



Root traits as tools for creating phosphorus efficient crop varieties

Tara Singh Gahoonia¹ & Niels Erik Nielsen

The Royal Veterinary and Agricultural University, Department of Agricultural Sciences, Plant Nutrition and Soil Fertility Laboratory, Thorvaldsensvej 40, DK-1871 Frederiksberg, Copenhagen, Denmark. ¹Corresponding author*

Received 29 July 2002. Accepted in revised form 6 June 2003

Key words: breeding, mycorrhiza, organic acids, phosphatase, root hairs, root morphology

Abstract

This paper provides a brief assessment of the genetic variation in root properties (root morphology, including root hairs), mycorrhizal symbiosis, uptake kinetics parameters and root-induced changes (pH, organic acids and acid phosphatase) in the rhizosphere of various crop species and their genotypes and then briefly discusses the opportunities and challenges of using such knowledge for enhancing P efficiency of future crop genotypes by genetic means. Wide genotypic variation and heritability of root morphology, root hair length and density and thereby P acquisition provide opportunities for selection and breeding for root characteristics for increasing P acquisition. The progress is challenged by the concerns of high carbon cost of larger root systems and by the lack of cost effective methods to determine root length of a large number of genotypes under field conditions. The carbon cost of root hairs is low. Furthermore, low cost methods now exist to compare root hair formation of field grown genotypes. The development and application of sophisticated methods has advanced our knowledge on the role of mycorrhizal symbiosis in P acquisition and also on the molecular basis of fungi and plant interactions. However, extensive studies to explore genotypic variation in mycorrhizal responsiveness are rare, which makes it difficult to assess, how mycorrhizal symbiosis can be manipulated through breeding efforts. The promising variation found in P uptake kinetics parameters of crop genotypes in few studies indicates that more genotypes may be screened by relatively simple nutrient solution culture techniques. The genetic manipulation of the overall differences in cation-anion uptake, which is the main cause of rhizosphere pH change, may be difficult. For manipulation of rhizosphere pH, agronomic measures such as applications of ammonium or nitrate fertilisers may be more useful than breeding approaches. Also it seems difficult to assess what kind of genetic analysis should be performed to support the breeding efforts. Phosphorus mobilisation effect of pH depends on soil P compounds, therefore will differ with soil type. Both the enhanced release of organic acids and higher acid phosphatase activity in the rhizosphere may be useful for increasing P acquisition from inorganic and organic P pools, respectively. Modification of these traits by genetic means should be considered. For successful breeding programmes, the role of various root traits needs to be targeted in an integrated manner and then methods need to be developed for studying their importance under natural soil conditions, so that the genotypic variation can be explored and their ecological significance in P acquisition can be established.

Introduction

Phosphorus (P) is a non-renewable resource and wasteful use is difficult to justify. The uneven development in global economy has contributed to the uneven distribution of P in agricultural soils of the world. In

the past decades, high import of P in animal feeds and generous applications of P fertilisers have resulted in accumulation of P in European soils to an extent that high P fertiliser doses pose a particular threat to the environment due to runoff losses, which can result in alga blooms (Turner et al., 2002). The soils of developing countries remained P-poor and increasingly

* FAX No: +45-35283468. E-mail: tsg@kvl.dk

depleted. The depletion was enhanced by export of crops and animal feeds because enough P fertilisers were not available or affordable by the resource poor farmers (Sanchez and Leakey, 1997). For securing food to the increasing population in developing countries, the need for P fertilisation is expected to increase (Brynes and Bumb, 1998). However, even when P fertilisers are applied to replenish soil fertility, about 70–90% of the P fertilisers is adsorbed and becomes 'locked' in various soil P compounds of low solubility without giving any immediate contribution to crop production (Holford, 1997). This suggests that P fertilisation alone is not a cost-effective way of increasing crop productivity in many P limiting soils (Tilman et al., 2002). New crop varieties with improved root traits, able to unlock and absorb P from bound soil P resources may be of additional value for increasing the efficiency of fertilisation (Abelson, 1999). Although the variety of differences in P uptake and their link to the size of root systems were reported many years ago (Lyness, 1936; Smith, 1934), breeding for efficient root systems has received little attention (Clarke and McCaig, 1993). This is despite the fact that wide variation has *indeed* been reported in the ability of crop genotypes to perform and produce economic yields under P-limited soil conditions (Clark, 1991).

Based on the nutrient contents of most well-nourished young plants (5% N, 0.5% P and 5% K), the ratio between optimal inflow of N, P and K into young plants would be 1:0.1:1 (Nielsen, 1996), whereas, even in most fertile soils, the ratio between availability (mobility) of soil solution N, P and K in the rhizosphere is 1:0.001:0.1 respectively (Barber, 1995). Hence, rhizosphere soil is particularly important for supplying less mobile nutrients such as P (Gahoonia et al., 1992, 1994) for crop growth. A variety of root properties (root morphology, root diameter, root hairs), mycorrhiza symbiosis, P uptake kinetics parameters and rhizosphere processes (rhizosphere pH, phosphatase activity and root-released organic acids) cause variation in P uptake among the plant species and their genotypes. The various root-induced chemical (Hinsinger, 1998) and biological processes (Richardson, 2001) in relation to plants' ability to mobilise nutrients have been critically reviewed. In this paper, we will concentrate on genetic variation in such processes in relation to plants' ability to mobilise and absorb soil P. Any decision to make use of root properties and root induced rhizosphere processes for improving P acquisition through breeding efforts would require that (a) the desired trait is easily iden-

tifiable and it has a clear relation to P acquisition, (b) there exists a large genetic variation and (c) the mode of inheritance is known, for transferring it from one individual to another by genetic means.

This paper aims to synthesise the existing knowledge about intraspecific genetic variation in root traits and to discuss the possibilities and limitations with regards to the decision supporting criteria and then suggest how the accumulated knowledge can be used as a tool for selecting and breeding P efficient crop varieties.

Root size and morphology

Larger root system provides greater root-soil contact, which is particularly important for uptake of P. Mobile nutrients, like nitrate, can be depleted at low rooting density, while for less mobile ions like P uptake is often closely related to root length (Atkinson, 1991). Brück et al. (1992) by using a 'Rootless' (a maize mutant that produce drastically less crown roots) and a 'Normal' (produces normal crown roots) maize line documented the outstanding importance of root size on P uptake at low P supply.

Large differences in root morphology and distribution exist between genotypes of plant species (Atkinson, 1991; Nielsen, 1983; O'Toole and Bland, 1987; Römer et al., 1988). Leon and Schwang (1992) found that yield stability of oats and barley cultivars was related to their total root length. Barraclough (1984) found that total root length of winter wheat was positively correlated to grain yield. This raises the possibility that selection and breeding of crop genotypes with extensive root systems may contribute to more efficient use of soil P resources and yield stability. However, few breeders have paid attention to the possibility of manipulating root length for making crop genotypes P efficient. This may be partly due to the difficulties and expense of studying root systems. The methods available (Smit et al., 2000) to measure root systems are time consuming and prone to errors, limiting their reliability and widespread use for comparing the root systems of a large number of crop genotypes. Model simulations of plant root systems for predicting optimum root architecture for P acquisition in low P conditions (Lynch 1995) are promising. However, validation of such models through field experiments still remains a problem as development of root systems is influenced by a large number of soil conditions and climate factors (Hoad et al., 2001), which additionally

obstruct screening of genotypes for root systems. Increasing root size may also divert assimilates from top to roots and so change the root/shoot ratio. The amount of carbon transferred below ground may be up to 50% of the total net assimilates of aboveground production (Linjeroth et al., 1994; Swinnen, 1994). It is difficult to know whether larger root size of plants absorbing higher amounts of P is the result or the cause of larger root size. The P uptake per plant correlates not only with root size but also with shoot size (Caradus and Snaydon, 1986). Thus, the apparent importance of root size in determining P uptake may be simply a result of root size being a component of total plant size.

Yadav et al. (1997) by studying a double haploid population of 105 lines derived from a cross between indica and japonica rice, reported that the main Quantitative Trait Loci (QTLs) were common for root thickness and maximum root length. Based on this, they suggested that there is a possibility of modifying several aspects of root morphology simultaneously. The reported heritability of root length is 0.14–0.51 in wheat (Porceddu et al., 1978), 0.83 in oats (Barbour and Murphy, 1984) and 0.35 in rice (Ekanyake et al., 1985), which are high enough to consider worthy for manipulation of root length.

Root diameter

Root diameter is important because it defines the volume of soil, which can be contacted by roots by investing a given amount of photosynthate (Atkinson, 1991). A smaller root diameter can contact a larger soil volume per unit root surface area. Direct measurements of root diameter are rare in the literature (Woodfield and Caradus, 1990). This may be partly due to that even modern image analysis protocols (Bouma et al., 2000) for measuring root diameter are prone to errors. Specific root length (SRL, m g^{-1} root) is the frequently used indicator of root fineness. The variation in SRL between plant species is well documented (Atkinson, 1985; Fitter, 1985), but the variation between genotypes and its link to P uptake is rarely investigated (Nielsen and Schjørring, 1983). Crop genotypes with thinner roots may be more effective in absorbing P. However, the maintenance carbon cost of producing finer roots may be higher as these will have to be replaced more frequently (Persson, 1982). Woodfield and Caradus (1990) reported high heritability (0.54) of root diameter of white clover. However, with our present understanding and limited data on intra-specific variation in root diameter,

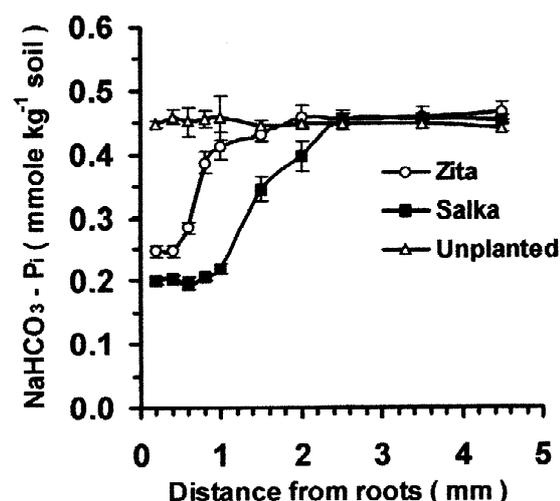


Figure 1. Depletion of inorganic P (extracted with 0.5 M NaHCO₃) at varying distances from the roots of two barley cultivars, Salka (long root hairs) and Zita (short root hairs) (Gahoonia and Nielsen, 1997).

Table 1. Root hair lengths (RHL in 0P) and grain yield of barley cultivars in the field at low (0P, P-limited), moderate (10P) and high (20P) levels of phosphorus (P) fertilisation. (0P = no P since 1966, soil solution P = 3 μM ; 10P = 10 kg P ha⁻¹ year⁻¹, soil solution P = 6 μM ; 20P = 20 kg P ha⁻¹ year⁻¹; soil solution P = 10 μM)

Barley genotypes	RHL mm	Grain yield,	Grain yield,	Grain yield,
		tons ha ⁻¹ 0P	tons ha ⁻¹ 10P	tons ha ⁻¹ 20P
Pongo	1.34 ± 0.1	5.81 ± 0.09	5.72 ± 0.09	5.70 ± 0.14
Linus	1.30 ± 0.1	5.86 ± 0.13	5.84 ± 0.33	5.83 ± 0.16
Tofta	0.91 ± 0.03	5.26 ± 0.09	5.39 ± 0.19	5.35 ± 0.21
Cecilia	0.40 ± 0.02	3.90 ± 0.14	4.91 ± 0.53	5.27 ± 0.47
Meltan	0.57 ± 0.02	5.02 ± 0.45	6.54 ± 0.08	7.07 ± 0.44
Scarlett	0.56 ± 0.03	5.06 ± 0.05	6.30 ± 0.07	6.71 ± 0.09

neither a clear picture on the link of root diameter with P uptake can be given, nor genetic analysis can be warranted.

Root hairs

Root hairs enhance the effectiveness of roots to exploit rhizosphere soil for P; because of their geometrical arrangement on root surface and their ability to increase effective root surface area many times (Föhse et al., 1991; Gahoonia et al., 1997). Among all possible ways of increasing effective root surface area, change in root hair morphology is considered least meta-

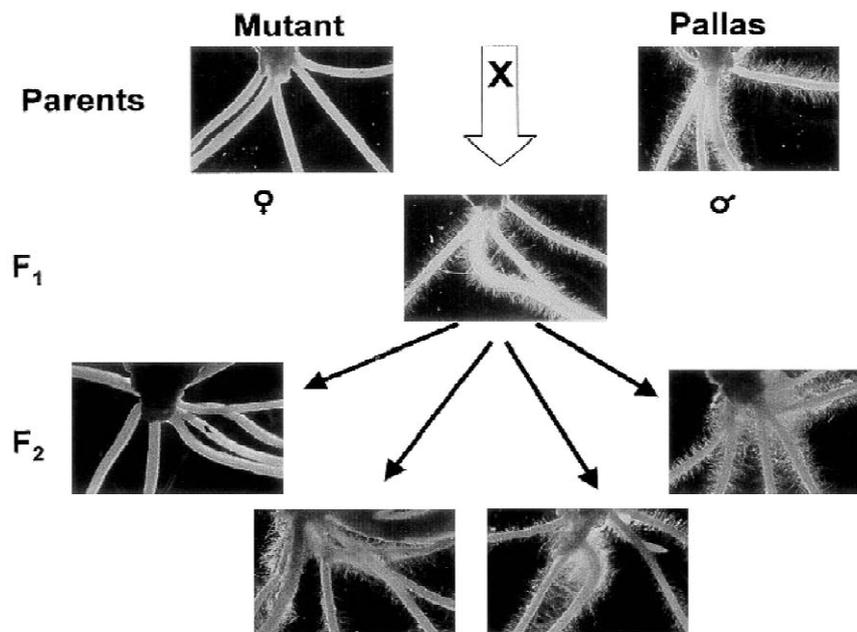


Figure 2. The root hair-less barley mutant was crossed with wild type (spring barley cultivar Pallas). The segregation ratio of 1:3 in selfed F₂ generation, suggests that root hair trait is inherited in Mendelian manner (Gahoonia et al., 2001).

bologically costly (Hetrick, 1991; Röhm and Werner, 1987). The role of root hairs in P uptake is now well documented (Bates and Lynch, 2000; Gahoonia and Nielsen, 1998). A considerable intraspecific variation in root hair formation has been found among genotypes of white clover (Caradus, 1979), common bean (Yan et al., 1995), cowpea (Krasilnikoff et al., 2001) and wheat and barley (Gahoonia et al., 1997, 1999). For example, the barley genotype Salka with longer root hairs (1.00 ± 0.26 mm) exploited the rhizosphere soil for P twice as effectively (Figure 1, Gahoonia and Nielsen, 1997) than Zita with shorter root hairs (0.63 ± 0.24 mm). Salka also absorbed more P than Zita when grown in a low-P field and also produced higher shoot biomass (Gahoonia et al., 1999). Our more recent studies showed that barley cultivars with long root hairs (Pongo, Linus and Tofta) maintained stable economic grain yield both in P-limited ($3 \mu\text{M}$ P in soil solution) and high-P ($10 \mu\text{M}$ P in soil solution) field soils. In contrast cultivars with short root hairs (Cecilia, Meltan and Scarlett) produced lower grain yields in P-limited soil and they produced high grain yields only when P fertilisers were applied (Table 1).

Root hairs are the easily assessable and visible component of root-soil interface. Recent advancements in methods (Gahoonia and Nielsen, 1997) have facilitated studies of root hair formation of field grown

genotypes. Genetic analysis of root hair formation has progressed with the help of root hair *defective* mutants of model plant *Arabidopsis* (Schiefelbein and Somerville, 1990) and *Arabidopsis* genes with roles in root hair development have been reported (Grierson, et al., 2001). The availability and characterisation of a root hairless barley mutant (*brb*) has opened new possibilities of genetic analysis of cereal root hairs (Gahoonia et al., 2001). The cross between the mutant and wild type (Pallas, a spring barley cultivar) revealed that root hairs follow the Mendelian mode of inheritance (Figure 2; Gahoonia et al., 2001), indicating that *brb* is a single gene mutation. The realised heritability of root hair length in white clover ranged from 0.33 to 0.44, at which selection for increased root hair length was possible (Caradus, 1979). All this shows that it should be possible to select and also breed new crop genotypes for longer root hairs to improve P uptake.

Mycorrhiza symbiosis

Mycorrhizal fungi (VAM), by their hyphae extending plant root systems help plants to exploit much greater volume of soil for P (Kothari et al., 1990; Smith and Read, 1997). Mycorrhizal colonisation usually represents a significant photosynthetic carbon cost and host genotypes appear to regulate such carbon

cost by reducing the degree of colonisation (Graham and Eissenstat, 1994). Even though the occurrence of mycorrhizae in agro-ecosystems is almost universal, its beneficial effect for P acquisition may be restricted, as the dependency of plant species on VAM colonisation varies, e.g. cereals being less dependent than legumes. Despite existence and application of sophisticated methods for determining mycorrhizal colonisation (Brundrett et al., 1996), the genotypic variation in degree of VAM colonisation is often not found. Investigations with 27 wheat lines (Kapulnik and Khusnir, 1991) and 10 barley cultivars (Jakobsen and Nielsen, 1983) did not show significant variation in mycorrhizal colonisation. In a recent study, Zhu et al. (2001) found lower mycorrhizal colonisation in modern wheat cultivars than in old cultivars, indicating that modern breeding programs may have reduced the VAM colonisation potential of modern wheat cultivars. Few studies (Harrison and van Buuren, 1995; Hetrick et al., 1995) enrich our understanding of genetic background of VAM symbiosis and explain how fungi and plants interact at the molecular level. A phosphate starvation gene *Mt4* in mycorrhizal roots has been reported (Burleigh and Harrison, 1998). It remains to be explored how such knowledge can be used in breeding programmes for increasing mycorrhizal colonisation of future crop plants. The potential value of breeding plants for greater mycorrhizal colonisation will also depend on the cost/benefit of VA mycorrhizae for the specific crop, soil and environmental conditions.

P uptake kinetics

Under conditions in which the rate-determining step is located in the plant root, the mean net influx (I_n) of phosphate into plant root can be expressed by (Nielsen, 1976)

$$I_n = \frac{I_{\max} (C_o - C_{\min})}{K_m + C_o - C_{\min}} \quad (1)$$

I_{\max} is the mean maximum influx, $\text{mol cm}^{-1}\text{s}^{-1}$, C_o is the concentration of P at root surface, mol cm^{-3} , C_{\min} is the concentration at which $I_n = 0$, K_m is the Michaelis-Mentes factor, mol cm^{-3} , $I_n = 1/2$ if $C_o = K_m + C_{\min}$.

The values of the parameters I_{\max} , K_m and C_{\min} differed among genotypes of maize (Nielsen and Barber, 1978) and barley (Table 2) and wheat (Pandey, 2001). For improvement in P efficiency, the genotypes should be identified for high I_{\max} , values and

root lengths and for low C_{\min} and K_m values. Lower C_{\min} reflects ability to absorb P at lower soil solution concentration, which may gain further importance in future low P input farming systems. Although the uptake kinetics parameters (I_{\max} , K_m and C_{\min}) of various crop genotypes can be determined relatively easily by nutrient solution culture techniques, their importance in soil-plant system is yet to be ascertained.

Root-induced pH change

Change in soil pH around roots has often been demonstrated; differences as large as 2 units have been found (Römheld and Marschner, 1986). The reasons for such pH changes can be many, including imbalance of cation-anion uptake (Hedley et al., 1982), which is particularly affected by sources of nitrogen (Gahoonia and Nielsen, 1992); enhanced efflux of protons as a result of P deficiency (Schjørring, 1986). Whatever the origin of pH changes, large variation is reported among plant species (Marschner and Römheld, 1983) and along the root system (Marschner et al., 1982), which affects the bioavailability of soil P (Grinstead, et al., 1983; Gahoonia and Nielsen, 1992). Soil phosphorus enters the roots mainly as H_2PO_4^- via the soil solution (Hendriks, 1967). The concentration of H_2PO_4^- in soil solution is pH related. Plant species or genotypes inducing rhizosphere acidification may absorb more P by this mechanism. Gollany and Schumacher (1993) demonstrated intraspecific variation in rhizosphere pH among genotypes of various plant species. Our studies (Gahoonia and Nielsen, 1996), however, showed that some wheat and barley genotypes differed significantly in the capacity to absorb inorganic P from the rhizosphere soil even when rhizosphere pH remained unchanged, suggesting that in addition to root-induced pH change, other major mechanisms may be involved in causing variation in P acquisition between cereal genotypes.

Overall cation-anion balance of the plants plays a major role in the rhizosphere pH change (Hedley et al., 1982; Nye, 1981). This suggests that if the ability of the plants to change rhizosphere pH has to be manipulated genetically, their cation-anion balance has to be modified, which may be difficult. The effect of rhizosphere pH change in mobilisation of P also depends on soil type (Gahoonia et al., 1992; Armstrong and Helyar, 1992). In a luvisol, where phosphate was mainly bound to calcium, rhizosphere pH decrease enhanced the mobilisation of P, whereas in an Oxisol,

Table 2. Phosphorus uptake kinetic parameters of barley genotypes (Nielsen and Schjørring, 1983)

Genotype	Root length (m g ⁻¹)	Inflow I _{max} (pmol P cm ⁻¹ sec ⁻¹)	Uptake capacity K _m (μmol P)	Minimum concentration C _{min} (μmol P)
Salka	65	0.08	2.9	0.02
Rupal	46	0.10	3.6	0.04
Nuremberg	68	0.11	3.6	0.06
Zita	57	0.12	4.7	0.03

where P was mainly bound to Al and Fe, the same plant with same type of nitrogen fertilisation mobilised more P with rhizosphere pH increase (Gahoonia et al., 1992). Hence, it is worth considering whether agronomic means, such as applications of ammonium (in moderately alkaline soils) or nitrate (in acid soils) fertilisers may be more effective than breeding and genetic manipulation of the ability of crop plants to change rhizosphere pH. For genetic manipulation, it is also difficult to envisage what kind of genetic analysis should be performed and which genes should be targeted to manipulate this ability in new crop genotypes.

Phosphatase activity

Increased phosphatase activity in the rhizosphere soil seems to increase the hydrolysis of soil phosphate esters (Bielski and Johnson, 1972), releasing inorganic P for plant uptake (Tarafder and Jungk, 1987). However, if plants continue to grow poorly in P deficient soils, despite marked increase in root exocellular phosphatase activity (Alexander and Hardy, 1981), it may be argued that P mobilised through the increased phosphatase activity is not able to compensate for an inadequate supply of inorganic P. Asmar et al. (1995) found significant variation in root-induced phosphatase activity between barley genotypes and the genotype inducing higher activity indeed mobilised more organic P from the rhizosphere soil, reopening the debate on the role of phosphatase in P nutrition and bioavailability of soil organic P. Depending on the soil type, the organic phosphorus content may constitute 20 – 80% of the total phosphorus (Dalal, 1977). Therefore, there is great interest for enhancing the ability of plant roots to secrete phosphatase and increase plant availability of P from soil organic P. Although the rela-

tionship between rhizosphere phosphatase activity and depletion of rhizosphere organic P is often reported (Asmar et al., 1995; Tarafder and Jungk, 1987), any evidence, showing that the level of acid phosphatase or phytase are limiting the mobilisation of P from soil organic P pools, is yet to be obtained. The extent of intraspecific variation in rhizosphere phosphatase activity is yet unexplored and the genetic analysis of acid phosphatase activity is only at the initial stage. The existence and characterisation of an *Arabidopsis* mutant missing one acid phosphatase isoform (Trull and Deikman, 1998) could be a valuable tool for making progress in this direction.

Organic acids

Main organic acids released from roots of axenic grown plant species are anions such as citrate, malate and fumarate and oxalate (Hocking, 2001; Jones, 1998), but quantitative determination of root-released organic acids by growing plants in soil still remains a major challenge. Addition of chemical forms of these acids, especially citrate, to soils enhanced the solubility of soil P from insoluble and adsorbed P fractions (Gerke, 1992). Review of the literature (Ryan et al., 2001) strongly suggests that organic acids exuded from roots can benefit the P nutrition of plants. The information on the role of the organic acids in P mobilisation and plant uptake has been mainly obtained using plant species such as white lupin (Johnson et al., 1996; Neumann et al., 1999) and rape (Hoffland et al., 1992). The variation in root release of organic acids in relation to P acquisition is rarely investigated between crop genotypes (Gahoonia et al., 2000; Gaume et al., 2001), which limits the possibilities of using root-released organic acids as selection cri-

teria and further dissection of genetic background of organic acids release in the rhizosphere.

There is scope of changing root exudation of organic acids through genetic engineering. De La Fuente et al. (1997) introduced a citrate synthase gene from *Pseudomonas aeruginosa* in tobacco and reported that it increased the root citrate efflux fourfold, which enhanced the ability of transgenic tobacco to acquire P from P-poor alkaline soils. Later Delhaize et al. (2001) engineered the same transgenic tobacco lines to produce 100-fold more *P.aeruginosa* citrate synthase protein. This had no effect on internal citrate concentration or its efflux in the rhizosphere. These conflicting findings have raised controversy about the usefulness of enhancing citrate synthase.

While assessing the role of organic acids for P acquisition, it should also be kept in mind that their release to the rhizosphere will also enhance the growth of microbial biomass (Jensen and Sørensen, 1994). The consumption of organic acids by the microorganisms might reduce their effectiveness in dissolving strongly bound P in rhizosphere soil. However, as long as there is net presence of organic acids (i.e. more produced than consumed) they will be useful in mobilising P from strongly bound P pools.

Perspectives of integrated approach

The existence of genotypic variation in P uptake and variable performance of crop genotypes in P limited-environment provide an opportunity for saving non-renewable P resources through selection and breeding of P efficient cultivars. Many P-stress adapted plant genotypes might have emerged by the natural evolutionary process and also through the unconscious breeding efforts in the past decades. To accelerate this process, the emerged genetic variability should be explored and then used in targeted plant breeding to tailor new crop genotypes for fitting them in P limiting soils. As P is mobilised from rhizosphere soil, favourable root traits like long root hairs and root-induced processes for dissolving bound soil P should be central for creating new crop genotypes. The intensity and expression of such traits often increases when P is limiting. Hence plant genotypes seem to have developed response mechanisms that help them to extract phosphorus more effectively from P-deficient soils. The specific processes involved in P efficiency also show that a basic understanding of plant–soil interactions in the rhizosphere will be necessary to develop mean-

ingful screening techniques for identification of crop genotypes adapted to low P conditions. The selection and breeding of P efficient genotypes may be a successful strategy for maintaining high yields at moderate P deficiency in soils.

Most benefits of breeding for P efficiency may be expected if P absorption and P dissolving root traits, as illustrated in Figure 3, are considered in an integrated manner, because search for a single trait will be a less efficient strategy. Phosphorus is found in soils as inorganic (Pi, mainly bound to Ca^{2+} , Al^{3+} , Fe^{3+} ,) and organic form (Po), of which nearly one half may be phytate (*myo*-inositol hexaphosphate) and its derivatives (Dalal, 1977). However, P enters into plant roots only as inorganic P, mainly as H_2PO_4^- and in a smaller amount as HPO_4^{2-} . To be useful in plant nutrition, the soil organic P esters must, therefore, undergo enzymatic hydrolysis, in soil solution outside the root cells, to form the orthophosphate anions, H_2PO_4^- or/and HPO_4^{2-} (Figure 3). The rate of hydrolysis may to be limited either by the availability of substrate (P-ester bonds) or the availability of phosphatase enzymes, or by the combined effect of both. Plant roots can release carboxylates, organic acids (Ryan et al., 2001), including citrate (Gahoonia et al., 2000), which has the potential to open the organic P ester complex into the rhizosphere soil solution (Figure 3; Gilbert et al., 1999). Other studies have shown (Asmar et al., 1995; Kandeler et al., 2002), that roots can also induce high activity of rhizosphere acid phosphatase (Apase in Figure 3) e.g. *orthophosphoricmonoester phosphorhydrolyases EC 3.1. 3.2*; including phytase, *myo-inositol hexakisphosphate phosphohydrolase EC 3.1. 3.8*, catalysing the hydrolysis of enzyme available P esters into Pi. Phytase possesses the potential to dephosphorylate phytate to yield Pi and *myo*-inositol.

Extracellular Apase and phytase activity may be associated to root and soil particles or dissolved in the soil solution of the rhizosphere (Asmar, 1997). A preliminary assessment of the ability of transgenic *Trifolium subterraneum* (transformed with phytase gene from *Aspergillus niger*) by Richardson et al. (2001), indicated that presence of extracellular phytase improved the P nutrition of plants when supplied with phytate and grown in agar under sterile conditions. It can be envisaged that crop varieties with long root hairs will inject both extracellular enzymes as well as citrate into rhizosphere soil at longer distance away from the root surface and induce the transformation of Po to Pi and plant uptake.

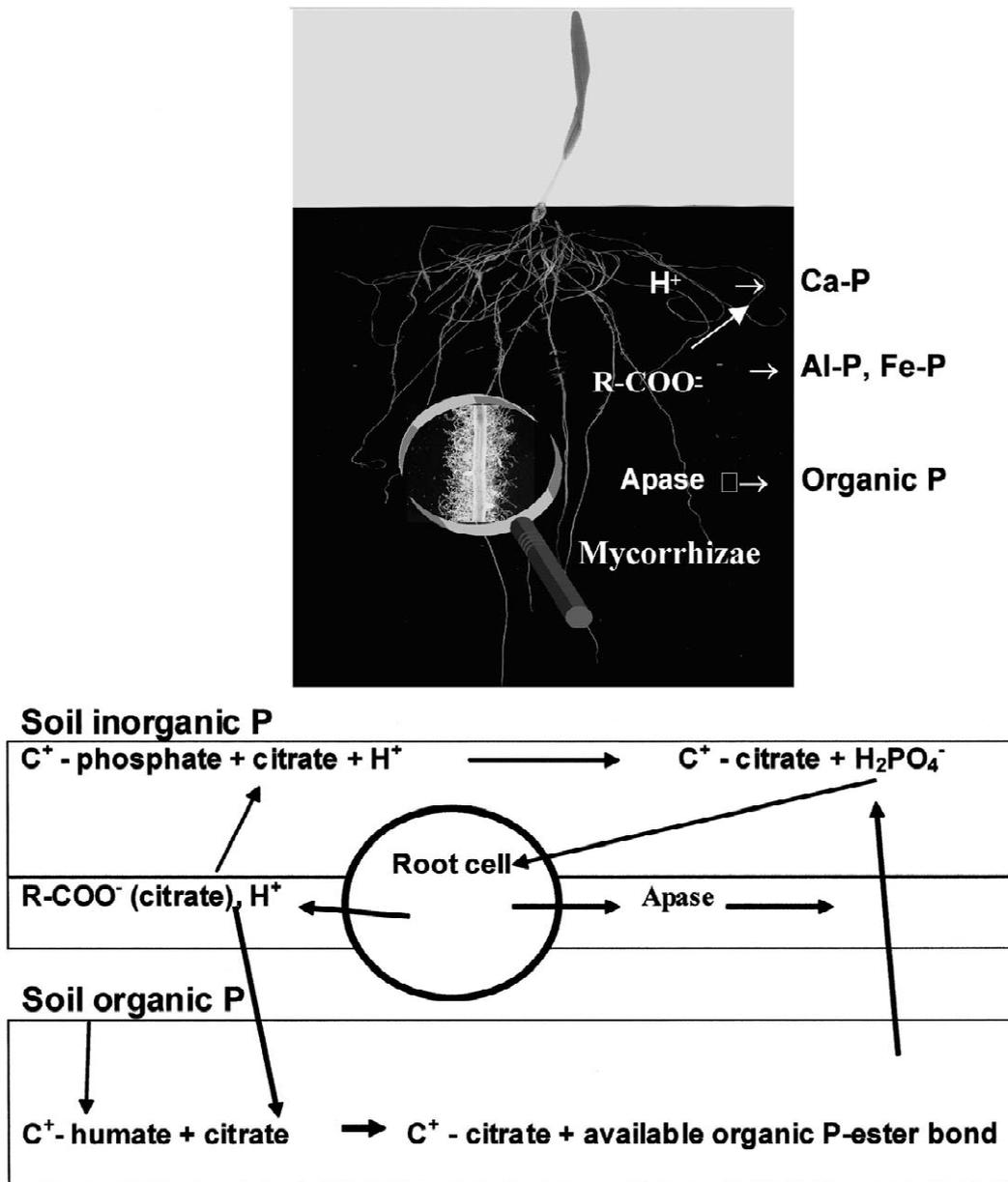


Figure 3. Illustration of important root traits mobilising soil phosphorus. C^+ denotes Ca^{2+} , Al^{3+} , Fe^{3+} , which form sparingly soluble compounds with inorganic phosphate and form strong complex with organic matter. H^+ causes rhizosphere acidification. $R-COO^-$ denotes carboxylate group, exemplified here with citrate. Apase denotes Acid phosphatase, which includes phytase.

In earlier studies (Avers, 1958; Dosier and Riopel, 1977) higher phosphatase activity was found in root epidermis cells (trichoblasts), which can differentiate into root hairs than in other (atrachoblasts) root cells. Our study also supported that root with root hairs may indeed have higher rhizosphere Apase activity (Gahoonia et al., 2001).

Variation in extracellular root phytase activity of barley genotypes is reported (Asmar, 1997) and wheat genotypes appear to differ in utilising phytate as P source (Osborne and Rengel, 2002). The fact that root hairs are part of the root and also simulation calculations (Hoffland, 1992) hint a link between the presence

of root hairs and organic acids in the rhizosphere, which has not been studied experimentally yet.

A wide variation in root hairs length of barley varieties exists and it was linked to stable grain yield in P-limited soil (Table 1). The varieties with longer and more root hairs may also have higher Apase activity, perhaps also higher release of organic acids into the rhizosphere. Therefore, they may possess the potential to utilise more of the soil inorganic well as organic P. It is envisaged that such knowledge will be of dual advantage for management of soil P in environmentally friendly agriculture. First, the presence of abundant root hairs expands the effective root surface area and thereby P uptake. Second, root hair release of citrate and Apase may enhance the transformation of Po into Pi, and utilisation of organic soil P within the root hair zone. Consequently, due to the synchronised and synlocalised liberation of P and its uptake and the acquisition efficiency is ultimately enhanced.

Acknowledgements

The financial support of the Danish Ministry of food, Agriculture and Fisheries, under the Research Programme: *Development of Future Crop Plants* is thankfully acknowledged. We thank the anonymous reviewers for their valuable comments. Thanks to Dr P Hinsinger, INRA, France, for his valuable suggestions for improving the manuscript.

References

- Abelson P H 1999 A potential phosphate crisis. *Science* 283 (5410), p. 2015.
- Alexander I J and Hardy K 1981 Surface phosphatase activity of Sitka spruce mycorrhizas from a serpentine soil. *Soil Biol. Biochem.* 13, 301–305.
- Armstrong R D and Helyar K R 1992 Changes in soil phosphate fractions in the rhizosphere of semi-arid pasture grasses. *Aust. J. Soil Res.* 30, 131–143.
- Asmar F, 1997 Variation in activity of root extracellular phytase between genotypes of barley. *Plant Soil* 195, 61–64.
- Asmar, F Gahoonia T S and Nielsen N E 1995 Barley genotypes differ in extracellular phosphatase activity and depletion of organic phosphorus from rhizosphere. *Plant Soil* 172, 117–122.
- Atkinson D 1985 Spatial and temporal aspects of root distribution as indicated by the use of a root observation laboratory. *In Ecological Interactions in Soil*. Eds. A H Fitter, D Atkinson, D J Read and M B Usher. (pp. 43–66). Blackwell, Oxford.
- Atkinson D 1991 Influence of root system morphology and development on the need for fertilizers and the efficiency of use. *In Plant Roots: The Hidden Half*. Eds. Y Waisel, A Eshel and U Kafkaki. (pp 411–451). Marcel Dekker, Inc. New York, Basel, Hongkong.
- Avers C J 1958 Histochemical localization of enzyme activity in root epidermis of *Phleum pratense*. *Am. J. Bot.* 45, 609–613.
- Barber S A 1995 *Soil Nutrient Bioavailability – A mechanistic approach*. 2nd Edition John Wiley & Sons, Inc. New York.
- Barbour W and Murphy C F 1984 Field evaluation of seedling root length selection in oats. *Crop Sci.* 24, 165–169.
- Barraclough P B 1984 The growth and activity of winter wheat roots in the field – Root growth of high yielding crops in relation to shoot growth. *J. Agric. Sci.* 103, 439–442.
- Bates T R and Lynch J P 2000 The efficiency of *Arabidopsis thaliana* (*Brassicaceae*) root hairs in phosphorus acquisition. *Am. J. Bot.* 87, 964–970.
- Bielecki R L and Johnson P N 1972 The external location of phosphatase activity in phosphorus deficient *Spirodela oligorrhiza*. *Aust. J. Biol. Sci.* 25, 707–720.
- Bouma T J, Nielsen K L and Koutstaal B 2000 Sample preparation and scanning protocol for computerised analysis of root length and diameter. *Plant Soil* 218, 185–196.
- Brundrett M, Bougher N, Dell B, Grove T and Malajczuk N 1996 Working with mycorrhizas in forestry and agriculture. Australian Center for International Agricultural Research, Canberra. ACIAR Monograph 32. 374 pp.
- Brück H H, Becker H C and Sattelmacher B 1992 Phosphate efficiencies of two maize inbred lines. *In Root Ecology and its Practical Applications*. 3rd. ISRR Symp., Wien. Eds. L Kutschera, E Hübl, E Lichtenegger, H Persson and M Sobotik (pp. 193–196).
- Brynes B H and Bumb B L 1998 Population growth, food production and nutrient requirements. *In Nutrient Use in Crop Production*. Ed. Z Rengel. Food Product Press, New York, London.
- Burleigh S H and Harrison M J 1998 Characterization of the *Mt4* gene from *Medicago truncatula*. *Gene* 216, 47–53.
- Caradus J 1979 Selection for root hair length in white clover (*Trifolium repens* L.). *Euphytica* 28, 489–494.
- Caradus J and Snaydon R W 1986 Plant factors influencing phosphorus uptake by white clover from solution culture. I. Population differences. *Plant Soil* 93, 153–163.
- Clark R B 1991 Physiology of cereals for mineral nutrient uptake, use and efficiency. *In Crops as Enhancers of Nutrient Use*. Eds. V C Baligar and R R Duncan. (pp. 131–183). Academic Press, Inc. San Diego, USA.
- Clarke J M and McCaig T N 1993 Breeding for efficient root systems. *In Plant Breeding: Principles and Prospects*. Eds. M D Hayward, Bosemark N O and Romagosa. Chapman and Hall, London.
- De la Fuente J M, Ramirez-Rodriguez V, Cabrera-ponce J L and Herrera-Estrella L 1997 Aluminium tolerance in transgenic plants by alternation of citrate synthesis. *Science* 276, 1566–68.
- Dalal R 1977 Soil organic phosphorus. *Adv. Agron.* 29, 83–117.
- Delhaize E, Hebb D M and Ryan P R 2001 Expression of a *Pseudomonas aeruginosa* citrate gene in tobacco is not associated with either enhanced citrate accumulation or efflux. *Plant Physiol.* 125, 2059–2067.
- Dosier L W and Riopel J L 1977 Differential enzyme activity during trichoblast differentiation in *Elodea canadensis*. *Am. J. Bot.* 64, 1049–1056.
- Ekanayake I J, O’Toole J C, Garrity J P and Masajo T M 1985 Inheritance of root characters and their relations to drought resistance in rice. *Crop Sci.* 25, 927–933.
- Fitter A H 1985 Functional significance of root morphology and root system architecture. *In Ecological Interactions in Soil*. Eds. A H Fitter, D Atkinson, D J Read and M B Usher. (pp. 87–106). Blackwell, Oxford.

- Föhse D, Claassen N and Jungk A 1991 Phosphorus efficiency of plants. Significance of root radius, root hairs and cation-anion balance for phosphorus influx in seven plant species. *Plant Soil* 132, 261–272.
- Gahoonia T S and Nielsen N E 1992 The effect of root induced pH changes on the depletion of inorganic and organic phosphorus in the rhizosphere. *Plant Soil* 143, 185–191.
- Gahoonia T S and Nielsen N E 1996 Variation in acquisition of soil phosphorus by wheat and barley genotypes. *Plant Soil* 178, 223–230.
- Gahoonia T S and Nielsen N E 1997 Variation in root hairs of barley cultivars doubled phosphorus uptake from soil. *Euphytica* 98, 177–182.
- Gahoonia T S and Nielsen N E 1998 Direct evidence on participation of root hairs in Phosphorus (^{32}P) uptake from soil. *Plant Soil* 198, 147–152.
- Gahoonia T S, Raza S and Nielsen N E 1994 Phosphorus depletion in the rhizosphere as influenced by soil moisture. *Plant Soil*, 159, 213–218.
- Gahoonia T S, Care D and Nielsen N E 1997 Root hairs and acquisition of phosphorus by wheat and barley cultivars. *Plant Soil* 191, 181–188.
- Gahoonia T S, Claassen N and Jungk A 1992 Mobilisation of phosphorus in different soils by ryegrass supplied with ammonium and nitrate. *Plant Soil* 143, 241–248.
- Gahoonia T S, Nielsen N E and Lyshede O B 1999 Phosphorus acquisition of cereal cultivars in the field at three levels of P fertilization. *Plant Soil* 211, 269–281.
- Gahoonia T S, Asmar F, Giese H, Nielsen G G and Nielsen N E 2000 Root released organic acids and phosphorus uptake of two barley cultivars in laboratory and field experiments. *Eur. J. Agron.* 12, 281–289.
- Gahoonia T S, Nielsen N E, Joshi P A and Jahoor A 2001 A root hairless barley mutant for elucidating genetics of root hairs and phosphorus uptake. *Plant Soil* 237, 211–219.
- Gaume A, Machler F, De Leon C, Narro L and Frossard E 2001 Low -P tolerance by maize (*Zea mays* L.) genotypes: Significance of root growth and organic acids and phosphatase root exudation. *Plant Soil* 228, 253–264.
- Gallany H T and Schumacher T E 1993 Combined use of colorimetric and microelectrode methods for evaluating rhizosphere pH. *Plant Soil* 154, 151–159.
- Gerke J 1992 Phosphate, aluminium and iron in the soil solution of three different soils in relation to varying concentrations of citric acid. *Z. Pflanzenernähr. Bodenkd.* 155, 339–343.
- Gilbert G A, Knight J D, Vance C P and Allan D L 1999 Acid phosphatase activity in phosphorus deficient white lupine roots. *Plant Cell Environ.* 22, 801–810.
- Graham J H and Eissenstat D M 1994 Host genotype and the formation and function of VA mycorrhizae. *Plant Soil* 159, 179–185.
- Grierson C S, Parker J S and Kemp A C 2001 *Arabidopsis* genes with roles in root hair development. *J. Plant Nutr. Soil Sci.* 164, 131–140.
- Grinsted M J, Hedley M J, Nye P H and White R E 1983 Plant induced changes in the rhizosphere of rape (*Brassica napus* var. Emerald) seedlings. I. pH change and the increase in P concentration in the soil solution. *New Phytol.* 91, 19–29.
- Harrison M J and Van Buuren M L 1995 A phosphate transporter from the mycorrhizal fungus *Glomus versiformae*. *Nature* 378, 626–629.
- Hedley M J, Nye P H and White R E 1982 Plant induced changes in the rhizosphere of rape (*Brassica napus* var. Emerald) seedlings. II. Origin of pH change. *New Phytol.* 91: 31–44.
- Hendrix J E 1967 The effect of pH on the uptake and accumulation of phosphate and sulphate ions by bean plants. *Am. J. Bot.* 54, 560–564.
- Hetrick B A D, Wilson G W T, Gill B S and Cox T S 1995 Chromosome location of mycorrhizal responsive genes in wheat. *Can. J. Bot.* 73, 891–897.
- Hetrick B A D 1991 Mycorrhizas and root architecture. *Experientia* 47, 355–362.
- Hinsinger P 1998 How do plant roots acquire mineral nutrients? Chemical processes involved in the rhizosphere. *Adv. Agron.* 64, 225–265.
- Hoad S P, Russell G, Lucas M E and Bingham I J 2001 The management of wheat, barley and oat root systems. *Adv. Agron.* 74, 193–246.
- Hocking P J 2001 Organic acids exuded from roots in phosphorus uptake and aluminium tolerance of plants in acid soils. *Adv. Agron.* 74, 63–97.
- Hoffland E 1992 Quantitative evaluation of the role of organic acid exudation in the mobilization of rock phosphate by rape. *Plant Soil* 140, 279–289.
- Hoffland E, van den Boogaard R, Nelemans J A and Findenegg G R 1992 Biosynthesis and root exudation of citric and malic acid in phosphate starved rape plants. *New Phytol.* 122, 675–680.
- Holford I C P 1997 Soil phosphorus, its measurements and its uptake by plants. *Aust. J. Soil Res.* 35, 227–239.
- Jakobsen I and Nielsen N E 1983 Vesicular Arbuscular Mycorrhiza in field-grown crops. I. Mycorrhizal infection in cereals and peas at various time Soil depths. *New Phytol.* 93, 401–413.
- Jensen L S and Sørensen J 1994 Microscale fumigation-extraction and substrate- induced respiration methods for measuring microbial biomass in barley rhizosphere. *Plant Soil* 162, 151–161.
- Johnson J F, Allan D L, Vance C P and Weiblen G 1996 Root carbon dioxide fixation by phosphorus-deficient *Lupinus albus*. Contribution to organic acid exudation by proteoid roots. *Plant Physiol.* 112, 19–30.
- Jones D L 1998 Organic acids in the rhizosphere- a critical review. *Plant Soil* 205, 25–44.
- Kandeler E, Marschner P, Tschirko D, Gahoonia T S and Nielsen N E 2002 Microbial Community Composition and Functional Diversity in the Rhizosphere of Maize. *Plant Soil* 238, 301–312.
- Kapulnik Y and Kushnir U 1991 Growth dependency of wild, primitive and modern cultivated wheat lines on vesicular-arbuscular mycorrhiza fungi. *Euphytica* 56, 27–36.
- Kothari S K, Marschner H and Römheld V 1990 Contribution of the VM mycorrhizal hyphae in acquisition of phosphorus and zinc by maize grown in a calcareous soil. *Plant Soil* 131, 177–185.
- Krasilnikoff G, Gahoonia T S and Nielsen N E 2001 Phosphorus uptake from sparingly available soil-P by cowpea (*Vigna unguiculata*) genotypes. In *Integrated Nutrient Management in Africa*. Eds. B Vanlauwe, J Diel, N Sanginga and R Merckx. CABI Publishing, Wallingford, UK.
- Leon J and Schwang K U 1992 Description and application of a screening method to determine root morphology traits of cereals cultivars. *Z. Acker. Pflanzenbau* 169, 128–134.
- Linjertho E, Kuikman P and Vanveen J A 1994 Carbon translocation to the rhizosphere of maize and wheat and influence on the turnover of native soil organic matter at different soil nitrogen levels. *Plant Soil* 161, 233–240.
- Lynch J P 1995 Root architecture and Plant productivity. *Plant Physiol.* 109, 7–13.
- Lyness A S 1936 Varietal differences in phosphorus feeding capacity of plants. *Plant Physiol.* 11, 665–683.

- Marschner H and Römheld V 1983 *In vivo* measurements of root induced pH change at soil-root interface. Effect of plant species and nitrogen source. *Z. Pflanzenphysiol.* 111, 241–251.
- Marschner H, Römheld V and Ossenberg-Neuhaus H 1982 Rapid method for measuring pH and reducing process along the root of intact plant. *Z. Pflanzenphysiol.* 105, 407–416.
- Neumann G, Massonneau A, Martinoa E and Römheld V 1999 Physiological adaptations to phosphorus deficiency during pro- teoid root development in white lupin. *Planta* 208, 373–382.
- Nielsen N E 1976 A transport kinetic concept of ion uptake by plants. III. Test of the concept by results from water culture and pot experiments. *Plant Soil* 45, 659–677.
- Nielsen N E 1983 Plant parameters controlling the efficiency of nutrient uptake from soil. *In Efficient Use of Fertilizers in Agri- culture. Developments in Plant Soil Sciences, Vol. 10, (pp 352). Martinus Nijhoff / or W. Junk pub.*
- Nielsen N E 1996 Bioavailability of Nutrients in Soil, p. 411–427. *In Roots and Nitrogen in Cropping Systems of the Semi-Arid Tropics.* Eds. O Ito, C Johansen, J J Adu-Gyamfi, K Katayama, J V D K Kumar Rao and T J Rego. International Crop Research Institute for the Semi-arid Tropics, India (ISBN 4-906635-01-6).
- Nielsen N E and Barber S A 1978 Differences among genotypes of corn in the kinetics of P uptake. *Agron. J.* 70, 695–698.
- Nielsen N E and Schjørring J K 1983 Efficiency and kinetics of phosphorus uptake from soil by various barley genotypes. *Plant Soil* 72, 225–230.
- Nye P H 1981 Changes of pH across the rhizosphere induced by roots. *Plant Soil* 61, 7–26.
- Osborne L D and Rengel Z 2002 Growth and P uptake by wheat genotypes supplied with phytate as the only P source. *Aust. J. Agri. Res.* 53, 845–850.
- O'Toole J C and Bland W L 1987 Genotypic variation in crop plant root systems. *Adv. Agron.* 41, 91–145.
- Pandey R 2001 Physiological Basis of Phosphorus Efficiency Differences in Wheat (*Triticum aestivum*). Ph.d Thesis. Indian Agriculture Research Institute, New Delhi.
- Persson H 1982 The importance of fine roots in boreal forests. *In Root Ecology and its Practical Applications. International Symposium at Gumpenstein, Austria.* (pp. 595–608).
- Porceddu E, Martignano F and Scaracia Mugnozza G T 1978 Interpopulation variation in coleoptile and primary root length in durum wheat. *In Proc. 5th Int. wheat Genet. Symp., Indian Society of Genetics and Plant breeding, New Delhi,* (pp. 852–860).
- Richardson A E 2001 Prospects of using soil microorganisms to improve the acquisition of phosphorus by plants. *Aust. J. Plant Physiol* 28: 897–906 2001.
- Richardson A E, Hadobas P A and Simpson R J 2001 Phytate as a source of phosphorus for the growth of transgenic *Trifolium subterraneum*. *In Plant Nutrition – Food Security and Sustainability of Agro-Ecosystems* Eds. W Horst et al. (pp. 560–561). Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Römer W J, Augustin J and Schilling G 1988 The relationship between phosphate absorption and root-length in nine wheat cultivars. *Plant Soil* 111, 199–201.
- Röhm M and Werner D 1987 Isolation of root hairs from seedlings of *Pisum sativum*. Identification of root hair specific proteins by *in situ* labelling. *Physiol. Plant.* 69, 129–136.
- Römheld V and Marschner H 1986 Mobilization of iron in the rhizo- sphere of different plant species. *In Advances in Plant Nutrition, Vol. 2.* Eds. B Tinker and A Läuchi. (pp. 155–204). Praeger Scientific, New York.
- Ryan P R, Delhaize E and Jones D L 2001 Function and mechanism of organic anion exudation from plant roots. *Annu. Rev. Plant Physiol.* 52, 527–560.
- Sanchez P A and Leakey R R B 1997 Land use transformation in Africa: Three determinants for balancing food security with natural resources utilization. *Eur. J. Agron.* 7, 15–23.
- Schiefelbein J W and Somerville C 1990 Genetic control of root hair development in *Arabidopsis thaliana*. *Plant Cell* 2, 235–243.
- Schjørring J K 1986 Nitrate and ammonium absorption by plants growing at a sufficient or insufficient level of phosphorus in nutrient solution. *Plant Soil* 91, 313–318.
- Smit A L, Bengough A G, Engels C, Noordwijk M van, Pellerin S and van de Geijn S C (Eds.) 2000. *Root Methods: A Handbook.* Springer Verlag, Heidelberg. 565 pp.
- Smith F 1934 Response of inbred lines and crosses in maize to vari- ation of nitrogen and phosphorus supplies as nutrients. *J. Am. Soc. Agron.* 26, 785–804.
- Smith S E and Read D J 1997 *Mycorrhizal Symbiosis.* 2nd Ed Academic Press London, 605 pp.
- Swinnen J 1994 Rhizodeposition and turnover of root-derived or- ganic material in barley and wheat under conventional and integrated management. *Agric. Ecosyst. Environ.* 51, 115–128.
- Tarafder J C and Jungk A 1987 Phosphatase activity in the rhizo- sphere and its relation to the depletion of organic phosphorus. *Biol. Fertil. Soils* 3, 199–204.
- Tilman D, Cassman K G, Matson P A, Naylor R and Polasky S 2002 Agricultural sustainability and intensive production practices. *Nature* 418, 671–677.
- Trull M C and Deikman J 1998 An *Arabidopsis* mutant missing one acid phosphatase isoform. *Planta* 206, 544–550.
- Turner B L, Paphazy M J, Haygarth P M and McKelvie I D 2002. Inositol phosphates in the environment. *Philos. Trans. Royal Soc. London series B-Biol. Sci.* 357, 449–469.
- Vance C P 2001 Symbiotic nitrogen fixation and phosphorus acquisi- tion. *Plant nutrition in a world of declining renewable resources.* *Plant Physiol.* 127, 390–397.
- 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 progenies on mid-parent. *Euphytica* 46, 211–215.
- Yadav R, Courtois B, Huang N and McLaren G 1997 Mapping genes controlling root morphology and root distribution in double- haploid population in rice. *Theor. Appl. Genet.* 94, 619–632.
- Yan X L, Lynch J and Beebe S 1995 Genetic variation for phos- phorus efficiency of common bean in contrasting soil types. I. Vegetative response. *Crop Sci.* 35, 1086–1093.
- Zhu Y-G, Smith S E, Barritt and Smith F A 2001 Phosphorus (P) efficiencies and mycorrhizal responsiveness of old and modern wheat cultivars. *Plant Soil* 237, 249–255.