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Tackling phosphorous deficiency in rice by a loaded PSTOL

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ABSTRACT: *Currently plant breeding has become highly challenging to meet the demands of the burgeoning world population. Huge yield targets are to be reached with limited resources in a changing climate with problem soils for world food security. Farmers have to invest huge amounts of money on fertilizers to replenish the nutrients in the soil. This is increasing the input costs of agriculture and thereby creating burden on the farming community. Fertilizer costs have increased drastically because of huge demand and less supply. Phosphorus is one the most essential macro-nutrient required for growth and high yield of crop plants. It is mostly bound to the rocks in the form of organic phosphate and is not available to the plants. Most of the phosphorus applied as fertilizer gets leached into water bodies necessitating the repeated application. Global conflicts are likely to emerge because of the concentration of the rock phosphate, a non-renewable source of phosphorus with few nations and they are playing their monopoly in regulating the fertilizer prices. In this scenario of limited phosphorus resources, an alternative method to minimize the fertilizer application and to enhance the phosphorus utilization would be a right approach for enhanced crop yields. This review focuses on the research work carried out by various research groups across the globe to enhance phosphorus utilization with more emphasis on rice crop which is a staple food for more than two-thirds of the world population. India is rich in diverse germ-plasm and many potential genes which are successful in molecular breeding programs are of Indian origin. Many quantitative trait loci (QTL) for phosphorus uptake (Pup) were identified and successfully implemented in rice breeding programs. Sequential achievements in the discovery of the phosphorus uptake QTL's/ genes is elaborated. More insights into phosphorus metabolism have paved way for the discovery of phosphorus starvation tolerance genes (PSTOL) which were mined from aus-type rice varieties from Assam of Eastern India. It was found that these genes were highly conserved in rice germ-plasm and impart drought tolerance to plants by having long roots. We speculate, translational research with this gene will be highly promising and remunerative.*

Key words: *Rice, Phosphorus, Pup1, PSTOL1, Transgenic.*

INTRODUCTION

Rice is the most important food crop for more than two-thirds of the world population. Global rice demand is estimated to raise from 439 million tons in 2010 to 496 million tons in 2020 as per the projections of Food and Agricultural Policy Research Institute (FAPRI). This is an overall increase of 26% in the next 25 years. World food production will need to increase by 70% by the year 2050 in order to meet growing demand (FAO, 2013). The yield of rice must rise faster than in the recent past if world market prices are to be stabilized at affordable levels for the billions of consumers. Globally, farmers need to produce at least 8–10 million tons more rice each year at an annual increase of 1.2–1.5% over the coming decade, equivalent to an average yield increase of 0.6 t/ha during the next decade (IRRI, 2014) to meet the food security.

Rice is grown in a wide range of environments, but more than 40% of global rice production is from rain-fed ecosystem with limited control on water which is often associated with drought, flood or other calamities. Moreover 60% of rain-fed soils are deficient in one or more nutrients (Haefele and Hijmans 2007). In these soils, phosphorus (P) is one of the most important macro nutrients that is limited in availability. Worldwide, rice was harvested from about 162 million hectares in 2010 and out of that 91% was from Asian countries (IRRI, 2014). Rice yield will have to continue to grow faster than at present because of pressure on rice lands in the developing world from urbanization and climate change.

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Moreover, it was estimated that about 100 million ha of land suited to rice production in South and Southeast Asia is currently unused because of the problem soils (Senadhira 1994). These problem soils contain toxic minerals or deficient in one or more nutrients. If these problem soils can be rectified, global hunger, food security and poverty can be addressed with increased rice yields.

PHOSPHORUS DEFICIENCY AFFECTS PLANT GROWTH AND YIELD

Phosphorus (P) is of unequivocal importance for the production of food crops. It is often referred as the "energizer" since it helps to store and transfer energy during photosynthesis. It is a vital component of ATP, the 'energy currency' of the cell. It forms the basic component of many organic molecules, nucleic acids and proteins (Lea and Miflin 2011). Phosphorus is an essential nutrient and forms a part of several key plant structure compounds and plays a role as catalyst in the conversion of numerous key biochemical reactions in plants. There is no substitute for P in food production and it is considered as the most limiting mineral nutrient for plants across all arable land (Kochian, 2012). It has several important roles in physiology of plants (Fig. 1)

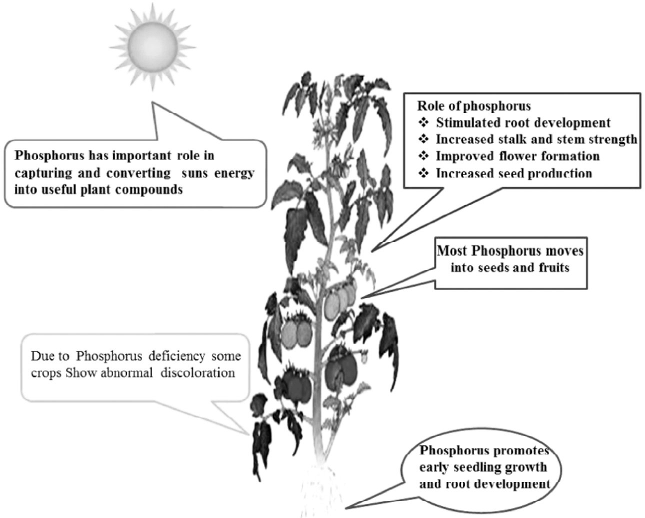

Figure 1: Role of Phosphorus in plants (Modified from photo source http://cliparts.co/clipart/487152).

Most of the P is tightly bound to the soil. It is present either as unavailable form or slowly available form, which is not immediately accessible by plants (Fig.2). Only 1% of P is present as available form in the soil solution which is found in irrigated ecosystems, where rice is grown in water logged conditions. There is a scarcity of P in most of the soils and hence soils are to be replenished with P fertilizers regularly, but this increases the input costs of agriculture. Moreover, fertilizers have environmental concerns. Phosphorus due to its insoluble nature binds to the soil leading to deficiencies of copper, iron, manganese and zinc. Phosphorus fertilizers leach away through water or soil, get bound to rocks and become unavailable to the plants necessitating repeated applications of P fertilizer to the soil.

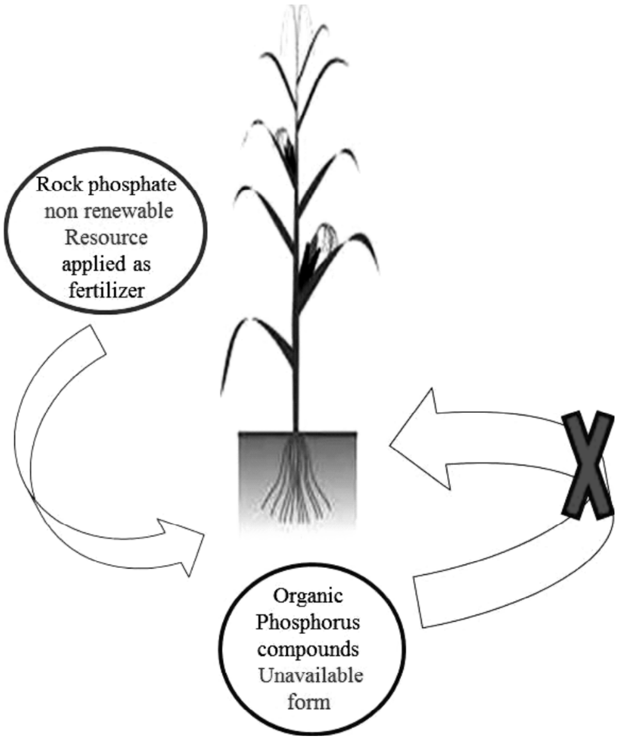

Figure 2: Non-availability of Phosphorus from the soil (Modified from photo source *http://cliparts.co/clipart/17592*).

DEPLETING GLOBAL PHOSPHOROUS RESERVE

Estimates suggest that, during the period 2008 to 2012, global fertilizer demand has increased by 1.7% annually, amounting to 15 million tons (Mt), of which 69% is utilized in Asia alone. The potential balance of phosphate is expected to rise from 2.1 million tons in 2012 to 3.8 million tons in 2016. The ratio of potential phosphate balance (H_3PO_4 based) to global phosphate demand is likely to grow from about 5 per cent in 2012- 2013 to 8 per cent in 2015-16 (FAO 2012). In addition, fertilizer prices are increasing due to high energy costs and because of limited natural resources. Geopolitical conflicts are likely possible since mineable P reserves are heavily concentrated in countries like Morocco holding about 75% of the global share, followed by China 6% and Algeria 3% (Jasinski, 2013). Worldwide P reserves are expected to be exhausted in 40-400 years, because of the estimated worldwide demand for this scarce mineral (Vaccari, 2009; Cooper *et al.,* 2011; MacDonald *et al.,* 2011; Sattari *et al.,* 2012;Obersteiner *et al.,* 2013; Cordell and White, 2013).

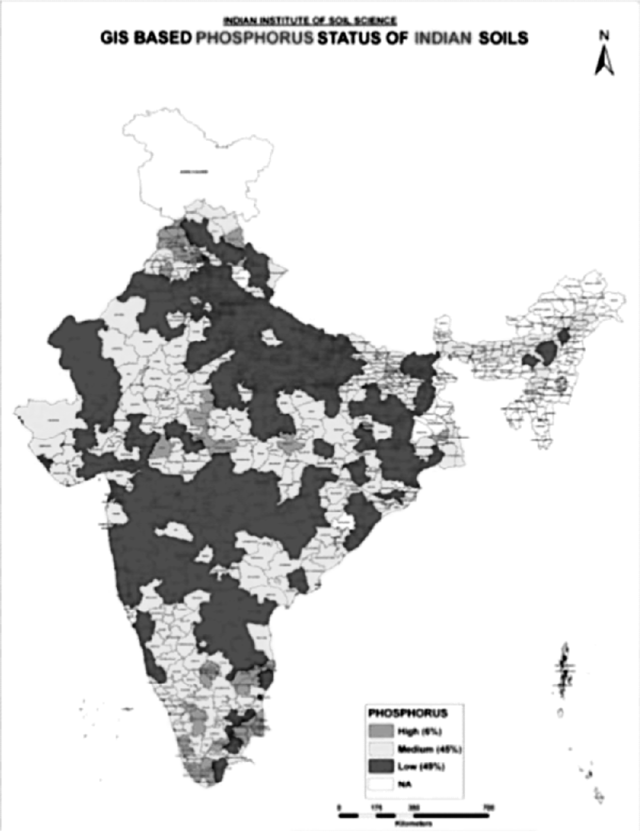

Figure 3: Phosphorus deficient soils in India
(Source: ICAR-Indian Institute of Soil Science, Bhopal)

In Sub-Saharan-African countries fertilizer application rates are very low below 5kg P ha-1 (Obersteiner et al., 2013), thus leading to P deficits of the agricultural production system (MacDonald et al., 2011). Phosphorus imbalances in the world, with too much P in some countries and too little or inaccessible P in others, will require different measures and breeding strategies (MacDonald et al., 2011). Therefore, there is a strong need for developing rice varieties that are P efficient. Keeping in view that 49% of rain-fed rice growing regions in India are deficient in P (Fig.3), crops that produce more with less external input and tolerant to phosphorus starvation is of priority.

RICE GENETICS AND GENOMICS FOR ADAPTIVE TRAITS

Recent advances in rice genomics and marker aided breeding are encouraging. Submergence tolerant rice varieties were developed by IRRI with *SUB1*-QTL conferring tolerance in flood prone regions. Efforts were made to introgress multiple stress tolerant genes into popular high yielding varieties, which are expected to provide an yield advantage of about 2 t/ha. (Sarkar *et al.,* 2009, Singh *et al.,* 2009). Rice genome sequence is available and several QTLs were identified which are associated with stress tolerance genes. Phenotypic variation in many quantitative traits can be explained with few loci of large effect, with the remainder due to numerous loci of small effect (Remington & Purugganan, 2003~ Mackay, 2004~ Roff,2007). To understand these effects QTL mapping can be done using molecular tags such as AFLP or more commonly, SNPs.

Keeping in view that most of the rice growing soils are P deficient and fertilizer applications did not show long standing benefits in fields, there was an immense search for phosphorus related genes and QTLs. 'Phosphorus uptake' was the most important quantitative trait locus which was first mapped (Wissuwa et al., 1998). It was identified in a breeding program in the backcross inbred lines obtained from the cross between *japonica* cultivar- Nippobare with *indica* land race from Assam-Kasalath, genotyping was done by RFLP markers. QTLs were identified for P uptake, internal P-use efficiency, dry weight and tiller number. P uptake QTL is located on chromosome 12 and showed major effect. However, QTLs like P-use efficiency and P-uptake are indirectly related. Number of tillers produced under P-deficiency is considered as an indirect indication for P-deficiency tolerance. It was found that *indica* alleles increased P-uptake but not P-use efficiency; this is due to indirect relation between the two QTLs. Plants expressing the P-uptake – QTL were found to have slightly higher dry weight compared to P-use efficiency.

PUP1 IS THE PHOSPHOROUS UPTAKE QTL IN RICE

Genes responsible for phenotypic traits can be mapped to genetic chromosomes by analysing near isogenic lines (NILs). These are lines of genetic codes that are identical except for differences including a specific gene or QTL of desired interest at a few specific locations or genetic loci. NILs are very useful for comparative physiological and biochemical studies of the function of a single gene. They are used to confirm the position and fine map a putative QTL. They are also used in the study of potential P-uptake (Yano and Sasaki 1997). The major QTL for phosphorus uptake was mapped on the long arm of chromosome 12 and is referred as *Pup1*. The phenotypic variation obtained was mainly due to the *Pup1* without any epistatic interactions. This potential QTL was made more effective for Marker assisted breeding programs by clearly defining the boarders of the QTL by an efficient method called substitution mapping (Wissuwa *et al.,* 2002).

Ni *et al.,* (1998) have identified the phosphorus deficiency tolerance QTL in the breeding program by single seed descent method through recombinant inbred lines obtained from the cross between P deficiency tolerant cultivar IR20 and P-deficiency sensitive variety IR55178-3B-9-3 and named the QTL as PHO, genotyping was done by both AFLP and RFLP markers. QTLs were identified for P uptake, tillering ability, shoot dry weight and root dry weight. P uptake QTL is located on chromosome 12 and showed major effect. This QTL had explained 78.8% of the total phenotypic variation for phosphorus uptake, i.e., under P deficiency improved line has enhanced P uptake by 170% and grain yield by 250% (Wissuwa *et al.,* 2001a). *Pup1* region in the chromosome contain the transcription factor gene OsPTF1 which confers tolerance to P deficiency (Yi et al., 2005). Moreover, the research experiments showed that the *Pup1* enables the plants to increase P uptake by 3- to 4-fold primarily because it conferred strong and high root growth rates despite of P deficiency in soils (Ismail *et al.,* 2007).

PUP1 GENE EXPRESSION IS TISSUE SPECIFIC

Root morphological and physiological studies indicated that the *Pup1* gene expresses in root tissue where it either leads to higher root growth per unit P (higher internal efficiency) or improves P uptake per unit root size (external efficiency) (Wissuwa 2003). Therefore, varieties with *Pup1*locus might contain the morphologically and physiologically favorable root structure for the efficient usage of P uptake. It was observed in experiments that rice with *Pup1* extract up to 3 times as much naturally occurring soil phosphorus, tripling the grain yield and dry weight (Fredenburg 2006). Heuer *et al.,* (2009) sequenced the *Pup1*region and confirmed that 278-kbp sequence of Kasalath rice variety was significantly different from the syntenic regions in Nipponbare rice variety due to large insertions or deletions (INDELs) that is directly linked with P deficiency tolerance. It is reported that the impact of *Pup1* on enhancing yield in P-deficient soil under drought stress is significantly high (Bernier *et al.*, 2009; Venuprasad *et al.,* 2009). Chin *et al.,* (2010) reported that *Pup1*nearisogenic lines (NILs) developed from Kasalath and Nipponbare population showed significant association between *Pup1* gene and yield/ root related traits.

Pup1 has been successfully introgressed into two irrigated rice varieties, namely IR64 and IR74 and three Indonesian upland varieties, namely Dodokan, Situ Bagendit, and Batur (Chin *et al.,* 2011). Tyagi *et al.,* (2012) have identified four genotypes containing *Pup1*, Sahbhagi Dhan, Dagaddeshi, Pynthor and Paijong, adapted to North Eastern and Eastern part of India, as potential donors for rice breeding for P deficiency tolerance. According to the haplotype analysis conducted by Tyagi *et al.,* (2012), in aus-type varieties, *Pup1* is equally present in 80–90% of the upland and lowland/irrigated varieties.

PHOSPHORUS-STARVATION TOLERANCE 1 (*PSTOL1*) IS A PHOSPHOROUS SENSOR

Underlying *Pup1* is a single kinase gene, OsPupK46-2 which is located in the indel which is closely associated with P deficiency and is highly conserved in the drought tolerant accessions in the rice germ-plasm (Chin *et al.,* 2010, 2011). This gene underlying the *Pup1* locus increases early root growth and P acquisition efficiency under low-P conditions in several different genetic backgrounds and is subsequently named Phosphorus-starvation tolerance 1 (*PSTOL1*), which encodes a serine/ threonine kinase of the LRK10L-2 subfamily (Gamuyao *et al.,* 2012). The overexpression of *PSTOL1* in two transgenic rice varieties enhanced the grain yield over 60 % under low-P conditions due to larger root system which improved the uptake of P and other nutrients. OsPupK20-2, which codes for a dirigent protein in root development is present in the down-stream of the *PSTOL1*gene which plays a role in lignification of rice roots in response to drought and P-stress. Kinases play an important role in sensing and signaling of P homeostasis, hence *PSTOL1* is considered as a suitable candidate gene for P metabolism. This is confirmed by the GUS expression analysis in the stem nodes which is a potential region for crown root formation in rice. Transgenic expression studies indicate that *PSTOL1*gene has to be expressed above certain threshold levels and it is independent of P availability in the soil.

CONCLUSION

It is evident that certain QTLs/genes are highly conserved and inherited through natural selection to sustain the problem soils which are deficient in one or more nutrients and to adopt in the stress prone environment. This provides a wide platform for the researchers to screen the germ-plasm including land races and wild varieties which are found to be rich sources of stress tolerant genes. The strategy was successful in the case of *SUB1* gene which gives tolerance for submergence and recently with *PSTOL1*gene which can survive P starvation. This is successfully implemented to screen several varieties and the best performing varieties were chosen for successful breeding programs to enhance the yield in the present day consequences of climate change and problem soils with ever increasing need for more yields with limited resources and increasing population. This review covers the sequence of successful research activities carried out by various research groups across the world in identifying the QTL and finally ending up with the invention of new gene which improves yield under phosphorous limited conditions, thus minimizing the input costs of agriculture and playing a pivotal role in international food security.

AUTHOR CONTRIBUTION

RR and PKD conceived the idea; SKR and PKD designed the concept. SKR summarized and wrote the manuscript with input from other authors.

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