Bioengineering and management for efficient phosphorus utilization in crops and pastures

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Phosphorus (P) is one of the three macronutrients for plants. Because of its low mobility and high fixation in soils, low P availability is a worldwide constraint for crop productivity. Molecular biology provides great opportunities to improve P efficiency in plants. However, transgenic plants cannot be commercialized before integrating all the knowledge on bottlenecks for improving P efficiency of crops/pastures. This review intends to summarize the main strategies of bioengineering to improve P efficiency of crops/pastures, including conventional and molecular assisted breeding, identification and application of key genes for biotech plants. It highlights recent advances in the understanding of improving P efficiency through the integration of bioengineering with P fertilization and cultivation management.

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Improving P efficiency via conventional and marker assisted selection breeding

It is well documented that genetic variations for PE exist among plant species and genotypes within a species, indicating that it is possible to improve plant PE through conventional and marker assisted selection (MAS) breeding. MAS is a process using markers (morphological, biochemical or one based on DNA/RNA variation) to select the traits of interest during breeding. Root traits are thought to be crucial for P uptake. This ‘root breeding’ strategy has proven effective in improving PE of some crops (e.g. soybean) [5]. In order to overcome limitations of genetic variations within the same species, transferring alien genes from other species even pastures to crops through chromosome engineering is an effective approach in improving PE of crops. For example, wheat lines carrying 1BL/1RS, 1AL/1RS or 1DL/1RS wheat–rye chromosomal translocations (the long arm of wheat chromosomes 1A, 1B, and 1D is replaced by the short arm of rye chromosome 1R, respectively) exhibited increased PE and higher yield [6]. However, the root traits associated with PE are hidden in soils, therefore root breeding through conventional phenotype selection is time consuming and complicated. MAS breeding might be better as demonstrated by recent achievements in rice [7*]. Through the major quantitative trait locus (QTL) controlling P uptake, phosphorus uptake (Pup1), improved PE and yield of rice genotypes have been successfully
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Figure 1

World primary crop and biotech crop planting area, along with global annual consumption of total chemical fertilizers and P fertilizer over the period from 1996 to 2010. World primary crop area was the total planting area of main crops. The total chemical fertilizers were the sum of fertilizer N, P and K. (Data of GM crops are from http://www.isaaa.org/resources/publications/pocketk/16/default.asp. The other data are from FAOSTAT [http://faostat.fao.org/], accessed January 10, 2012).

developed. However, most identified QTLs made small contributions to PE, and thus were impractical for MAS breeding [8,9].

Improving P efficiency via transgenic modification
Plants have evolved multiple adaptations to low P availability for efficient utilization of P from soils. Understanding the mechanisms and identifying genes involved in these adaptations promises to facilitate improvement of PE in crops and pastures through transgenic modification.

Optimizing root architecture for Pi acquisition
Because of the immobility and heterogeneous distribution of Pi in soils, root architecture traits that establish the framework of root system and enhance topsoil foraging are particularly important for Pi acquisition (Figure 2) [4]. The architectural traits mainly include shallower root angles, enhanced adventitious rooting and lateral root branching, greater root hair density, and more cluster root formation [4,10,11]. However, the underlying molecular mechanisms of root architecture adaptation to P deficiency remain unclear [4,5]. Recently, a β-expansin gene, GmEXPB2 from soybean was shown to be critical for regulating root architecture responses to Pi starvation in crops, and overexpressing GmEXPB2 could successfully improve PE in soybean [12**]. In pasture plants, two glycerophosphodiester phosphodiesterase genes, LaGPX-PDE1 and LaGPX-PDE2 from white lupin, were reported to participate in acclimation to P deficiency through the regulation of root hair development and enhanced glycerophosphodiester turnover [13]. Many other genes have been identified to mediate root architectural changes in crops and pastures, such as crown rootless 5 (OsCRL5) and expansin 17 (OsEXP17) in rice [14,15*], but they need to be tested for any contributions to improving PE. Therefore, while progress has been realized, improving PE through transgenic modification of root architecture has a long way to go.

Releasing Pi from insoluble P pools in rhizosphere
Since over 99% of soil P exists as poorly available forms to plants [16], an important trait for optimal PE complementary to the development of optimal root architecture is the ability to stimulate release of Pi from insoluble P pools in the rhizosphere. It has been well documented that secreted protons (H+), organic acids (OAs) and phosphatases play vital roles for plants to mobilize and utilize the fixed P in soils (Figure 2).

Since H+-ATPases mediate ATP-dependent H+ extrusion to the extracellular space, the genes affecting H+-ATPases should be crucial for H+ secretion from roots, as well as subsequent P acquisition. In support of this, knockout of OsAδ, a P-type H+-ATPase gene, which affected transcripts of several other H+-ATPases, inhibited P acquisition in rice mutants [17].

It is generally assumed that secreted OAs could mobilize insoluble P through both rhizosphere acidification and
A model for improved P efficiency in crops and pastures through transgenic engineering. The genes in parenthesis have been used in transgenic modification for improving P efficiency in crops, pastures. The first two letters of the gene label represent the abbreviated species name, except ath-miR399d from Arabidopsis. Os: Oryza sativa; La: Lupinus albus; Po: Penicillium oxalicum; Ta: Triticum aestivum; Pv: Phaseolus vulgaris.

chelation of metal ions, and that this process is mainly determined by activities of OA transporters and enzymes for OA metabolism, such as phosphoenolpyruvate carboxylase (PEPC), malate dehydrogenase (MDH), and citrate synthase (CS) [18]. A number of reports provided support for OA roles in improved P acquisition from insoluble sources through overexpression of OA related genes, such as PoMDH from Penicillium oxalicum in tobacco and aluminum activated malate transporter1 from *Triticum aestivum* (*TaALMT1*) in barley [19,20]. These results indicated that some OA-related genes might be useful for biotech improvement of PE.

Organic P often accounts for 30–70% of total soil P, which is not directly available to plants unless hydrolyzed by phosphatases [21]. Enhanced exudation and activities of phosphatases, therefore, become potential hotspots for improving PE through genetic engineering. It has been documented that overexpressing the *purple acid phosphatase* 3, *PePAP3* in bean results in increased utilization of extracellular ATP [22]. In addition, another class of enzymes, phytases from microorganisms and plants has been used to improve phytate-P utilization in crops and pasture plants. Instances of this strategy include overexpression of a *phytase* gene, *phaA* from *Aspergillus niger* in cotton [23], *EcAPPa* from *Escherichia coli* in potato [24], *AtPAP15* from Arabidopsis in soybean [25], *MtPHY1* and *MtPAP1* from *Medicago truncatula* in clover [26], and *LaSAP2* from white lupin in tobacco [27], suggesting that increased activities of root secreted phytases could enhance phytate-P utilization in crops and pasture plants through genetic modification.

**Enhancing expression of high affinity Pi transporter**

Phosphate acquisition and translocation in plants are mediated by Pi transporters (PT). Among them, members from the high affinity PT family, Pht1, mainly function in Pi acquisition from soils and translocation from roots to other parts of plants [28]. Although overexpressing *NtPht1:1, OsPht1:2* or *OsPht1:8* facilitated Pi acquisition in transgenic rice plants, their biomass and yield were not coincidently improved due to the toxicity of excess P at high P level [29–31], suggesting that overexpressing *Pht1* in crops should be integrated with soil/farm management in order to improve crop PE (Figure 2).

**Modifying regulators in P signaling network**

Plant responses to P deficiency are coordinately regulated by an elaborate signaling network involving many actors, including phytohormones, sugars, miRNAs, transcription factors, and other regulators [32**,]. Among these participants, miRNAs, SPX (SYG/PHO81/XPR1) domain containing proteins, and several transcription factors are major components for regulating P homeostasis in plants [33]. Interestingly, overexpression of a transcription factor, *PTF1* (*Pi starvation induced transcription factor 1*), enhanced PE in both rice and maize [34,35**] (Figure 2). Conversely, overexpressing a transcription factor, *phosphate starvation response 2* (*OsPHR2*), a major component in P signaling pathways in rice, resulted in
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increased P concentration but inhibited plant growth, which might be caused by excessive P amounts in leaves [36]. Similar results were also observed in modifying SPX and miR399. Suppressing OsSPX1 in rice, and overexpressing ath-miR399d from Arabidopsis in tomato led to excessive P accumulation in leaves, and subsequently inhibited plant growth [37,38]. Therefore, improving PE through transforming the critical genes in P signaling networks requires more insights into the physiological and molecular connections between components.

Improving P efficiency via better P fertilization and cultivation management

To feed the increasing population, large-scale fertilization and excessive consumption of natural P resources are increasing, and thus result in severe environmental problems [39]. Improving P utilization only from the plant side is not enough to optimize P utilization and solve the environmental problems caused by soil P accumulation. For this, soil-testing based upon ‘building-up and maintenance’ was developed and widely used in developed countries and some fast developing countries (e.g. China) [40]. The principle of this approach is to adjust soil P levels from those threatening environmental damage or P deficiency to the levels ensuring stable crop yield. This method is particularly useful for controlling high soil P accumulation and reducing environmental risks. The rhizosphere-based P management is an alternative approach to improve PE and crop yield through exploitation of biological potentials for efficient mobilization and acquisition of P by crops, and reducing the reliance on application of chemical fertilizer P [41]. The match between demand and supply can be strongly improved by positioning mineral fertilizers close to the expanding root system [42], and thus rhizosphere-based P management is a priority for improving P fertilizer use efficiency.

Soil P exists in various forms that require different biochemical or chemical reactions to release Pi. It is well known that plant species vary in utilization of different P forms, and this kind of biodiversity can be applied to improve PE of crops/pastures through cultivation management, such as intercropping. Complementarity can occur within intercropped species for different soil P pools. Legumes generally have higher capacities to mobilize soil Pi than cereals [43], and thus are successfully used to improve Pi uptake and yield of cereal crops grown on P deficient soils through legume-cereal intercropping [43,44]. The cultivation management can also be used to improve the productivity and sustainability of pastureland which often receives low or no input of fertilizers.

Perspectives

Some progress has been made to improve PE in crops and pasture plants mainly through the three strategies discussed in this review. However, any individual strategy might have disadvantages. For example, incompatibility and lack of genetic variability severely inhibit wide application of conventional and MAS breeding to improve PE. Although these problems could be solved using genetic engineering techniques, selecting the suitable candidate genes is a big challenge, and also the suitable candidate genes might be variety or species dependent. Additionally, P fertilization management could affect dynamics of rhizosphere P, and subsequently affect P acquisition efficiency in plants. Moreover, competition might also influence the P uptake of intercropped species. Therefore, more intensive studies are required to further elucidate the critical procedures mediating superior PE in plants and P fertilization as well as cultivation management in the field.

In conclusion, through integration of breeding, genetic engineering, P fertilization and cultivation management, optimum PE and productivity of crops/pastures can be synchronously achieved with the better understanding of plants, soils and management.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


This paper defines a core set of Pup1 markers, and identifies sequence polymorphisms suitable for single nucleotide polymorphism marker development for high-throughput genotyping. Furthermore, using a marker-assisted backcrossing approach, Pup1 was introgressed into different rice varieties. These results suggest that Pup1 is effective in different genetic backgrounds and environments and it has the potential to significantly enhance grain yield under field conditions.

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In this work, a phosphate starvation-induced vegetative β-expansin gene, GmEXPB2, was cloned. Furthermore, it demonstrates that GmEXPB2 may enhance P acquisition through transgenic expression by regulating adaptive changes of the root system architecture. This finding has great agricultural potential for improving crop P uptake on both low-P and P-fertilized soils.


This study demonstrates that a root hair-specific expansin, OsEXP4A17 was identified from a rice mutant with short root hairs, which is required for root hair elongation. These results suggest that members of the root hair EXPA subclade play a crucial role in root cell elongation in Graminaceae.


In this study, a novel purple acid phosphatase (PAP), PvPAP3, was purified in bean. Furthermore, overexpressing PvPAP3 enhanced root growth and P uptake when ATP was supplied as the sole external P source. These results suggest that PvPAP3 might function in the adaptation of bean to P deficiency, possibly through enhancing utilization of extracellular ATP as a P source.


This study demonstrates that increased P acquisition and yield was observed in transgenic soybeans with overexpressing an Arabidopsis purple acid phosphatase (AtPAP15) containing a carrot extracellular targeting peptide. This is the first report on the improvement of P efficiency in soybean through constitutive expression of a plant acid phosphatase gene.


In this work, the authors integrate and discuss the present knowledge of the molecular mechanisms and networks with regard to phosphate sensing and signaling in plants.


This work shows that a phosphate starvation responsive transcription factor, ZmPTF1 was cloned from maize. Furthermore, overexpressing ZmPTF1 improves phosphate efficiency of maize through regulating carbohydrate metabolism and root growth. This research provides a useful gene for transgenic breeding of maize with superior phosphorus efficiency.


In the study, suppression of OsSPX1 resulted in over-accumulation of phosphorus in rice. However, overexpression of OsSPX1 suppressed expression levels of 10 phosphate starvation-induced genes in rice.
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These results suggest that OsSPX1 acts via a negative feedback loop to optimize growth under phosphate-limited conditions.


In this work, plant growth responses to phosphorus stress of two maize varieties intercropped with soybean were examined in the field and in a transparent gel system. Results showed that plant roots could integrate information on phosphorus status and root behavior of neighboring plants. This study provides new insights into the dynamics and complexity of root behavior and kin recognition among crop species in response to nutrient status and neighboring plants.