A review on Improving Phosphorus use efficiency of upland rice with emerging opportunities of breeding

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Abstract
Phosphorus is an essential macro nutrient for plant growth, especially in nucleic acids and metabolic pathways. It is important nutrient for root development as well as plant growth. Phosphorus deficiency is very common in upland rice, and its damage occurs throughout crop cycle. Improving Phosphorus Use Efficiency is required in rice crop especially in upland rice as phosphorus uptake is difficult due to aluminium toxicity, P-fixation which occurs commonly in acid soil and slow diffusion rate. Through this paper we are reviewing the importance of Phosphorus Acquisition Efficiency (PAE), and Phosphorus Use Efficiency (PUE). Phosphorus uptake 1 (Pup1) a major QTL has been mapped from the population resulting from a cross between Nipponbare x Kasalath. Phosphorus-Starvation Tolerance 1 (OSPSTOL1) is a gene which shows Phosphorus tolerance in Pi deficient soils found within the QTL Pup1.

Keywords: Phosphorus Use Efficiency, Phosphorus Acquisition Efficiency, Inorganic Phosphorus (Pi), Root System Architecture, Quantitative Trait Loci

Introduction
Phosphorus is a natural element which is considered to be an important macronutrient for plant growth. It is known by the symbol P. In NPK we can understand that Phosphorus importance comes next after Nitrogen. Phosphorus is an essential macronutrient for plant growth, particularly in DNA and RNA, which is the main component of a gene. It is also important for phospholipids, and ATP [1]. Phosphorus is also required for signalling pathways such as protein kinases. Phosphorus is taken up by the plants through the process of diffusion. But due to high fixation and slow rate of diffusion in soils, phosphorus becomes unavailable to the plant. Uptake from soil can be difficult because it is usually get converted to organic form and adsorption also occurs in the soil which becomes an important limiting factor in achieving optimal yields in agriculture [3]. In acid upland soils, P becomes unavailable because of high P fixation capacities and it is also bound with Iron (Fe) whereas, in alkaline soils uptakes of P from the soil is difficult because P is bound to calcium [3]. In addition, organic material present in the soil (e.g. from manure or crop debris) can also bind phosphate, in particular phytate (inositol compounds). Phosphorus deficiency is widespread in all major rice ecosystems and is the major growth limiting factor in acid upland soils because of large P fixation. When P deficiency is severe, plants may not flower at all and grain formation may not occur. Phosphorus deficiency is fairly common in irrigated rice, and its damage occurs throughout crop cycle (www.knowledgebank.irri.org). Deficiency of phosphorus can cause reduction of primary root growth while increase in lateral root length as well as root hair length and density. Crops usually show no obvious symptoms of phosphorus deficiency other than stunting growth in the early stages of plant growth. Phosphorus deficiencies late in the growing season affect both seed development and normal crop maturity (www.cropnutrition.com). By the application of Pi-containing fertilizer, phosphorus deficiency in the soil can be obtained. Pi is usually obtained from a reserve resource which is limited. Available phosphate rock reserves are located in Morocco which has the highest amount of phosphate rock followed by USA and China [4]. Overuse of phosphorus fertilizers results in low phosphorus use efficiency (PUE). It also cause serious environmental consequences and accelerates the depletion of phosphorus mineral reserves. To avoid phosphorus depletion, P uptake by harvested crops and P fertilization should be in equilibrium [5][6]. As the plant ages the demand for phosphorus increases [7], therefore, it is necessary to check the growth condition of a plant at different stages of plant growth.

Upland rice refers to rice grown on both flat and sloping fields that are prepared and seeded under dry land conditions and depend on rainfall for moisture [8]. Phosphorus Use Efficiency (PUE) is defined as the amount of P accumulated in the tissue.
per biomass unit (shoot and/or root) or grain produced [9]. Internal PUE is calculated as the ratio of plant biomass produced per unit P; e.g. shoot dry weight (SDW) per mg phosphorus taken up [10] which is the inverse of tissue P concentration. For the external Phosphorus uptake, that is, Phosphorus Acquisition Efficiency (PAE), the amount of P taken up by the plant is calculated as mg phosphorus per plant. However, it does not include effects on biomass production per plant. Plants producing equal biomass at lower P concentrations when comparing with other plants at higher P concentrations would be considered to have high PUE [11].

The importance of root for Phosphorus nutrients
Root System Architecture (RSA) is very important for the uptake of plant macronutrients which will improve crop yield and productivity. This parameter will determine how much P is taken up by the root biomass. Total Phosphorus in the plants is distributed between shoots and roots, initiating the accumulation of biomass cycle. The amount of P distributed to root tissue will determine root biomass accumulation and on root PUE.

Root hairs, shallow root system are of great importance to nutrient uptake, particularly for weakly mobile nutrients such as Pi (inorganic phosphate) because they help in increase the root surface area and enable plants to explore more in the soil rhizosphere. Since, phosphorus is not distributed evenly in the soil, and because of slow diffusion rate the ability of the plants to access P from the soil is greatly dependent on the ability to develop a dense shallow root system [12] such as in common bean and maize. In response to low P conditions some varieties of maize and bean alter rooting angle and lateral root proliferation in response to low P conditions [13].

Two important traits such as lateral root growth and root hair length are also important for P uptake. Traits that are under genetic control include basal-root geotropism, adventitious-root formation and lateral branching. Genetic variations in root length and density are very necessary for weakly immobile nutrients such as Phosphorus and Potassium [5].

Lateral root growth is favoured over primary root growth in low phosphate condition, through increase in lateral root density and length [14]. Lateral root length and the total lateral root number were found to be associated with phosphorus uptake. This shows that elongation and development of lateral root were important to the ability of P uptake from growth medium where P supply was less [15]. Since, phosphorus is more abundantly available in the top soil. Therefore, adaptations of root architecture with increase root density in the upper parts of the soil will result in high P uptake efficiency. Shallower growth of axial roots will develop greater length of roots in the top soil. Formation of more adventitious root from stems, e.g. crown roots from basal stem nodes in maize, phosphorus uptake in the soil will be high [16]. However, in a study by [17] it has been reported that increase in adventitious root formation reduces lateral rooting. But not all crops have the ability to modify root hairs formation for increasing P uptake capacity. Root growth is directly limited by Phosphorus rather than by assimilate availability [18]. Root System Architecture is very important quantitative trait characterized by multiple genes. In different crop species several quantitative trait loci (QTL) associated with RSA under nutrient limitation have been identified but only few nucleotides underlying QTL have been identified. The problem lies with the size and complexity of crop genomes in addition of small effects of the QTL on root development [19].

Aluminium toxicity the problem for root growth and development
In acid soils, where pH is below 5, Al is solubilized into the toxic Al^3+ ion, and for many crop species the primary effect of Al^3+ toxicity is to inhibit root growth [20] leading to problems with nutrient and water uptake impacting crop productivity as a whole. A consequence of reduced root growth is poor exploration of soil, and this is particularly important for the uptake of an immobile nutrient such as Pi. Al toxicity in the soil which is the main constraints in rice production in Asia [21] inhibits root growth, and it is for this reason that progress in enhancing PUE can only be achieved if plants are also tolerant of Al toxicity or any other major stress-limiting root growth. In north eastern states of India where majority of soil conditions are acidic acid tolerant variety should be grown under such conditions for improving tolerance to Al toxicity and better Phosphorus uptake.

Two different concepts related to Phosphorus uptake and use in Crop Plants:
1. Phosphorus Acquisition Efficiency (PAE)
Phosphorus efficiency has typically been described by two separate terms: Phosphorus Acquisition Efficiency (PAE) and internal Phosphorus Use Efficiency (PUE) [22]. Phosphorus Acquisition Efficiency (PAE) relates to Phosphorus uptake from the soil and may be affected at the genotype level by differences in root size, root architecture or rhizosphere interactions that enhance P bioavailability [6]. It is commonly expressed as the relative difference of P acquired under low and high P availability conditions. It is highly desirable to enhance Pi uptake from the soil, however, care has to be taken that nutrients removed from fields are replenished regularly to maintain soil fertility and prevent soil degradation. For low-input agricultural systems, enhancing Pi uptake from outside source will not have a long lasting benefit and might deplete soil reserves faster. This would be very critical for soils with low total P content, and therefore for these regions crops that require less Pi per unit harvested grain would be a more promising approach.

When Phosphorus becomes unavailable or when it is present on a fixed source plants try to adapt by for obtaining phosphorus from the soils. In this case root exudates are released in the rhizosphere. Root exudates can improve P availability by mobilizing sparingly available P forms in the soil solution. These root exudates which are localized in the root tip include low molecular weight organic acids (LMWOAAs), such as carboxylates and corresponding carboxylic acids which constitute the major fraction of exudates in the rhizosphere during P deficiency [23]. Citrate, oxalate, malate and acetate are found to efficient in mobilizing P depending on the pH soil mineralogy and anion concentration [24]. Other exudates include proton extrusion,
phosphatase exudation. The exudation of phosphatases was mostly associated with the cell wall [25]. It is a hydrolytic enzymes response by plants to P-deficiency. This enzyme enhanced both external PUE and internal PUE [26].

Plants also adapt to low Phosphorus by association with symbiotic and non-symbiotic microorganisms present in the rhizosphere. In fact exudation got enhanced when microorganisms were present in plant growth solution [27, 28]. Plant growth promoting rhizobacteria enhance nutrient bioavailability [29]. It is known that there is a close relationship between root exudates and microbial diversity [30], but it is still unknown at what level they influence each other. For efficiency of P uptake released, high-affinity transporters, transport proteins located in cell membranes, are important and are also up-regulated at low P.

2. Phosphorus Use Efficiency (PUE)
Phosphorus Use Efficiency (PUE) on the other hand measures how efficiently the P taken up is utilized to accumulate either grain or vegetative biomass. PUE can be determined by the ability of the plants to take up P from the soil and the efficiency of allocation/mobilization of P within the plant for sustaining biomass production which is why it is considered tissue specific biomass and P allocation patterns may affect the P use efficiency in plants. PUE is determined by the size of different pools of Phosphorus how it is mobilize over different plant tissues. This will determine the economic use of Phosphorus in plants. Grain PUE and straw PUE are considered to be important component traits of PUE. Based on the P stored in a specific part of plant these two traits are independent from other traits based on the total P in an entire plant. Therefore, one must improve grain PUE and straw PUE independently [31].

Plants also developed adaptive mechanisms to reprioritize internal utilization under P deficiency [32] (Irfan et al., 2020). Increasing the activities of enzymes such as induced intracellular acid phosphatases involved in P scavenging and recycling and by altering respiratory electron transport and other metabolic pathways can improve P mobilization internally under P deficiency [33, 34, 35, and 36]. Gene expression in response to P deficiency should be considered an important technique because having acquired this knowledge one might allow the development of crops with improved PUE.

Phosphorus use efficiency of crops is, not just determined by the uptake efficiency but also by the utilization inside the plant once the P is taken up. A new technique for developing crops that grow and yield well in soils low in plant-available P is best way of improving crop yields in low-input farming systems [37]. In such cases P-efficient crops would ideally combine high P uptake with efficient use of Phosphorus in biomass accumulation. Rice breeding programs in Africa are already using high internal PUE genotypes as donors in their efforts to develop cultivars better adapted to the low-input agriculture practiced in much of the continent. As reported by [8] in their study on Phosphorus use efficiency on upland rice it has been found that tillers, dry root and shoot weight, and P uptake in root increased significantly up to the medium P level, and when P was further increased in the growth medium, there was no statistically significant increase.

Breeding for Phosphorus Use efficiency in rice crops:
Approach has been made to improve phosphorus use efficiency through plant breeding strategies and newly developed biotechnological methods. Selection for crops with improve PUE is hindered by the environment and variation in soil conditions which in turn mask the genetic variation in the genotype. Breeding for Phosphorus Use Efficiency is complicated as it is based on quantitative characters which are controlled by many genes [26]. It is important to break down this complex set of traits into component traits. PUE can be dissected into traits that are more feasible to screening and selection. Conventional breeding method has been enhanced by molecular techniques such as marker assisted selection and genomics. In this way complex trait can be broken down into component trait that can be localized on the genome, i.e. by utilizing genetic linkage maps based on DNA markers and statistically locating sites on these maps linked to desirable traits, quantitative trait loci (QTL). However, despite the presence of genotypic variation for PUE and associated QTLs, there has been little progress in breeding crops with high PUE. Root traits such as lateral branching and root hair density are very important characteristics for improving PAE. Other physiological traits, which are important for improving PUE, are root exudation of acids and enzyme phosphatases enabling accumulation in the soil, and architectural traits primarily of the root. In order to improve Phosphorus Use efficiency these traits must show possible inheritance to bring an elite plant material. For assessing both phenotypic traits and genotypic traits it is recommended that plants should be grown in a manner where these traits can be easily access. RSA can be dissected, principally as a result of the availability of amenable marker systems. However, phenotyping Root System Architecture (RSA) is a complex task which requires a growth system, root imaging system and software tools [38]. Root System Mark-up Language (RSML): a system that enables root system architecture information storage based on the MTG formalism and XML standards [39] has been introduced. Root can be easily imaged for studying root behaviour using a gel-based growth platform. [40] Identified a root growth system in which the roots grow in a solid gel matrix that was used to reconstruct 3D root architecture in situ. The growth behaviour of root in gel and in soil might not be comparable. When conducted phenotypic studies in rice for RSA, result shows that depth and average root radius are the two major traits which shows variation which suggest that there is a strong genetic component underlying rice RSA [41]. Understanding genetic regulation of root development requires knowledge of the molecular regulatory network of root development in rice [42].

Major QTL detected in Rice: Research and findings
Identification of QTL for traits that are positively or negatively correlated with PUE is an important step for developing improved rice variety with high efficiency of PUE. QTL for Phosphorus uptake (Pup1) in rice was identified during the genotypic screening studies. It is a major quantitative trait locus (QTL) located on rice (Oryza sativa) chromosome 12 and has been found to be associated with tolerance of phosphorus (P) deficiency in soil [43, 44].
Phosphorus uptake1 (Pup1) confers tolerance of phosphorus deficiency in soil and is currently one of the most promising QTLs for the development of tolerant rice (Oryza sativa) varieties. Pup 1 has a significant impact in P-deficient soil. Pup1 rice breeding lines are currently being evaluated in low-input upland rice growing areas, mainly in South East Asia and Africa, to evaluate their performance in different target environments. Pup1 gene was introgressed into two irrigated rice varieties and three Indonesian upland varieties adopting marker-assisted backcrossing approach and it was found that it has high yield potential under different environmental conditions. Backcrossing Near Isogenic Lines (NIL) C443 line to Nipponbare secondary mapping population was developed to fine-map the QTL for major P uptake, close to 80% of the genotypic variation at Pup1 locus for tiller number was observed between F2 families. In a crossing between a variety Nipponbare and Kasalath a major quantitative trait loci for P uptake had been identified in the resulting population. This quantitative trait locus was transferred after three generation of backcross to ‘Nipponbare’ which shows better performance to the parent (Nipponbare) in P uptake and grain yield. These results confirmed the presence of genetic variation between the parent and the developed cultivar resulting from the cross. Precision mapping of the Pup1 locus on chromosome 12 had been carried out in the donor variety Kasalath, revealing that Pup1 was located on a low recombination frequency region which indicates that insertions/deletions (INDELs) is likely responsible for the observed low recombination frequency and many genes located in this region are unknown.

Using two contrasting varieties Gimbozu and Kasalath a cross was performed developing hybrid population. From this population QTL analysis for induced root elongation under P deficiency condition was detected under genetic linkage map, indicating single QTL for the elongation response was detected on chromosome 6 which will be useful for improving nutritional disorder in rice under low P. F3 lines developed from a cross between NERICA 10 (P tolerant variety) and Hitomebore (P sensitive variety) which results into four QTL detected on chromosomes 4, 6 and 11 for P deficiency tolerance. From a Recombinant Inbred Lines (RILs) population developed from a cross between IR 20 and IR55178-3B-9-3, four QTL has been detected on chromosome 4, 6 and 11, one for Acid Phosphatase (AAP) and three for Phosphorus stress induced Psi-AAP respectively under two different Phosphorus condition (deficient and sufficient) in rice crop. Other RILs developed in rice from a cross between indica variety Dasanbyeo and japonica TR22183 in which six panicle length QTLs, qPL1.4, qPL2.1, qPL2.2, qPL4.1, qPL9.2, and qPL11.2 have been detected on chromosomes 1, 2, 4, 9, and 11 respectively and prove to be beneficial for improving rice varieties under tropical conditions. QTL for grain yield (qtl12.1) under drought condition results from a mapping population in a cross between Vandana and Way Rarem, this QTL improved grain yield under drought condition in upland rice varieties. Three QTL related to tolerance to low Phosphorus using genetic linkage map from a double haploid population and Single Seed Distribution has been located in which two QTLs affected relative Phosphorus uptake and one for Phosphorus utilization efficiency. QTL for adaptive traits has been detected from introgression lines which suggest that rice plant develop different mechanisms in low and high Phosphorus supply levels. Seventeen QTL were detected for plant yield and component traits under Phosphorus deficiency from a Double Haploid Population result from a cross between two japonica varieties IRAT 109 (P tolerant and Yeufu (P sensitive) 48 QTL have been detected in Introgession lines (ILs) developed from a cross between Minghui 86 and Yetuozaiz. These QTL have been identified under low phosphorus treatment. From backcross recombinant inbred lines (BILs) population which was formed from an interspecific cross (Oryza sativa L. × O. rufipogon Griff.) eight QTLs were detected which contribute to phenotypic variation.

**Studied on Phosphorus Starvation Tolerance gene (OsPSTOL1)**

OsPSTOL1 has been identified as the causal gene within a major QTL Pup1 which significantly enhanced grain yield in highly Pi-deficient soil. OsPSTOL1 codes for a protein kinase which is present only in phosphorus tolerant. Therefore, molecular markers need to be highly specific to the target gene to avoid nonspecific amplification when used in breeding programs. In fact, the first set of Phosphorus-Starvation Tolerance 1’ (OsPSTOL1)-specific markers produced unreliable results when tested in accessions of the African rice Oryza glaberrima for example, but this eventually led to the identification of a novel OsPSTOL1 allele in the rice variety CG14, the founder parent of NERICA (new rice for Africa) upland breeding lines.

OsPSTOL1 gene has been introgressed in two local varieties namely, ASD 16 and ADT 43 which harbour bacterial blight and blast resistance (R) genes through Marker Assisted Backcrossing (MABC) shows that OsPSTOL1 gene is responsible for enhancing the root system architecture and morphology under low P condition by improving root morphology and root hair density in rice plants.

**Phosphate Transporters and their post translational control**

Most of the plants absorbed Pi either in the form of HPO₄²⁻ or H₂PO₄⁻ forms from the soil solution. Within the plant, most phosphorus is stored in the vacuole. However, phosphate efflux from the vacuole is insufficient to balance the cytosolic phosphate concentration during phosphorus starvation. Under phosphorus-deficient conditions, the cytosolic phosphate concentration decreases rapidly due to insufficient phosphate efflux from the vacuole, which triggers plant adaptive responses to facilitate phosphorus acquisition and translocation. There are thirteen families of phosphorus transporters in rice. In rice, PHT11 and PHT13 have been shown to be specifically expressed in roots colonized by arbuscular mycorrhiza. Two other PHT1 family members, OsPHT1; 2 and OsPHT1;6, have been functionally characterized, and OsPHT1;6 emerges as a promising target, as outlined in more detail below. The transcription of PHT1 transporters is induced by phosphorus starvation. They
are responsible for phosphate uptake from the soil and transport to the shoot and the mycorrhizal symbiotic interface. In rice, two transporters have been functionally characterized in more detail, identifying OsPHT1; 2 as a low-affinity Pi transporter and OsPHT1;6 (OsPT6) as a high affinity Pi transporter, with OsPT6 being expressed in the epidermis, cortex and stele of young lateral seedling roots. Despite the complexity of the regulation of Pi uptake, the overexpression of OsPT6 alone revealed promising results in two independent studies. In rice, OsPT6 was cons titutively expressed using the ubiquitin promoter, and plants were tested in Pi-deficient soil and under field conditions. The authors report that the transgenic plants had a higher Pi content over a range of different Pi levels and, furthermore, that the transgenic plants had a higher grain yield under field conditions, mainly attributed to an increased tiller number. A comparative study using a set of seven diverse rice genotypes furthermore revealed a relatively higher OsPT6 gene expression in the irrigated variety IR66, which also showed a strong Pi starvation-induced increase in root growth. In agreement with that, in two independent field experiments IR66 maintained its grain yield under Pi deficiency, whereas other varieties had a reduced grain yield by up to 50%.

Discussions
Based on the study through researches and findings from work related to Phosphorus Use Efficiency (PUE) and its associated traits we understand that Pi is second most important nutrient next to Nitrogen. However, due to the high fixation of Phosphorus with other compounds in acid soils and slow diffusion rate its absorption by roots becomes very difficult for plants. To improve PUE we also need to consider PAE traits such as root system architecture, root length and microbial association in the rhizosphere. In fact, PAE traits are more concerned for absorption of Phosphorus uptake from the soil and should be focus in breeding programs. On the internal PUE which is tissue specific determines how P is being taken up and utilized to accumulate either grain or vegetative biomass. Breeding crops for PUE has been taken up and has a major problem as it is a quantitative trait which needs to be divided into small component traits. Major QTL PUP1 derived from Kasalath variety is a major breakthrough in search for Phosphorus tolerant in rice crops. After these findings, search for other QTLs has been initiated from different derived population results from different cross in japonica rice varieties. Despite these efforts, these QTL are not as promising as PUP1. More research on these QTL is needed to understand and to find out the effective QTL which will confer tolerance to low Phosphorus in the soil. OsPSTOL1 another tolerance gene has been found only in P tolerant varieties which codes for protein kinase. This gene is found to improve root morphology of crops which will in turn improve Phosphorus uptake in the soil.

Conclusions
From this review, we clearly understood how important Phosphorus is for plant growth and development. In upland rice where P is bound to other compound especially in acid soils it became unavailable to the plant. Therefore, researchers have studied how to improve Phosphorus Use Efficiency through different modifications such as the root architecture which is difficult because results of the study conducted on PUE clearly show that genotypic comparisons of PUE are biased if genotypes differ in P uptake. Study on Root traits such as lateral branching and root hair density for improving PUE have been studied in breeding programs. However, these characters are difficult to monitor and using them for selection in breeding programs is certainly not straightforward. A major QTL for P uptake (Pup1) in rice was identified during the genotypic screening studies by crossing the modern variety ‘Nipponbare’ and high P uptake variety ‘Kasalath’. The major quantitative trait locus (QTL) Phosphorus uptake1 (Pup1) confers tolerance of phosphorus deficiency in soil and Pup1 rice breeding lines are currently being evaluated in low-input upland rice growing areas. Another major gene OsPSTOL1 has been identified as the causal gene within a major QTL enhancing plant growth in highly Pi-deficient soil. To enhance P use efficiency in agriculture, different aspects from land use, preventing Erosion, reducing P removal from the field, maintaining soil quality, improving fertilizer recommendation, fertilizer placement methods, improving crop genotype and promoting mycorrhizas should be studied despite focusing on breeding efforts because these factors are primary in improving phosphorus use efficiency. There is also very less research done especially related to upland rice. Therefore, more studies should be done to find out ways and methods in enhancing the internal P use efficiency using breeding methods combined with biotechnology tools.

References
8. Fageria NK, Baligar VC. Upland Rice Genotypes


44. Wissuwa M, Ae N. Further characterization of two QTLs that increase phosphorus uptake of rice (Oryza sativa L.) under phosphorus deficiency. Plant and Soil. 2002; 237:275-286.
47. Chin JY, Gamuyao R, Dalid C. Developing Rice with High Yield under Phosphorus Deficiency: Pup1 Sequence to Application. 2011; 156:1202-1216.
66. Ai P, Sun S, Zhao J. Two rice phosphate transporters, OsPht1; 2 and OsPht1; 6, have different functions and kinetic properties in uptake and translocation. Plant Journal. 2009; 57:798-809.