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INCREASED EXPRESSION OF PHOSPHATE TRANSPORTER OSPT2, OSPT6 AND A NOVEL GENE NRR NOT ENHANCES THE P-UPTAKE AND ACQUISITION IN RICE (*ORYZA SATIVA* L.) IN P-STRESS CONDITION

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ABSTRACT

Phosphorus (P) is one of the most important macronutrients in the plant lifecycle. Lack of phosphate (inorganic phosphate, Pi) inhibits plant growth. Here, we report on the expression patterns, and the field validation of two members of this family OsPT2 and OsPT6 along with a novel gene NRR, responds to macronutrient deûciency and regulates root growth. The study showed that the expression of OsPT2, OsPT2 and NRR are much higher in Satabdi, a P-deficient non-tolerant variery, whereas expression is low in P-deficient tolerant genotype, Gobindobhog in P-srtess condition. Thus, OsPT2 , OsPT6 and NRR alleles of Gobindabhog were suitable for introgression into non-tolerant cultivars.

INTRODUCTION

Phosphate (Pi) is a constituent of key molecules such as ATP, nucleic acids, and phospholipids, which play crucial roles in energy transfer, metabolic regulation, and protein activation. Plants have evolved a number of different adaptive strategies to maximize Pi acquisition under Pi-limiting conditions. Many high-afûnity Pi transporter genes are expressed predominantly in roots and are induced by Pi depletion which indicate that they are involved in P-uptake and acquisition through the roots in P-strress condition (Seo *et al.*, 2008, Jia *et al.*, 2011, Sun *et al.*, 2012). Higher the root/shoot ratio, root hair proliferation and increased in length, and lateral root number, increase the surface area for absorption, thereby increasing P-uptake efûciency and acquisition under Pi-limiting conditions (Poirier and Bucher 2002).

In plant, PTs have two forms based on phosphate absorption kinetics and affinity to target phosphate (Furihata *et al.*,1992). 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 (Daram *et al.*, 1998; Rae *et al.*, 2003). Among all the known PTs, members belonging to the Pht1 family, which are presumed as high-afûnity PTs, were studied more intensively (Paszkowski, 2006). In addition to the identiûed thirteen putative high-afûnity Pi transporter genes belonging to the Pht1 family OsPT1 to OsPT13 genes (Paszkowski *et al.*, 2002), all of the other 13 OsPT genes from OsPT14 to OsPT26 have been identiûed in the rice (*Oryza sativa*) genome (Liu *et al.* 2011). These 26 genes are distributed on 11 rice chromosomes: chromosome 3 contains five genes; chromosomes 4 and 9 each contain four genes; chromosomes 1 and 6 each contain three genes; chromosomes 2 and 10 each contain two genes; and chromosomes 5, 8, 11, and 12 have a single gene each and out of the 26 coding sequences of OsPT genes, 11 have no intron, and other coding sequences are disrupted by introns, with numbers varying from 1 to 10. (Liu *et al*,; 2011). Ai *et al.*, (2009) demonstrated that two Pi starvation-responsive Pht1 members in rice, OsPT2 and OsPT6, have different functions and kinetic properties in Pi uptake and translocation. OsPT2 is broadly involved in Pi uptake and translocation through the plants. However, OsPT6, unlike other Pht1 members, is a low-afûnity Pi transporter that might mainly play roles during the Pi translocation process (Ai *et al.*, 2009). Overexpression of OsPT2 can cause over-accumulation of shoot Pi in rice and thus a Pi toxicity phenotype (Liu *et al.*, 2010). Although the functions and regulatory mechanisms of plant Pht1 genes have been widely studied a large amount of work is needed to decipher the biological roles of each member. Another novel rice gene, NRR (nutrition response and root growth) responds to macronutrient deficiency and regulates root growth. NRR is alternatively spliced, producing two 5′-coterminal transcripts, acting as the key components, modulate the rice root architecture with the availability of macronutrients (Zhang *et al.*, 2012).

The objective of this study is to investigate the effects of OsPT2, OsPT6 and NRR genes in Pi acquisition of rice in P-deficent situation.

MATERIALS AND METHODS

Total RNA of two genotypes were extracted using RNeasy plant mini kit (Qiagen) treated with RNase free DNase, from root of the seedlings grown in Yoshida (1971) culture solution supplemented with 10mg of inorganic P/l (P-sufficient solution) and 0.05mg of inorganic P/l (P-depleted solution), according to the manufacturer's instructions. Transcript level of OsPT2, OsPT6 and NRR were measured by quantitative RT-PCR as described previously (Bhattacharyya *et al.* 2003). First-strand cDNA was synthesized from 5 μg of total RNA using oligo-dT(18) primer and Super Script First-Strand Synthesis system for RT-PCR (Applied Biosystem). Quantitative real-time PCR was performed in 20 μl reaction volume containing 2 μl cDNA, 75ng each gene-specific primers, and SYBR Premix using Step One (Applied Biosystem) model. Normalization of target gene expression with housekeeping gene (*β-tubulin*) was useful in order to compensate sample to sample variations and to ensure the experimental reliability.

RESULTS AND DISCUSSION

In this study, we investigated the expression patterns of two Pi transporters and a novel gene NRR (Nutrient response and root growth) (Zhang *et al.*, 2012) from *Oryza sativa* in response to Pi depletion. Result showed that OsPT2 and OsPT6 gene have a different response to Pi deûciency, including a different expression pattern. Gobindobhog genotype, the aromatic Bengal land races was considered as P-deficiency tolerant due to its higher P-acquisition efficiency when grown on P-deficient soil (Sarkar *et al.*, 2011). On the other hand, Satabdi, a popular cultivar in the Gangetic plain of West Bengal does not possess such ability, thus considered as non-tolerant genotype. It has been observed that Gobindabhog accumulated approximately 27 mg P/plant when grown on P-deficient soil which was even higher than its accumulation when grown on P-sufficient soil (Table1). These two genotypes were grown in hydroponics with 10mg/l (P+) and 0.5mg/l (P-) of P for 14 days. Length and dry mass weight of root and shoot of ten days old seedlings were recorded. It had been observed that root and shoot length, secondary root and dry mass weight of root were increased in Gobindobhog, in P-deficient condition than P- sufficient condition. Contrarily, trend was opposite in case of Satabdi genotypes (Fig. 2). Weight of five seedlings was considered as weight of single root was too little for measurement. So, in P-deficient situation, Satabdi unable to increase its root dry weight but Gobindabhog made it almost double.

Activation level of three genes, NRR, OsPT2 and OsPT6 were higher in Satabdi in P-deficient condition. The yield of these two genotypes in P-deficient situation (Table1) reveals that Gobindobhog gives better yield than the poular cultivar Satabdi, due to higher P-uptake efficiency in P-deplete condition. Though Ai *et al.*, (2009) reported that OsPT2 is broadly involved in Pi uptake and translocation through the plants and OsPT6 might mainly play roles during the Pi translocation process and overexpession of OsPT2 can cause over-accumulation of shoot Pi in rice and thus a Pi toxicity phenotype (Liu et al., 2010), we have found that relative expression of both low affinity and high affinity Pi transporter OsPT2 and OsPT6 respectively was high in Satabdi genotype, having low P-uptake ability in P-deficient condition but the same expression is high in P-deficient tolerant genotype, Gobindobhog (Table 3). On the other hand NRR (LOC_Os05g51690) (nutrition response and root growth) responds to macronutrient deûciency and regulates root

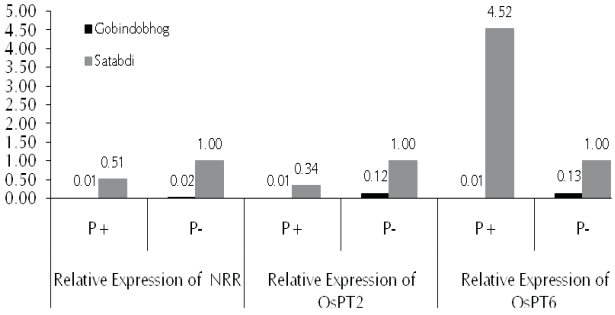

Figure 1: Relative expression of NRR, OsPT2 and OsPT6 of Gobindobhog and Satabdi

Table 1: P accumulation ability (mg/plant) and dry mass weight (mg/plant) in P-sufficient (P+) and deficient (P-) soil and yield on deficient soil of two genotypes

Genotype	P-accumulation (mg/plant)		Dry mass weight of aerial part (mg)		Yield/ sq m
	P+	P-	P+	P-	(P-deficient condition)
Gobinobhog	28.47 ± 2.19	27.72 ± 3.92	42.81 ± 12.16	40.49 ± 7.62	612 ± 28.6
Satabdi	13.38 ± 1.13	6.91 ± 1.22	21.22 ± 1.49	12.18 ± 2.07	392 ± 11.7

Table 2: Root-shoot length, secondary roots and root dry weight of selected two rice genotypes grown in P-sufficient and deficient solution

Genotype	Shoot length (cm)		Root length (cm)		Secondary root		Root dry weight	
	p+	p-	p+	p-	p+	p-	p+	p-
Gobindobhog	12.80	13.70	11.90	14.00	9.00	11.00	0.0084	0.0094
Satabdi	9.50	5.00	11.30	4.10	2.67	1.33	0.0019	0.0011

Table 3: Relative expression of NRR, OsPT2 and OsPT6 of P-deficient tolerant and non-tolerant rice genotypes

Genotype	Relative Expression of NRR		Relative Expression of OsPT2		Relative Expression of OsPT6	
	P+	P-	P+	P-	P+	P-
Gobindobhog	0.005	0.020	0.0109	0.1235	0.0050	0.1258
Satabdi	0.507	1	0.3352	1	4.5224	1

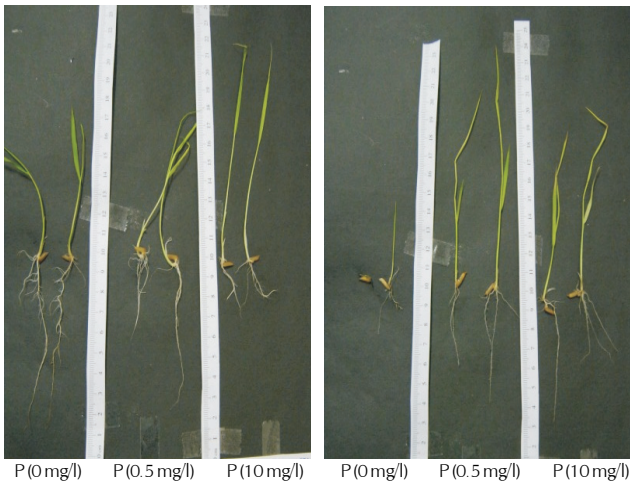

Figure 2: Seedling root growth of two rice genotypes in P-sufficient (10mg/l), deficient solution (0.5mg/l) and zero P solution after 14 days

growth. Zhang *et al.*, in 2012 reported that overexpression of NRR in rice exhibited signiûcantly retarded root growth. So, NRR gene played a negative regulatory roles in rice root growth. The similar result also found in our experiment also. Here we have observed that the relative expression NRR gene was higher in Satabdi genotype rather than Gobindobhog genotype in P-deficient condition (Table 3) and retarded root growth pattern was present in Satabdi genotype in P-deficient condition. The rice root architecture comprising root length, secondary root number, root hair, root dry weight with the availability of Phosphorus was also observed (Table 2) and it has been found that higher relative expression of NRR gene in rice exhibited retarded root growth (Fig.2). So, OsPT2 , OsPT6 and NRR alleles of Gobindabhog were suitable for introgression into non-tolerant cultivars through marker assisted breeding to make suitable for P-deficient soil.

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