexity. mRNAs that are produced *de novo* when the plant is stressed will be encoded by genes whose promoters fall into this class, and thus isolation of genomic clones that hybridize to these mRNAs will allow isolation and characterization of P-stress-responsive promoters.

Development of a comprehensive and well-characterized set of promoters and/or other gene sequences whose expression is controlled by the status of P stress in

the roots will be the core of an essential tool-kit. These cassettes will allow the targeted expression of other chimeric genes, including those for any of the exudates listed here, or for those involved in root morphogenesis, to occur only under P-stress conditions. This targeted expression is essential for effective use of any transgenic intervention, whether for experimental or ameliorative purposes. Cassettes can be prepared that will facilitate the use of these approaches by all investigators.

2.3 Output Three. Isolation, manipulation, and provision of gene sequences (e.g., promoters) that are responsible for restricting expression of genes to P limitation, root, and cell-types (cassettes). R Jefferson, Centre for the Application of Molecular Biology to International Agriculture(CAMBIA), Australia.

Activity 2.3.1. mRNA populations will be prepared from roots of both dicot and monocot species that have been grown under either phosphate-surplus or severely phosphate-limiting conditions, and used to generate cDNA and subsequently probes (RT-APPCR) for genomic libraries. These probes will be used to obtain cloned genomic fragments containing genes whose transcription is associated with either the stressed or unstressed condition.

Activity 2.3.2. These clones will be characterized further to ensure that the induction is restricted to roots, and exclusively to phosphate-deficiency stress, by hybridizing to mRNA populations isolated from roots subjected to a variety of such other stresses as water, nitrogen, or toxic cations.

Activity 2.3.3. The genes will be sequenced, and their promoter used to structure cassettes that can direct the expression of reporter genes in the first instance to occur only under these conditions.

Redox exudates. While various organic acids secreted by plant roots have been implicated in P availability, the role of root-exuded reductants has not been studied in this regard. Because many soil types contain P in nonavailable ferric phosphate pools, reductants exuded by roots could play a vital role in dissolution and mobilization of P from these ferric phosphate pools by destabilizing ferric phosphates through reduction of ferric iron to ferrous iron in these pools. Additionally, root-cell plasma membrane reductases are linked to the production and release of root reductants. Furthermore, stress induced root ethylene has been implicated in activation of root-cell plasma membrane reductases that control release of root reductants.

Factors controlling root cell plasma membrane reductase activity may be central to P-stress-induced mechanisms controlling P acquisition by plants dependent on P supplied from ferric phosphate pools, whether organic or inorganic in origin.

2.4 Output Four. Clarification and confirmation of the importance and nature of reductants exuded by roots and root-cell plasma membrane reductase activity on P acquisition by roots.

Activity 2.4.1. Two near-isogenic lines of *Pisum sativum* already developed that differ only in control of reductase enzyme will be evaluated for their P solubilizing activity. Leachates will be analyzed by biochemical and physical methods to determine both the redox characteristics of the compounds and their effects on differing Fe complexes that are found in agricultural soils. R Welch, USDA-ARS, Ithaca, USA.

Activity 2.4.2. Pigeonpea, a very P-efficient species, will be used to study the influence of root-cell membrane reductase activity on P dissolution and mobilization from iron phosphate sources using known inhibitors and promoters of root reductase activity. R Welch, USDA-ARS, Ithaca, USA.

Exuded enzymes

5 Output Five. Evaluation and elaboration in the rhizosphere by plants, of a suite of the microbial phytase enzymes responsible for organic phosphate hydrolysis.

Activity 2.5.1. Identification and cloning of genes encoding microbial enzymes responsible for hydrolysis of diverse organic-P sources. A Richardson, CSIRO Department of Plant Industry, Australia.

Activity 2.5.2. Preparation of coding cassettes, and insertion of these cassettes into controlled-expression vectors, from Output 3, to develop transgenic model test plants to assess for P-acquisition ability under controlled P-conditions. A Richardson, CSIRO Department of Plant Industry, Australia.

Activity 2.5.3. Transfer promising gene constructs to agriculturally relevant species for evaluation under field conditions. A Richardson, CSIRO Department of Plant Industry, Australia.

III. Cropping systems research component

The immediate objective is to increase uptake of P from sparingly available sources by improving cropping system technology.

The focus will be on the role of legume species as agents to increase available P for other crop components in the cropping system. This is particularly necessary in light of the evidence that shorter season, high yielding cereal varieties have higher soil fertility requirements, and that the physiological options for increasing cereal P uptake are fewer.

Attempts will be made to identify situations where species and varieties being studied can be compared to corroborate basic mechanisms being studied in other components of the project. It is recognized that some legumes in those widely used systems, e.g., *Phaseolus* or species of lupins, may not have a significant impact on P release from sparingly available sources. This information is essential to provide a basis for assessing the value of system adjustments.

Ten cropping systems were selected, which are listed below together with proposed centers for the research:

Phaseolus/maize	EMBRAPA CNPAC/CIAT
Soyabean/maize	EMBRAPA CNPAC/IITA
Phaseolus/rice	EMBRAPA CNPAC/CIAT
Cajanus/millet	ICRISAT (IAC)
Cajanus/sorghum	ICRISAT (IAC)
Vigna/millet	ICRISAT (ISC)
Stylosanthes/maize	EMBRAPA/CIAT
Arachislrict	KKU, Thailand
Cicer/sorghum	ICRISAT (IAC)
Arachis/sorghum	ICRISAT (Mali)
Lupin/wheat	Uni/DOA Australia

Lupinus albus/wheat rotations are likely to be more interesting than *Lupinus angustifoliusl* wheat as regards bringing slowly available P within the biological cycle to benefit the rotation. Most of the lupin grown in Western Australia is *L. angustifolius* and more *L. albus* is grown in eastern Australia.

3.1 Output One. Assess existing information and data. Considerable research relevant to the problem has been conducted for other purposes (e.g., N effects) without attention to the impact of various cropping systems on P availability. It is important to revisit existing data sets, which may have been collected for other experiments, and evaluate them for their potential to contribute to knowledge on P mobilization.

Activity 3.1.1. Collect and review relevant published and unpublished data from completed and ongoing studies. In collaboration with an appointed person at each location, review data and provide a standardized output of these data relevant to the subject matter. Conduct a workshop (working group with multi-discipline approach) to evaluate existing knowledge and plan future studies.

3.2. Output Two. Incorporate additional measurements and analyses into ongoing relevant studies.

Activity 3.2.1. Intensive short-term (3-year) studies (doctoral or postdoctoral) will be made in each cropping system using existing experiments/projects. Dynamics of the following P parameters will be determined: (1) organic and inorganic P forms, (2) soil solution P concentration, (3) plant P uptake, (4) root growth, (5) rhizosphere and bulk soil pH, and (6) mycorrhizal spore count and root infection. For each cropping system, three rotation treatments will be used with zero P input and two levels each of industrial phosphate (SSP) and locally available rock phosphate. W A Payne and J H Williams, ICRISAT (ISC) and M M Anders et al., ICRISAT (IAC); H Marschner, University of Hohenheim, Germany; others to be identified.

Activity 3.2.2. Through samples collected in long-term ongoing studies, provide additional analysis for (1) organic P, (2) inorganic P, and (3) P uptake and contents of plant parts.

Activity 3.2.3. Quantity of P recycling in selected systems.

Activity 3.2.4. Evaluate the contribution of difference (if any) in seed P content on acquisition of P in systems.

3.3 Output Three. Incorporate outputs from linked studies (Sections I, II, III) into putative improved systems to evaluate their contribution to P utilization in these systems.

Activity 3.3.1. These new studies are dependent on outputs from I, II, and IV being available and would be conducted at the identified locations. These studies will focus on and complement information gathered in activities 3.2.1.

3.4 Output Four. Predict spatial and temporal impact of improved technologies.

Activity 3.4.1. Incorporate subroutines for root and soil P properties into existing crop and system models to predict benefits of improved technologies on cropping systems in the four soil environments.

3.5 Output Five. Implementation of P-efficient sustainable systems.

Activity 3.5.1. As sustainable P-efficient systems are identified, these will be tested on-farm and promoted within the relevant target areas.

IV. Mycorrhizae

Mycorrhizal contributions to phosphorus acquisition

Mycorrhizae could be the most important untapped and poorly understood resource for P acquisition in agriculture. While it has become widely accepted that mycorrhizal populations associated with roots of crop plants play a ubiquitous and critical role in P acquisition from sparingly available sources, our progress in understanding and utilizing this resource has been minimal. The fundamental reason underlying the disappointing progress is the lack of methodology suitable for either identification or evaluation of mycorrhizal species and strains under field conditions. Mycorrhizae are largely obligate symbionts and commensal organisms; culturing them has proven extremely difficult, and may never be a viable option. There are no criteria for assessing or assigning species or strain designations other than extremely crude morphological criteria. Until this constraint is overcome, our ability to make any intelligent use of mycorrhizae in sustainable systems development will be minimal.

Methodology is urgently required that will allow species and strains to be unambiguously identified at low cost and with a high throughput. Methodology for marking strains to begin an understanding of gene flow, competition, and persistence in the field is essential. Additionally, methodology for investigating the function of mycorrhizae in their symbioses is required.

Output One. Development of simple methodology for species and strain identification among field mycorrhizal populations. R Jefferson, CAMBIA; D Beck, CIAT.

Output Two. Development of marking technologies for assessing presence, competition, and persistence amongst field mycorrhizal populations. R Jefferson, CAMBIA; D Beck, CIAT.

References

Ae, N., Arihara, J., and **Okada, K.** 1991. Phosphorus uptake mechanisms of pigeonpea grown in Alfisols and Vertisols. Pages 91-98 *in* Phosphorus nutrition of grain legumes in the

semi-arid Tropics. (Johansen, C., Lee, K.K., and Sahrawat, K.L., eds.). Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Al-Abbas, A.H., and Barber, S.A. 1964. A soil test for phosphorus based upon fractionation of soil phosphorus: I. Correlation of soil phosphorus fractions with plant-available phosphorus. Soil Science Society of America Proceedings 28: 218-221.

Barber, S.A. 1979. Growth requirement for nutrients in relation to demand at the root interface. Pages 5-20 *in* The soil root interface (Harley, J.L., and Scott-Russell, R., eds.). London: Academic Press.

Barrow, N.J. 1993. Plant nutrition—from genetic engineering to field practice. Dordrecht, The Netherlands: Kluwer Academic.

Bassam, N.El., Dambroth, M., and Loughman, B.C. 1990. Genetic aspects of plant mineral nutrition. Dordrecht, The Netherlands: Kluwer Academic.

Flach, E.N., Quak, W., and Diest, A. van. 1987. A comparison of the rock phosphate mobilizing capacities of various crop species. Tropical Agriculture (Trinidad) 64: 347-352.

Hoffland, E., Van den Boogard, R., Nelemans, J., and Findenegg, G. 1992. Biosynthesis and root exudation of citric and malic acids in phosphate starved rape plants. New Phytologist 122: 675-680.

Khasawneh, F.E., and Doll, E.C. 1978. The use of phosphate rock for direct application of soils. Advances in Agronomy 30: 159-206.

Koala, S., Sims, J.R., El-Altar, H., and El-Halfawi, M. 1988. Phosphorus deficiency in the semi-arid tropics and implications for grain legume production. Pages 205-216 *in* World crops: cool season legumes (Summerfield, R.J., ed.). London, UK: Kluwer Academic.

Randall, P.J., Delhaize, E., Richards, R.A., and Munns, R. 1993. Genetic aspects of plant mineral nutrition. Dordrecht, The Netherlands: Kluwer Academic.

Tandon, H.L.S. 1987. Phosphorus Research and Agricultural Production in India. New Delhi, India: Fertilizer Development and Consultation Organization.

Concluding observations

The first day of the Workshop, devoted mainly to presentation of prepared papers by invited participants, proceeded smoothly as all participants were familiar with this format. Difficulties became apparent on the second day when the participants faced the challenge of converting a wealth of component-oriented knowledge on physiological, biochemical, and genetic aspects of phosphorus (P) nutrition of plants into forms suitable for practical application in enhancing sustainability of cropping systems. Although most participants envisioned long-term practical application of their research endeavors, explanations of the mechanics of how to achieve this were not readily forthcoming. This clearly highlighted that elegant scientific understanding does not automatically 'trickle down' to find practical application. On the contrary, a concerted effort is needed to think through how basic knowledge may be practically applied.

However, despite these initial difficulties, research areas worthy of being pursued were identified by the assembled group. The areas were root manipulation, exudate modification, mycorrhizal enhancement, and cropping systems adjustment. In the afternoon of the second day, working groups on these four topics were established and participants divided themselves among them according to their expertise. Apart from deciding on these topics, it was also significant that the group decided that the project should primarily target legumes as these plants in general appeared to have most favorable adaptations for P acquisition. Thus, legumes seem important not only in terms of ensuring sustainability of the cropping systems because of their ability to add fixed nitrogen to the system but also in terms of acquiring P from sparingly available sources.

The process of finalizing a viable global project proposal is in hand. This general approach of a global research consortium is indeed now becoming popular on the international agricultural research scene. It fits very well with the recent Consultative Group on International Agricultural Research (CGIAR) approach of developing ecoregional and systemwide initiatives, the main aim of which is to gain synergies in the research effort and hasten impact of research in alleviating current problems in world agriculture. It is hoped that this global consortium on enhancing the ability of plants to acquire P will lead the way towards a global strategy for most efficient use of the planet's P resources for agriculture.

Participants

N Ae
Soil Biochemistry
National Institute of Agro-Environmental Sciences
Kannondai, Tsukuba
Ibaraki 305
Japan
Fax +81 298 38-8199

B S Ahloowalia
Plant Breeding and Genetics Division
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture
International Atomic Energy Agency
Wagramerstrasse 5, P 0 Box 100
A-1400 Vienna
Austria
Fax +43 1 234564

J Arihara
Hokkaido National Agricultural Experiment Station
1 Hitsujigaoka, Toyohira-ku
Sapporo 062
Japan
Fax +81 11 859 2178

D Beck
Department of Microbiology
Centro Internacional de Agricultura Tropical (CIAT)
Apartado Aereo 6713
Cali
Fax +57 23 647243
Colombia
E-mail ciat-bean@cgnet.com

J R Caradus
AgResearch
Grasslands Research Centre
Tennent Drive, Fitzherbert West
Private Bag 11008
Palmerston North
Fax +64 6 356 1130
New Zealand
E-mail caradusj@agresearch.cri.nz

E Delhaize
CSIRO Division of Plant Industry
G P 0 Box 1600
Canberra, ACT 2601 Fax +61 6 246 5000
Australia E-mail manny@picanpi.csiro.au

R A Jefferson
Centre for the Application of Molecular Biology to International Agriculture (CAMBIA)
G P O Box 3200
Canberra, ACT 2601
Australia Fax +61 6 246 5303

J L Karmoker
Botany Department
Dhaka University
Dhaka 1000
Bangladesh Fax +88 2 863057

E A Kueneman
Field Food Crops Group
Food and Agriculture Organization of the United Nations
Via delle Terme di Caracalla
00100 Rome Fax +39 6 52256347
Italy E-mail eric.kueneman@ fao.org@ internet

J F Loneragan
School of Biological and Environmental Sciences
Murdoch University, Perth
Western Australia 6150
Australia Fax +61 9 310 3505

H Marschner
Institut fur Pflanzenernahrung
Universitat Hohenheim
Fruwirthstrasse 20
70599 Stuttgart 70
Germany Fax+49 0711/459 3295

S Mori
Laboratory of Plant Nutrition and Fertilizer
Faculty of Agriculture
The University of Tokyo
1-1 Yayoi
Bunkyo-ku, Tokyo 113
Japan
Fax +81 3 3812 0544

P J Randall
CSIRO Division of Plant Industry
G P 0 Box 1600
Canberra, ACT 2601
Australia
Fax +61 6 246 5000

R N Roy
Integrated Plant Nutrition Systems Group
Plant Nutrition Service
Food and Agriculture Organization of the United Nations
Via delle Terme di Caracalla
00100 Rome
Italy
Fax +39 6 52253152

F W Smith
Division of Tropical Crops and Pastures
CSIRO Cunningham Laboratory
306 Carmody Road
St. Lucia, Queensland 4067
Australia
Fax +61 7 371 3946

R M Welch
US Department of Agriculture
Agriculture Research Service
US Plant, Soil and Nutrition Laboratory
Cornell University
Tower Road, Ithaca
New York 14853-2901
Fax +1 607 255 2459
USA
E-mail rmwl@cornell.edu

Observers

T Otani
National Institute of Agro-Environmental Sciences
Kannondai, Tsukuba
Ibaraki 305
Japan Fax +81 298 38 8199

T Karasawa
Hokkaido National Agricultural Experiment Station
1 Hitsujigaoka, Toyohira-ku
Sapporo 062
Japan Fax +8111 859 2178

ICRISAT participants

ICRISAT Asia Center, Patancheru 502 324, Andhra Pradesh, India

M M Anders
Principal Scientist (Agronomy)
Agronomy Division

O Ito
Principal Scientist (Agronomy)
and Team Leader (GOJ Special Project)
Agronomy Division

C Johansen
Director
Agronomy Division

J V D K Kumar Rao
Senior Scientist (Physiology)
Soil and Agroclimatology Division

K K Lee
Principal Scientist (Microbiology)
Soils and Agroclimatology Division

R C Nageswara Rao
Scientist (Physiology)
Agronomy Division

S N Nigam
Principal Scientist (Breeding)
Genetic Enhancement Division

T J Rego
Senior Scientist (Soil Science)
Soil and Agroclimatology Division

0 P Rupela
Senior Scientist (Physiology)
Agronomy Division

J G Ryan
Director General

B Seeling
Research Fellow
Soils and Agroclimatology Division

K K Sharma
Scientist (Cell Biology)
Cellular and Molecular Biology Division

G V Subbarao
Visiting Scientist
Agronomy Division

S P Wani
Scientist (Microbiology)
Soils and Agroclimatology Division

ICRISAT Sahelian Center, B P 12404, Niamey, Niger

K Anand Kumar
Director
Genetic Enhancement Division

K Harmsen
Executive Director
Western and Central Africa Region

W A Payne
Principal Scientist (Physiology)
Agronomy Division

J H Williams
Principal Scientist (Physiology)
Agronomy Division

Observers

ICRISAT Asia Center

F R Bidinger
Principal Scientist (Physiology)
Agronomy Division

C T Hash Jr
Principal Scientist (Breeding)
Genetic Enhancement Division

L J Reddy
Senior Scientist (Breeding)
Genetic Enhancement Division

Onkar Singh
Senior Scientist (Breeding)
Genetic Enhancement Division

H D Upadhyay
Senior Scientist (Breeding)
Genetic Enhancement Division

H A van Rheenen
Principal Scientist (Breeding)
Genetic Enhancement Division

About ICRISAT

The semi-arid tropics (SAT) encompasses parts of 48 developing countries including most of India, parts of southeast Asia, a swathe across sub-Saharan Africa, much of southern and eastern Africa, and parts of Latin America. Many of these countries are among the poorest in the world. Approximately one-sixth of the world's population lives in the SAT, which is typified by unpredictable weather, limited and erratic rainfall, and nutrient-poor soils.

ICRISAT's mandate crops are sorghum, pearl millet, finger millet, chickpea, pigeonpea, and groundnut; these six crops are vital to life for the ever-increasing populations of the semi-arid tropics. ICRISAT's mission is to conduct research which can lead to enhanced sustainable production of these crops and to improved management of the limited natural resources of the SAT. ICRISAT communicates information on technologies as they are developed through workshops, networks, training, library services, and publishing.

ICRISAT was established in 1972. It is one of 16 nonprofit, research and training centers funded through the Consultative Group on International Agricultural Research (CGIAR). The CGIAR is an informal association of approximately 50 public and private sector donors; it is co-sponsored by the Food and Agriculture Organization of the United Nations (FAO), the United Nations Development Programme (UNDP), the United Nations Environment Programme (UNEP), and the World Bank.

ICRISAT

International Crops Research Institute for the Semi-Arid Tropics
Patancheru 502 324, Andhra Pradesh, India

ISBN 92-9066-296-4 Cat.Code CPE 094 053-94