



Prospects for using conventional techniques and molecular biological tools to enhance performance of ‘orphan’ crop plants on soils low in available phosphorus

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Abstract

Molecular biology, combined with Mendelian and quantitative genetics in quantitative trait locus (QTL) mapping and marker-assisted selection (MAS), provides powerful new tools to facilitate efficient genetic manipulation by plant breeders of complex traits such as drought tolerance and phosphorus (P) acquisition ability. This paper examines current opportunities for genetically manipulating the ability of crop plants to more efficiently acquire (i.e. access and take up) essential soil nutrients, using as examples P and several of the crops in the genetic improvement mandate of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) – chickpea (*Cicer arietinum* L.), groundnut (*Arachis hypogaea* L.), pearl millet [*Pennisetum glaucum* (L.) R. Br.], pigeonpea [*Cajanus cajan* (L.) Millsp.], and sorghum [*Sorghum bicolor* (L.) Moench]. It is concluded that for at least some of these important, but often academically and economically orphaned tropical food, forage and feed-grain crops, the genetic variation and molecular tools that we will need already exist or can be expected to become available in the very near future. With appropriate, targeted research, these tools can permit empirical exploration of the potential for marker-facilitated mapping and manipulation of major genes that can contribute to enhanced ability of these crops to acquire P from sources with limited availability. With these tools, delivery of new versions of currently popular high-yielding, high quality, disease resistant crop cultivars, having genetically improved ability to acquire P currently in soils but unavailable for crop growth, could take as little as five to seven years. Sustainable use of such improved cultivars would require their utilization as components of integrated soil fertility management systems.

Introduction

Sustainable increases in global production of food and feed-grain crops – both legumes and cereals – will increasingly require integration of improved cultivars and cultural practices to economically achieve improvements in crop productivity and in stability of this productivity. This can be brought about in favorable environments in part by increasing the amounts of soil nutrients available to crops by application of mineral

and organic fertilizers, and selection for short-statured crop genotypes that are more responsive to fertilization and less prone to lodging. However, high rates of fertilization in such environments can result in pollution of surface and sub-surface water supplies since much of the applied fertilizer nutrients are not taken up by the crop and instead leach into the subsoil or are carried away with soil particles in runoff water. In environments less favorable for agricultural production, moisture and soil toxicity constraints can interact very strongly with soil nutrient availability (Brück et al., 2000; Payne et al., 1995; Schaffert et al., 2000;

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Zaongo et al., 1994), sometimes making it difficult to obtain economic responses to individual fertilizers or other soil amendments (Subba Rao et al., 2000). Phosphorus (P) uptake by plants is reduced to near zero during water stress. The consequences of this on plant growth have been clearly outlined by Sinclair and Vadez (2000). The problem of drought stress is often complicated by soil fertility stress that impedes deep root development (e.g. Payne et al., 1995; Zaongo et al., 1994). This is a frequent occurrence and of particular concern across the tropics – from arid regions to sub-humid regions. Aluminium (Al) toxicity frequently occurs across the tropical acid soil savannas (Schaffert et al., 2000). A high level of aluminium saturation in the soil solution inhibits cell multiplication and division in growing roots, thereby greatly restricting root growth and consequently water and nutrient uptake. Many tropical soils, including those of the acid savannas in Africa and Latin America and of other regions, are low in available P and have a high potential for P fixation due to mineral compositions high in iron (Fe) and Al oxides (Weir, 1972, 1977). In these soils, P is one of the least available essential elements. In fact, although the total P present can be as high as in more productive soils having limited P fixation potential, P in these soils is often available only in micronutrient concentrations. These soil constraints, due to their nature, cannot be resolved completely by the simple application of irrigation water and other soil amendments. Enhancing crop growth under such conditions requires improving the ability of crop plants to acquire (i.e. gain access to and take up) nutrients from the soil, as there appears to be little opportunity to enhance efficiency of biomass production from a fixed amount of available nutrients (Sinclair and Vadez, 2000). It will be necessary to breed agronomically productive plant genotypes with the ability to increase the proportion of total soil nutrients available for crop growth. Fortunately, there appear to be exploitable levels of genetic variation in many crop species for root traits associated with superior ability to acquire nutrients that are growth limiting. Some plants with such root traits can perform close to their maximum capacity under conditions of low nutrient availability, and for this paper will be defined as being more nutrient efficient than plant genotypes requiring higher nutrient availability levels for near maximum productivity.

In both favorable and less favorable agricultural production environments, genetic improvements in the ability of crops to acquire soil nutrients – both naturally occurring and applied – will be critical to achiev-

ing sustainable increases in agricultural productivity. Genetic enhancement of root traits (Lynch, 1995), including root-induced changes in the chemistry of the rhizosphere that can enhance nutrient availability (Hinsinger, 1998), will be critical to improving crop acquisition of soil nutrients.

This paper will explore the potential to enhance the ability of crops to access sparingly available P sources – such as rock phosphate and that which is fixed by iron and aluminium oxides in heavily weathered tropical soils – using genetic variation already present in the germplasm collections of each of these crops and their wild relatives.

Importance of phosphorus in crop growth

The essential physiological roles of P in crop growth are reviewed in considerable detail by Sinclair and Vadez (2000), so only a brief summary is provided here. P is an integral component of the biochemical compounds that make life possible – playing essential structural, biochemical and physiological roles in crop growth. It is a component of nucleic acids and phospholipids, and plays an especially important role as the energy ‘currency’ of nucleotide-based metabolic pathways (that often involve membrane processes) in which energy storage and transfer occur. Stable cytoplasmic concentrations of inorganic P (Pi) often appear necessary for sustaining optimal levels of enzymatic activity. This requirement is normally met in plants by using vacuole-stored P as a buffer for cytoplasmic P concentrations. However, developing plant cells lack mature vacuoles, and hence a stored P reserve to permit growth under P-deficient conditions. Because of this, developing cells in growing meristems are particularly sensitive to P deficiency. Reduced rates and extents of leaf area expansion, and through this – and other processes – reduced rates of increase in individual plant photosynthetic capacity, can be the first and most dramatic responses of crops to P deficits. Of course, these responses have direct negative consequences for subsequent crop growth rates and biomass production via their effects on plant energy metabolism.

Options for increasing growth in low-phosphorus conditions

As the role of P in life processes appears to have been optimized during the course of evolution, there is

likely to be little opportunity for reducing the amount of it that is required for plant growth. Therefore, in order to increase plant productivity, ways must be sought to increase the amount of P available for plant growth. Conceptually, this can occur either by moving stored P to the most rapidly growing plant tissues, or accessing additional P from the soil. Opportunities for enhancing the mobility of P between plant parts appear to be limited as it is generally present as P_i or in mobile compounds that are readily available for transfer between plant parts (Sinclair and Vadez, 2000), so that phosphate harvest index is commonly quite high. Indeed, phosphate harvest indices are sufficiently high [≥ 0.6 in pearl millet (Wani et al., 1990) and ≥ 0.8 for some grain legumes] that the fraction of total crop plant P that is allocated to harvested portions cannot likely be increased substantially (see Inthapanya et al., 2000; Sinclair and Vadez, 2000). Apart from addition of P to the cropping system in the form of crop residues (Buerkert et al., 1998; Hafner et al., 1993; Ikpe et al., 1999), farmyard manure and/or livestock urine (Ikpe et al., 1999), rock phosphate and/or mineral fertilizers (Batiano et al., 2000; Brück et al., 2000; Ikpe et al., 1999; Inthapanya et al., 2000; Subbarao et al., 2000), it should be possible to improve the ability of plant roots to acquire P that is already present in soils but not currently accessible (Subbarao et al., 1997a,b). There appear to be several mechanisms by which plant roots could do this. These range from increasing the soil volume explored – effectively increasing root surface area by increasing root branching and/or length and/or enhancing symbiotic relationships with soil micro-organisms (Tinker, 1984) such as vesicular-arbuscular mycorrhizae (VAM) (Gavito and Miller, 1998; Kaeppler et al., 2000; Krishna et al., 1985; Li et al., 1991; Marschner and Dell, 1994; Smith et al., 1992) – to increasing the solubility of P that is normally sorbed to soil particles in the vicinity of root surfaces and hence unavailable for uptake by plants. Several of these mechanisms are dealt with in greater detail by others later in this publication.

How phosphorus is acquired by plants – the role of root exudates

Soluble P in the rhizosphere is readily recovered by plant roots (Hinsinger, 1998). However, P commonly occurs at very low concentrations in the soil solution. Most P present in tropical soils is found in soil organic matter, as a component of sparingly soluble phosphate

minerals, or sorbed to Fe and Al oxides (Hafner et al., 1993; Morris et al., 1992; Tiessen et al., 1993). Such forms of P, that are not soluble, are not directly available for uptake by roots.

Severe decreases in soil solution P concentration of the rhizosphere can shift the adsorption–desorption and dissolution–precipitation equilibria. However, because P sorption onto soil constituents is often poorly reversible, and most phosphate minerals in soils have low solubility, solution P concentrations must reach very low levels before significant equilibria shifts occur. Some plant species have very low solution P concentration requirements for near-maximum plant growth, as little as $0.1\text{--}0.5\ \mu\text{M}$ for P-efficient grasses (Breeze et al., 1984; Föhse et al., 1988). However, even for such species, the rate of desorption of soil P or the rate of dissolution of P-bearing soil constituents must be larger than the fluxes of P uptake if P availability is not to limit growth. Surprisingly, several studies (for a summary see Hinsinger, 1998) indicate that P sometimes accumulates in the rhizosphere rather than being depleted. In *Lolium*, proton excretion can serve as the driving force for root-induced dissolution of rock phosphate that proceeds at a faster rate than P uptake. Modeling results indicate that with diffusion of protons away from roots and diffusion of dissolved P towards the roots, P can be expected to be depleted in the immediately vicinity of the roots and accumulate a short distance away. Small amounts of root growth into this P-accumulation zone could then result in substantially increased amounts of P available for uptake. Efficient uptake of the mineral nutrient from soil solution then occurs, resulting in large changes in ionic concentrations that again shift the equilibria involved in soil nutrient dynamics. Thus P uptake itself is not only the ultimate stage of the nutrient acquisition process, but can be regarded as the crucial driving force that enhances the proportion of soil P available for plant growth (Hinsinger, 1998).

As dynamics of the various forms of P_i are strongly pH-dependent, pH of the rhizosphere (to an even greater degree than that of the bulk soil solution) is a critical factor that can influence whether roots have access to P present in the soil. Root-mediated pH changes are the most documented chemical changes that have been observed to occur in the rhizosphere (Hinsinger, 1998). Root-induced acidification of the rhizosphere occurs when plant roots compensate for an excess uptake of cations over anions from the soil solution by releasing excess positive charge in the form of protons. The ability of some plants to utilize

P supplied in the form of phosphate rock is related to their capacity for proton excretion, and proton excretion is stimulated in some species under conditions of P-deficiency. Therefore, proton excretion by plant roots can be regarded as an adaptive strategy for P acquisition (Hinsinger, 1998).

Conversely, phosphate ions are strongly sorbed to Fe- and Al-oxides in acid soils (Ae et al., 1990; Weir, 1972, 1977). The charge of these P-sorbing minerals is pH dependent. Hydroxyl or bicarbonate ions excreted into the rhizosphere by plant roots can desorb some phosphate from these soil minerals, rendering P available for uptake. Thus both increases and decreases in rhizosphere pH induced by roots can enhance P nutritional status of plants, depending on the forms of Pi that are predominant in the soil. However, the consequences of root excretion of protons or hydroxyls is probably much greater than that indicated by rhizosphere pH changes as the critical factor influencing acquisition of P by plant roots is the actually flux of proton or hydroxyl equivalents excreted at the soil-root interface and not the resulting rhizosphere pH change (Hinsinger, 1998).

Exudation of organic acids from roots of P-deficient seedlings has also occasionally been reported to result in pH changes in the rhizosphere. However, the more important role of such organic acid exudation may be for the resulting organic anions to complex with Al or Fe, resulting in increased solubilization of P-Al and P-Fe compounds. Citrate, malate and oxalate anions, which can be exuded by roots of several acid soil tolerant grass and cereal genotypes, appear to be especially effective in forming complexes with Al and hence facilitate access to Al-bound P (Bolan et al., 1994; Jones, 1998; Pellet et al., 1995, 1996), while piscidic acid and its derivatives exuded from pigeon pea roots enhance the ability of this legume to access Fe-bound P (Ae et al., 1990).

Finally, there are several additional roles that root exudates can play in P acquisition. Phosphatase and phytase ectoenzymes can play important roles in catalytic hydrolysis of organic P and subsequent acquisition of soil P by plant roots. Such ectoenzymes can be produced by root-associated microbes that feed on root exudates, or can be excreted into the rhizosphere by the roots themselves in the absence of microbes. Stimulation by P-deficiency of direct excretion of phosphatase by plant roots has been demonstrated, so release of such enzymes can be considered another adaptive response to nutrient stress. Further, exudation of high-molecular-weight polysaccharides (commonly

referred to as mucilage) can contribute to ion exchange capacity of root cell walls. In acid soils, Al can be detoxified by massive adsorption on mucilage, contributing to desorption of anions, such as phosphate, from soil particles (Hinsinger, 1998).

Thus, there is overwhelming evidence that associations of plant roots with VAM, and the exudates from plant roots, can contribute to significant increases in the supply of P to plant roots under conditions where only a limited portion of total soil-P is soluble. There are, therefore, two sets of target traits for assessment and possible utilization by plant breeders and physiologists. However, each set of traits is complex, and difficult to measure. Like other root-related traits, expression of phenotypic differences take place underground and out of sight of the breeder, so they will likely be expensive to assess and perhaps not possible to deal with in conventional plant breeding programs. Fortunately, there is a rapidly emerging set of tools suitable for dealing with traits such as these that are economically valuable but too complex, too difficult, too tedious and/or too expensive to accurately assess repeatedly on the large numbers of progenies required in an applied plant breeding program. These tools – quantitative trait locus (QTL) mapping and marker-assisted selection (MAS) – are the result of the melding together of Mendelian, molecular and quantitative genetics. They offer crop agronomists, breeders, nutritionists, physiologists, . . . , a new set of tools with which to effectively address complex target traits like enhanced drought tolerance or P-acquisition ability (Jones et al., 1997; Mohan et al., 1997; Prioul et al., 1997; Schneider et al., 1997). Conventional applied plant breeding approaches may also have potential (Bramel-Cox et al., 1991), but would require more cost-effective phenotypic screening procedures to permit repeated and accurate assessment of P-acquisition ability differences between large numbers of progenies.

QTL mapping and MAS

Molecular markers are rapidly being adopted by crop improvement researchers globally as an effective and appropriate tool for basic and applied studies addressing biological components in agricultural production systems (Jones et al., 1997; Mohan et al., 1997; Prioul et al., 1997). Molecular markers offer specific advantages in assessment of genetic diversity and in trait-specific crop improvement (Edwards, 1992;

Kochert, n.d.; Paterson et al., 1991). Use of markers in applied breeding programs can range from facilitating appropriate choice of parents for crosses, to mapping/tagging gene blocks associated with economically important traits (often termed QTLs) (Doerge et al., 1997). Gene tagging and QTL mapping in turn permit marker-assisted selection (MAS) in backcross (Frisch et al., 1999a,b; Ribaut et al., 1997), pedigree (Mohan et al., 1997), and population improvement programs. Plant numbers required to obtain the desired segregants can be readily predicted (Hash, 2000; Hash et al., 2000; Sedcole, 1977; Stam and Zeven, 1981), allowing well-structured plant breeding programs to be designed. MAS appears to be especially useful for crop traits that are otherwise difficult or impossible to deal with by conventional means (Moreau et al., 1998, 1999). The near-isogenic products of a marker-assisted backcrossing programme in turn provide genetic tools for crop physiologists and crop protection scientists to use in improving understanding the mechanisms of tolerance to various abiotic stresses such as extremes of temperature and water and/or nutrient availability (Jones et al., 1997; Prioul et al., 1997), in addition to mechanisms of resistance to biotic production constraints such as diseases, insect pests, nematodes, and parasitic weeds like *Striga*. QTL mapping of yield and quality components, and the components of other physiologically or biochemically complex pathways, can provide crop breeders with a better understanding of the basis for genetic correlations between economically important traits (linkage and/or pleiotropic relationships between gene blocks controlling associated traits; e.g. flowering time and biomass; inflorescence size and inflorescence number). This can facilitate more efficient incremental improvement of specific individual target traits like P-acquisition ability. Further, specific genomic regions associated with QTLs of large effect for one target trait can be identified having minimal effects on otherwise normally correlated traits, permitting an improvement in the first trait that need not be accompanied by counterbalancing reductions in others. Finally, these molecular marker tools can also be used in ways that allow more effective discovery and exploitation of the evolutionary relationships between organisms, through comparative genomics (Devos and Gale, 1997, 2000; Devos et al., 2000; Gale and Devos, 1998).

Opportunities

We would now like to switch gears, and look at opportunities to use these new genetic tools in ICRISAT mandate crops and related species, to identify and manipulate gene blocks contributing to within-species differences in P-acquisition ability, building on the recent examples of Wissuwa et al. (1998) in rice (*Oryza sativa* L.) and Kaeppler et al. (2000) in maize (*Zea mays* L.). In order for such an approach to be successful, we first require heritable genetic variation for the trait(s) of interest, effective screening procedures for efficient detection of these genetic differences (at least once, and under conditions that ultimately are relevant to farmers' fields in the breeding programme's target environment), adequate levels of marker polymorphism, and potential parents of mapping populations that differ in the both the trait(s) of interest (in economically important levels) and in marker genotype (at least in the vicinity of gene blocks contributing to the traits of interest. Therefore each of these is examined in turn.

Availability of genetic variability

There are considerable numbers of publications reporting genetic variability for various traits demonstrated to have, or with circumstantial evidence for, association with enhanced P-acquisition ability (Ae et al., 1990; Graham and Eissenstat, 1994; Kaeppler et al., 2000; Lynch, 1995; Mucchal et al., 1996; Pellet et al., 1995, 1996; Raghothama, 1999; Ryan et al., 1995; Subbarao et al., 1997a,b; Wani et al., 1990; Wissuwa et al., 1998). It appears that at least some genotypes of groundnut, pigeonpea and rice have the ability to access and take up Fe- and/or Al-bound P (Otani and Ae, 1996b; Subbarao et al., 1997a,b). Genotypic differences have been detected between pigeonpea cultivars for P solubilizing activity of root exudates (Ishikawa et al., 2000; Subbarao et al., 1997a). Similarly, substantial genetic differences have been detected for root growth in chickpea (Ali et al., 2000) and pearl millet (Krishna et al., 1985), and for both P-use efficiency (Batiano et al., 2000; Wani et al., 1990) and response to mycorrhizal colonization (Krishna et al., 1985) in pearl millet. In some cases (e.g. Kaeppler et al., 2000; Wissuwa et al., 1998), QTLs associated with enhanced P uptake have already been mapped.

Availability of reliable screening procedures for accurate phenotyping

Reliable screening procedures for phosphorus uptake in controlled conditions are now available (Kaeppler et al., 2000; Otani and Ae, 1996a,b; Subbarao et al., 1997a,b). Similarly, a system permitting rapid assessment of root volume on large numbers of plants under field conditions has recently been described (van Beem et al., 1998) and is now being evaluated for improving drought tolerance in maize (Bänziger et al., 2000; Mugo et al., 1999). Additional procedures that could be used in phenotypic characterization of factors contributing to genetic variation in P-acquisition ability are described by others in this publication. Among this set of procedures, we believe there will undoubtedly be some that can be used to assess genetic variation in P-acquisition ability that is relevant to the specific causes of non-availability of P in a particular target environment, and can be used on large enough numbers of mapping progenies, with high enough heritability, to permit QTL detection in mapping populations that are segregating for the trait and having adequate marker polymorphism, as has already been done for rice (Wissuwa et al., 1998) and maize (Kaeppler et al., 2000; Reiter et al., 1991).

Availability of molecular biology tools

Extensive research in recent years has demonstrated genomic synteny across the grasses (Devos and Gale, 1997, 2000; Gale and Devos, 1998; Van Deynze et al., 1998). Once suitable mapping populations for P acquisition and related target traits are identified or developed in pearl millet and sorghum, we can begin by attempting marker saturation in genomic regions related to those in which QTLs for P uptake have already been detected in rice (Wissuwa et al., 1998) and maize (Kaeppler et al., 2000). For pearl millet, we already have available a short genetic linkage map based on RFLP and STS markers (Devos et al., 1995; Liu et al., 1994), and this has recently been tied to the linkage maps of foxtail millet and rice (Devos et al., 2000) and hence other well-studied cereals and forage grasses. Levels of marker polymorphism in pearl millet are very high, even between elite inbred parental lines of hybrids adapted to India. Unfortunately, we do not yet have large sets of STMS or other micro-satellite-based markers, but these are under development (Katrien Devos, pers. comm.). In the case of sorghum, several molecular-marker based genetic linkage maps are available (Peng et al., 1999; Subudhi and Nguyen,

2000). Although the level of marker polymorphism in sorghum appears to be much less than that in pearl millet, it should be adequate as more STMS and other micro-satellite-based markers become publicly available over the next couple of years (Bhatramakki et al., 2000; Boivin et al., 1999; Brown et al., 1996; Kong et al., 2000; Tamarino et al., 1997). The required molecular tools for the legumes in ICRISAT's genetic enhancement mandate are currently under development. In case of chickpea, STMS markers that have recently been developed at the University of Frankfurt (Hüttel et al., 1999; Winter et al., 1999) are now permitting detection of polymorphism where little could be detected with previously available molecular marker systems (Jagdish Kumar, pers. comm.). Similar PCR-compatible co-dominant marker systems based on simple sequence repeats are currently under development for pigeonpea in a DFID-supported collaboration between ICRISAT and the University of Birmingham (Paula Bramel, pers. comm.), but still need to be developed for groundnut (SL Dwivedi, pers. comm.).

Current availability of mapping populations

Pearl millet

Several pearl millet mapping populations of moderate size (120–275 progenies) have been developed as sets of F₄ progeny bulks and their F₃ testcrosses derived from individual skeleton-mapped F₂ plants (Hash and Bramel-Cox, 2000; Hash and Witcombe, 1994). These now involve some 10 pairs of genetically diverse inbred lines, of Asian, African and American origin, selected for QTL mapping of disease resistances (Jones et al., 1995), abiotic stress tolerances (Howarth et al., 1997; Yadav et al., 1999, 2000), grain and stover yield and quality components (Hash and Bramel-Cox, 2000; Hash et al., 2001) and morphological markers. Several of these populations have parents of contrasting Indian and West African origin (e.g. PT 732B × P 1449-2; H 77/833-2 × PRLT 2/89-33; 841B × 863B; and W 504 × P 310-17) that could be expected to differ in their ability to be colonized by VAM (Graham and Eissenstat, 1994; Krishna et al., 1985). All ICRISAT pearl millet mapping population parental lines have recently been testcrossed to a set of four genetically and phenotypically diverse elite male-sterile lines to allow ready assessment of differences for such additional secondary target traits as P-acquisition ability in both inbred and hybrid form. Originally conceived as a quick way to assess opportunities for using existing

skeleton-mapped populations for mapping components of stover yield and quality (Hash et al., 2002), this line \times tester set should also permit rapid evaluation of the potential for using these populations to map QTLs for many other traits including components of improved P acquisition.

Sorghum

Several skeleton-mapped sorghum random inbred line (RIL) sets are also available, with ICRISAT and several other agencies globally. However, the array of tropically adapted genotypes in these is not so broad as those used in developing the pearl millet mapping populations, and the degree of marker polymorphism in these is markedly less than that commonly detected in pearl millet. Thus, there is a need to increase the diversity of tropically-adapted sorghums to be included in future mapping populations (see sorghum recommendations in Ribaut and Poland, 2000).

Groundnut

Adequate mapping populations derived from crosses of cultivars are not yet available due to the low levels of marker polymorphism in cultivated groundnut; however, the cross of a cultivated tetraploid with an artificial allotetraploid (developed from an interspecific cross of two wild diploids) which shows good levels of RFLP marker polymorphism and linkage map development is underway at the University of Georgia (A. Paterson, pers. comm.). There are also sets of progenies derived from backcross introgression of segments from wild relatives into cultivated groundnut (Garcia et al., 1995; Nalini Mallikarjuna, pers. comm.) that could be screened for both phenotypic and marker polymorphism (Tanksley and McCouch, 1997).

Chickpea

In chickpea, several mapping populations have been developed, including derivatives of both cultivated \times wild and cultivated \times cultivated crosses (Jagdish Kumar, pers. comm.). These are available in the form of RIL sets. RAPD and STMS markers are being used to develop a genetic linkage map that can readily be used for skeleton map transfer to populations based on other crosses. New mapping populations developed with drought tolerance as a primary target trait might also reveal useful differences in P-acquisition ability as one of the drought-tolerance mechanisms being pursued is increased root length and root volume. It is also likely that differences exist for production of acidic

root exudates (e.g. Ali et al., 2000; Rengel, 2000a) and for ability to establish successful associations with VAM (Graham and Eissenstat, 1994; Rengel, 2000b), although screening of mapping populations and their parents for these traits in P-limited growing conditions has not yet been taken up in this species.

Pigeonpea

Mapping populations are not yet available for this crop, but are under development (Paula Bramel, pers. comm.).

An aside: Negative consequences of improving phosphorus uptake ability?

Some will express concern that crop genotypes that are more efficient at acquiring P might be less responsive to applied P and, therefore, at least initially, contribute to abuse of the soil ecosystem by farmers using this as an excuse to mine soil P rather than replenish that which is removed with each harvest. However, Buah et al. (1998) have observed that specific selection for high N-use efficiency in grain sorghums did not diminish responsiveness to applied N, and Akin-toye et al. (1999) report similar results from maize. In case of improved P-use efficiency proposed here (to be achieved via improved P-acquisition ability), mechanisms based on altered root architecture (rooting depth, degree of branching, intensity of root hair development, ...) and root exudates appear unlikely to be associated with reductions in responsiveness to applied P. However, for mycorrhizae-based mechanisms of improved P acquisition, it is likely that at least in some crop \times VAM combinations these might not be expressed when higher levels of applied P were available (Kaepler et al., 2000; Koide and Schreiner, 1992), and/or in years of limited soil moisture availability (Hafner et al., 1993). Some host plant genotypes having superior ability to establish associations with mycorrhizae under low-P conditions might indeed be less responsive to applied P than those lacking this mechanism of tolerance to low P-availability. This potential lack of responsiveness is likely to be less problematic under conditions, such as those in the Sahel, where subsistence food crops like pearl millet will for the foreseeable future be receiving at most a modest application of sparingly soluble rock phosphate, or more often, being used to harvest residual P fertility following fertilization of a prior cash crop such as cotton (*Gossypium* sp.) or groundnut.

A more troubling concern is the observation by Buerkert et al. (1998) that increased P uptake (as a result of application of P or crop residues) had negative effects on the nutritional quality of pearl millet grain produced in the Sahel. The first observation that increased grain yield as a result of increased P uptake was accompanied by a reduction in grain protein content, is not unexpected. As levels of the limiting nutrient for crop growth and grain yield (in this case P) are raised, other factors (nutrients, water, light, ...) are expected to become limiting. This could be overcome to some degree by accompanying the P application with a modest dose of N (in organic or inorganic form). In any case, any increase in grain availability as a result of increased P uptake will directly contribute to an increase in the availability of carbohydrates (and thereby energy) for consumption by people and their livestock. Human diets that are more protein-deficient are unlikely to result from such changes provided that total per capita caloric intake increases, although the possibility of micronutrient deficiencies can not be ruled out (Welch and Graham, 1999, 2000).

The observation that increased P uptake leads to an accumulation of the anti-nutritional factor phytate (Buerkert et al., 1998), is also less alarming than it might initially appear. This is so despite the fact that in most crops, the vast majority of P present in grain is stored in the form of phytate. Phytate levels are strongly dependent on P fertilization and the total P concentration and it is therefore unlikely to be possible to increase P uptake without some increase in grain phytate concentration (except under carefully controlled laboratory conditions where water, light and nutrient availability might be carefully balanced). Appropriate non-lethal low phytate mutants, such as that recently reported in rice (Larson et al., 2000), could provide a genetic solution, but are not yet available in the orphan crops in ICRISAT's genetic improvement mandate. However, the primary reason that increased phytate accumulation should not be considered alarming is that milling, fermentation, and other traditional steps in preparation of food from grains high in phytate can all greatly reduce the levels of this anti-nutritional factor (Abdalla et al., 1998a,b; Kheterpaul and Chauhan, 1991; Simwemba et al., 1984).

Conclusions

From the above review of the literature, it appears that for at least some of the academically and economically orphaned tropical food and feed grain crops in the crop improvement mandate of ICRISAT, the genetic variation and molecular tools already exist that can permit plant breeders to enhance the P-acquisition component of efficiency in low-nutrient environments, in a manner similar to that demonstrated for drought tolerance in common bean by Schneider et al. (1997). Where not yet already in place, these genetic tools can be expected to become available in the very near future. Therefore, it is our belief that with appropriate, targeted research these tools can permit empirical exploration of the potential for marker-facilitated mapping and manipulation of major genes that can contribute to enhanced P-acquisition ability of these crops. With these tools, delivery of new versions of currently popular high-yielding, high quality, disease resistant crop cultivars, having genetically improved ability to acquire P currently in soils but unavailable for crop growth, could take as little as 5 – 7 years. Of course, sustainable use of such improved cultivars would require that they be used as components of integrated soil fertility management systems.

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