

Review

Integrating molecular tools with conventional breeding strategies for improving phosphorus acquisition by legume crops in acid soils of Sub-Saharan Africa

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Leguminous crops are key components of low input agricultural cropping systems, and play an important role in ensuring food security in many societies in Sub-Saharan Africa (SSA). However, legume crop productivity in SSA is frequently limited by mineral nutrient deficiencies (particularly phosphorus, P). A common remedy for P deficiency is the application of P-fertilizers or in the case of low input cropping systems the reliance on symbiotic relations between crops and beneficial soil bacteria (rhizobia) and fungi (mycorrhizas). More recently, identification of legume species and genotypes with high efficiencies of P uptake and P use has been the focus of improvement programs using conventional breeding techniques. Due to inherent time limitations in conventional breeding approaches, progress in improving legume P uptake and P use efficiencies has been slow. Advances in attaining this goal could be by integrating molecular tools with conventional improvement strategies. A consideration of molecular and physiological mechanisms underlying differences in P uptake and P use efficiencies can result in more precise targeting of genetic variation and improvement through marker-assisted selection and other conventional techniques. This article discusses the potential for improving legume crop P uptake and P use efficiency in low-P, acid soils of SSA by integrating physiological and genomic tools, with conventional crop improvement in acid soils.

Key words: Legume, phosphorus uptake, breeding, comparative genomics, crop improvement, Sub-Saharan Africa.

INTRODUCTION

Land degradation and soil fertility depletion are among the major causes of low agricultural productivity which in turn leads to food insecurity and natural resource destruction in many parts of Sub-Saharan Africa (SSA) (Sanchez, 2010). These factors have important economic and social implications, and are responsible for the gap between food production and population growth in SSA, as well as low productivity leading to poor nutrition and health. Therefore, a significant investment in advanced

technologies that allow crop plants to adapt to inherent soil limitations to productivity as a complement to the conventional approach of adapting the production environment (soil) to meet crop requirements are needed to sustain and improve yields. This approach is particularly relevant in the smallholder cropping systems that dominate food production in SSA. Intercropping with legume crops is a key feature of these smallholder cropping systems, and legume crops play an important role not only in the overall productivity of these systems, but also in human health and nutrition (Kamanga et al., 2010).

Fabaceae (Legume family) is one of the most species-rich and economically important plant families because

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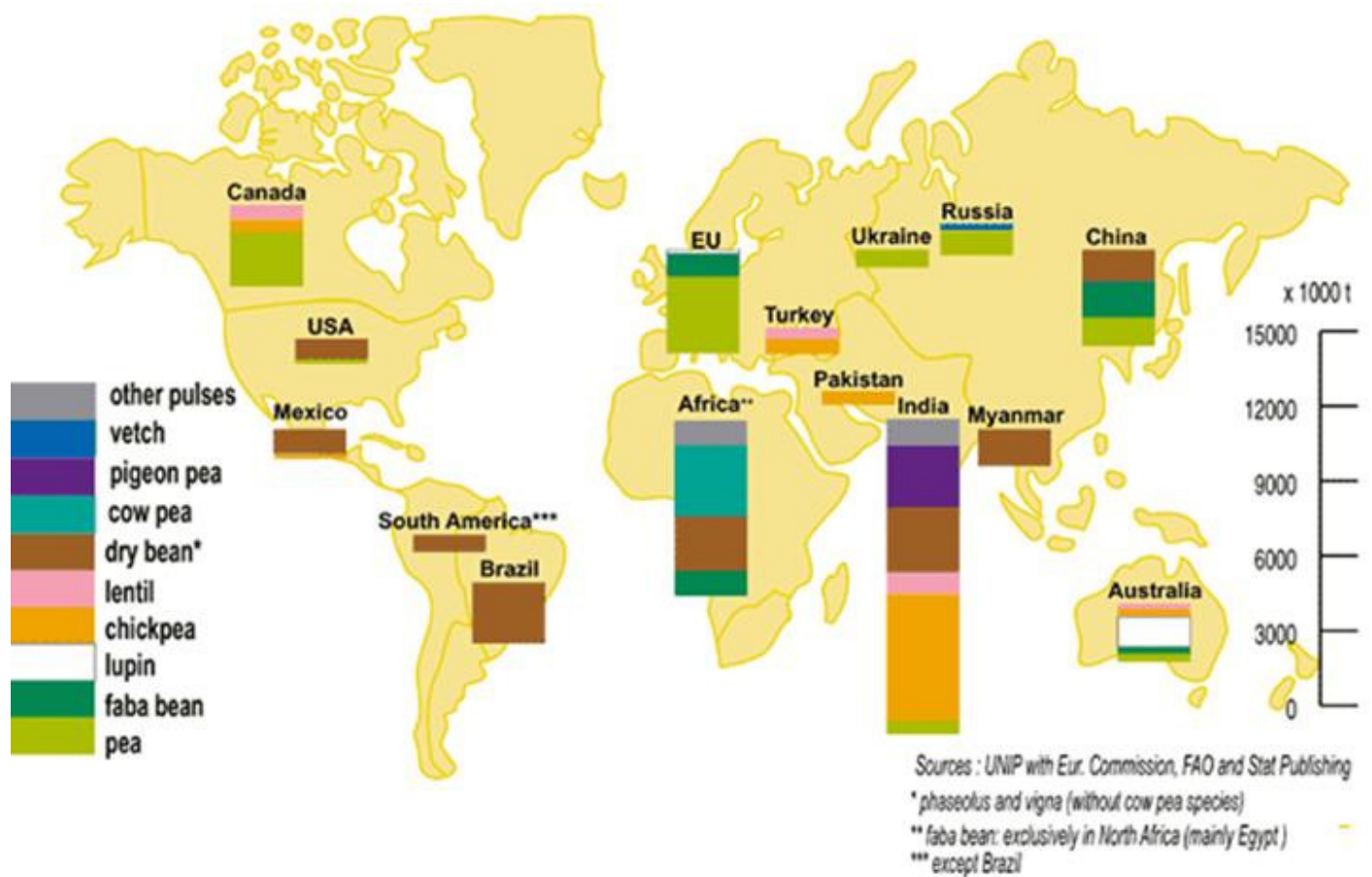


Figure 1. Market and consumption of grain legumes in the world. There are some grain legumes like cowpea that are produced uniquely in Africa.

they are an important source of food, fodder, energy and cover crops. Some of the most important commercial legume species in SSA (Figure 1) include the common bean (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*), soybeans (*Glycine max*), peas (*Pisum sativum*), groundnut (peanut, *Arachis hypogaea*), and alfalfa (*Medicago sativa*). Their ability to fix atmospheric nitrogen (N) makes them key components of low-input cropping systems as a sustainable approach for improving soil N status (Ghosh et al., 2006). In this regard, they are ideal crops in intercropping production systems. Legume seeds and foliage also constitute an inexpensive and, in some cases, the only source of high-quality proteins in many low-income societies (Vance et al., 2000; Schneider, 2002; Xinshen et al., 2003).

However, in most parts of SSA, and the tropics in general, low phosphorus (P) availability is a major constraint for legume crop establishment, nitrogen fixation, growth, and productivity (Hague et al., 1986; Sanchez et al., 1997; Bouhmouch et al., 2005; Sanchez, 2010). Unlike N, which some crops can obtain through

symbiotic biological fixation, P must be supplied from mainly commercial fertilizers, plant and animal manures, industrial and domestic wastes, and soil parent materials (Brady and Weil, 2001; Havlin et al., 2005). Highly weathered acid soils that fix large quantities of P dominate arable lands in SSA (Table 1, Scherr, 1999), and include Aridisols (36.8%), Alfisols (21.0%), Ultisols and Oxisols (22.6%), Entisols (14.1%), while Inceptisols and Vertisols cover ca. 2.5 and 2.1%, respectively (Hague et al., 1986; Aubert and Tavernier, 1972). The widespread P deficiency in these soils is evidenced by the positive crop response to fertilizer P additions (Hague et al., 1986; Smit et al., 2009). However, in many instances, most of the applied P is rapidly fixed by Fe- and Al-oxides in these soils, or bound in sparingly soluble P pools not immediately for plant uptake (Sample et al., 1980). Additionally, soil erosion and P removal in harvested crops also contribute to the P deficiency problem. Maintaining a sufficient supply of plant available P in arable lands is, therefore, a key prerequisite for optimal productivity and quality.

Table 1. Global estimates of soil degradation in agricultural land (from Scherr, 1999).

Agricultural land (million hectares)	Region							World
	Africa	Asia	South America	Central America	North America	Europe	Oceania	
Total	187	536	142	38	236	287	49	1475
Degraded	121	206	64	28	63	72	8	562
Percent	65	38	45	74	26	25	16	38

In Africa, highly weathered acid soils that fix large quantities of Phosphorus dominate arable lands.

Low soil solubility and mobility of P, and high P sorption in soils make plant available P, usually as H_2PO_4^- or HPO_4^{2-} orthophosphate ion (Marschner, 1995; Havlin et al., 2005) one of the major limitations to plant growth. In most arable soils, as well as in nutrient-poor grassland and forest soils, over 70% of phosphate is present in highly unavailable organic forms (Macklon et al., 1994) and only about 25% of the P applied in fertilizers in tropical soils is recovered by crops (Baligar and Bennett, 1986). Because of low available P in many soils, plants have evolved a variety of P uptake and utilization strategies including: (1) formation of mycorrhizal associations between roots and symbiotic fungi to increase soil exploration and uptake of water and immobile nutrients, notably P, (2) increasing the length and density of root hairs to increase the effective absorptive area of roots, and reduce the diffusive pathway distance for P to reach the root surface, (3) modifications in root architecture and branching patterns to thoroughly explore the soil, (4) exudation of organic acids, H^+ and phosphatases to solubilize and release organic and inorganic P from the soil, and (5) increasing the concentration of phosphate transporters in the root cell plasma membranes (Kochian, 2000; Lambers et al., 1998; Marschner, 1995; Havlin et al., 2005). These varied mechanisms for increase P uptake by plants have led to genetic variation among species for P uptake and P use efficiency. Such variation presents a unique opportunity for breeding programs to further improve on these traits and thereby ensure sustainable productivity of many cropping systems in P-deficient soils.

Farmers in SSA routinely incorporate legume crops in farming systems as an intercrop or in rotation to improve associated crop yields (Jemo et al., 2006; Wendt and Atemkeng, 2004; Niang et al., 2002). Until recently, little attention had been given to the P nutrition of legume crops despite the pivotal role that P plays in legume crop growth and productivity. In an intercrop study on low P soils including maize and Faba bean, Li et al. (2007) used permeable and impermeable root barriers to demonstrate that superior yields of intercropped maize compared to monocropping resulted from its uptake of P mobilized by acidification of the rhizosphere through Faba bean root release of organic acids and protons.

These enhancements in P nutrition seem to be greater when symbiotic associations between crop and microbes exist. For instance, Jemo et al. (2007) demonstrated that inoculation of *Macuna* (*Macuna pruriens*) with Bradyrhizobia and arbuscular mycorrhizal fungi on acid soils significantly increased P-uptake and P-use efficiency. Numerous recent studies have now confirmed that useful genetic variation for P uptake and P-use exists among various economically important legumes species. For instance, large genotypic differences P acquisition, P-use efficiency and important traits for P uptake (root length and root hair densities) have recently been reported among cowpea genotypes (Krasilnikoff et al., 2003; Saidou et al., 2007; Abaidoo et al., 2007). Similarly, genotypic differences in P acquisition and P-use efficiency have also been demonstrated among pigeonpea [*Cajanus cajan* (L.) Millsp.] genotypes, with short-duration genotypes accumulating more P, producing more total dry matter, and producing more dry matter per unit of absorbed P than the medium- and long-duration genotypes (Vesterager et al., 2006). These authors also reported positive correlations between P uptake and P-use efficiency. These findings of considerable genetic variability in P uptake-efficiency among legume genotypes have made it possible for conventional legume improvement programs to select and breed for this important trait as a means to improve productivity. Modern molecular techniques such as marker-assisted selection (MAS) can greatly expedite this process and ultimately assist in narrowing the gap between food production and demand in SSA.

Only recently, have researchers begun to use molecular genetics as a priority tool to enhance P uptake (Muchhal et al., 1996) with biochemical, physiological, and morphological plant response to P stress the subject of many recent reviews (Vance et al., 2003). This review presents opportunities, gaps and potentials of integrating molecular biological tools with traditional crop improvement efforts, to understand and exploit underlying genetic mechanisms of P-deficiency adaptation for food legume improvement in acid soils in SSA. We have therefore: (1) highlighted the importance of P in legume production, its deficiency, and strategies to improve availability in acid soils (2) elaborated on the importance

of microbial P turnover in preventing P sorption and enhancing P availability in acid soils (3) surveyed the literature on molecular biological tools and materials important in breeding legumes for adaptation to low-P (4) presented a brief overview of plant biology research carried out on some model legumes [common bean (*P. vulgaris* L.), barrel medic (*M. truncatula* Gaertna) and white lupine (*Lupinus albus*)] with respect to P-stress adaptation and (5) concluded with a future outlook. This summary will enable legume plant breeders to use QTL markers in selecting and developing P-efficient legume plants, which would in turn, benefit low-input agricultural systems and enhance environmentally friendly cropping in intensively cultivated systems.

PHOSPHORUS AVAILABILITY IN SSA SOILS

A predominance of high activity Al and Fe in most SSA soils is a result of excessive weathering of basic cations (Ca and Mg) due to frequent heavy rainfall that usually exceeds evapotranspiration, with a resultant drop in soil pH commonly below 5.5. Among the trio of major plant nutrients, P is commonly the most limiting in these soils (Buehler et al., 2002), existing in inorganic and organic forms. Because of its particular chemistry, orthophosphate (HPO_4^{2-} , H_2PO_4^-) which is the preferred inorganic form taken up by plants reacts readily and forms relatively low soluble, high energy bonds with positively charged Al and Fe at low pH and Ca at high pH (Kideok and Kubicki, 2004). Even when total soil P may be high, >80% still exists in unavailable forms to plants (Rengel and Marschner, 2005). Rhizosphere pH is further reduced when N-fixing legumes take up cations and excrete H^+ from roots to maintain internal electroneutrality. Therefore since legume nodules are sinks for P with a concentration higher than that of other organs and require much ATP for nitrogenase functioning (Gniazdowska et al., 1998) different strategies have to be employed to enhance P uptake and use efficiency (Vance et al., 2003).

PLANT STRATEGIES FOR IMPROVING P-AVAILABILITY IN ACID SOILS

A variety of adaptive strategies to improve P acquisition and use involve changes in root morphology and architecture (Yan et al., 2004; Beebe et al., 2006; Ochoa et al., 2006) root proliferation and elongation (Bates and Lynch, 2000) as well as changes in shoot and flower development (Bucciarelli et al., 2006). Some plants may excrete hydrogen ions (H^+) to acidify the rhizosphere which could result from surplus uptake of nutrient cations or rather, from light induced photosynthesis observed

with cowpea seedlings (Rao et al., 2002). Phosphorus-stressed plants also tend to allocate a greater proportion of biomass to root dry matter compared to P-sufficient plants (López-Bucio et al., 2003). Some P-stressed plants exude organic acids (Wang et al., 2007) and phosphatase enzymes (Tomasi et al., 2008) into the rhizosphere, or produce cluster roots (Lynch and Brown, 2001) enhance symbiotic relationships with soil organisms (Brundrett, 2002) such as vesicular-arbuscular mycorrhizas (VAM) (Kaeppeler et al., 2000). But at what cost are root exudation and root development for example, to the overall yield of the legume crop? For inherently P deficient or P depleted soils, excretion of organic ions can significantly reduce plant yields costing up to 70% of the C source (Johnson et al., 1996a). Kuzyakov and Domanski (2000 and 2002) reported that of the total C allocated below-ground, 7 to 13% is ultimately found in roots, 2 to 5% exuded and 7 to 14% used up in root respiration for maintenance, root growth and ion uptake. This is an important consideration in selecting P adaptive traits because P stress and sugar signaling are related (Müller et al., 2007) whereby, a proportionate increase in the amount of sugars is translocated to roots (Hernández et al., 2007) despite reduced demand of photosynthate and higher sugar levels (Morcuende et al., 2007).

MICROBIAL P TURNOVER

Albeit methodological challenges for assessing microbial turnover of soil organic P, the importance of biological process in enhancing soil P availability to crops, in subsistence farming especially, is well documented (Tiessen and Shang, 1998; Buehler et al., 2002; Oberson and Joner, 2005; Steffens et al., 2009). For example, the incorporation of P into microbial cells prevents P sorption thereby maintaining it in easily mineralizable form. Since food legumes supply large amounts of organic residues above and below ground, and organic matter boosts soil microbial activity, the microbiologically-driven processes in soil P dynamics are enhanced, and the microbial P pool increased in legume cropping systems. However, as more genes regulating arbuscular mycorrhizal fungi (AMF) and plant P uptake remain uncharacterized (Tesfaye et al., 2007), there is still a paucity of research findings to determine that mycorrhization of legumes significantly contributes to P uptake. Yan et al. (2006) showed no variation in P uptake amongst mycorrhizal and non-mycorrhizal soybean genotypes. However, breeding legumes to establish symbiotic relationships with Rhizobia and AMF in low pH soils, for the purpose of enhancing P uptake in SSA, has the dual advantage of enhancing soil N fertility following the formation of N-fixing root nodules of host plant roots.

In acid soils, nodulation of a range of pasture and crop

legumes (e.g. subclover, lucerne, white clover, pea, cowpea, bean, etc.) is reduced, mainly because of sensitivity of early nodulation events, such as attachment, root hair curling and initiation of infection thread formation (Vlassak and Vanderleyden, 1997). *Nod* gene expression, more notable in acid-sensitive than in acid-tolerant rhizobial strains, may account for these deleterious acidity-related effects (McKay and Djordjevic, 1993). Several of the genetically identified symbiotic genes have been cloned (Geurts et al., 2005) facilitated by the development of model systems (*Medicago* and *Lotus*) for which efficient molecular tools became available. These genetic tools have facilitated the cloning of orthologous genes from pea and soybean (Kaló et al., 2005). Improvements in the symbiotic relationship between rhizobia and legumes may be brought about by introducing the association of legume-rhizobia and plant growth-promoting rhizobacteria, legume-rhizobia and helper bacteria or legume-rhizobia and “arbuscular mycorrhizas” AM. Legume-AM symbiosis is an exquisite, highly regulated interaction between the legume host and AM, requiring coordinated expression of genes from two vastly different organisms. A complete review of AM symbiosis is beyond the scope of this paper, but can be referenced to several important and recent reviews (Kuster et al., 2007; Oldroyd et al., 2005). With advances in the development of molecular tools, genes responding to AM can be characterized, with the ultimate aim of identifying genetic strategies that regulate AM symbioses and P acquisition.

MOLECULAR TOOLS AND PLANT BREEDING

Conventional phenotypic selection for specific traits requires the evaluation of the trait from multiple environments over several years; this is often very expensive, time consuming, and labor intensive (Yuan et al., 2002). On the other hand, molecular marker technology is a powerful tool for selecting specific traits (Babu et al., 2003). Recently, a number of studies on quantitative trait loci (QTL) analysis relating root morphology and physiology to P nutrition in plants were reported (Beebe et al., 2006; Li et al., 2007; Chen et al., 2009; Cichy et al., 2009; Li et al., 2009). The first QTL descriptive analysis began by relating P stress to root weight in field-grown maize (Reiter et al., 1991) and subsequently to root architecture traits such as root hair length, and lateral root branching and length (Zhu et al., 2005a, b). Meanwhile, QTL analysis of root traits and P efficiency in legumes started much later.

Quantitative trait loci 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; for example, 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. It has been shown in maize and common beans, that the root architecture is closely related to the crop's P-acquisition efficiency under various P levels (Lynch, 2001; Rubio et al., 2003; Zhu et al., 2005a, b). The studies with recombinant inbred lines (RILs) of maize and common beans identified QTLs affecting root development under P-stressed condition (Zhu et al., 2005; Beebe et al., 2006; Ochoa et al., 2006; Zhu et al., 2006). These findings provide a foundation for molecular marker development in breeding to develop new varieties with enhanced tolerance to P-stressed conditions. Therefore, RILs provide a powerful and useful genetic tool for mapping and marker development required for the selection of a new variety with the improved genetic traits of interest (Zhuang et al., 2002; Andaya and Mackill, 2003; Liu et al., 2008). The RILs are a population of homozygotes generated by a cross between two inbred parental lines followed by repeated selfing or crossing between progenies (Broman, 2005). Each RIL is a new inbred line and harbors a unique mosaic combination of two parental chromosomes which is a useful source for the genetic mapping (Broman, 2005).

Doubled Haploid (DH) technology allows breeders to generate a population of homozygous progenies in a single generation from heterozygous parents thus saving cost, and time needed for the generation of genetic materials for further analysis such as genetic mapping (Baenziger et al., 1989; Murigneux et al., 1993; Smith et al., 2008). The technology is often designed to segregate for several traits of importance at the same time and superior individuals from the populations progressed in breeding programs where developed markers can be applied directly. Double Haploidy lines can be generated either by anthers culture or by crossing with other species whose genomes are excluded from the embryos followed by chemical treatment to duplicate the haploid genome (Collins and Sadasivaiah, 1972; Devaux, 1988; Wan et al., 1989; Devaux et al., 1993; Thomas et al., 1997). This process results in a complete pair of homozygous genomes in each DH line in a single generation, which is not only more efficient but also reliable and predictable than conventional self producing segregating progeny (Choo et al., 1985; Bernardo, 2003; Bonnet et al., 2005; Smith et al., 2008). To accelerate variety breeding in order to meet consumer demands, the use of DH technology and molecular markers in practical breeding has been promoted for varied agronomic and end-use quality traits in several crops including barley, wheat, rapeseed, oat and rye (Manninen et al., 2004; Marwede et al., 2004; Tuvešson et al., 2007; Amar et al., 2008).

Because the practical use of markers is not evident in all crops due to limited access to trait-linked markers, there is a global call for collaboration and technology transfer in the improvement of DH protocols in recalcitrant crops.

The functional genomics approach such as transcriptomics and metabolomics provides opportunities to discover gene(s) whose expression level change against biotic and abiotic stress. The RNA interference (RNAi) approach that abolishes or reduces the expression level of a target gene at the RNA level is useful to identify the function of the identified target gene (Miki and Shimamoto, 2004; McGinnis et al., 2005; Li et al., 2006). Hernandez et al. (2007) investigated the changes of transcripts under the P stress condition and discovered genes whose expression levels are up- or down-regulated. The functionality of those genes for the P efficiency can be investigated by RNAi that adopts an intrinsic cellular surveillance system that protects an organism from the invasive or parasitic genetic materials such as virus and transposable elements Hannon, 2002). In addition, the functional genomics approach can be used to develop gene-specific molecular markers applicable to the selection of a new variety with improved P efficiency.

As molecular genetics advances, a vast amount of molecular data will be available for breeders to incorporate into the conventional breeding program. The use of molecular genetic information together with MAS will improve the breeding program especially for the crop traits that are difficult to improve by conventional approach (Dekkers and Hospital, 2002). Furthermore, 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 et al., 2000).

INFORMATION AND MATERIAL RESOURCES

The completion of the *Arabidopsis thaliana* genome sequence in 2002 (Arabidopsis Genome Initiative, 2000) followed by progress on genome sequencing for rice in 2002 (Yu et al., 2002) caused much excitement among researchers. These landmark efforts were followed by advances in characterization of genomes of other crops including corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), soybean, and barril medic (Ware et al., 2002; Lunde et al., 2003; Young et al., 2003). There is therefore a rich library of research information that allows plant researchers to explore new paradigms to address fundamental and practical questions in a multidisciplinary manner. The new genetic tools for studying abiotic stress tolerance implored by the International Centre for Tropical Agriculture and the International Centre for Research in the Semi-Arid Tropics present opportunities

to identify and manipulate gene blocks contributing to within-species differences building on the examples of drought tolerance in bean (Broughton et al., 2003) and P-acquisition ability (Kaeppler et al., 2000) in maize, respectively. The success (Figure 2) of these approaches requires (1) heritable genetic variation for the trait of interest (2) 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 program's target environment) (3) adequate levels of marker polymorphism, and (4) potential parents of mapping populations that differ in 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). In what follows, genetic variation for P-stress tolerance and effective screening procedures are further discussed.

P-stress tolerance variability

Abounding research has established an association of genetic variability for various traits with enhanced P-acquisition ability albeit some circumstantial evidence (Kaeppler et al., 2000; Subbarao et al., 1997a, b; Wissuwa et al., 1998). Genotypic differences have been detected between pigeon pea cultivars for producing P solubilizing root exudates (Ishikawa et al., 2002; Subbarao et al., 1997a) and some between genotypes of groundnut and pigeon pea with apparent relative abilities to access and take up Fe- and/or Al-bound P (Subbarao et al., 1997a, b). Similarly, substantial genetic differences have been detected for root growth in chickpea (Ali et al., 2002) and pearl millet (Krishna et al., 1985), as well as for both P-use efficiency (Bationo et al., 2002) and response to mycorrhizal colonization (Krishna et al., 1985) in pearl millet. A reverse genetic tool, TILLING (Targeted Induced Local Lesions In Genomes), has been developed (Perry et al., 2003) that allows the identification of all alleles of a gene of interest from large ethylmethane sulfonate-mutagenized populations (McCallum et al., 2000). The identification of alleles with a weak or wild type-like phenotype can then be used, in addition to the knockout phenotype, to obtain insight into the function of a gene of interest. In some cases (e.g. Kaeppler et al., 2000; Wissuwa et al., 1998) QTLs associated with enhanced P uptake have already been mapped.

Available and reliable screening procedures for accurate phenotyping

There exist reliable screening procedures for P uptake in controlled conditions (Kaeppler et al., 2000; Subbarao et al., 1997a, b). Similarly, a system permitting rapid assessment of root volume on large numbers of plants

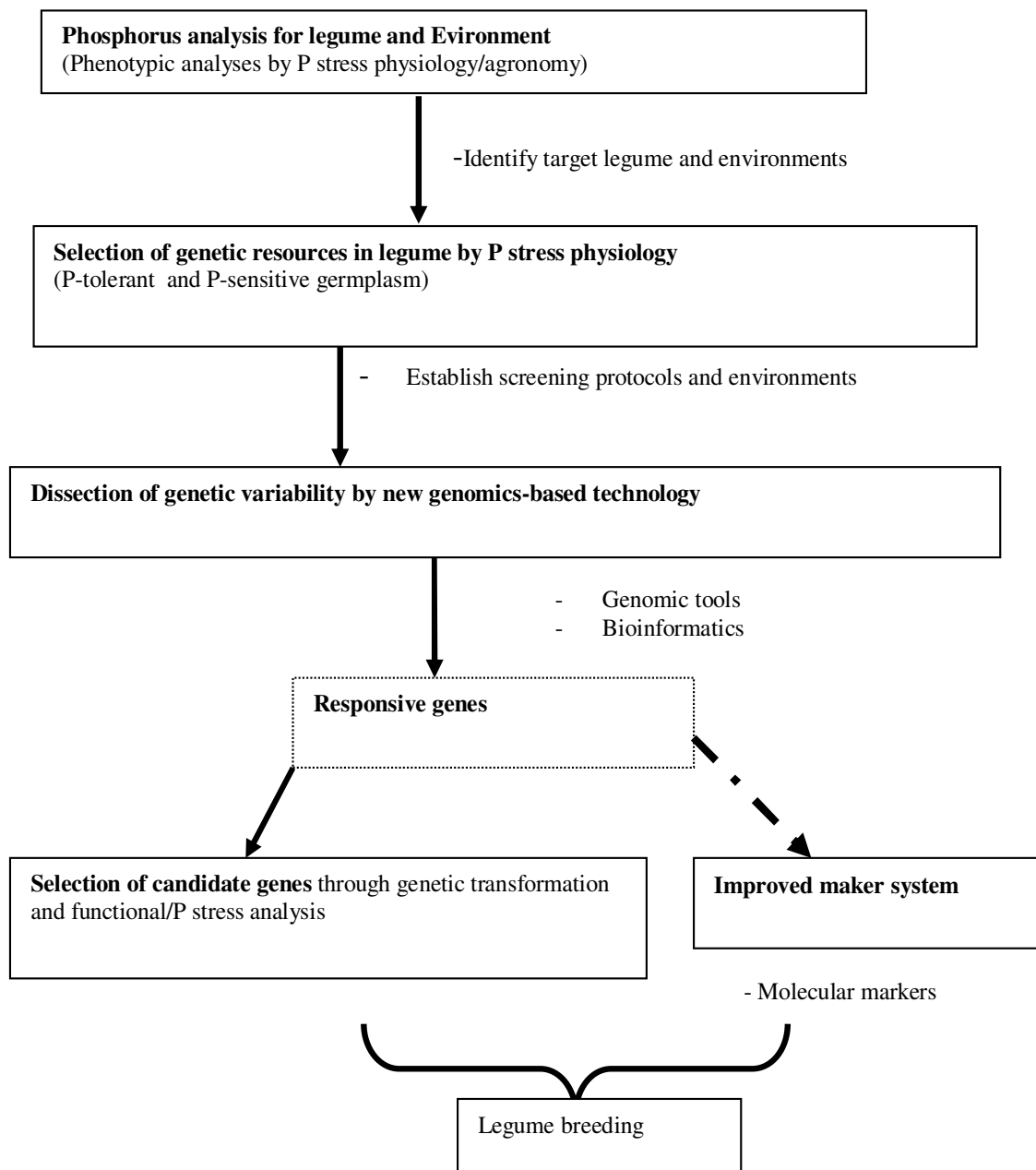


Figure 2. The top-down (phenotype to gene) approach for developing P- stress tolerance in food legumes. Research carried out on the physiology and agronomy of food legumes permits the analysis of P-stress and selection of promising genetic material. This research combined with genomic tools leads to subsequent identification of candidate genes for P-stress tolerance in food legume that can be exploited by legume breeders.

under field conditions has been described (Van Beem et al., 1998) and evaluated for improving drought tolerance in maize (Mugo et al., 1999). Additional procedures that could be used in phenotypic characterization of factors contributing to genetic variation in P-acquisition exist. Among this set of procedures, it is evident that some can be used to assess genetic variation in P-acquisition.

Others may be relevant to the specific causes of non-availability of P in a particular target environment, 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

(Kaeppeler et al., 2000).

MODEL LEGUME RESEARCH ON P-STRESS ADAPTATION

Phosphorus stress studies on legumes have mainly focused on, common bean, white lupine, and to a lesser extent barrel medic, and soybean (*G. max*). In what follows, a brief review on plant biology research related to P-stress adaptation is presented for common bean, white lupine and barrel medic.

Common bean *P. vulgaris* L

Common bean, the most important food legume worldwide has information on genetic variability for the capacity to produce grain in low soil P conditions (Broughton et al., 2003; Ochoa et al., 2006). The genetics of inheritable traits conferring low soil P tolerance in bean has also been reported in Africa (Kimani et al., 2007). For promising genomics approaches, the ability to transform a crop is the preferred method for providing “proof of concept” (Meagher, 2002; Wang et al., 2003) since transformation allows confirmation of the function of candidate genes. For crops such as common bean, where a reliable transformation system is lacking, the ‘proof of concept’ approach cannot be applied directly, and testing has to be conducted in other species that have high transformation efficiencies. If tests are successful, the candidate gene(s) can be transferred using conventional breeding methods combined with MAS.

An account of bean improvement from classical to molecular breeding for both abiotic and biotic stresses has been provided by Miklas et al. (2006). In addition, the QTL identification approach is now used to analyze P stress tolerance and adaptation in common bean and *Arabidopsis* (Beebe et al., 2006; Ochoa et al., 2006). By employing the composite interval mapping approach, Beebe et al. (2006) used RILs from a cross between two bean genotypes with contrasting total P accumulation in low P conditions, to identify QTLs for P accumulation and associated root architectural traits in common bean. They found a total of 26 individual QTLs. The P accumulation QTLs often coincided with QTLs for basal root development, indicating that basal roots appear to be important in P acquisition. Similarly, Ochoa et al. (2006) generated RILs from a cross of two common bean accessions with contrasting root architecture traits for adventitious roots and identified 19 QTLs for adventitious root formation, screening 86 F5:7 RILs under P stress and P-sufficient conditions. Furthermore, QTL analysis applied to RIL of a cross of G19833 and DOR 364 common beans showed that root hair formation and root

organic acid exudation are important traits for marker-assisted selection and breeding of P stress tolerance and adaptation (Yan et al., 2004). Moreover, several thousand Expressed Sequence Tags (ESTs) derived from P-stressed common bean roots have been characterized (Ramírez et al., 2005) and a limited number (575) is registered in the NCBI database: <http://www.ncbi.nlm.nih.gov/dbEST>. It is also worth mentioning that Hernández et al. (2007) have completed a P stress root transcriptome survey in common bean, identifying some 125 genes responsive to P stress. This information will go a long way to facilitate genetic improvement for low P adaptation in low-input agricultural systems.

Barrel medic (*M. truncatula*)

Bucciarelli et al. (2006) reported that barrel medic, a model legume for plant biology research responds to soil P deficiency by delaying (1) leaf development and leaf expansion along the main and axillary shoots; (2) axillary shoot emergence and elongation, resulting in stunted plants; and (3) timing and frequency of flower emergence. It was also observed that P-stressed barrel medic formed shorter petioles and shorter blade lengths relative to plants in P-sufficient conditions. These morphological changes require more supporting evidence to attribute overall delay in whole plant development as a P stress response. However, the plastic nature of plant morphological traits (Beebe et al., 2006; Ochoa et al., 2006) coupled with the lack of a standardized approach to describe plant growth and phenotypic responses to P stress (Bucciarelli et al., 2006), makes result comparisons from different laboratories difficult. Changes in root architecture are often associated with plant adaptation to P stress. However, Bucciarelli et al. (2006) reported no root architecture differences between barrel medic plants grown under P-sufficient and P-deficient conditions until 28 days after planting, when lateral root length and number of P-limited plants showed a decline. On the contrary, alfalfa (*M. sativa*) roots show changes in architecture when grown under P stress. Genetic regulation of root architecture changes due to P stress within and among species is not fully understood and thus, offers a fruitful area of emphasis for future research. According to Tesfaye et al. (2007), the sequencing of the genomes of *Medicago* and lotus will soon be completed.

Given the conserved synteny among legume genomes, using positional cloning, it should be possible to identify specific genes that contribute to QTLs affecting adaptation to P stress in *Medicago*. Phytochromes have also been proven to play a role in legume adaptation to P stress. It is noteworthy that barrel medic, bean and lupine plants exposed to P stress have increased density and length of root hairs. Some 40 genes are suggested to be

involved in root hair development (Grierson et al. 2001). Amongst these, a key gene, 1-aminocyclopropane-1-carboxylic acid oxidase, in ethylene biosynthesis is over represented in the ESTs derived from P-stressed roots of *Medicago*, lupine and bean (Graham et al., 2006). This indicates that ethylene production and/or plant responsiveness to ethylene plays a role in root adaptation to P deficiency.

White lupine (*Lupinus albus*)

White lupine is a non-mycorrhizal species that adapts to P deficient soil. Lupine displays a highly synchronous suite of molecular and biochemical adaptations to P stress by developing proteoid (cluster) roots, increasing organic acid exudation, and enhancing the expression of many genes, such as secreted acid phosphatase (LaSAP1) and Pi transporters LaPT1 (Uhde-Stone et al., 2003a, b; Vance et al., 2003,). A recent bioinformatic analysis of legume gene indices (*Medicago*, *Glycine*, *Phaseolus*, and *Lupinus*) queried for genes overrepresented in P-stressed tissue revealed the annotation of several putative transcription factor genes, including WRKY, MYB, and zinc finger family of genes (Graham et al., 2006). In addition, leaves and root tissues showed non-overlapping sets of transcription factor genes (Wu et al., 2003). Tesfaye et al. (2007) have also observed the PHR1 imperfect palindromic consensus sequence motif within the 5' upstream region of many P stress-induced genes, including the white lupine LaPT1 and LaSAP1 (Liu et al., 2001; Müller et al., 2007). Also noteworthy is that contigs encoding orthologs of PHR1 occur in the common bean, barrel medic, and soybean gene indices. Auxin (principally indole-3-acetic acid) has been implicated in the regulation of many aspects of plant growth and root development, including P stress-induced proteoid (cluster) root development (Gilbert et al., 2000). More so, exogenous application of auxin to P-sufficient white lupine mimics proteoid cluster root formation as seen under P-deficient conditions (Gilbert et al. 2000). Auxin transport inhibitors added to P-deficient plants dramatically reduced the formation of cluster roots. Phosphorus deficiency stress is also known to stimulate ethylene production in lupine and other plant species (Gilbert et al. 2000), and *Arabidopsis* (Ma et al., 2003). Stimulation of ethylene production results in an increase in root hair density and length (Grierson et al., 2001; López-Bucio et al., 2003) characteristic of lupine plants exposed to P stress. These results strongly suggest that cluster root development in response to P deficiency in white lupine is controlled by auxin and ethylene availability. The role of cytokinins in root growth and P deficiency stress is not resolved. In P-stressed lupine proteoid roots, CKX gene expression showed a 3- to 5-fold increase in expression (Vance et al., 2003). Moreover, application of cytokinin to P-deficient white

lupine inhibits proteoid root formation, and kinetin content is increased in proteoid roots (Neumann et al., 2000). Aloni et al. (2006) have proposed a mechanism for lateral root initiation in P-sufficient plants that involves the interaction of auxin, cytokinin, and ethylene. They propose that factors that modulate root tip cytokinin production allow ethylene and auxin to increase at lateral root initiation sites, giving rise to new laterals.

CONCLUSION

Legume contribution of soil nutrients in cropping systems is known as well as legume production constraints especially in acid soils where Al toxicity and P deficiency are common. With the availability of a wide array of genomic and bioinformatic research platforms, P stress research is advancing toward an exciting phase geared towards signal transduction, regulation of developmental plasticity, gene function, and increased efficiency of use. Information from the literature indicates that for some food legumes, genetic variation and molecular tools already exist that can permit plant breeders to enhance the P-acquisition component of efficiency in low-nutrient environments. Where not yet already in place, these genetic tools can be expected to become available in the very near future. To promote sustainable agriculture, plant breeders are expected to identify mechanisms in plants that improve P acquisition and exploit these P stress adaptations to produce plants that rely on low energy consumption, and are efficient in acquiring and utilizing soil P. Most importantly, comparative genomics utilizing the integrated information from different plants is expected to provide a common language to aid knowledge transfer among different species, knowing well that improving soil P availability in legumes enhances the practice of economical and environmentally friendly agriculture.

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