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Full Length Article

Cytokinin Interaction to Cope with Phosphorous Starvation in Rice (*Oryza sativa*)

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Abstract

Phosphorous (Pi) acquisition in limiting environment is a complex phenomenon and different plants adopt different strategies for their survival. Efforts for crop improvements in terms of Pi acquisition in phosphorus deficient environment are hindered by involvements of several complex cross-talks; therefore we examined the effect of phosphorous deficiency and involvement of cytokinin pathway in rice plants. Japonica rice seedlings were grown on three Pi concentrations including, i) P_c: control seedlings grown on normal Pi concentration (45 μM), ii) $P_{1/2}$: Pi deficient conditions with 22.5 μM Pi and iii) P_0: Pi starved conditions. Our data showed significant increase ($p < 0.05$) in shoot and adventitious root lengths under Pi deprived conditions along with thin and comparatively longer primary roots than P_c. Despite of physiological responses, up-regulation of phosphate transporter proteins and their respective genes in roots has confirmed the enhanced phosphorous absorption and mobilization through roots in response to Pi deficiency. Reduced cortex cell number and enlarged size of root apical meristem region in phosphorous deficient/starved plants were complemented with addition of exogenous cytokinin (0.1 μM BA), provides an evidence of involvement of root tips in Pi sensing due to increased surface area and cytokinin pathway interaction to facilitate Pi acquisition. Similarly, down regulation of type-B response regulator (OsRR6 and OsRR9/10) and up-regulation of isopentenyltransferases (OsIPT7) in the phosphorous deficient rice roots have implicated antagonistic interaction of cytokinin with Pi signaling pathways in this particular rice variety. Rice varietal screening can help to elucidate differential modifications in general responses under Pi deficient conditions to improve future agronomic practices and future biotechnological strategies of crop improvements. © 2018 Friends Science Publishers

Keywords: Phosphate transporter; Phytohormone; Root apical meristem; Response regulator

Introduction

Plants require phosphorous as an essential macronutrient, which take part in important cell processes for growth and development like energy metabolism, signal transduction and synthesis of important cellular components (Raghothama, 1999; Li *et al.*, 2012). Plant roots mainly absorb inorganic phosphate (Pi), which is most readily accessible but least available form of phosphorous in the soil. To confront low-Pi stress in growth media or soil, plants adapt many strategies at morphological, physiological and biochemical levels (Miura *et al.*, 2005; Jiang *et al.*, 2007; Teng *et al.*, 2013). Phosphorous deficiency have been studied in many plants like, *Arabidopsis* (Poirier and Bucher, 2002; Sanchez-Calderon *et al.*, 2005; Svistoonoff *et al.*, 2007), wheat (Shukla *et al.*, 2016), rice (Insalud *et al.*, 2006; Oono *et al.*, 2013), maize (Li *et al.*, 2012), barely (Schünmann *et al.*, 2004; Vysotskaya *et al.*, 2016) and lentil (*Lens culinaris*) (Sarker and Karmoker, 2009).

Phosphorous deficiency mainly induces root architecture modifications like changes in root/shoot ratio, length of primary root, lateral root length/number and induction of adventitious roots to increase the absorptive capacity of roots (Martín *et al.*, 2000; Li *et al.*, 2012). However, these root traits differ significantly in response to Pi availability in different species or sometimes among species (López-Arredondo *et al.*, 2014). Low-Pi availability in *Arabidopsis* is usually shown with reduced primary root length while number of lateral roots and root hairs were increased (Bates and Lynch, 1996; Sanchez-Calderon *et al.*, 2005). Absence of Pi from the nutrient medium have inhibitory effects on whole plant growth in barley; root length was maintained but number of lateral roots significantly declined (Vysotskaya *et al.*, 2016). Pi deficiency in lentil (*Lens culinaris Medik*) led to an increase in primary root, number of lateral roots and their length. Moreover, rise in the root meristem volume of lentil seedlings was also observed (Sarker and Karmoker, 2009). While low-Pi treatment to maize plant retarded shoot growth and promoted root growth. Lateral root formation was also inhibited in low-Pi medium in maize (Li *et al.*, 2012).

Rice (*Oryza sativa*) is one of the most important food

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crops in the world. Phosphorous starvation in rice led to many morphological adaptations such as root elongation, induction of adventitious root growth, alteration in lateral root length and number along with modifications at physiological and biochemical levels for efficient Pi uptake and transport (Oono *et al.,* 2013). Nutrient deficiencies effect phytohormones biosynthesis as well as their signaling (Kiba *et al.,* 2011; García *et al.,* 2015; Pozo *et al.,* 2015). Recently, researchers are exploring the role of different phytohormones in many nutrient deficiency responses (including nitrogen (N), phosphorus (P), potassium (K), boron (B) and many others nutrients) in plants (Wang *et al.,* 2006; Liu *et al.,* 2009; Vysotskaya *et al.,* 2016; Zhou *et al.,* 2016). In phosphorous deficiency, the role of phytohormones such as ethylene, cytokinin, abscisic acid (ABA) and auxin is elucidated (Martín *et al.,* 2000; Wang *et al.,* 2006; Vysotskaya *et al.,* 2016; Giri *et al.,* 2018). Cytokinin (CK), a structurally diverse hormone, plays key role in growth and development by involvement in nutrient signaling and homeostasis, regulates many cellular processes like cell division, chloroplast biogenesis and root or shoot morphogenesis (Kieber and Schaller, 2014). It has been reported that cytokinin have crucial role in low Pi stress by regulating phosphate absorption and translocation and by down regulating many genes in response to Pi starvation (Martín *et al.,* 2000; Hou *et al.,* 2005). In *Arabidopsis*, Pi deprivation causes increase in root to shoot growth ratio that was associated with a decrease in cytokinin contents and the expression of *AtIPS1* and many other Pi starvation-genes were repressed by exogenously applied cytokinin in response to Pi depletion (Martín *et al.,* 2000), suggesting the important role of cytokinin in Pi limited environment. The role of cytokinin in phosphorous limiting conditions in cereals crop might provide a method to cope with stress condition.

Phosphorous (Pi) acquisition in limiting environment can lead different plants to adopt different strategies at physiological, biochemical and molecular level for survival. Therefore we examined the effect of phosphorous deficiency and involvement of cytokinin pathway in rice plants. We also studied the effect of Pi deficiency on cell size and number of root apical meristem of rice seedlings and the influence of cytokinin on them. The level of different phosphate transporters and response regulator genes in Pi starved tissue was also determined to find evidences and nature of interaction between Pi starvation and cytokinin signaling pathway.

Materials and Methods

Plant Material and Growth Conditions

We used japonica rice cv. Kitaake (*Oryza sative* L.) to study the effect of phosphorous deficiency and cytokinin signaling in rice. Ten days old seedlings grown on agar plates were used to study in-depth mechanism of Pi deficiency on plant morphology.

Sterilized rice seeds were germinated on half-strength Kimura B nutrient solutions, which consist of $(NH_4)_2SO_4$, KNO_3, $MgSO_4$, **KH_2PO_4**, NaEDTA-Fe, $Ca(NO_3)_2$, $MgSO_4$, H_3BO_3, $MnCl_2$, $ZnSO_4$, $CuSO_4$, and $(NH_4)_6Mo_7O_{24}$ (Yoshida *et al.,* 1976; Ma *et al.,* 2001). KH_2PO_4 concentrations were adjusted for phosphorous deficient and starved treatments. Three Pi concentrations; P_c *i.e.*, control with normal/sufficient phosphorous concentration (45 μM), $P_{1/2}$ *i.e.*, Pi deficient condition (22.5 μM), and P_0 (Pi starved conditions) were used.

Seeds were inoculated on large square plates (120x120 mm) containing half strength Kimura B nutrient solution based on the MES salts and 0.8% agar, incubated at 37°C over night for germination and then shifted to 25°C,12/12 h light /dark cycle vertically. At 10th day images were scanned to observe the difference in seedlings growth. Images were then processed and analyzed by ImageJ 1.50 b (National Institutes of Health, Bethesda, MD, USA).

For molecular analysis, rice seedlings were germinated for 21 days in hydroponics containing Kimura B nutrient media based on the MES salts, with three Pi concentrations (P_c, $P_{1/2}$ and P_0). Fresh solution was replaced on every third day and the plants were kept at 25°C with a 12-h photoperiod in a growth chamber. After 21 days, root and shoot samples were taken and stored in liquid nitrogen at -80°C until analysis.

Microscopy of Root Apical Meristem

Root apical meristem of the rice seedlings were analyzed using chloral hydrate. The cells of root cortical layer was counted in a line starting from quiescent center till the cell length increased than its width, using a Nikon Eclipse 90i optical microscope with Nomarski optics and a 20X resolution objective lens (Perilli and Sabatini, 2010).

Western Blotting

Pi uptake from the soil mainly relies on PHT1 (phosphate transporter1) proteins, therefore, the effect of Pi deficiency on PHT1 proteins trascripit level was evaluated. Protein extraction was done by using SDS Extraction Buffer (containing 10 m*M* EDTA, 120 m*M* Tris-HCl, 4% SDS, 10% β-ME (β-mercaptoethanol), 5% Glycerol and Coomassie Brilliant Blue dye). For all three (P_c,$P_{1/2}$, and P_0) treatments 0.1g plant tissue and 4X buffer volume was used for extraction and 30 μL was loaded on 12% SDS-PAGE. Protein-specific primary antibodies, PHT1 (1:2000), created by the Heckathorn lab (for protocol see Mishra *et al.,* 2012) were used. Stabilized Goat Anti-Rabbit HRP Conjugated (PIERCE) secondary antibody (1:10,000) and SuperSignal™ West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific) (1:5 dilutions) were used in western blot analysis. Images were analyzed by chemidoc instrument.

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Quantitative Real-Time PCR (RT-qPCR)

We also analyzed the transcript levels of five Pi-transporter genes OsPT1, OsPT2, OsPT4, OsPT6 and OsPT8 to check the differences in the phosphate acquisition at molecular level in roots and shoots of 21 days old rice seedlings grown hydroponically in the selcted Pi concentrations *i.e.*, P_c, $P_{1/2}$ and P_0. Rice Ubiquitin 5 (UBQ5) was used as an internal control gene for expression analysis. Root and shoot samples were saved separately for total RNA isolation by E.Z.N.A. Plant RNA kit (Omega Bio-Tek, GA, USA). For DNase treatment, TURBO DNA-free kit (Amibion by Thermofisher Scientific) was used according to manufacturer's instructions. First strand cDNA was synthesized using standard protocol of BIORAD iScriptc DNA synthesis kit for RT-qPCR according to manufacturer's instructions. cDNA, prepared from 2 μg RNA, and was diluted to five folds to be used for real time PCR. For RT-qPCR three technical replicate of each cDNA sample were analyzed with the BIORAD iTaq Universal SYBR Green supermix with CFX384 Real-Time PCR system (Bio-Rad). The gene-specific primers were designed using Integrated DNA technologies (IDT) Primer Quest SM and they are listed in Table 1.

Results

Changes in Rice Root Architecture and Plant Physiology in Pi-Depleted Conditions

Ten days old rice seedlings were examined for effect of Pi starvation on selected treatments including $P_{1/2}$ and P_0 in comparison to control seedlings (P_c) (Fig. 1A). Significant changes in plant morphology was observed. Shoot length was increased in response to lower concentrations of Pi and interestingly P_0 samples ($p \leq 0.05$) of selected rice variety had longest, pale and weak shoots (Fig. 1B), because Pi deficiency or depletion caused reduction in the photosynthetic rate (P_N) in rice seedlings. To support this, the maximum efficiency of PSII (F_v/F_m) and its comparison with the control plants showed significant decline in the given Pi-deficient or Pi-depleted rice leaves. Primary root length slightly increased with decreasing phosphorous concentration ($p = 0.09$). Average length of three longest adventitious roots was also measured from each set of seedlings for comparisons. More importantly, significant increase in adventitious root lengths ($p \leq 0.01$) was also observed in P_0 seedlings as compared to control (P_c) seedlings (Fig. 1C).

Effect of Pi Deficiency with and Without Cytokinin Treatments on Root Apical Meristem

Effect of Pi deficiency on the size of root apical meristem of rice seedlings in the presence and absence of cytokinin were examined using primary root tips of treated and control seedlings. Root apical meristem consists of three different zones: stem cell niche, division zone and the elongation zone and all of these distinct zones were found in present study. Cortex cells were counted to determine the size of root apical meristem starting from the quiescent center to the first elongated cell entering the elongation zone using microscopy of ten days old rice seedlings grown on large square kimura agar plates in five different conditions (P_c, $P_{½}$, P_0, P_c+BA and P_0+BA). P_c seedlings had highest cell count i.e., 40 along with more layers in the rice root cortex than Pi deficient ($P_{½}$) seedlings (cell count 33.8). Similarly, the cell size also increased in that region ($P_{1/2}$) while the number of cell layers was fewer than control. In P_0, the cell sizes were enlarged and well organized into cell layers along with significant decline in cell count i.e., 31.3 as compared to P_c (Fig. 2 A&B). Interestingly, when exogenous cytokinin (0.1 µM BA) was applied to control sample (P_c), the number of cells is reduced from 40 to 35.9 but when BA is applied to P_0 samples the cell count only drop to 32 as compared to P_c (Fig. 2 A&B). Exogenous cytokinin application inhibits the cell division at root apical meristem as evident from decrease in cell number at root apical meristem of control seedlings (Fig. 2 B).

Effect of Pi Deficiency on PHT1 Expression

We noticed three bands of approximately 60 kDa, 45 kDa and 33 kDa on western blot with varied expression in our conditions in root and shoot samples seperatly. Estimated size of mature PHT1 in rice is approximately 60 kDa, while other two bands may represents its partial translational products. PHT1 expression was minimum in control samples, while several fold increase in PHT1 protein expression was observed with gradual decrease in the Pi concentartions to Pi starved (P_0) samples, provides the evidences of increased Pi aquisitions under given treatments on rice seedlings (Fig. 2C). Enhanced accumulation of PHT1 protein in phosphorous limitation is also related to help in accelerated phosphorous uptake in plants.

Induced Expression of Rice Phosphate Transporter Genes (OsPTs) in Response to Low Phosphorous Show Enhanced Pi Acquisition

In roots, relative expression of OsPT2 and OsPT4 increased with decreasing Pi concentrations in the solution. Pi deficient ($P_{1/2}$) and Pi starved (P_0) plants had 1.5 and 3 folds induction respectively as compared to P_c (control) plants, while OsPT1, OsPT6 and OsPT8 did not showed any significant change(< 2fold) (Fig. 3A). In shoots OsPT2 showed ~2 fold induction in P_0 plants as compared to P_c plants. OsPT6 level was slightly increased in shoots at Pi-deficient samples, while OsPT1, OsPT4 and OsPT8 levels were not changed significantly (Fig. 3B).

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Table 1: List of Genes and Primers used for study

Internal Control Gene	GENE	FORWARD PRIMER	REVERSE PRIMER
	UBQ5	ACCACTTCGACCGCCACTACT	ACGCCTAAGCCTGCTGGTT
Pi Starvation Responsive Genes	*OsPT1*	GTGAGTGAGATGTGTGTATATG	GGTCGCTACAAACAATCAA
	OsPT2	CAGGCTAAGACGCAATG	GTGATGTCGGTGTAGTAAAG
	OsPT4	CACGGGTTACTGTTGATATT	GTAGGCGATGTTATTGTTATT
	OSPT6	CATCTTCACCAGCATCAA	AAGACGGTGAACCAGTA
	OsPT8	GCCTTCACCTTCTTCTTC	CGAACGATCCGATGATG
Cytokinin Pathway genes	*OsRR 6*	CATCACCGACTACTGGATGC	GGGATCTCCTTGAGCTGAGA
	OsRR9/10	TCATGAGGACAGCCCAATTTCTA	TGCAGTAGTCTGTGATGATCAGGTT
	OsIPT7	GGCGATCGACGACATCA	CCCAGGCGTCGGACA
	OsCKX2	GTGGCCGGGATAGCCTAC	AGTGCCGCTTCTGCCACT

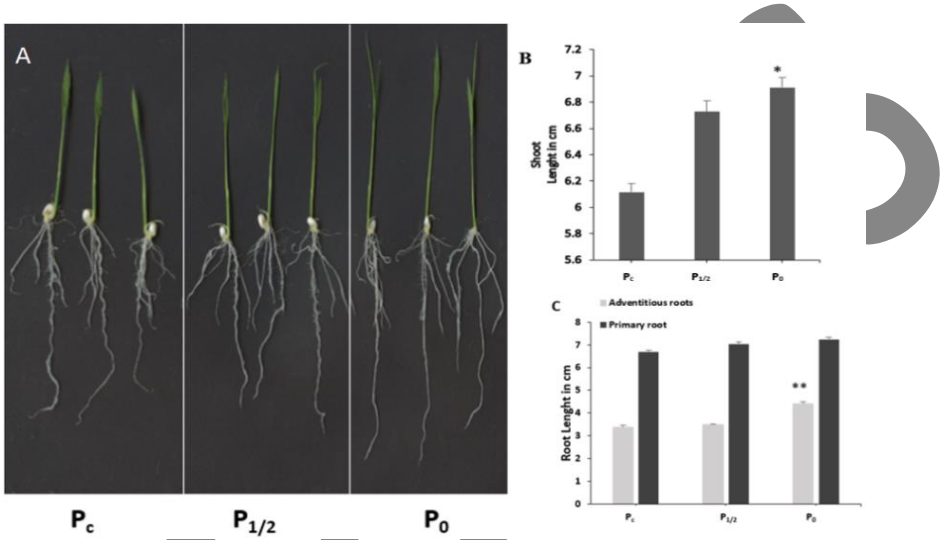

Fig. 1: Effect of phosphorous deficiency on rice plants. A: Ten days old rice seedlings grown on half strength Kimura B nutrient media (based on the MES salts and 0.8% agar) with (control) and without phosphorous. Three representative rice seedlings are shown from each treatment i.e., P_c (control), $P_{1/2}$ (Pi deficient) and P_0 (Pi starved). B: Comparison of shoot length for three treatments. C. Comparison of root (primary and adventitious) lengths analyzed with ImageJ software is shown here for eachtreatments. Comparison of three longest adventitious roots length from all replicate for three treatments is given here. Error bars represent the means ±SD of three replicates with 07 seedlings in each replicate. Asterisks (*) represents significant differences from P_c (*; $P \leq 0.05$, **; $P \leq 0.01$ from One-way ANOVA with post-hoc Tukey HSD Test)

Induced expression of OsPT2 and OsPT4 in rice roots under Pi deprived conditions showed increased phosphate absorption and transport as evidant by increased OsPT2 trasctripts in shoots.

Effects of Pi Deficiency on Cytokinin Signaling Pathway Gene Expression

In order to evaluate the role of cytokinin signaling in Pi limiting environment, we analyzed the transcript levels of four important genes from cytokinin signaling pathway, comprising of two type-B response regulators (OsRR6 and OsRR9/10), an isopentenyltransferase (OsIPT7) and a cytokinin oxidase/dehydrogenase gene (OsCKX2). All of these four genes are downstream components of cytokinin two-component signaling pathway. Expression data showed suppressed level of OsRR6 and OsRR9/10 in root samples with decreasing Pi concentrations ($P_{1/2}$ and P_0) in comparison to P_c, while OsIPT expression level was slightly upregulated in Pi starved root samples as compared to P_c (Fig. 3C). On the other hand, in shoot samples no remarkable difference in expression of all four selected genes was observed (Fig. 3D). Alteration in expression of cytokinin signaling key players in roots, implicates the involvement of cytokinin pathway in Pi stress.

Discussion

Although phosphorus has a great impact on overall plant growth but plant roots are highly influenced by the availability of phosphorus. Plant's ability to cope with Pi deficient conditions varies in different plants or genotypes (López-Arredondo *et al.,* 2014). We studied the effect of Pi deficiency on rice growth and involvements of cytokinin pathway in Pi induced responses.

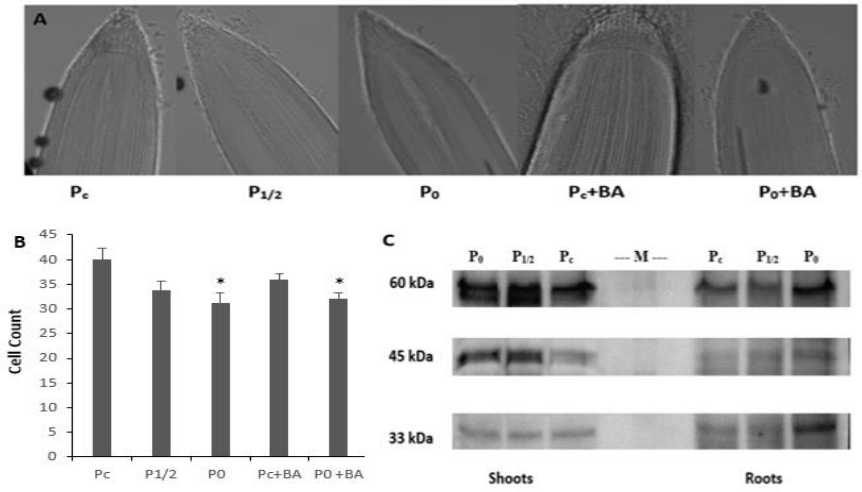

Fig. 2: Effects of phosphorous deficiency on root apical meristem and PHT1 transcription. A: Microscopic examination of cell count and cell size in root apical meristem of primary root tips of 10 days old seedlings under Pi deficiency with and without cytokinin. Images of root apical meristem by 20X objective of a Nikon Eclipse 90i optical microscope. B: Bar graph showing trend of cell count under Pi limiting conditions with and without 0.1 µM BA (exogenous cytokinin, 6-Benzyl-aminopurine) in root apical meristem. The means (n=10) is presented here. Error bars represent SE. Asterisks (*) represents significant differences from Pc (*; $p \leq 0.05$ from One-way ANOVA with post-hoc Tukey HSD Test). C: Western blot analysis of PHT1 antibody on shoot and root total protein extracts reveal the presence of potential multimers. Rice seedlings were grown for 21 days in Kimura B nutrient media with three different phosphorous concentrations these are P_c(control),), $P_{1/2}$ (Pi deficient) and P_0 (Pi starved). From left to right, first 3 lanes are of shoot samples (P_0, $P_{1/2}$ and P_c) in the center marker (M) (Bio-Rad Precision Plus Protein Standards) is loaded, then three samples of roots (P_c, $P_{1/2}$ and $P_{0)}$ were loaded

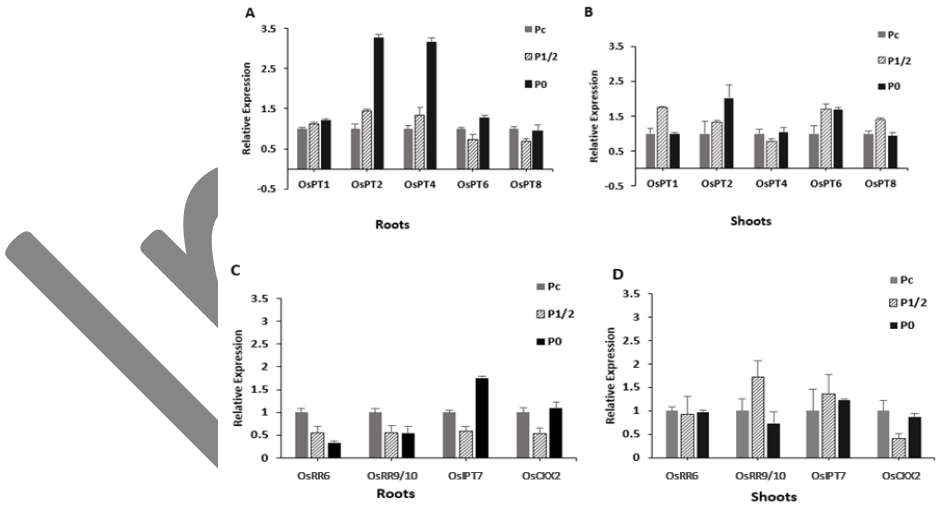

Fig. 3: Relative expression of Pi transporter genes and key cytokinin signaling players in rice roots and shoots under limited Pi availability. A and B: Expression pattern of five OsPTs genes (OsPT1, OsPT2, OsPT4, OsPT6 and OsPT8) in rice roots and shoots is shown here for three different Pi level (P_c, $P_{1/2}$ and P_0). CandD.Transcript levels of two response regulator genes OsRR6 and OsRR9/10, along with OsIPT7 and OsCKX2 genes is shown here for 21 days old rice seedlings (roots and shoots) treated with three Pi concentration (Pc, $P_{½}$ and P_0). Ubiquitin 5 is internal control gene

Phosphorous deficiency in rice (Kitaake cv.) led to an increase in overall plant growth and less vigor in present experimental conditions. Shoot and adventitious root lengths were significantly increased (p-value <0.05) in Pi-starved conditions as compared to control plants. The increase in the length of the primary root of rice (p-value 0.09) in P_0 conditions was also observed but the roots were very weak, showing some adaptive mechanisms in this particular rice

variety. Our findings were totally in conjunction with other studies, when Japonica rice varieties showed promoted growth of primary as well as adventitious roots in case of less phosphorous availability (Yi *et al.,* 2005; Zhou *et al.,* 2008; Dai *et al.,* 2012). Similar type of root elongation is reported in response to Pi deficiency in many other plants species including maize and lentils (Kirk and Du, 1997; He *et al.,* 2003; Sarker and Karmoker, 2009; Li *et al.,* 2012). Elongation of primary and adventitious roots in Pi limiting environment may help the plant to increase the root surface area for efficient Pi uptake, as LePT1 protein accumulation was seen in different parts of Pi starved tomato roots, except the extreme tip regions, suggesting that plants enhances their ability to forage phosphorous all along the roots in response to Pi-starvation (Muchhal and Raghothama, 1999). Li *et al*. (2012) also found that in low Pi solution the length and growth of primary root was promoted in maize while shoot length, number of lateral root and lateral root primordia were retarded. In case of rice, we observed a significant increase in shoot growth, although the plants were pale and weak in comparisons to control plants. Shimizu and co-authors (2004) screened ~62 rice varieties for root elongation by phosphorous deficiency, and observed a clear difference in root length among varieties at two different Pi concentrations (Shimizu *et al.,* 2004). All of these morphological differences in response to limited phosphorous conditions among and between plants species, are might be due to different experimental designs and genotypes used (Li *et al.,* 2012).

Root architecture modification in response to low phosphorous mainly depends on cellular activity occurring at root apical meristem. Which is controlled by a number of hormonal and nutrient interactions depending on the environmental stimuli (Street *et al.,* 2015). The present study showed a significant decline in cell count at root apical meristem of rice seedlings with eliminating the phosphorus from growth media. These results indicate that low Pi inhibits the cell division at root apical meristem. Reduced number of cortex cells at root apical meristem had bigger cell sizes to increase the surface area for Pi foraging, which might result in longer root than the normal growth conditions.

Cytokinin is known to reduce the cell proliferation at root apical meristem (Stenlid, 1982), as appeared by a decrease in cell count at root meristem after BA application to control sample, but surprisingly it did not cause any further drop in number of cells at root meristem in P0 samples. Interestingly, in present work both Pi deprivation and cytokinin application exhibited decrease in cell count at root apical meristem but their effects on primary root length were opposite, as Pi starvation increased the root length while exogenous cytokinin application decreases the root length. This contrast behavior needs attention!

Phosphorous uptake and transport carried out through special protein carriers called phosphate transporter proteins (PHTs or PTs). In rice, the size of PHT1 members usually ranges from 508-582 amino acids with an estimated molecular weight around 60 kDa (Available at: https://www.ncbi.nlm.nih.gov/protein/?term=OsPHT1). To explore the clues of transcriptional and post-translational regulation of phosphate transporter proteins in response to low-Pi, we used PHT1 antibodies synthesized and used by in Heckathorn lab (Mishra *et al.,* 2012). Three bands of approximately 33 kDa, 45 kDa and 60 kDa sizes were observed on the blot and protein accumulation was increased with decreasing Pi concentration in the medium, reveal that these proteins are primarily involved in Pi deficiency responses in plant to enhance their capacity for phosphorous uptake. As the estimated size of OsPHT1 is 60 kDa, the other bands might represent partial translational products of PHT1 on the blot. Similar results were also reported in other plants like *Arabidopsis*, Tomato and *Medicago* (Muchhal and Raghothama, 1999; Chiou *et al.,* 2001; Nussaume *et al.,* 2011). In tomato a band of approximately 60 kDa size was observed on western blot, while in other plants such as *Medicago* and *Arabidopsis*, a band of ~45 kDa was observed instead of ~60 kDa on western blot for phosphate transporter proteins when plants were subjected to Pi deficiency (Muchhal and Raghothama, 1999; Chiou *et al.,* 2001; Nussaume *et al.,* 2011). While, Chiou *et al.* (2001) also observed an additional 33 kDa band on the blot and suggested that it is a partial translational product. We also observed presence of multiple bands on blot, which was in accordance with the previous reports (Chiou *et al.,* 2001; Nussaume *et al.,* 2011).

Among two families of Pi transporters (*Pht1* and *Pht2*), *Pht1* gene family is studied extensively and in rice *Pht1* has 13 members (OsPT1-OsPT13) (Goff *et al.,* 2002; Paszkowski *et al.,* 2002). Among these OsPT1, OsPT2, OsPT4, OsPT6 and OsPT8 are associated with direct Pi uptake in low Pi stress (Ai *et al.,* 2009; Yang *et al.,* 2012; Ye *et al.,* 2015). We here examined the transcript levels of these transporters in Kitaake rice root and shoot parts under three Pi concentrations (control, deficient and Pi starved levels). Expression levels of OsPT2 and OsPT4 increased significantly, OsPT1 slightly induced and OsPT6 and OsPT8 did not show any induction in roots when grown in Pi limiting environment (Fig. 3A). While in shoot, among the five Pi transporter studied, only OsPT2 level was upregulated in Pi starvation. In a similar study on Japonica rice (cv. Zhonghua 10), Dai and colleagues (2012), found OsPT2 level was upregulated in shoots and roots of OsMYB2P-1-overexpressing (an R2R3 MYB transcription factor induced by Pi starvation) plants. They also found that out of 13 OsPTs examined only OsPT2, OsPT6, OsPT8 and OsPT10 expression level was increased in roots of wild type rice under Pi deficiency. While in another study from rice, Seo *et al*. (2008) examined expression pattern of nine OsPTs in roots and shoots under low Pi availability. They reported upregulation of OsPT2, OsPT4, OsPT6, OsPT7, OsPT8, and OsPT12 in roots and OsPT1 gene constitutively expressed in the shoot and slightly elevated in roots when treated with Pi deficiency (Seo *et al.,* 2008). Up regulation of phosphate transporters in roots and shoots under limited Pi conditions

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revealed that these transporter enhance the phosphorous absorption and its transportation through roots. The higher expression of OsPT transcripts and proteins under low-Pi stress is evident for the transcriptional regulation of these transporters for efficient phosphorous acquisition and transport in plants (Muchhal and Raghothama, 1999; Smith *et al.,* 2000). Our results are consistent with previous findings where decrease in cytokinin contents have been shown in Pi starvation due to its involvement in signaling of plant Pi concentration or plant sugar contents (Gessler *et al.,* 2004; Franco-Zorrilla *et al.,* 2005). Previous studies have shown that cytokinin and its receptors play vital role in suppressing the upregulation of many genes under Pi deficiency environment, implicating potential cross-talk between cytokinin and Pi signaling pathways (Martín *et al.,* 2000; Franco-Zorrilla *et al.,* 2005; Hou *et al.,* 2005; Wang *et al.,* 2006).

Nutrient deficiencies in plants alter phytohormones biosynthesis and their signaling pathways. In a recent study, the changes in growth and hormones levels of Pi deficient barley plants showed that the root architecture modification of phosphorous deficient plants are might be due to the interaction between phytohormones such as cytokinin, auxin and abscisic acid (Vysotskaya *et al.,* 2016). Similarly a significant role of auxin and *OsAUX1* in promoting root for Pi acquisition in rice has been reported (Giri *et al.,* 2018). Exogenous cytokinin treatment represses many of the Pi starvation responsive genes (Martín *et al.,* 2000; Hou *et al.,* 2005; Wang *et al.,* 2006). We analyzed the transcript levels of some important genes of cytokinin signaling pathway in rice roots and shoots under phosphorous starvation. We picked two type-B response regulator (OsRR6 and OsRR9/10), an isopentenyltransferases (OsIPT7) and a cytokinin oxidase/dehydrogenase (OsCKX2). No significant change in transcript levels of all four genes was observed in shoot samples, while response regulator (OsRR6 and OsRR9/10) levels declines with decreasing Pi concentration and OsIPT7 relative expression slightly increased in phosphorous starved rice roots. OsCKX2 level does not change with changing Pi level. Low Pi availability in plants suppresses cytokinin activity by decreasing its concentrations (Martín *et al.,* 2000; Wang *et al.,* 2006) and by downregulation of the expression of cytokinin receptor i.e., CRE1 (Kuiper *et al.,* 1988; Franco-Zorrilla *et al.,* 2002). The downstream molecules of CRE1 are AHPs (histidine-containing phosphor transfer proteins) and type-B RRs (Kieber and Schaller, 2014). The down regulation of type-B OsRRs in this study also suggest that cytokinin activity is suppressed in response to low Pi availability. The key biosynthetic enzymes for cytokinin are adenosine phosphate-isopentenyltransferases (IPTs), slightly increase level of OsIPT may also results from decreased CK content of cells. Wang *et al.* (2006) found that most of CK responsive genes remain unchanged in response to Pi starvation in rice. These finding suggest that a complex interaction between CK signaling and Pi deficiency responses may occur and needs further exploration.

Conclusion

Phosphorous deficiency in this particular rice variety (Kitaake cv.) showed several modifications in root architecture system at morphological and cellular level along with transcriptional regulation of Pi transporter genes/proteins. Crucial role of cytokinin in response to phosphorous deficiency was shown by compensated effect of Pi starvation on root apical meristem with exogenous cytokinin application and downregulation of key cytokinin signaling pathway players in phosphorous starved samples has implicated potential cross-talk between cytokinin and Pi signaling pathways.

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