Enhancing Phosphorus Uptake and Use Efficiency in Wheat for Sustainable Crop Production – Management and Genetic Options

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Abstract

Wheat, the second major cultivated food crop worldwide requires phosphorus (P) for its physiological and biochemical accomplishments. Phosphorus, a key element in the nutrient supply chain realizes a potential yield of wheat crop. Enhancement of P acquisition and utilization by plants is critical for economic, humanitarian and ecological reasons (Vance et al., 2003). Conservation strategies for P utilization include: development of P efficient cultivars and integrated application of inorganic P sources through highly soluble fertilizers coupled with biofertilizers such as arbuscular mycorrhizal fungi, and rhizobacteria like *Azotobacter*, which in turn will help in solubilizing phosphorus in rhizosphere and promote its uptake by plants.

In a study conducted earlier, thirty wheat genotypes were classified into eight different groups on the basis of their grain yield performance and P uptake which proved useful in identifying varieties suitable for cultivation in different soil P regimes and selection of parents for recombination breeding to develop P efficient cultivars. It was concluded that inter-mating between varieties belonging to HGY-HP (PBW 343 and WH 711) and HGY-LP (Raj 3765 and WH 283) would further expand genotypic variability and thus the frequency of recombinants exhibiting different grain yield and P uptake levels.

Background

Wheat, the second most cultivated crop worldwide provides 21 percent of the food calorie and 20 percent of the protein for more than 4.5 billion people in 94 developing countries (Braun et al., 2010). Phosphorus is a key substrate in energy metabolism in the form of ATP, biosynthesis of nucleic acid in the form of sugar-phosphate backbone and membranes in the form of a phospholipid bilayer. Thus, required to sustain plant life, P affects wheat growth and development throughout the season. An adequate amount of P improves wheat seedling establishment, encourages early season development of adventurous roots, improves winter hardiness and facilitates greater nitrogen uptake which results in higher grain protein.

Abundant P results in early proliferation of tillers which increases biomass and grain yield potential (Behl et al., 2005). Also, wheat with adequate P matures earlier and more uniformly. The uneven development in a global economy has contributed to the uneven distribution of P in agricultural soils of the world. Generous application of high-grade phosphorus fertilizers, following more than half a century, agricultural soils in Europe and North America now said to have surpassed critical phosphorus levels and thus requires light application to replace what is lost in the harvest (FAO, 2006). However, the situation in developing and emerging economies is contrastingly different (Cordell et al., 2009). There will be an estimated 2-2.5 billion new mouths to feed by 2050 (IWMI, 2006), mainly in urban slums of the developing world. For securing food, the need for P fertilization in the growing economies like India and China is expected to increase in the foreseeable future (Maene, 2007). Phosphorus fertilizers, derived from high grade rock phosphate reserves are non-renewable finite resources (Vance, 2001). The unique property of P to form insoluble complexes with free iron and aluminium oxides in acidic soil of tropical and subtropical agriculture and with calcium and magnesium compounds in calcareous soil, results in usually very low recovery (10-30 percent) of P fertilizer by crop plants (Holford, 1997). This warrants for more sustainable use of P resources in agriculture and improving the efficiency of P fertilizers used in different agricultural systems (Cordell et al., 2009), to extend the world P availability.

Developing P efficient cultivars (yield well under P deficient conditions) are not in themselves a sustainable solution to the problem, as it will not deny the ultimate need for P fertilizers (Sanchez, 2010). However, it is the most realistic approach to the problem of P deficiency in cultivated soil (Gunes et al., 2006; Liao et al., 2008). More P efficient cultivars provide opportunities for initial gain in crop productivity to be achieved that may consequently assist access to P fertilizers (Lynch, 2007).The distribution of improved cultivars to farmers is among the most cost-effective means to upgrade crop production (Byerlee, 1996). In this paper we review mechanisms underlying phosphorus uptake and transport in wheat plant and the potential of different approaches that leads to improved P use efficiency in agricultural systems.

How P is absorbed by wheat?

Wheat being a cereal produces two types of roots : seminal roots (also called as primary roots) develop at the nodes of the embryonic hypocotyl of the germinating cross and adventurous roots (also called nodal, secondary or crown roots) emerge from nodes at the base of the apical culm (main stem) and tillers only when the fourth main stem leaf appears. The roots generally considered as source of Pi for other plant parts, become a sink during Pi starvation. This appears to be a deliberate, adaptive response by the plant to promote root proliferation and thereby enhance soil exploration and Pi uptake (Raghothama, 1999). High-input semi-dwarf wheat is characterized by shallow root architecture i.e. seminal root dependence. In contrast, low input genotypes develop a large root system essentially based on adventurous roots.

Plant roots absorb inorganic P (Pi), from the soil solution mainly in the form of primary orthophosphate $(H_2PO_4)^{2-}$ and secondary orthophosphate $(HPO_4)^{2-}$ ions (Vance et al., 2003). Many other forms are also available. Soil phosphate ions move towards the root *via* diffusion (Marschner, 1995).Plants must maintain intercellular (cytosol) Pi level at millimolar range, even when the concentration of soil Pi are at micromolar level, to meet the high demand of Pi in the cells (Reisenauer,1966). This necessitates roots to acquire Pi against a strong concentration gradient (100- fold or higher). An energy mediated H^+/Pi co-transport process, driven by the plasma membrane H^+ extrusion pump such as the P-type H^+ATPase has been proposed for Pi uptake in plants (Ullrich-Eberius et al., 1981; Ullrich-Eberius et al., 1984). Two categories of transporters are expressed for phosphate acquisition and transport across the plants. High-affinity P transporters primarily involved in P acquisition from external soil solution to the cell cytoplasm and the low-affinity P transporters also involve in P uptake and vascular loading and unloading, internal distribution and remobilization of acquired P (Smith et al., 2001). Acquisition of P by high-P- affinity transporters is regulated by internal P in the plants whereas low-affinity transport system expressed constitutively. Phosphate acquired by root epidermal cells through the transporter mediated pathway and apoplasmic movement is loaded into the xylem for further transportation in different plant parts according to metabolic needs (Bieleski and Ferguson, 1983). The initial movement of the phosphate ions across the plasma membrane to the root epidermal cells and cortical cells and subsequent loading into xylem appears to be two major checkpoints of the regulation of ion transport across roots (Smith et al., 2003). Plants have developed adaptive responses to facilitate external Pi acquisition, limit consumption of Pi and adjust recycling internally to maintain constant cytoplasmic Pi concentration referred to as homeostasis, in case of inadequate Pi availability (Raghothama, 1999; Poirier and Bucher, 2002).

In wheat full length cDNA sequence of a high affinity phosphate transporter gene (*PHT1*), *TaPHT1.2* and partial sequence of seven other *PHT1*genes were cloned (Davies et al., 2002). A 579 bp of *TaPHT1.2* promoter is sufficient to drive gene expression in the roots of wheat and *Arabidopsis* under low P conditions (Tittarelli et al., 2007). The regulatory element in Pi response, P1BS, is also identified in *TaPHT1.2* transporter, *TaPHT1.2-D,* on long arm of wheat 4D (Miao et al., 2009).

Approaches for improving phosphorus use efficiency in wheat

Use efficiency of P (PUE) depends on external P availability and internal P requirements. The latter can be split into P uptake efficiency (Fohse et al., 1988) and P utilization efficiency. Phenotypic and genotypic adaptations influencing P acquisition under P stress mainly involve changes in root characteristics because of relative immobility of P in soil, with the highest concentration usually found in the top soil. Increased harvest index, P harvest index and low P.

P concentration in grain may improve P utilization efficiency in wheat (Batten, 1992). Being in additive mode, small optimization of the each component traits can trigger PUE of wheat as a whole. Achieving these changes can be brought about through the following approaches described below.

Screening and breeding of P efficient wheat lines.

Selection of P efficient lines in terms of yield is complicated as almost everything in the genome contributes either directly or indirectly to yield. If the selection pressure for P or any other nutrient is strong enough then efficient genotypes may be selected based on yield, but there will be possibility of the strong influence of interactions that would affect the results under field conditions (Ortiz-Monasterio et al., 2001). Screening is further complicated in the field because of the uneven distribution of P. Genotypes can be screened in uniform soils under greenhouse experiment. However, growing conditions are less realistic and ranking may not be closely related to P efficiency obtained in the field (Gunes et al., 2006). Special attention should be given to growth conditions in screening wheat for P efficiency. Green house experiments however, can act as a primary screening tool to reduce the number of genotypes.

The geometry of the root system is essential for improvement of P uptake as it may be directed to maximize root per unit soil volume i.e. root length density. Wheat genotypes with higher root length density are able to take up more P (Manske et al., 2000a). Root diameter, root hair abundance (Jones et al., 1989) and high ratio of root to shoot growth-rates (Fohse et al., 1988) are other determinants of P uptake efficiency in wheat. Genotypes with thinner roots showed improved P uptake (Manske et al., 1996). Manipulation in root hair morphology is the least metabolically costly way of increasing root surface area. Root hair length plays a significant role in P acquisition (Gahoonia et al., 1997). Root hair density among bread wheat varied considerably and positively correlated with P uptake at anthesis when grown on a P deficient calcareous Ardisols (Manske, 1997). Wide genotypic variation and heritability of root morphology, root hair length and density provide opportunities for selection and breeding for root characteristics for increasing P acquisition (Gahoonia and Nielsen, 2004).

Root induces changes in the PH of their rhizosphere, which affects the bioavailability of soil P (Grinsted et al., 1983). The concentration of orthophosphate ions in the soil solution is pH influenced. Plant species or genotypes inducing rhizosphere acidification may absorb more P by this mechanism (Gahoonia and Nielsen, 2004). Organic acids especially citrate and malate ions are the major wheat roots exudates responsible for this adaptation (Manske and Vlek, 2002; Khademi et al., 2010).The consumption of organic acid 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 (Gahoonia and Nielsen, 2004). Exudation promotes the solubility of soil P from inorganic and adsorbed P fractions (Neumann and Romheld, 1999). Under conditions of Fe deficiency, roots release considerable amounts of chelating exudates (phytosiderophores), which form plant-available Fe-phytosiderophore complexes (Aciksoz et al., 2011) and thus, release bound Pi from the Fe-P complex, in to the rhizosphere. Depending on the soil type organic P may constitute 30-80 percent of the total phosphorus (Dalal, 1977). Plants can only absorb organic P compounds after they are hydrolyzed to inorganic P, preferably close to the root surface in order to prevent sorption by the soil particles. Organic P can be digested by root-surface-bound or excreted phosphatase (Seeling and Jungk, 1996). Genotypic variation in root phosphatase, excreted or bound at the root surface, exists (McLachlan, 1980). Results from a set of genotype also indicate the ability of wheat roots to utilize organic P through root phosphatase activity (Tabatabai and Bremner, 1969).

Root modification systems however, usually require additional carbon input. In wheat over 50 percent of the carbohydrate are translocated to roots for root growth and maintenance and for the absorption of ions, of them anion uptake only costs about 20 percent of the translocated carbohydrate (Manske and Vlek, 2002). If the demand for carbohydrates in large root systems is not compensated for by improved P and water acquisition, as in case of sufficient nutrient and water supply, the roots themselves may limit yield (Manske et al., 2001) and study of wheat root traits may be very labour intensive. Techniques like minirhizotron, image analysis system and root washing machines have been developed and although they are often precise and faster, they are expensive and unsuitable for screening large number of segregating population (Manske et al., 2001). Indirect selection for above ground traits related to root growth could be a possibility to select genotypes with improved root systems for low input conditions. The number of spikes m^{2} is positively correlated with root length density, especially at low P (Manske et al., 2000a). Nonetheless, assessing root traits is essential for improving P acquisition efficiency under low P conditions, root analysis of large population can be done in nutrient solution. Hayes et al., (2004), by using only two wheat cultivars concluded that screening in nutrient solution is not reliable for P efficiency differences found in soil culture.

On the other hand, in a glasshouse comparison of 73 types of bread wheat and durum wheat, at similar concentrations of shoot P, genotypes showed considerable variation in shoot dry weight (Ozturk et al., 2005) suggesting useful variation in internal P utilization efficiency. More effective translocation of assimilates into kernels may improve the P utilization (Horst et al., 1996) because developing kernels are strong sink for assimilates. Given the small margin to breed for higher harvest index (HI), selection for low grain P content may improve P utilization efficiency. Selection for wheat genotypes that removes small amounts of P from the soil due to their low P grain concentration can contribute to sustainable land use (Schulthess et al., 1997). Opportunity exists to manipulate P grain composition. Nearly all the genotypic variations in seed total P are due to a variation in phytate, a mixed cation salt of phytic acid. Non-phytate P tends to remain constant (Raboy et al., 1991; Raboy, 2003). High phytate grain concentration reduces P bioavailability in monogastric animals and also contributes to poor availability of essential micronutrients (Liu et al., 2006). However, low P in seed can impact on seedling establishment (Bolland and Baker, 1988; Derrick and Ryan, 1998) especially under low input P conditions and agronomic involvements like P seed enrichment or P placement (Rebafka et al., 1993; Sekiya and Yano, 2010) may be required to overcome any restrictions to seedling growth. Moreover, more detailed experiments are needed for better understanding of role of P translocation within the plants and re-translocation to meristems (Ozturk et al., 2005) and to grain in improved P efficiency in wheat.

Any wheat breeding programme for higher grain yield, selects indirectly for improved P utilization (Egle et al., 1999), especially under low P regimes. Modern breeding had increased P use efficiency through better utilization for grain formation i.e. higher P harvest index (Horst et al., 1993), further improvement in P use efficiency can be realized by increasing either ears per plant or grains per ear (Wang et al., 2010). In a Metroglyph analysis of spring wheat Gill et al. (2004), classified 30 genotypes in to eight different groups on the basis of their grain yield performance and phosphorus uptake and concluded: low P requiring genotypes can be grown on soils marginally deficient in available P to get maximum phosphate use efficiency which in turn results in better cost benefit ratio; Inter-mating between varieties belonging to high grain yield-high phosphorus (HGY-HP; PBW 343 and WH 711) and high grain yield-low phosphorus (HGY-LP; Raj 3765 and WH 283) group would further expand genotypic variability and thus frequency of recombinants exhibiting different grain yield and P uptake levels.

Breeding of new improved cultivar relies on screening of genotypes varying considerably for associated traits. As, traits most likely associated with P use efficiency has been identified in wheat, it seems feasible to combine root traits and internal P utilization efficiency into agronomically elite commercial cultivars and perform early generation selection under both low and high P conditions. The later will select for high yield potential and former for adaptation to low P (Manske et al., 2000a). Phenotypic complexity of roots and shoot responses to P limiting conditions reflects the polygenic nature of the process. Variation for complex phenotypic traits are frequently controlled by many genetic loci, scattered throughout the genome (Price, 2006). Quantitative trait loci analysis is based on statistically significant association of phenotypic differences for the trait of interest with molecular markers that constitute the genetic map (Doerge, 2001). Molecular markers found linked to the target trait can be used in marker-assisted selection (for reviews, see Gupta et al., 1999; Varshney et al., 2007) as given challenges and the time scale, breeding for improved cultivars can no longer rely on 10-year cycles and all technologies that shorten selection cycles must be mobilized (Paux et al., 2011). Despite QTLs related to P deficiency tolerance has been identified in wheat (Su et al. 2006, 2009; Li et al. 2007; Guo et al., 2011) their utilization in marker aided selection is a remaining challenge.

Transgenic approach

Exudation of organic acids and phosphatase into the rhizophere has been proposed to increase P acquisition efficiency (PAE) in wheat plants. Transgenic barley expressing *TaALMT1* aluminium activated malate transporter gene, enhanced expression of which improved Al^{3+} resistance in transgenic wheat (Pereira et al., 2010), showed improved P nutrition and grain production when grown on an acidic soil (Delhaize et al., 2009). These finding provides an opportunity to manipulate P deficiency and aluminium toxicity tolerance in wheat under acidic soil using *TaALMT1* as a candidate. Phytases, a special type of APases with the capability to hydrolyze phytate and its derivates, predominant inositol phosphates present in seeds and soil. Wheat engineered for *Aspergillus* phytase-encoding gene (phyA), accumulates significant amount of *Aspergillus* phytase in grains may be of relevance for improving the phytate-phosphorus digestibility in non-ruminants including humans. Given soluble P, high affinity phosphate transporters play an important role in Pi uptake and translocation (Zeng et al., 2002; Davies et al., 2002; Tittarelli et al., 2007; Miao et al., 2009) and offer the possibility to enhance the P nutrition of wheat by gene technology approach.

Integrated approach

An integrated approach that enhances availability and acquisition of native P includes integrated use of inorganic P sources through highly soluble fertilizers coupled with inoculation of plant growth promoting rhizobacteria (PGPR) like *Azotobacter*, *Azospirrilum* or mycorrhizae (Fig 1).

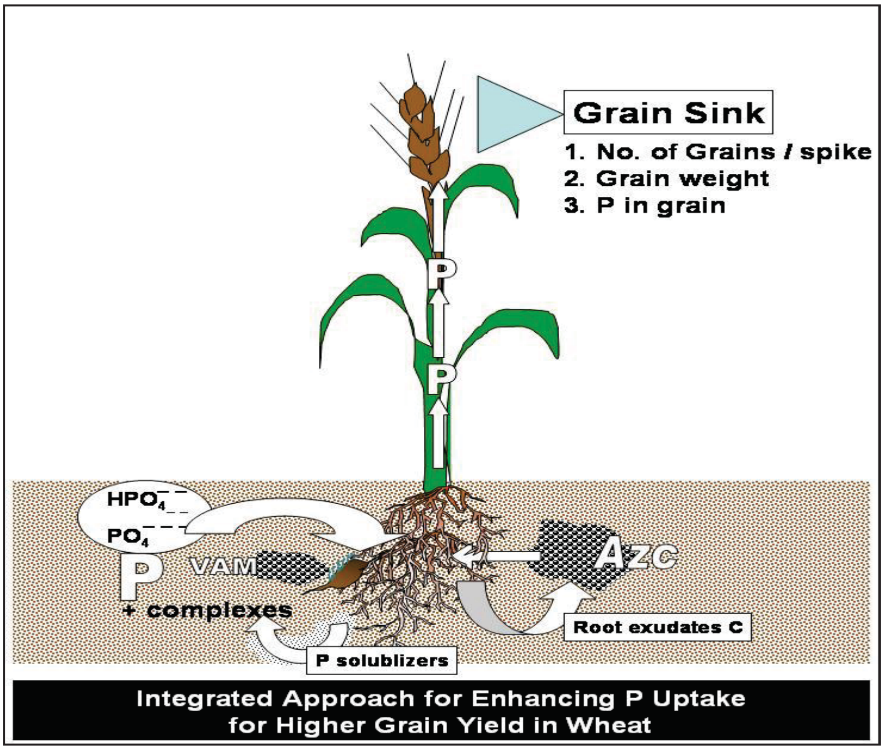

Fig.1, adopted from Behl et al., 2005

This approach can also compliment the products of plant breeding and gene technology besides improving the efficiency of applied P fertilizers. PGPR enhance the capacity of plants to acquire P from soil through the various mechanisms includes: increased lateral root growth by hormonal stimulation like production of indole-3-acetic acid (IAA) (Barbieri et al., 1986), gibberellin or enzymes that alter plant ethylene precursors such as ACC-deaminase (Richardson et al., 2009; Hayat et al., 2010), solubilization and mineralization of fixed P from inorganic and organic forms via efflux of organic anions and protons, production of siderophores and release of phosphatase (Richardson et al., 2009). Genotypic differences for *Azotobacter* infection (Kumar and Narula, 1999) increase P (Kumar et al., 2001) and micronutrients uptake (Singh et al., 2004) exists among Indian bread wheat. Solubilized P can be absorbed both directly by plants or *via* arbuscular mycorrhizal fungal hyphae. Mycorrhizal symbiosis is based on the mutulastic exchange of carbon from the host plant in return for P (Schweiger and Jakobsen, 2000) and other nutrients from the fungus (Marschner and Dell, 1994).Wheat roots excrete carbonaceous exudates which could help in proliferation of VAM and *Azotobacter chroococcum* (Azc) (Manske et al., 2000b). Inoculation of Azc also compliments wheat-VAM interaction due to its nitrogen fixation, phytohormone production and phosphate solubilizing properties (Behl et al., 2003). Thus, inoculation of VAM with phytohormone and vitamin producing Azc could result in improved plant growth promoting effects of diazotrophs in rhizosphere. Behl et al., (2003), reported the effect of cultivar and dual inoculation of Azc and arbuscular mycorrhiza fungi (AMF, *Glomus fasciculatum*) on AMF infection in four wheat varieties and their three crosses under low input conditions. Comparative evaluation of treatment averages viz. control (common in other two treatments also), AMF and AMF + Azc revealed that inoculation of Azc led to increase in AMF infection in roots.

High-affinity phosphate transporters localized in root-mycorrhiza interface in cortical cells, co-ordinately expressed in response to P deficiency and interaction with mycorrhizal fungi in wheat (Glassop et al., 2005; Bucher, 2007). However, this information requires further basic research for better understanding of mechanism underlying mycorrhizal Pi uptake pathways, for adoption in breeding programme. Regardless of substantial potential, with an exception of commercialization of fungal-based inoculants in North America and recently in Europe and Australia, large scale application of microbial products remains limited due to inconsistent response to inoculants under different environments as a consequence of complex plant-microbe interaction in soil environment which has proven difficult to manage (Richardson, 2001; Richardson and Simpson, 2011).

Conclusion and Outlook

P nutrition is critical for reaching the attainable yield potential of wheat. Given that genetic variability exists among bread wheat genotypes and alien species, there is some scope for pyramiding genes for P acquisition and utilization in agronomically superior genotypes possessing commensurate ontogenic and structural features to support sinks for grain yield with efficient use of Pi, through combination of conservation strategies discussed in this review. Plant breeding under the umbrella of Plant Genomics will lead to the identification of genes regulating adaptation to P stress through the development of well-defined Recombinant Inbred Lines (RILs) and Near Isogenic Lines (NILs) having QTLs for P tolerance coupled with next generation sequencing (Vance, 2010). P nutrition must be a part of an integrated nutrient management strategy that considers the importance of bio-inoculants, organic manures and other nutrients.

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