Research Article

Analysis of Expression of Phosphorus Deficiency Tolerance Related Genes in Root and Leaf Tissue in Indica Rice Genotypes

P.H. Janjal*, Shubha Banerjee and Satish B. Verulkar

Department of Plant Molecular Biology and Biotechnology, IGKV, Raipur (CG)

Abstract

Expression of 13 Pi deficiency induced and utilization related genes was characterized in root and leaf tissue of 20 rice genotypes indicating differential genotype and tissue specific expression was observed. The genes *OsPHR2, OsPHR3, OsPTF1, OsPTF2, PT1CD, OsPT2, KN1 and KN2* were significantly up regulated in shoot tissues of R-RF-78, SL0-16 and HYL-27 genotypes under P deficiency. The NRR (nutrient response and root growth) locus related genes (*RH3, Hd3a, Ehd1, RFT1*) known to be associated with yield attributing factors (heading date etc.) were also up regulated in these genotypes. The root and shoot traits were also recorded indicating significant variation and general reduction in plant height, number of leaves/plant, number of tillers/plant, root and shoot dry weight in P deficient condition. However, the genotypes namely R-RF-78, HYL-27, SLO-16, Mahamaya, G-8, G-102 and Dagaddeshi, showed Pi deficiency induced increase in root length, volume, surface area and dry weight under P deficiency. The correlation of gene expression analysis and phenotypic data for root and shoot traits under P deficiency indicated these genotypes as P deficiency tolerant rice genotypes.

Keywords: Phosphorus deficiency, Rice, NRR, Gene expression

***Correspondence**

Author: PH Janjal

Email: phjanjal@gmail.com

Introduction

Rice (*Oryza sativa* L.) is the main staple food crop worldwide and it is grown in a variety of environments, covering a wide range of latitudes and altitudes. Total soil phosphorus is often hundred fold more than the fraction of inorganic phosphorus available for uptake by crop plants often most of the phosphorus applied to field form complexes with iron and aluminum in acidic and calcium in alkaline soil and thus becomes unavailable to plants [1]. Pi is the most readily accessed form of P by plants [2]. Phosphorous deficiency inhibits Plant height whereas it induces maximum root length and root-shoot ratio. Also it increases the concentration of starch in roots whereas it has no effect on the content of starch in shoots or roots [3]. P deficiency at whole-plant level is a complex multi-genic trait governed by interactions between genetic, environmental, and management factors (G × E × M) and then highlighted the potential and limitations of root architectural and crop simulation models for evaluating the utility of root adaptive traits for P uptake and crop yield [4]. A few genes such as *PHR1*, *PHR2*, *OsPTF1*, *OsSPX1*, *OsSPX2*, *OsSPX3*, *OsIPS1* and *OsIPS2* have been reported in P deficiency signaling [6]. Screening for P-efficient rice varieties has its own problems, as the phenotypic screening, particularly in acidic soils is often limited due to other stresses (e.g. Toxicity of Iron, Toxicity of aluminum) which retard root growth and restrict phenotyping [5].

The uptake, translocation and remobilization of Pi are regulated by complex molecular mechanism by the action of Pi transporters (PTs) and other genes. Expressions of these PSI genes are regulated by the Pi Starvation Response Regulator (OsPHR2)-mediated transcriptional control and PHO2-mediated ubiquitination [6]. Rice has 13 known P transporters, of which 12 are expressed, and an additional 13 putative P transporters have recently been identified. Till now, only two transporters have been functionally characterized, that reveals OsPT2 encodes a low-affinity and OsPT6 encodes a high affinity transporter [4]. In rice roots the expression of NRR is greatly influenced by the deficiency of major nutrients. If NRRa is over expressed in rice then it may retard root growth. Both NRRa and NRRb plays negative regulatory roles in rice root growth and NRRa & NRRb genes acts as the key components and modulate the rice root architecture with the availability of macronutrients. OsPTF1 is a basic helix-loop-helix domain for tolerance to inorganic phosphate (Pi) starvation in rice. Over expression of OsPTF1 enhance tolerance to Pi starvation in transgenic rice [7]. The Phosphate Starvation Response Regulator 1 from Arabidopsis (PHR1) plays an important role in Pi starvation signaling. There are two homologous proteins of PHR in rice viz. *OsPHR1* and *OsPHR2*. Over expression of *OsPHR2* in rice mimic the Pi starvation signals. It induces PSI gene expression and result in the enhancement of Pi acquisition. In case of Arabidopsis and rice the transcript level of PHO_2 is negatively regulated by Os*PHR1 and OsPHR2* through miR399- dependent RNA cleavage [7].

Experimental

A set of 20 rice genotypes were grown in soil filled glass rhizotron for 60 days supplied with standard rice nutrient solution (Yoshida solution)[8]. Seeds were sown in glass plate filled with soil. Two Phosphorous treatments were applied in the present experiment: (1) Control (sufficient P supply, complete standard solution; (2) Low P (deficient P supply, $1/10^{th}$ of the standard solution). Each treatment had three replicates. The pH of nutrient solution was adjusted to 5.0 by adding 1 mol/L HCL or NaOH.

Phenotypic evaluation

Eight quantitative morphological characters were measured in each plant i.e. Plant height (cm), number of leaves/plant, number of tillers/plant, root length (cm), root volume (cm^3), root surface area(cm^2), root dry weight (g) and shoot dry weight (g).

RNA extraction; cDNA synthesis and semi quantitative RT–PCR

Total RNA was extracted from root and leaf samples using RNAzol reagent (Sigma, St Louis, USA) and cDNAs were synthesized using Thermo Scientific VersoTM cDNA Synthesis Kit as per manufacturer's instructions. The first strand cDNA synthesis was carried out using 1 μg RNA in a tube containing 5x cDNA synthesis buffer (4 µl), dNTP Mix (2 µl), anchored Oligo-dT Primer (1 µl), RT Enhancer (1 µl), verso enzyme mix (1 µl), water (PCR grade 11 µl) and template RNA (2 µl). Tubes were incubated in PCR machine on reverse transcription cycling program at 42ºC for 30 min. The cDNA was subsequently used as a template for semi quantitative RT-PCR after dilution. PCR reactions consisted of around 1,000 ng/µl cDNA 2 µl, PCR buffer (10X) 2 µl, dNTP mix (2 mM) 2 µl, Primer Forward (10 µM) 1 µl, Primer Reverse (10 µM)1 µl, Taq polymerase (1 U/ µl) 1 µl and Nanopure water 11.0 µl with a thermal cycling profile of initial denaturation at 94◦C for 4 min followed by 35 cycles consisting of denaturation at 94ºC for 30 s, annealing at 55–60ºC for 1min, extension at 72◦C for 1 min and a final extension of 7 min at 72ºC. Normalization of target gene expression with housekeeping gene (β actin) was carried out to compensate sample to sample variations and ensure experimental reliability. Scoring was carried out using 1.5% agarose gel (Ultra Pure Agarose 1000; Sigma,). The gel was stained with ethidium bromide (0.5 μg/ml) and visualized under UV light and the image was captured using Biorad gel documentation unit.

Results and Discussion

Phenotypic evaluation

The observations for phenotypic parameters viz, plant height, number of leaves per plant, total tillers per plant, root length, root surface area, root volume, shoot dry weight and root dry weight in rhizotrons at 60 DAS indicated significant variation under P deficient and P sufficient condition. The genotypes R-RF-78, Sahabhagi Dhan, Mahamaya, SLO-16, HYL-27, G-8, G-93, G-102, Dagaddeshi and G-108 showed increase upto 3% to 78% in plant height, 14% to 100% in number of tillers/ plant under P deficiency. It was reported that tillering ability is the best marker of phosphate deficient tolerant rice cultivars. There is an increase of about 3% to 181% in total number of leaves/ plant, 1%-100% in root dry weight, 4%-72% in shoot dry weight under P deficiency. It has been reported that exogenous sucrose can increase the biomass of rice and promote the absorption of phosphorus in rice under phosphate deficiency [9]. The genotypes R-RF-78 (573.75%) and G-8 (304.17%) showed significant increase in root length, surface area and volume (Fig.1). The genotypes Mahamaya, HYL-27, G-8, G-93, G-102, Swarna, Dagaddeshi and MTU-1010 also showed significant increase in root surface area. Higher Pi uptake ability of plants under P deficient conditions may be attributable to the larger root surface area [10].

Semi-quantitative RT-PCR analysis

The expression patterns under P-deficient and P-sufficient conditions were evaluated by semi quantitative RT-PCR using cDNA samples with 13 genes, Pi deficiency responsive transcription factors encoding genes (*OsPTF1*, *OsPTF2*) Pi starvation signalling protein encoding genes (*OsPHR1*, *OsPHR2*, *OsPHR3*) Pi transporter protein genes (*OsPT1*, *OsPT2)* and putative genes co-localized to Pup-1 QTL (*KN1* and *KN2*, designed by PRIMER 3.2 software) as well as genes (*RH3*, *Hd3a*, *Ehd1*, *RFT1*) related to NRR.

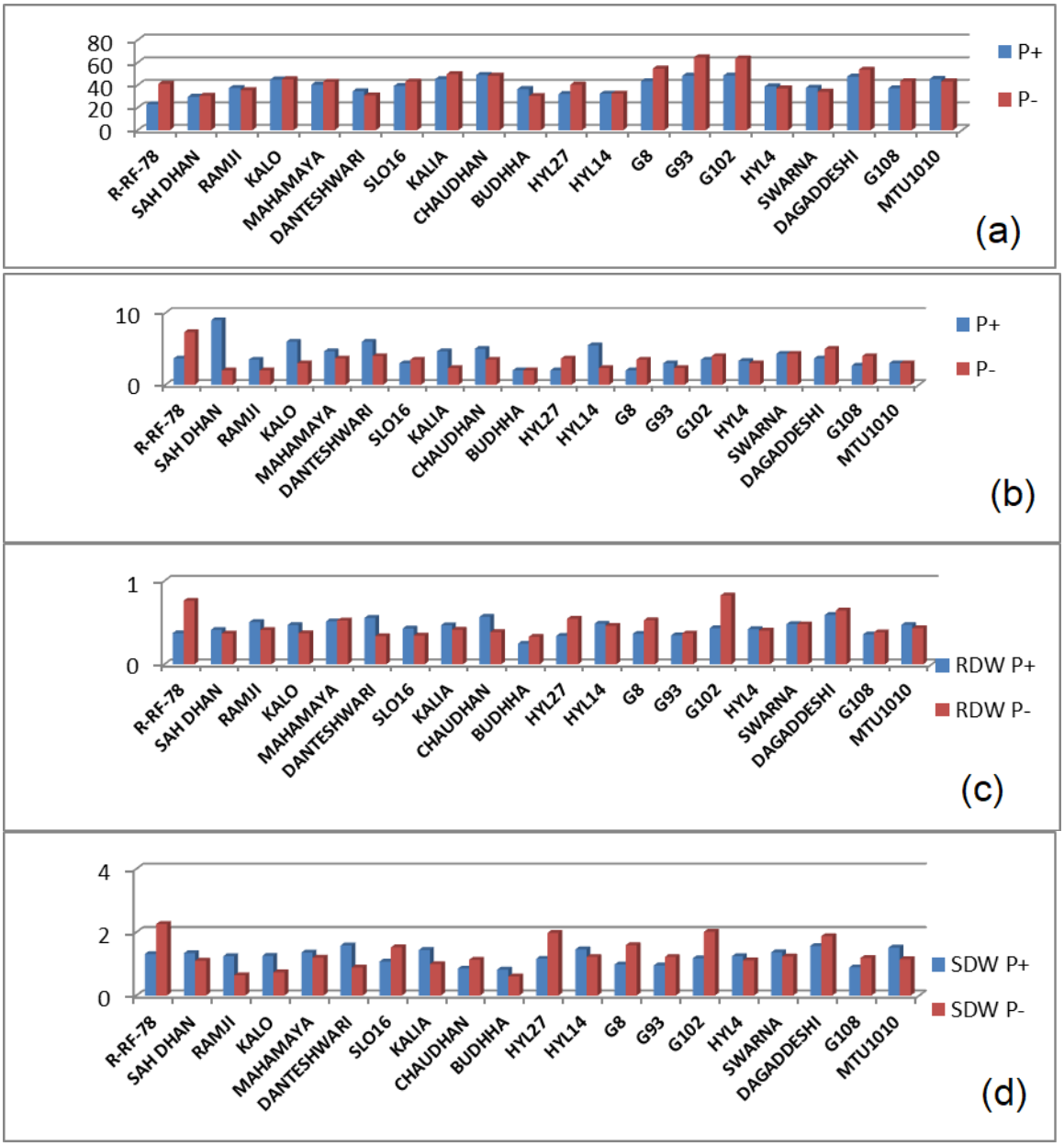

Figure 1 Variation of a. Plant height b. Number of tillers/plant c. Root dry weight d. Shoot dry weight under P sufficient and deficient condition

Expression Patterns of OsPHR1, OsPHR2 and OsPHR3

The *OsPHR1* gene did not show any expression in the root tissues of any genotypes under both P supplemented and P deficient condition. In shoot tissues *OsPHR1* gene showed detectable expression in genotypes RRF-78, Danteshwari, Kalia and G-108 under P deficiency, while in P supplemented condition there was no expression in shoot. The gene *OsPHR2* showed differential expression in root and shoot tissues of all the genotypes under P supplemented and deficient condition. The gene *OsPHR2* expressed at very low level in Ramjiyawan, Kalia and G-8 in root tissues under P supplemented condition, while no expression in root tissues of any genotype was observed under P deficient condition (Fig.2). Moderate to high level of expression was observed in leaf tissues of R-RF-78, SLO-16, Chau Dhan, HYL-27 and HYL-4 genotypes; hence the gene was up regulated in shoot tissues of these five genotypes and down regulated in shoot tissues of Mahamaya and roots of Ramjiyawan, Kalia and G-108 under P deficiency. The gene *OsPHR3* expressed in root tissues of only G-8 in P sufficient and P deficient condition. Whereas the *OsPHR3* gene was up regulated in root tissues of only Chau dhan under P deficiency. The gene *OsPHR3* was up regulated in shoot tissues of R-RF-78, Mahamaya, SLO-16, Chau dhan and G-108 in P deficient condition with high level of expression in Mahamaya.

Expression Patterns of OsPTF1 and OsPTF2

The *OsPTF1* is known to be responsible for tolerance to Pi starvation in rice and it is reported to be induced in the rice roots under Pi deficient conditions [11]. *OsPTF1* gene showed differential expression under P supplemented and deficient condition. It was up regulated in shoots of RRF-78, SLO-16, Chau dhan, Buddha and HYL-27 and was highly up regulated in HYL-4 under P deficient condition. *OsPTF1* did not show any expression in roots in P sufficient and P deficient condition. The genotypes showing up regulation of *OsPTF1* showed increase in number of tillers per plant under Pi-deficient conditions. increased root and shoot dry weight, longer root length and larger root surface area were observed in the genotypes RRF-78, SLO-16 and HYL-27 under P deficiency which shows higher expression of *OsPTF1* gene which implies that the over expression of *OsPTF1* enhanced tolerance to Pi deficiency. The *OsPTF2* gene also showed differential expression in leaf tissues. It showed lower expression in Sahabhagi Dhan in P sufficient condition which was still down regulated under P deficiency. The *OsPTF2* was up regulated in RRF-78, Mahamaya, Buddha, HYL-4 and G-108 in P deficiency. Among selected 20 rice genotypes Mahamaya, Buddha and G-108 showed significantly higher expression of *OsPTF2* under P deficiency in leaf tissues (Fig.3). In roots no expression was observed in *OsPTF2* under both P sufficient and deficient condition. Over expression of *OsPTF1* triggers the expression of plant PHO genes involved in the effective utilization of absorbed Pi in plants as a Pi starvation signal.

Expression Patterns of PT1CD and OsPT2

Uptake of phosphate at the roots is regulated by membrane-spanning phosphate transporter (PT) proteins. In plant, *PT*s are classified into two forms based on phosphate absorption kinetics and affinity to target phosphate i.e. High-affinity *PTs* and Low-affinity *PTs.* High-affinity *PTs* are induced under phosphate deficient conditions particularly in the roots, whereas low-affinity *PTs* are expressed constitutively in the aerial parts of plants. In our study the *PT1CD* gene expressed differentially in shoot tissues under both P supplemented and P deficient condition. The gene *PT1CD* was up regulated in the shoot tissues of R-RF-78, Ramjiyawan and Mahamaya under P deficient condition. While *PT1CD* gene was down regulated in shoot tissues of Sahabhagi Dhan under P deficient condition. In root the *PT1CD* gene showed genotype specific expression where it expressed in root tissues of Ramjiyawan, Kalia, Buddha and G-108 under P sufficient condition. The gene *PT1CD* expressed in root tissues of Kalia, Chau dhan, Buddha, G-8, HYL-4, Swarna and G-108 under P deficiency. *PT1CD* gene was up regulated in the root tissues of the genotypes Chau dhan, HYL-4 and Dagaddeshi under P deficiency (Fig.4). Whereas in the genotypes Ramjiyawan, Mahamaya, Danteshwari, SLO-16, Kalia and Buddha the gene *PT1CD* was down regulated in P deficient condition. The gene *OsPT2* was up regulated in the genotypes RRF-78, Mahamaya, Buddha, HYL-27, HYL-4 and G-108 under P deficiency. In Sahabhagi Dhan *OsPT2* gene was down regulated under P deficiency. *OsPT2* gene did not show any expression in root tissues under both P sufficient condition and P deficient condition.

Expression Patterns of KN1 and KN2

The gene *KN1* and *KN2* showed differential expression under P deficient and P sufficient condition in shoots of different rice genotypes. The *KN1* gene expressed at lower in shoot tissues of the genotypes Swarna and Dagaddeshi while it was expressed at high level in G-108, SLO-16, Buddha, HYL-27, G-93 and HYL-4 in P sufficient condition. Under P deficient condition the gene *KN1* was up regulated in RRF-78, Sahabhagi dhan, Mahamaya, SLO-16, Chau dhan, Buddha, HYL-27, HYL-14, HYL-4, G-108 and MTU-1010. In root the *KN1* gene expressed at lower level in the genotypes Ramjiyawan, Kalokuchi, Mahamaya, Danteshwari, SLO-16, Buddha, HYL-14, G-8, G-93, HYL-4 and Swarna under P sufficient condition. Under P deficient condition the gene *KN1* showed differential expression in shoot tissues as it was up regulated in Kalia, G-8 and MTU-1010. In Ramjiyawan, Mahamaya, Danteshwari, Chau dhan, Buddha, HYL-27 and HYL-4 the gene *KN1* expressed at lower level under P deficiency. The gene *KN2* also showed differential expression in shoot tissues as it expressed at lower level in Chau dhan, G-8, G-102, Swarna and G-108 under P sufficient condition and showed higher expression in SLO-16, Buddha, G-102, HYL-27, G-93, HYL-4 and Dagaddeshi. Whereas in P deficient condition the gene KN2 was expressed in almost all genotypes except Buddha and MTU-1010. The gene *KN2* showed higher level of expression in shoot tissues of Danteshwari, Chau dhan, HYL-27, HYL-4 and G-108 under P deficient condition (Fig.5). The gene *KN2* was up regulated in shoot tissues of Sahabhagi dhan, Ramjiyawan, Kalokuchi, Mahamaya, Kalia and HYL-14 under P deficient condition. In root tissues the *KN2* gene did not expressed in Sahabhagi dhan, Chau dhan, G-93, G-108 and MTU-1010 but showed lower level of expression in other genotypes under P sufficient condition. Higher expression in Ramjiyawan, SLO-16, Kalia and G-8 was observed in shoot tissues under P sufficient condition. Whereas under P deficient condition *KN2* showed lover level of expression in all genotypes except G-102 where it did not expressed. In the root tissues of

Mahamaya, Chau dhan, Buddha and HYL-4 the gene *KN2* showed relatively higher level of expression. Being *Pup*-1 QTL co localized gene, the expression pattern of *KN1* and *KN2* gene in Ramjiyawan and Kalokuchi is an agreement with their Pup 1 haplotyping analysis.

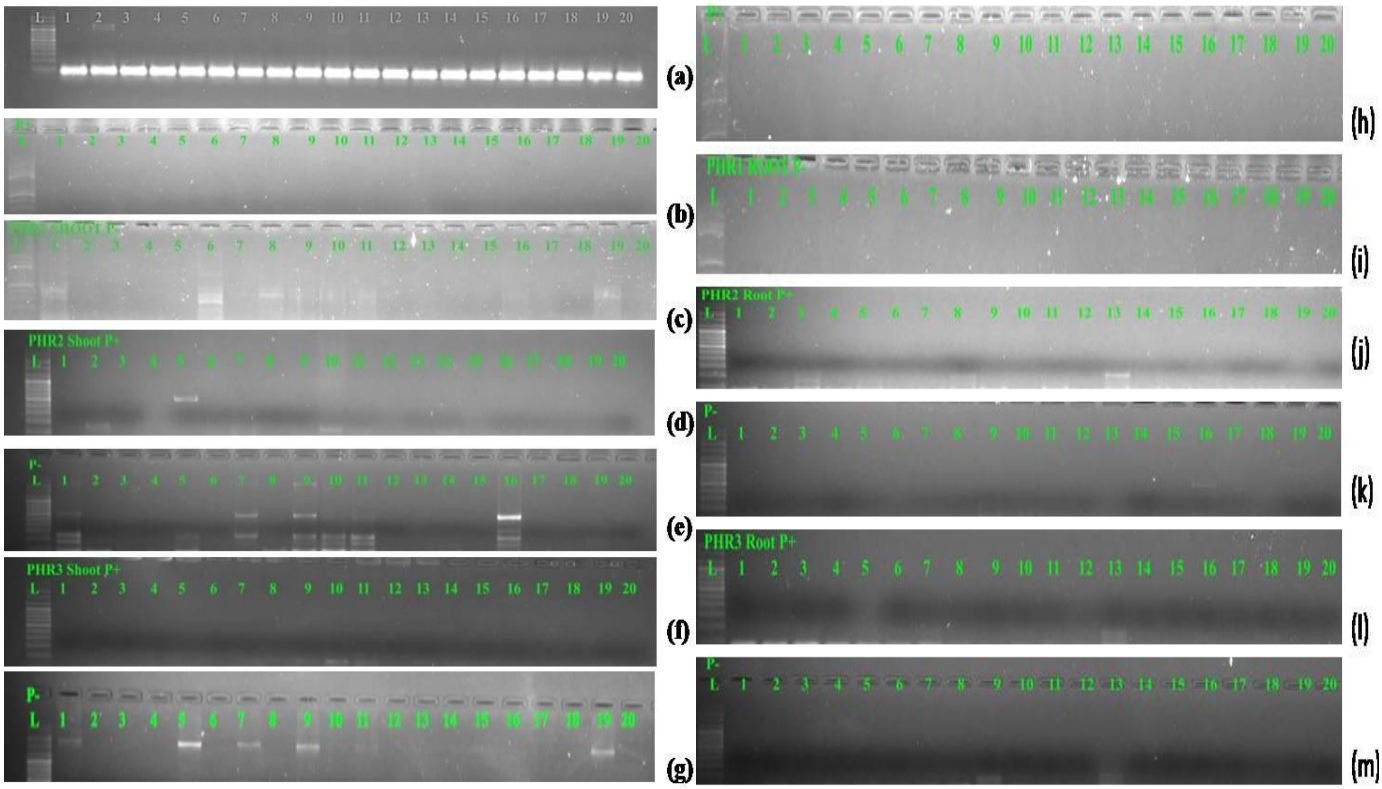

Figure 2 Levels of expression of, *OsPHR1*, *OsPHR2* and *OsPHR3* in root and shoot tissue; (a) Actin (b) *OsPHR1* shoot P+ (c) *OsPHR1* shoot P- (d) *OsPHR2* shoot P+ (e) *OsPHR2* shoot P- (f) *OsPHR3* shoot P+ (g) *OsPHR3* shoot P- (g) *OsPHR1* root P+ (i) *OsPHR1* root P- (j) *OsPHR2* root P+ (k) *OsPHR2* root P- (l) *OsPH3* root P+ (m) *OsPHR3* root P-

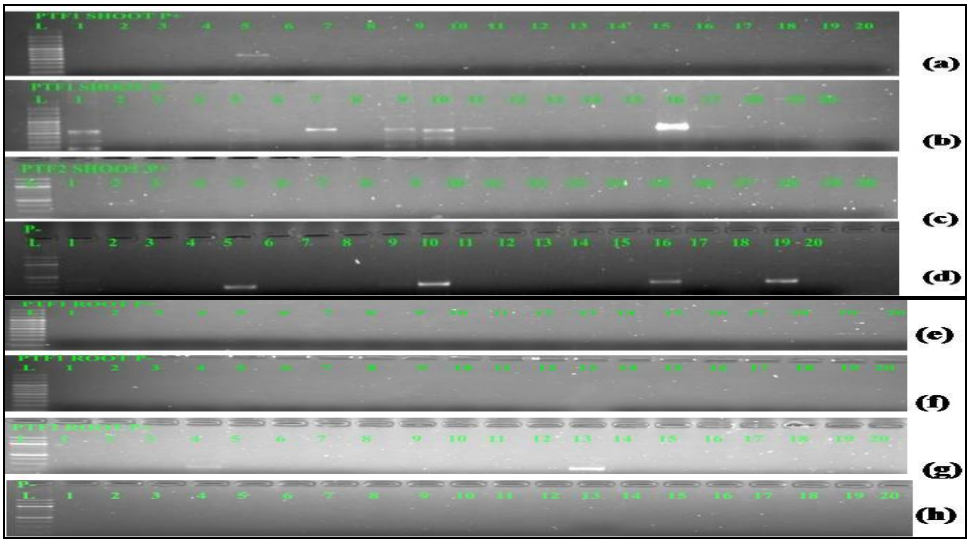

Figure 3 Levels of expression of, *OsPTF1* and *OsPTF2* in root and shoot tissue; (a) *OsPTF1* shoot P+ (b) *OsPTF1* shoot P- (c) *OsPTF2* shoot P+ (d) *OsPTF2* shoot P- (e) *OsPTF1*root P+ (f) *OsPTF1* root P-(g) *OsPTF2* root P+ (h) *OsPTF2* root P-

Expression pattern of NRR related genes

The gene *RH3* expressed at lower level in shoots of Sahabhagi dhan, Buddha, G-102, HYL-4 and G-108 under P sufficient condition and it was up regulated significantly under P deficiency in the shoot tissues of Mahamaya, SLO-16, Chau dhan, Buddha, HYL-27, G-8 and G-108. The gene *RH3* was down regulated under P deficiency in shoot

tissues of Sahabhagi dhan, G-102 and HYL-4. In root tissues the gene *RH3* expressed only in R-RF-78, G-8 and G-108 under P sufficient condition with significantly higher level of expression in of G-108 under P sufficient condition. In the root tissues the gene *RH3* was up regulated under P deficiency in Chau dhan only while it was down regulated in R-RF-78 and G-108 (Fig.6). The gene *Hd3a* showed differential expression in shoot tissues as it expressed only in Sahabhagi dhan, SLO-16, HYL-27, G-8, G-93, HYL-4 and Dagaddeshi under P sufficient condition. Under P deficient condition *Hd3a* gene was up regulated in shoot tissues of R-RF-68, Ramjiyawan, Kalokuchi, Danteshwari, SLO-16, Chau dhan, Buddha, HYL-27, G-93, Dagaddeshi, G-108 and MTU-1010. In shoot tissues of Sahabhagi Dhan and G-8 *Hd3a* gene was down regulated under P deficiency.

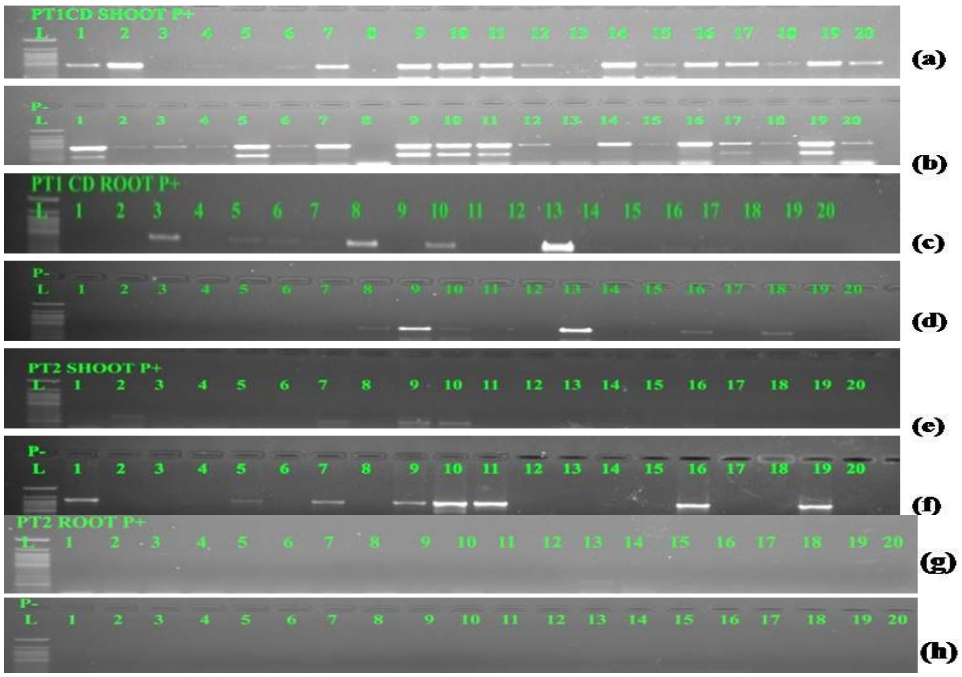

Figure 4 Levels of expression of *PT1CD* and *OsPT2* in root and shoot tissue; (a) *PT1CD* shoot P+ (b) *PT1CD* shoot P- (c) *PT1CD* root P+ (d) *PT1CD* root P- (e) *OsPT2* Shoot P+ (f) *OsPT2* shoot P- (g) *OsPT2* root P+ (h) *OsPT2* root P-

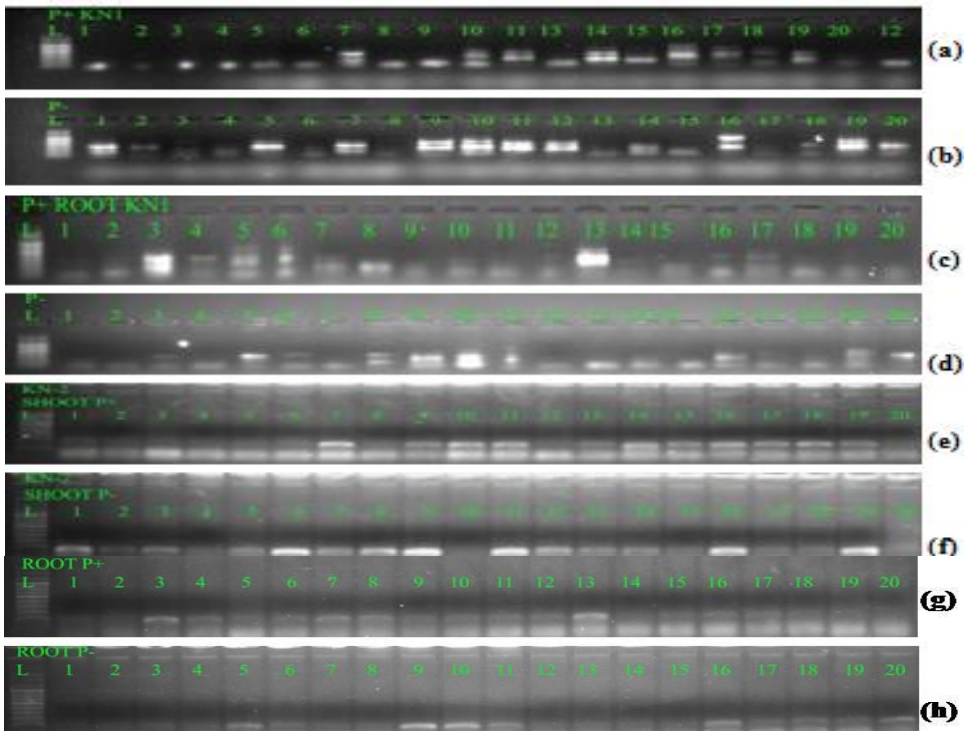

Figure 5 Levels of expression of *KN1* and *KN2* (a) *KN1* shoot P+ (b) *KN1* shoot P- (c) *KN1* root P+ (d) *KN1* root P- (e) *KN2* shoot P+ (f) *KN2* shoot P- (g)*KN2* root P+ (h) *KN2* root P-

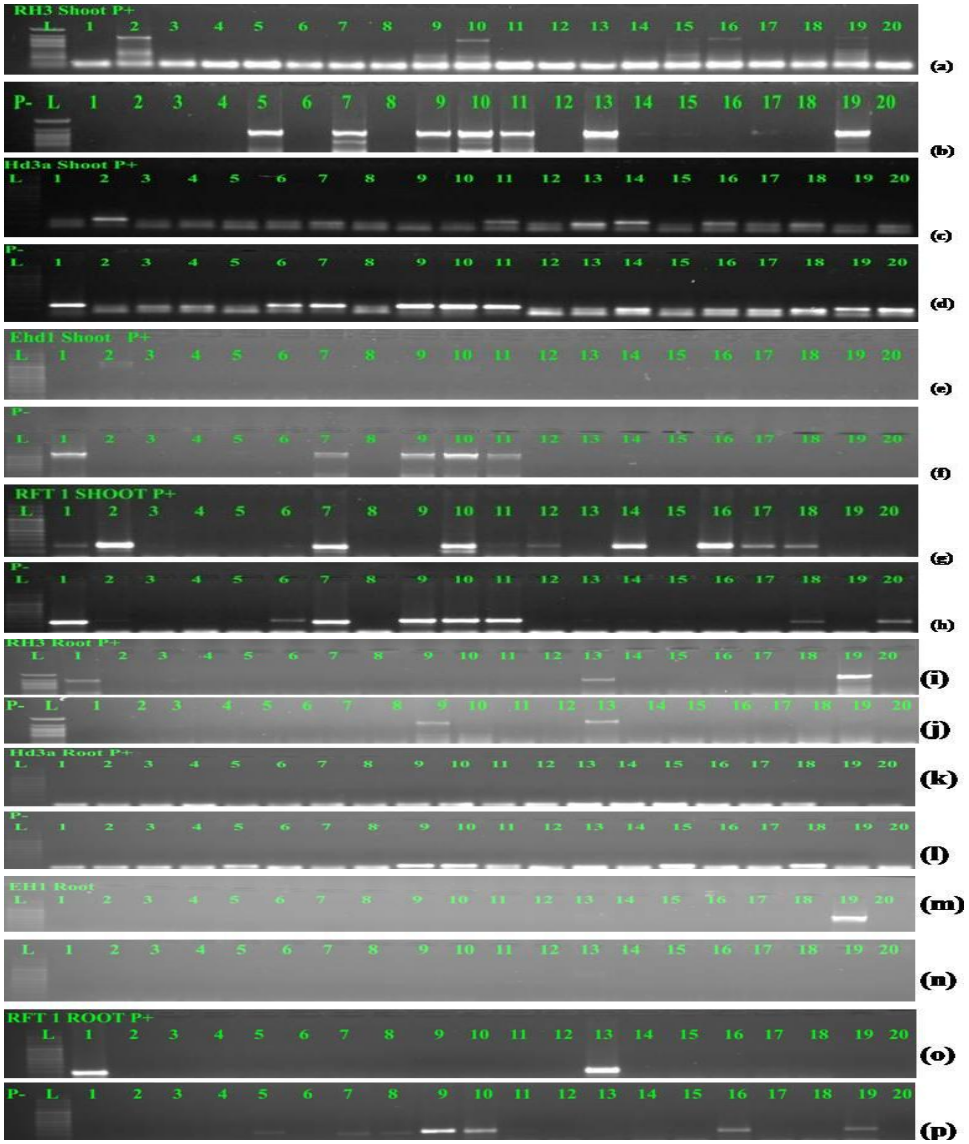

Figure 6 Levels of expression of (a) *RH3* shoot P+ (b) *RH3* shoot P- (c) *Hd3a* shoot P+ (d) *Hd3a* shoot P- (e) *Ehd1* shoot P+ (f) *Ehd1* shoot P- (g) *RFT1* shoot P+ (h) *RFT1* shoot P- (i) *RH3* root P+ (j) *RH3* root P- (k) *Hd3a* root P- (l) *Hd3a* root P+ (m) *Eh1* root P+ (n) *Eh1* root P- (o) *RFT1* root P+ (p) *RFT*1root P-.

The gene *Ehd1* did not expressed in shoot tissues under P sufficient condition, except Sahabhagi Dhan and Buddha. Whereas the gene *Ehd1* was up regulated in shoot tissues of R-RF-78, SLO-16, Chau dhan, Buddha and HYL-27 under P deficient condition. In shoot tissues of Sahabhagi Dhan *Ehd1* gene was down regulated under P deficiency. The gene *RFT1* showed lower level of expression in shoots of R-RF-78, HYL-27, HYL-14, Swarna and Dagaddeshi under P sufficient condition. Whereas in shoot tissues of Sahabhagi dhan, SLO-16, Buddha, G-93 and HYL-4 the gene *RFT1* showed higher level of expression under P sufficient condition. Under P deficiency the gene *RFT1* showed higher expression in Dagaddeshi and G-108. The gene *RFT1* was up regulated in R-RF-78, Danteshwari, Chau dhan, Buddha, HYL-27 and MTU-1010 under P deficiency. Whereas the gene *RFT1* was down regulated in shoot tissues of Sahabhagi Dhan, HYL-14, G-93, HYL-4 and Swarna. The genotypes R-RF-78, SL0-16 and HYL-27 showing increase in plant height, number of leaves, number of tillers per plant, root and shoot dry weight, root length, root surface area and root volume under P deficiency hence these genotypes were identified as tolerant to P deficiency.

Conclusion

The phenotypic observations under both P supplemented and P deficient condition indicated substantial variation among selected 20 rice genotypes for phosphorus deficiency tolerance. Significant increase in plant height, number of leaves per plant, root dry weight, shoot dry weight, root length and root surface was observed under P deficient

condition as compared to P supplemented condition. Results of expression analysis were correlated to increase in total tillers per plant as observed in the genotypes R-RF-78, SL0-16 and HYL-27.

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