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Genetic background influences phosphorous deficiency tolerance in rice (*Oryza sativa* L.)

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Abstract
Phosphorous insufficiency is the major constraint for rice yield. The increase in phosphorous fertilizer cost as well as the decline in rock phosphate reserves poses a greater threat in rice farming. Hence the development of phosphorous starvation tolerance cultivars found to be the best solution than application of fertilizers. Hydroponics experiment was carried out in 33 introgressed rice lines containing *PSTOL*1 gene along with parents to study their responses under phosphorous sufficient and deficient condition. Significant differences were observed for shoot length, root length, fresh and dry weights of root and shoot among the genotypes. Enzymatic activity was also differed significantly between genotypes of different genetic background but carrying phosphorous starvation tolerance gene. The present study showed the importance of selection of genetic background to exploit the potential of introgressed gene.

Keywords: Phosphorous, rice, hydroponics, genetic background

Introduction
Rice, the important cereal crop is considered to be the stable food for about half of the world population. Phosphorous referred as "king-pin" in Indian agriculture and also as "energy currency" of the plants (Dey *et al.*, 2017) [4] is one such macronutrient among 16 essential elements required for growth and root development, tillering, early flowering and ripening (Marschner, 2011) [12]. Approximately 80% of the applied inorganic P (Pi) is wasted by the processes such as fixation with calcium/magnesium in alkaline soils, iron/aluminium in acidic soils and slow diffusion, allowing only 20% of it to be used by the plant. As a result, P fertilizer usage must be enhanced (Herrera-Estrella and López-Arredondo, 2016; Plaxton and Tran, 2011; Vinod and Heuer, 2012; Yi *et al.*, 2005) [8, 14, 19, 21], predominantly in India where the P fertility of soils is extremely poor (Sanyal *et al.*, 2015) [17]. The inorganic P (Pi) which is liberated from the insoluble phosphorous the "labile compartment" can be used by the plants. Yet, this release is very slow and therefore P deficiency is widespread (Maathuis, 2009) [11]. Phosphorous scarceness causes a considerable reduction in photosynthesis rate. Under phosphate deficiency symptoms such as dark to blue green coloration of leaves, shoot, undersized growth and branching, reduced tillering, weaker and thin stems , delayed maturity ,imperfect pollination, smaller number of flowers, poor grain quality and low yield will be resulted (Kennelly *et al.*, 2012) [9]

However, it is estimated that by the year 2050 there will be no more P reserve present in the soil; hence a major *Pup*1 QTL which was found in the traditional 'Kasalath' aus type variety confers tolerance for phosphorous deficiency. Introgression of this QTL to local varieties is expected to boost rice productivity under low phosphorous condition (Gamuyao *et al.*, 2012) [6].

Selection of desirable background for transfer of genes of importance is essential for effective molecular breeding programme. Generally locally well adopted high yielding with good grain quality is preferred for introgression or gene transfer. Previous study in rice and other crops showed the effect of genetic background greatly influences the expression of gene of interest. In Arabidopsis, the genetic background plays a vital role in the expression of RPS-2 mediated resistance (Banerjee *et al.*, 2001) [1]. Similarly resistance gene expression of Xa26/Xa3 was found to be better in rice *japonica* background cultivars than in *indica* background cultivars (Sun *et al.*, 2004) [18]. Resistant varieties which are influenced by the host genetic background can be used as donor parents (Sakthivel *et al.*, 2017) [16].

Plants under low phosphorous availability generally secrets acid phosphatase. But the secretion ability differs depends on plant species (Yan *et al.*, 2001) [20]. With this background, present work was carried out to view the responses of the improved *phosphorus uptake* lines harbouring *PSTOL 1* gene for different genetic background.

Materials and methods

The experiment was carried out in the Department of Plant Breeding and Genetics, AC&RI, Madurai during 2017-2019**.** Thirty three BC_3F_4 improved lines *harbouring PSTOL1* from CB14001(ASD16/IRBB60), CB14002(ADT43/IRBB60) and CB14004 (ASD16 /IRBB60/ (BPT 5408×Tetep)) crosses along with three parental lines (ASD 16, ADT 43 and IR 64 *Pup*1) were evaluated for phosphorous deficiency tolerance under hydroponic condition. This lines were confirmed for the presence of *PSTOL1* gene using gene specific markers, K29-3 (co dominant) and K46-1 (dominant). The details for development of these lines through marker assisted selection were given in (Chithrameenal *et al.*, 2018). The details of homozygous improved lines carrying the *PSTOL*1 gene in the background of ASD 16 and ADT 43 were presented in the Table 1.

Table 1: List of materials used for hydroponics screening

S. No	Improved lines	Parentage
1	IL 1	BC_3F_4 CB 14001 / *Pup*-1
2	IL 2	BC_3F_4 CB 14002 / *Pup*-1
3	IL 7	BC_3F_4 CB 14002 / *Pup*-1
4	IL 10	BC_3F_4 CB 14002 / *Pup*-1
5	IL 11	BC_3F_4 CB 14002 / *Pup*-1
6	IL 13	BC_3F_4 CB 14004 / *Pup*-1
7	IL 14	BC_3F_4 CB 14002 / *Pup*-1
8	IL 15	BC_3F_4 CB 14002 / *Pup*-1
9	IL 16	BC_2F_5 CB 14002 / *Pup*-1
10	IL 18	BC_3F_4 CB 14002 / *Pup*-1
11	IL 19	BC_3F_4 CB 14002 / *Pup*-1
12	IL 28	BC_3F_4 CB 14002 / *Pup*-1
13	IL 29	BC_3F_4 CB 14002 / *Pup*-1
14	IL 30	BC_3F_4 CB 14002 / *Pup*-1
15	IL 31	BC_3F_4 CB 14002 / *Pup*-1
16	IL 34	BC_3F_4 CB 14002 / *Pup*-1
17	IL 35	BC_3F_4 CB 14004 / *Pup*-1
18	IL 39	BC_3F_4 CB 14002 / *Pup*-1
19	IL 44	BC_3F_4 CB 14002 / *Pup*-1
20	IL 49	BC_3F_4 CB 14002 / *Pup*-1
21	IL 52	BC_3F_4 CB 14004 / *Pup*-1
22	IL 62-2	BC_3F_4 CB 14002 / *Pup*-1
23	IL 62-4	BC_3F_4 CB 14004 / *Pup*-1
24	IL 63	BC_2F_5 CB 14002 / *Pup*-1
25	IL 65	BC_3F_4 CB 14002 / *Pup*-1
26	IL 66	BC_3F_4 CB 14002 / *Pup*-1
27	IL 67	BC_3F_4 CB 14002 / *Pup*-1
28	IL 69	BC_3F_4CB 14002 / *Pup*-1
29	IL70	BC_3F_4 CB 14002 / *Pup*-1
30	IL 72	BC_3F_4 CB 14002 / *Pup*-1
31	IL 79	BC_3F_4CB 14002 / *Pup*-1
32	IL 85	BC_3F_4 CB 14002 / *Pup*-1
33	IL 92	BC_3F_4CB 14002 / *Pup*-1

Molecular screening using DNA markers

Genomic DNA from the fresh leaf sample has been isolated using CTAB method and they are stored for -20°C for further usage. In the improved lines the target genes were confirmed using gene specific markers. The details for these markers were specified in Table 2. PCR conditions were maintained as: Initial denaturation 94°C for 5 minutes, followed by 35 cycles of Final denaturation 94°C for 30 sec, Annealing 58°C for 45 sec, Initial extension 72°C for 90 sec and a final extension by 72°C for 10 minutes.

Table 2: Details of the markers used for phosphorous starvation tolerance screening

Marker	Targeted gene	Primer sequence (5' - 3')	Chromosome	AT(°C)	Reference
K 29-3 (Co dominant)	*OsPSTOL*1	F: TTCGTCCAGATGCTGCTATG R: TCTTCGGTGTAATTGGCACA	12	58°C	(Chin *et al.*, 2010)
K 46-1 (Dominant)	*OsPSTOL*1	F: TGAGATAGCCGTCAAGATGCT R: AAGGACCACCATTCCATAGC	12		

F- Forward primer, R- Reverse primer

AT- Annealing temperature

Table2: Biometrical and enzyme activity of improved lines grown under hydroponic conditions at two levels of phosphorous treatments.

IL LINES	Root length (cm)		Shoot length (cm)		Fresh root weight (g)		Fresh shoot weight(g)		Dry root weight(g)		Dry shoot weight(g)		(molar para nitro phenol released per min per mg of fresh weight)	
	+P	-P	+P	-P	+P	-P	+P	-P	+P	-P	+P	-P	+ P	-P
IL 1	13.10	12.70	30.50	39.00	0.06	0.11	0.21	0.45	0.04	0.04	0.14	0.13	1.15	0.86
IL 2	20.70	20.90	43.30	42.00	0.08	0.11	0.33	0.44	0.03	0.06	0.22	0.19	1.08	1.77
IL 7	10.20	11.50	31.30	37.00	0.06	0.18	0.22	0.69	0.02	0.08	0.11	0.17	0.30	1.58
IL 10	15.50	18.90	47.60	32.30	0.20	0.10	0.44	0.25	0.05	0.04	0.13	0.10	1.00	1.18
IL 11	15.00	15.10	27.50	33.00	0.05	0.29	0.18	1.09	0.03	0.08	0.10	0.22	0.29	1.25
IL 13	15.80	18.20	56.10	68.60	0.11	0.31	0.39	0.49	0.05	0.18	0.20	0.33	0.28	1.20
IL 14	10.70	13.00	36.00	38.00	0.28	0.07	1.07	0.48	0.09	0.04	0.28	0.18	0.58	1.17
IL 15	17.30	17.20	40.80	33.50	0.06	0.13	0.41	0.39	0.04	0.08	0.20	0.18	0.21	0.41
IL 16	14.40	17.80	38.00	46.60	0.07	0.50	0.35	1.05	0.03	0.18	0.11	0.42	0.33	1.77
IL 18	12.50	20.80	35.50	33.80	0.05	0.10	0.14	0.16	0.02	0.07	0.08	0.10	0.68	1.19
IL 19	22.80	14.80	40.20	29.90	0.37	0.07	1.02	0.24	0.07	0.03	0.28	0.11	1.10	1.14
IL 28	14.30	15.00	50.80	39.30	0.20	0.13	1.32	0.78	0.07	0.08	0.50	0.49	0.93	1.54
IL 29	12.80	14.80	39.00	40.00	0.16	0.20	1.13	0.80	0.06	0.09	0.33	0.24	1.11	1.38
IL 30	20.00	22.00	41.80	44.80	0.06	0.04	0.14	0.22	0.04	0.03	0.08	0.15	0.25	0.38
IL 31	14.60	14.20	50.50	59.00	0.12	0.16	0.43	0.49	0.06	0.08	0.18	0.23	0.70	0.98
IL 34	13.80	15.40	32.00	37.80	0.08	0.23	0.22	0.92	0.03	0.09	0.07	0.29	0.66	1.73
IL 35	24.70	16.90	35.80	47.70	0.12	0.28	0.38	0.58	0.04	0.09	0.13	0.18	0.26	1.28
IL 39	11.90	14.00	38.00	41.80	0.05	0.12	0.31	0.59	0.03	0.06	0.17	0.25	0.77	1.17
IL 44	20.00	15.00	43.50	34.00	0.08	0.04	0.33	0.22	0.06	0.03	0.11	0.15	0.44	0.45
IL 49	14.50	15.30	32.00	32.80	0.07	0.06	0.21	0.45	0.03	0.05	0.13	0.20	0.09	0.66
IL 52	21.20	22.80	39.00	41.00	0.11	0.13	0.26	0.44	0.03	0.05	0.11	0.20	0.66	0.85
IL 62-2	14.30	12.50	46.00	41.50	0.10	0.32	0.54	1.40	0.06	0.18	0.22	0.45	0.94	1.34
IL 62-4	13.30	14.70	60.50	75.50	0.10	0.26	0.41	1.41	0.06	0.16	0.20	0.62	0.87	1.97
IL 63	14.80	16.80	39.30	44.30	0.11	0.12	0.32	0.56	0.07	0.06	0.07	0.22	1.14	1.59
IL 65	15.30	16.50	39.00	32.00	0.06	0.05	0.22	0.14	0.04	0.03	0.12	0.07	0.59	0.97
IL 66	12.80	19.80	40.10	34.00	0.04	0.13	0.16	0.15	0.03	0.07	0.05	0.08	0.89	0.29
IL 67	14.80	13.30	40.80	36.00	0.10	0.09	0.39	0.26	0.04	0.04	0.17	0.14	0.12	0.48
IL 69	14.00	14.40	36.50	43.40	0.05	0.10	0.28	0.39	0.02	0.05	0.12	0.19	0.15	0.82
IL70	15.70	16.10	40.00	35.00	0.07	0.12	0.37	0.35	0.04	0.06	0.17	0.19	0.22	0.54
IL 72	13.10	14.50	50.50	42.50	0.09	0.27	0.61	0.63	0.05	0.12	0.19	0.24	0.65	0.80
IL 79	14.30	16.50	34.30	36.30	0.03	0.07	0.12	0.21	0.02	0.05	0.06	0.14	0.75	1.58
IL 85	11.20	12.40	38.50	36.80	0.15	0.20	0.73	0.82	0.05	0.10	0.14	0.22	0.87	1.14
IL 92	14.00	13.70	41.00	38.00	0.08	0.11	0.43	0.69	0.05	0.07	0.15	0.27	0.52	0.97
ASD 16	16.80	17.80	45.80	29.80	0.15	0.11	0.72	0.42	0.06	0.04	0.21	0.10	0.60	0.81
ADT 43	13.30	17.00	34.30	33.00	0.08	0.19	0.40	0.50	0.04	0.09	0.17	0.20	1.84	2.07
IR 64 Pup1	18.80	16.80	46.30	45.00	0.18	0.11	0.62	0.79	0.06	0.06	0.22	0.27	1.11	1.70
Grand Mean	15.31	16.07	40.60	40.39	0.10	0.15	0.44	0.55	0.04	0.07	0.16	0.22	0.67	1.14
CD(0.05)	3.11	4.68	5.50	8.86	0.06	0.13	0.28	0.21	0.03	0.07	0.14	0.13	0.08	0.06
CD(0.01)	4.17	6.28	7.38	11.88	0.08	0.17	0.37	0.28	0.04	0.09	0.19	0.17	0.10	0.09
SEd	1.53	2.31	2.71	4.37	0.03	0.06	0.14	0.10	0.01	0.03	0.07	0.06	0.04	0.03

Screening of improved lines under hydroponic condition

Thirty three improved lines along with three parents were grown in hydroponics condition (with (100%) and without (0%) phosphorous). Hydroponics solution is prepared based on modified Yoshida solution (Yoshida, 1976)**.** The experiment was laid out in completely randomised design (CRD) with two replications. Seeds were germinated using roll towel method. After 11 days of germination rice seedlings were transferred to hydroponics solution trays. The pH of the solution was maintained daily at 5.0 using adjustment with 1N sodium hydroxide and 1N hydrochloric acid also the hydroponics volume to be maintained properly. Once in a week the nutrient solution has been changed. After 40 days of transplanting the plants were individually harvested and separated into root and shoot. Roots were cleaned with fresh water and the following parameters such as root length (cm), shoot length (cm), fresh root weight (g), fresh shoot weight (g) were taken and dried at 65 °C for 48 hrs followed by dry weights of root and shoot were measured.

Acid phosphatase activity

One gram of fresh tissue was taken from the fresh plant samples after 40 DAS. Fresh samples were ground in a cold mortar using 10 ml of 50 mM citrate buffer (pH 5.3). The extract was filtered and centrifuged at 10,000 rpm for 10 minutes. Now, the supernatant has been used as enzyme source.3 ml of the acid substrate incubated at 37 °C for 5minutes. 0.5 ml of enzyme extract will be added and thoroughly mixed. Then 0.05 ml was removed immediately and mixed with 9.5 ml of sodium hydroxide 0.085 N. This serves as a blank. Now the remaining solution (substrate + enzyme) is incubated for 15 min at 37 °C. In that 0.5 ml of the sample is drawn and mixed with 9.5 ml sodium hydroxide solution. Then the absorbance of blank and incubated tubes is measured at 405 nm followed by the standard curve is drawn. The enzyme activity is expressed as molar p-nitro-phenol released per min per mg of fresh weight (Sadasivam, 1996) [15].

Results and discussion

All the thirty three improved lines derived through marker assisted selection were confirmed for the presence of *PSTOL*1 gene (Figure 3) using gene specific markers. The performance of 33 improved lines with genetic background of ASD 16 and ADT 43 along with their parents grown under hydroponic conditions at two levels of P is given in the Table 2.Among the three parental lines, ASD 16, the popular rice variety with bold grains has high root length under Phosphorous deficient condition. It seems that most of the improved lines show increase in root length in phosphorous deficient condition when compared to phosphorous sufficient condition.

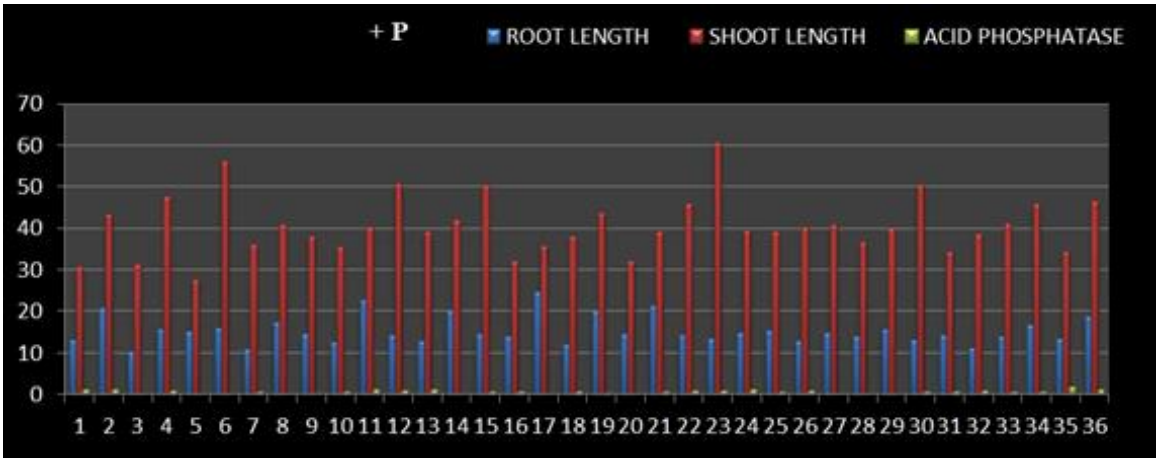
Lines with ASD16 genetic back ground had more root, shoot length when compared with ADT43 derived ones. IL 35 showed higher root length (24.7 cm) in phosphorous sufficient situation. Similarly IL 52 resulted with increased root length (22.8 cm) in deficient condition. On the other hand for shoot length traits, while comparing many lines contains higher shoot length in phosphorous sufficient condition than in deficient situation. Higher shoot length were observed in IL 62-4 in both phosphorous sufficient (60.5cm) and deficient (75.5cm) conditions. Variations for high root length and shoot length were given in Figure 2. Similar results obtained by (Yugandhar *et al.*, 2017) [22] such that root length was increased also shoot length decreased in phosphorous deficient condition. For fresh root weight parameter IL 19 has more root weight (0.37 g) in normal phosphorous application and IL 16 has about (0.50 g) of fresh root weight in deficient phosphorous condition. Conversely IL 28 not only has higher fresh shoot weight of about (1.32 g) in sufficient phosphorous application but also in dry shoot weight (0.50 g) in similar treatment. Similarly IL 62- 4 has greater fresh shoot weight (1.41 g) as well as higher dry shoot weight (0.62 g) in scarce phosphorous treatment. (Panigrahy *et al.*, 2014) [13] revealed that increase in root length, root/shoot fresh weight in phosphorous deficiency condition used as the indicators for P tolerance. In maize shoot weight were considered as parameter which is most sensitive to P deficiency (Fageria *et al.*, 1988) [5]. Here the growth parameters were mostly influenced by CB 14004 with the genetic background of ASD 16. The influence of genetic background for the expression of resistance was noticed in rice.

The rice lines having genetic backgrounds of Samba mahsuri, ASD 16, ADT 43 and IR 24 exhibited higher resistance to most of the pathotypes studied whereas the rice lines having ADT 47 background had shown more susceptibility and thus ineffectiveness of ADT47 for resistance transfer (Sakthivel *et al.*, 2017) [16].

Activity of acid phosphatase enzyme was studied in the shoots of 33 improved lines. It was significantly higher in phosphorous deficiency treatments when compared to phosphorous sufficiency treatment. The outperformance of ASD16 root length under phosphorous deficient condition implies the importance of phosphorous acquisition transporters present in the root system. Similarly the improved lines possessing the recurrent parent ASD16, under deficient phosphorous condition also may due to the expression of active 'P' transporters. Further in-depth study of 'P' transporters may reveal the difference. Among the lines IL 62-4 show higher enzyme activity in deficiency condition, which has the background of ASD16. Similar results were obtained in Nagina 22 mutant lines such that enzyme activity is highly significant in phosphorous deficient tolerant lines when compared with phosphorous deficiency susceptible lines (Panigrahy *et al.*, 2014) [13]. Distribution of mean values of 33 improved lines and their parents for root length, shoot length and enzymatic activity under two phosphorous levels normal (+ P) and low (- P) was given in the Figure 1.

The difference in expression of rice lines carrying same *PSTOL*1 gene confirmed through gene specific markers and back ground study by microsatellite markers might be due to influence of genetic back ground of recurrent parents. Such difference in expression of resistance genes was reported in rice by (Gautam *et al.*, 2015; Sakthivel *et al.*, 2017) [16].

Recent advances in molecular biology confirmed that plants have multiple transporters for Pi. Around four different transporter genes have been cloned from Arabidopsis. The multiple Pi-transporter genes are differentially expressed. Some are strongly up-regulated by Pi starvation, whereas the expression of others is constitutive (Leggewie *et al.*, 1997) [10].



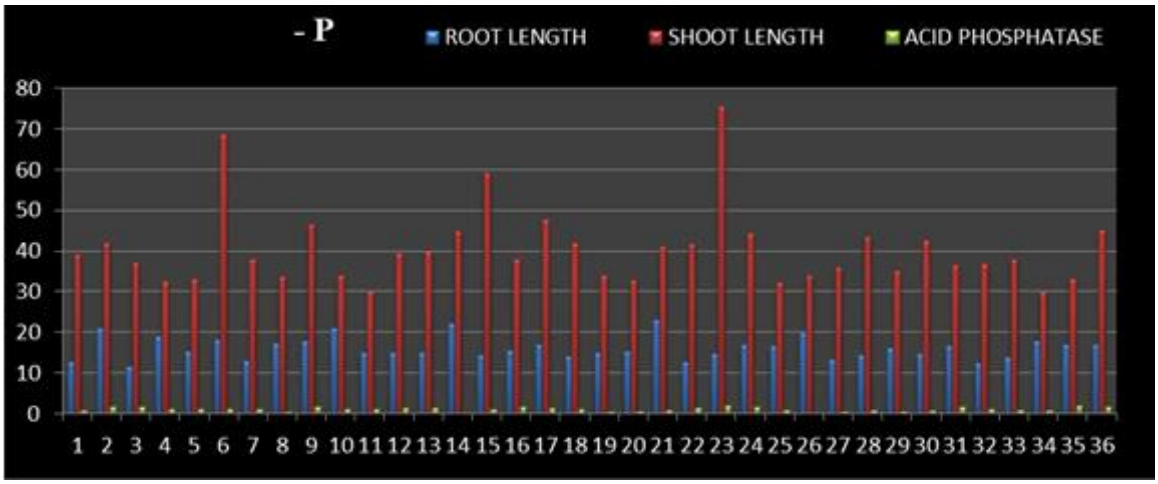
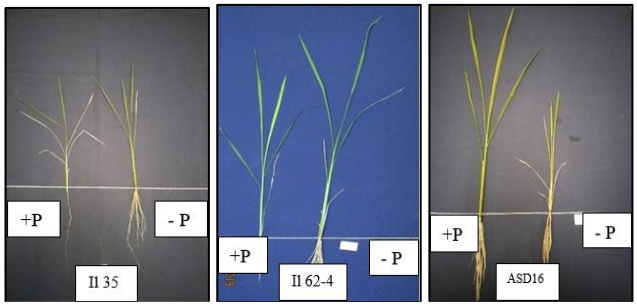
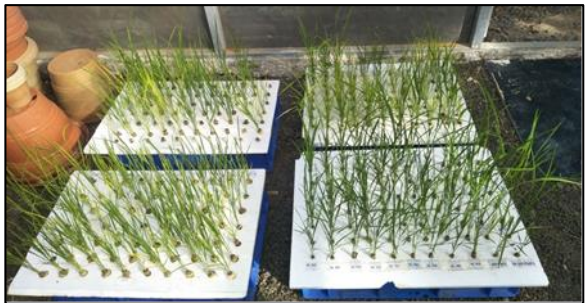


Fig 1: Distribution of mean values of 33 improved lines and their parents for Root length, shoot length, Enzymatic activity under two phosphorous levels normal (+ P) and low (- P)

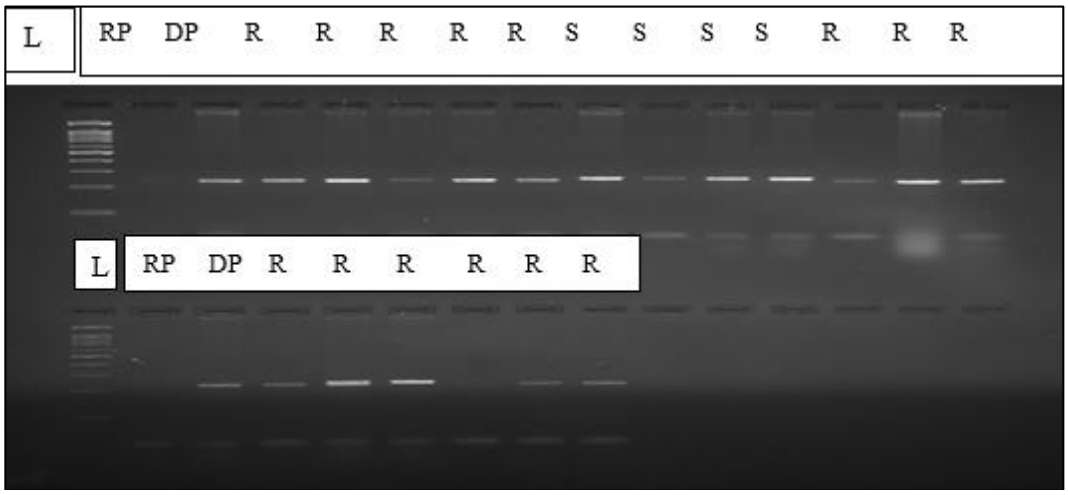


(a) (b) (c)

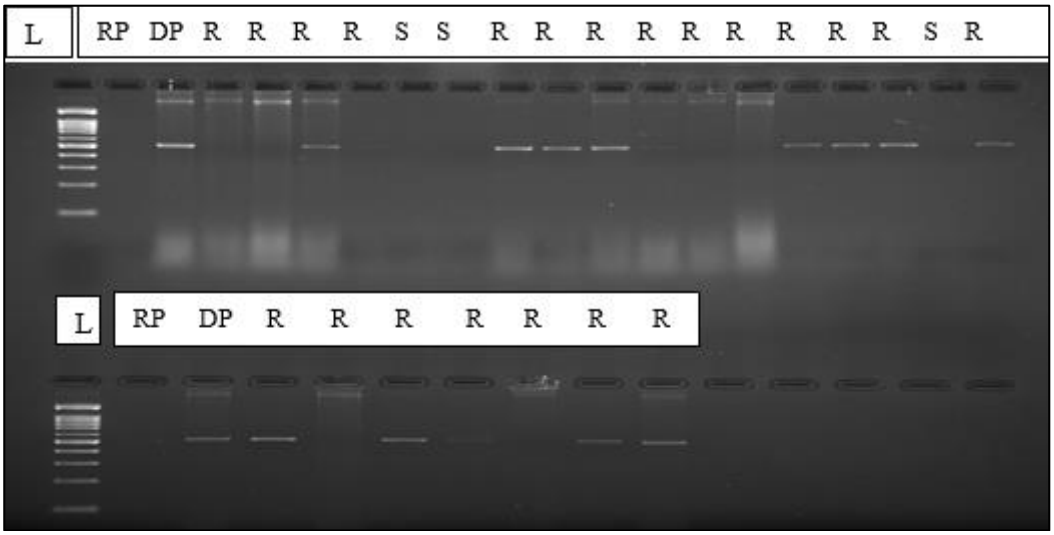


(d)

Fig 2a: IL 35 shows higher root length in +P; b: IL 62-4 shows larger shoot length in both +P and – P; c: ASD 16(control); d: Hydroponics in tray solution



(a)



(b)

Fig 3: a. represents gel image for co dominant marker K29-3 b. represents gel image for dominant marker K 46-1

Conclusion

This study reveals though all the introgressed lines possess *PSTOL*1 gene, yet the traits like root length, shoot length were not same. There may be some chance of background cultivar role for these differences. Significant differences were observed in each trait between these lines. (Sakthivel *et al.*, 2017) [16] reported that ASD 16 parentage possess complete resistance to all the pathotypes and for their virulence potential. The genotype, ASD16 has greater advantage over other lines while expressing either resistance or phosphorus tolerance gene. Also the Phosphorous acquisition transporters played a major role in ASD16. Here, high root length and shoot length in both the treatments were obtained in CB 14004 lines (IL 52 and IL 62-4) which have been developed with the background of ASD 16. It is clear that either cytoplasmic, nuclear gene content or promoter region drives for phosphorous starvation tolerance in ASD 16. Hence, ASD 16 influences the genetic background role for growth parameters and these lines may be used as donor parents for further breeding programme.

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