Received: 25 May 2022 | Revised: 04 Jul 2022 | Accepted: 08 Jul 2022

Electronic Journal of Plant Breeding

Research Article

Evaluation of recombinant inbred lines for low soil phosphorous tolerance derived from Rasi - a low soil phosphorous tolerant variety

M. Anila[1&2], Archana Giri [2] and R. M. Sundaram[1*]

[1]ICAR- Indian Institute of Rice Research (ICAR-IIRR), Rajendranagar, Hyderabad- 500030, Telangana, India
[2]Jawaharlal Nehru Technological University Hyderabad (JNTUH), Hyderabad, Kukatpally 500085, India
***E-Mail:** rms_28@rediffmail.com

Abstract
Rasi is a P efficient genotype identified by ICAR-IIRR, Hyderabad, lacking *Pup1,* the known major low soil P tolerance QTL-. In the present study, a set of 214 recombinant inbred lines (RILs) developed from cross Rasi x Improved Samba Mahsuri was evaluated in a low soil P plot (available P < 2 kg ha^{-1}) and normal soil P plot (available P >18 kg ha^{-1}) at ICAR- IIRR along with known tolerant and the sensitive checks. The RILs showed high genetic variability for the traits associated with low soil P tolerance. Stress indices were calculated based on the yield and the RILs were grouped into highly tolerant, tolerant and sensitive lines. Thirty-six RILs performing better than the tolerant checks and exhibiting excellent tolerance to low P and a yield reduction of < 40% were identified. These RILs can serve as donors for the new novel source for low soil P tolerance.

Keywords: Low phosphorous tolerance, Rasi, Improved Samba Mahsuri, Recombinant inbred lines, Stress indices

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world's most important cereal crops and feeding more than 60 per cent of the global population. It is a staple food and source of calories. Rice is grown in 117 countries and is consumed by ~ 3 billion people (Swamy *et al.,* 2020). Rice is cultivated in about 165.15 M ha around the world and India is the largest in terms of cultivation (45.77 M ha) and second largest in terms of production (124.4 million tonnes) in the year 2020-21 *(https://apps.fas.usda.gov/psdonline/circulars/production.pdf*). Rice production is adversely affected by various factors which include, rapidly changing climate, decreasing arable land, nutrient deficient soils, etc. which presents great challenges to scientists (Khush, 2005). Rice yield is majorly affected by both biotic and abiotic stresses. Among the various abiotic stresses, drought, salinity and nutrient deficiencies play an important role in limiting rice production significantly. Phosphorus (P) is an essential nutrient and no plant can produce a good yield if it suffers from P deficiency (Tandon, 1987). In rice, P deficiency is a major constraint on plant growth, development, productivity and yield worldwide (Fageria and Baligar, 1997; Wissuwa *et al.,* 1998; Dobermann and Fairhurst, 2000; Zhang *et al*. 2014). P deficiency causes stunted growth with reduced tiller number, spindly stem, narrow leaves and reduced grain number. The P deficient condition also causes a delay in flowering and maturity by one week to 10 days and in severe conditions the plants may not flower at all. Moreover, Indian soils are low (49.3 %) to medium (48.8 %) P deficient in nature and only 1.9% of the soils are rich in available P (Hasan, 1996; Wissuwa *et al.,* 1998; Tiwari, 2001; Muralidharudu *et al.,* 2011). To avoid yield losses, Indian farmers are applying more and more of P fertilizers to the soils leading to an increase in the cost of production

(Hasan, 1996; Tiwari 2001; Adavikolanu, 2014; Webeck *et al*., 2014).

To overcome the above-mentioned issues, breeding for P efficient genotypes, (Fageria and Baligar, 1997; Fageria *et al.,* 1988; Akinrind and Gaizer, 2006), improving the crop residue management, adoption of integrated nutrient management, development of low soil P tolerant rice varieties (Chin *et al.* 2010), identification of novel sources from the existing rice genotypes and new QTLs/ genes responsible for the low soil phosphorous tolerance (Wissuwa *et al.,* 1998) has become essential. P use efficiency in plants can be achieved by improved uptake of phosphate from soil (P-acquisition efficiency) or by improved productivity per unit P taken up (P-use efficiency). In general, low P tolerance is a complex quantitative trait and different studies revealed various phenotypic traits are correlated positively and negatively with tolerance to low soil P (Du *et al*., 2008; Islam *et al*., 2008; Krishnamurthy *et al*., 2014; Mukharjee *et al*., 2014; Panigrahy *et al*., 2014; Aluwihare *et al*., 2016; Tian *et al*., 2017; Wang *et al*., 2017). However, a QTL named *Pup1* is the only major QTL associated with low soil P tolerance identified so far and there is a looming need to identify additional, novel, non-*Pup1* type QTLs (and the genes underlying them) associated with low P tolerance in rice (Kale *et al.,* 2021). A rice cultivar, Rasi was earlier identified to show excellent tolerance to low soil P conditions, when screened under an acutely P-deficient plot at ICAR-Indian Institute of Rice Research (ICAR-IIRR), Hyderabad. Interestingly, it was also devoid of the popular QTL, *Pup1,* which is associated with low soil P tolerance based on genotyping with a set of functional markers specific for *Pup1* (Chin *et al.,* 2010), indicating novel non- *Pup1* type mechanism associated with tolerance. With these points in view, the present study was carried out to screen a recombinant inbred line (RIL) mapping population under both low soil P stress conditions and non-stress conditions to identify the lines which are highly tolerant lines for low soil phosphorous conditions for their possible use in future breeding studies.

MATERIALS AND METHODS

A total of 214 recombinant inbred lines (RILs) (F_{14} generation) were developed from the cross Rasi (low P tolerant) and Improved Samba Mahsuri (ISM) (low P sensitive), through a single seed decent method. These lines were screened for their tolerance levels in the low soil P plot of ICAR-Indian Institute of Rice Research (ICAR-IIRR). The plot has been maintained without the application of phosphorus fertilizer over the past 20 years (Krishnamurthy *et al.,* 2014) and is found to possess very low available P (*i.e.,* 3-5 ppm). The tolerant (Rasi and Swarna) and sensitive checks (ISM and MTU 1010) were included in the experiment for the evaluation of the mapping population in both low soil P plots (stress) and normal (Optimum) P plots.

The developed RILs were screened in the low soil P plot (available P < 2 kg ha^{-1}) and normal soil P plot (available P >18 kg ha^{-1}) of ICAR-IIRR, Hyderabad during *Kharif,* 2019. Seedlings were grown in a normal nursery bed following all the agronomic practices. Thirty days old seedlings were transplanted at a spacing of 15 x 20 cm and 10 hills per row in a low soil P plot and 20 hills per row in a normal soil P plot in two replications, along with the donor and the recurrent parents. Both the plots were nourished with a basal application of N, K, Fe and Zn except P fertilizer in low soil p plot along with a top dressing of N at the maximum tillering stage of the entire crop season. The soil pH, available N, P, K was measured as described by Jackson (1967), Subbaiah and Asija (1956), Olsen *et al*. (1954) and Jackson (1964), respectively. A total of fourteen parameters *viz.* days to 50% flowering (DFF), plant height (PH), the number of productive tillers per plant (NPT, nos.), flag leaf length(FLL), panicle length (PL), shoot length (SL), root length (RL), root volume (RV), dry shoot weight (DSW), dry root weight (DRW), root to shoot ratio (RSR), grain yield per plant (GY), thousand grain weight (TGW) and biomass (BM) were recorded. For measuring the root length, the plants were uprooted with most care to prevent any damage to the roots. The roots were thoroughly washed with running water to remove all the soil remains and the root length (cm) was measured from the roots crown to the tip of roots. The root volume was measured using water displacement method which is described by Anila *et al.* (2018). The data collected from both plots were analyzed using R studio software (version 3.6.3). To evaluate the performance of RILs to low soil P stress, stress indices *viz.* stress tolerance index (STI), tolerance index (TOL), yield reduction (YR), stress susceptibility index (SSI), yield stability index (YSI), yield index (YI) and per cent yield reduction (PYR), were calculated for the plants based on low and normal soil P response. The RILs were further grouped into highly tolerant, tolerant and sensitive genotypes by cluster analysis (DARwin6; Perrier and Jacquemoud-Collet, 2006) using the Euclidean distance, with UPGMA (Unweighted Pair Group Method using Arithmetic means) based on the calculated stress indexes.

RESULTS AND DISCUSSION

Analysis of the soils before the planting revealed that the soil pH was neutral (6.9). The presence of nitrogen (N), available phosphorus (P) and available potassium (K) was 126.3 kg ha-1, 1.36 kg ha-1 (very low) 587 kg ha-1 (high), respectively in the case of low soil phosphorous plot of ICAR- IIRR, Hyderabad. In case of the normal plot the available NPK was recorded as 130.4 kg ha-1, 18.3 kg ha-1 (medium) and 592 kg ha-1 (high) with a soil pH of 7.2 (neutral).

Under normal P conditions, the RILs showed normal growth and development. ANOVA revealed the presence of significant variability among the recombinant inbred lines for all the traits except for the root length, grain yield per plant and thousand seed weight but showed higher values in the normal soil P conditions w.r.t all the traits (**Table 1 and Fig. 1**). The days to 50% flowering among the RILs ranged from 80.25 and 117.33 with an overall mean of 97.13 ± 0.67 days. The plant height of the RILs varied from 49.46 to 131.46 cm with an overall mean of 84.14 ± 0.85 cm. The number of productive tillers per plant varied from 5.33 to 22.58 with an overall mean of 12.54 ± 0.22. The panicle length varied from 9.54 to 28.77 cm with an overall mean of 18.72 ± 0.2 cm and flag leaf length varied from 15.32 to 40.81 cm with an overall mean of 27.77 ± 0.3 cm. The shoot length varied from 50.8 to 114.81 cm with an overall mean of 72.27 ± 0.87 cm, the root length varied from 13.5 to 34.14 cm with an overall mean of 23.87 ± 0.29 cm and the root volume varied from 6.67 to 106.67 ml with an overall mean of 36.91 ± 1.42 ml. The dry shoot weight varied from 1.85 to 44.53 g with an overall mean of 17.53 ± 0.65 g and the dry root weight varied from 0.51 to 11.51 g with an overall mean of 3.68 ± 0.15 g. The root to shoot ratio varied from 0.09 to 0.63 with an overall mean 0.22 ± 0.01, grain yield of the plant varied from 1.06 to 26.12 g with an overall mean of 13.47 ± 0.34 g, the thousand seed weight varied from 12.39 to 24.61 g with an overall mean of 18.47 ± 0.17 g and the biomass varied from7.69 to 58.88 g with an overall mean of 31.04 ± 0.74 g in normal P conditions for the RILs.

Under stress conditions most of the RILs exhibited a delay in days to 50% flowering along with a reduction in the plant height, the number of productive tillers per plant, panicle length, flag leaf length, shoot length, root length, root volume, dry shoot weight, dry root weight, grain yield per panicle, thousand grain weight and biomass. ANOVA revealed the presence of significant variability among the recombinant inbred lines for all the traits except for the number of productive tillers, panicle length, root length and root to shoot ratio (**Table 1 and Fig.1**). The days to 50% flowering ranged from 84.17 and 132.67 with an overall mean of 108.53 ± 0.83 days. The plant height of the RILs varied from 38.93 to 80.35 cm with an overall mean of 60.71 ± 0.62 cm. The number of productive tillers per plant varied from 2.59 to 12.92 with an overall mean of 6.26 ± 0.14. The panicle length varied from 10.04 to 21.36 cm with an overall mean of 15.21 ± 0.15 cm and flag leaf length varied from 11 to 28.33 cm with an overall mean of 19.56 ± 0.26 cm. The shoot length varied from 43.15 to 90.4 cm with an overall mean of 67.31 ± 0.61 cm, the root length varied from 16.23 to 36.81 cm with an overall mean of 25.38 ± 0.26 cm and the root volume varied from 2.68 to 50.79 ml with an overall mean of 19.49 ± 0.67 ml. The dry shoot weight varied from 0.9 to 20.68 g with an overall mean of 6.13 ± 0.23 g and the dry root weight varied from 0.17 to 6.06 g with an overall mean of

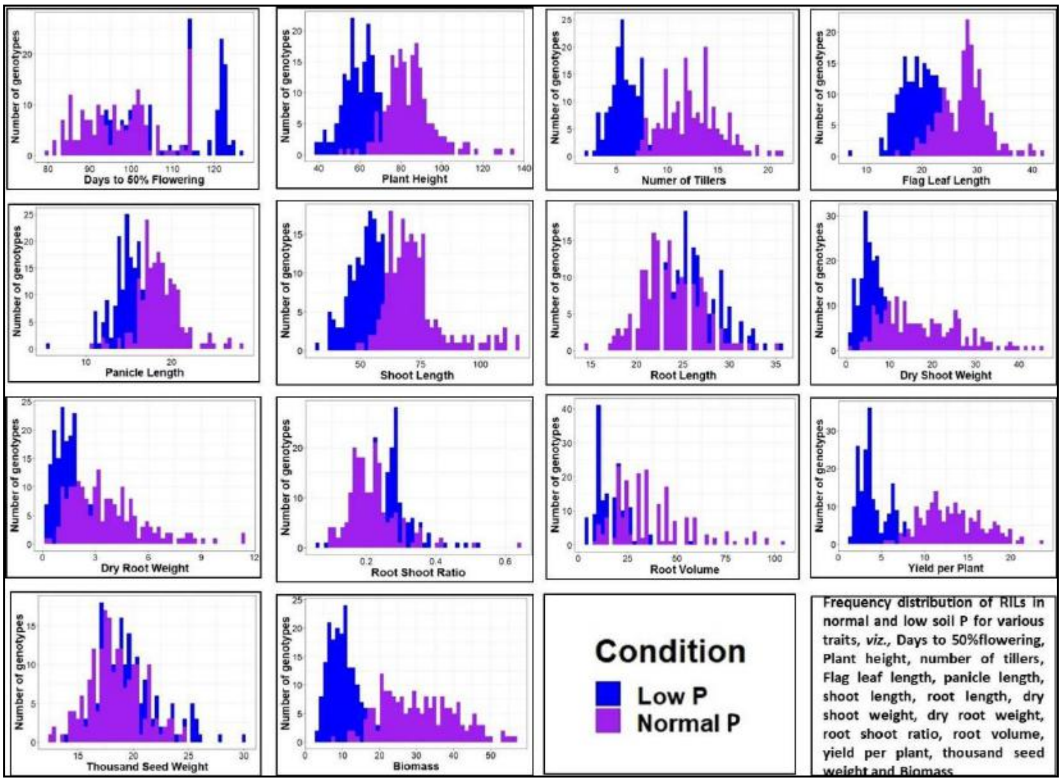

Fig. 1. Frequency distribution for various traits of RILs in Normal (N) and Low (L) phosphorous soil conditions of ICAR- IIRR, Hyderabad

Table 1. Analysis of variance for the various agro- morphological parameters recorded for RILs under normal P and low soil P conditions of ICAR-IIRR, Hyderabad

	Trait	DFF	PH (cm)	NPT (No.s)	PL (cm)	FLL (cm)	SL (cm)	RL (cm)	RV (ml)	DSW (g)	DRW (g)	R:S Ratio	GY (g)	TSW (g)	BM
1A- Normal Soil P condition	Range	80.25-117.33	49.46-131.46	5.33-22.58	9.54-28.77	15.32-40.81	50.8-114.81	13.5-34.14	6.67106.67	1.8544.53	0.51-11.51	0.090.63	1.0626.12	12.39-24.61	7.6958.88
	Minimum	80.25	49.46	5.33	9.54	15.32	50.8	13.5	6.67	1.85	0.51	0.09	1.06	12.39	7.69
	Maximum	117.33	131.46	22.58	28.77	40.81	114.81	34.14	106.67	44.53	11.51	0.63	26.12	24.61	58.88
	Mean	97.13	84.14	12.54	18.72	27.77	72.27	23.87	36.91	17.53	3.68	0.22	13.47	18.47	31.04
	S.E	0.67	0.85	0.22	0.2	0.3	0.87	0.29	1.42	0.65	0.15	0.01	0.34	0.17	0.74
	CV%	2.25	3.11	9.64	4.13	4.35	2.5	10.28	11.11	6.05	8.63	6.38	18.7	11.22	8.58
	CD Test@ 5%	8.48	10.08	4.69	3.01	4.66	6.98	9.57	15.79	4.16	1.25	0.05	10.04	8.05	10.52
	CD Test vs check @ 5%	6.92	8.23	3.83	2.46	3.81	5.70	7.82	12.90	3.40	1.02	0.04	8.20	6.57	8.59
	MSS Checks	553.89**	50.42 *	4.97	3.72 *	4.90	87.30	10.43	35.42	22.2 **	1.51 **	7.04	38.01 *	22.61 *	107.92**
	MSS Tests	92.50 **	144.85**	7.74 *	6.86 **	17.25 **	149.26**	12.26	370.35**	82.83 **	4.34 **	0.005 **	18.64	6.09	113.2 **
	MSS Test vs Checks	85.25**	146.33**	7.78*	6.91**	17.44**	150.24**	12.29	375.61**	83.79 **	4.38 **	0.01 **	18.33	5.83	113.29**
	Skewness	0.30	0.92	0.43	0.60	0.23	1.50	0.37	1.19	0.66	1.05	1.86	1.29	0.28	0.38
	Kurtosis	2.20	5.51	3.12	5.40	3.72	5.41	3.48	4.25	2.91	4.22	10.37	6.71	2.99	2.59
1B- Low Soil P condition	Range	84.17-132.67	38.9380.35	2.59-12.92	10.04-21.36	11-28.33	43.15-90.4	16.23-36.81	2.6850.79	0.9-20.68	0.17-6.06	0.040.51	0.859.69	10.47-28.03	1.78-28.12
	Minimum	84.17	38.93	2.59	10.04	11.00	43.15	16.23	2.68	0.90	0.17	0.04	0.85	10.47	1.78
	Maximum	132.67	80.35	12.92	21.36	28.33	90.40	36.81	50.79	20.68	6.06	0.51	9.69	28.03	28.12
	Mean	108.53	60.71	6.26	15.21	19.56	67.31	25.38	19.49	6.13	1.59	0.26	4.00	20.99	10.13
	S.E	0.83	0.62	0.14	0.15	0.26	0.61	0.26	0.67	0.23	0.07	0.01	0.14	0.28	0.3
	CV%	2.20	5.39	17.09	11.47	5.92	3.73	9.79	7.27	11.10	15.96	31.09	7.77	1.89	6.91
	CD Test@ 5%	9.28	12.63	4.13	6.74	4.47	9.67	9.56	5.48	2.61	1	0.32	1.22	1.52	2.71
	CD Test vs check @ 5%	7.57	10.31	3.37	5.50	3.65	7.90	7.81	4.48	2.13	0.82	0.26	0.99	1.24	2.21
	MSS Checks	864.33**	374.67**	17.64**	14.43*	21.67**	357.23**	53.05*	185.32**	29.45**	7.35**	0.02	14.6**	57.27**	85.47**
	MSS Tests	139.74**	75.31**	3.81	4.03	10.77**	73.55**	12.95	75.92**	11.12**	1.00**	0.01	3.52**	17.89**	18.66**
	MSS Test vs Checks	128.35**	70.61*	3.60	3.86	10.6**	69.09**	12.32	74.21**	10.83**	0.90**	0.01	3.35**	17.27**	17.61**
	Skewness	-0.08	-0.12	0.86	0.08	0.18	-0.12	0.15	0.45	1.17	1.32	0.47	0.80	-0.31	0.73
	Kurtosis	1.70	2.72	4.13	3.01	2.69	2.99	3.27	2.97	5.18	5.68	4.46	3.07	2.21	3.88

** significant at 1 % level, * significant at 5 % level
DFF- Days to 50% flowering; PH - Plant height; NPT- Number of productive tiller/plant; PL - Panicle length; FLL - Flag leaf length; SL- Shoot length; RL- Root length; RV- Root volume; DSW- Dry shoot weight; DRW - Dry root weight; R:S ratio- Root to shoot ratio; GY- Grain yield/Plant; TSW - 1000 grain weight; BM- Biomass

1.59 ± 0.07 g. The root to shoot ratio varied from 0.04 to 0.51 with an overall mean 0.26 ± 0.01, grain yield of the plant varied from 0.85 to 9.69 g with an overall mean of 4 ± 0.14 g, the thousand seed weight varied from 10.47 to 28.03 g with an overall mean of 20.99 ± 0.28 g and the biomass varied from 1.78 to 28.12 g with an overall mean of 10.13 ± 0.3 g in low soil P conditions for the RILs.

The phenotypic evaluation of the mapping population both under low P and normal soils paved a way in identification of highly tolerant, tolerant and sensitive lines among the RILs. Significant phenotypic variation in rice has been observed earlier for various traits which are related to the increased productivity in P poor soils (Fageria *et al.,* 1988; Akinrinde *et al.,* 2006; Aluwihare *et al.,* 2016). Most of the traits showed a normal distribution with skewness towards the tolerance end which led to the perception of identification one or more major QTLs associated with tolerance in Rasi.

A delay in flowering for about 4-15 days was observed in all the entries in low soil P conditions which could be a plant adaptive mechanism for effective/increased phosphorus acquisition and utilization (Nord and Lynch, 2008). In research conducted by several groups (Shepherd *et al.,* 1987; Chauhan *et al.,* 1992; Rodriguez *et al.,* 1998), phenological delays in plants following exposure to low P conditions have been recorded which is linked to the adaptation mechanism for maximum phosphorous uptake. Plant height is dramatically affected in soil P conditions about 11-51cm reduction in height was observed in the present study, which could be another adaptive mechanism that helps the plant to acquire more P for growth and maintenance, thus reducing the cell growth (Cancellier *et al*., 2012). Considerable decrease upto to 3-15 productive tillers was observed among the RILs under stress conditions in comparison to non-stress conditions. Studies carried out by Fageria *et al.* (2011), Fageria and Knupp (2013), Deng *et al.* (2020) and Kale *et al.* (2021) have observed a similar condition and thus the number of tillers can be used as one of the criteria to evaluate low soil P tolerance in rice. In addition to the above trait, flag leaf length, panicle length, root length and shoot length also decreased significantly (**Table 1**). Various studies carried out by Fageria *et al.* (1988), Dobermann and Fairhurst (2000), Chankaew *et al.* (2019) and Deng *et al.* (2020) and in other grasses by Kavanova *et al.*(2006), Grimoldi *et al.*(2005) observed a similar trend in these traits under low soil P conditions.

Dry shoot weight, dry root weight, root volume, and root to shoot ratio of the RILs in the present study showed a reduction up to 50% in the stress condition (**Table 1**) and these traits are considered the best metrics for recording the tolerance for low soil P conditions. In the present study there was a significant variation of these traits in stress and non-stress conditions. The studies carried by Fageria *et al*. (1988), Wissuwa and Ae (2001), Wissuwa (2005), Li *et al.* (2009), Chithrameenal *et al.* (2018), Deng *et al.* (2020) and Kale *et al.* (2021) suggested a similar scenario in the above traits. In the current study, the was a reduction up to 60% among the RILs in the stress condition and was observed to be greatly dependent on the grain filling stage in many stresses such as drought, N and P deficiency. (Yoshida, 1981; Choudhury *et al.,* 2007 and Fageria *et al.,* 2011). Wissuwa *et al.* (2009) have defined the importance of phosphorous to generate biomass and the capability of absorption of P from available sources. Results obtained from the current study showed that the traits recorded were found to be the best metrics for the screening of individuals for low soil phosphorous tolerance and the above RIL population can be used for mapping the new novel QTL/genes responsible for low soil phosphorous tolerance conferred by Rasi.

In order to assess the low soil P stress tolerance of recombinant inbred lines, stress tolerance indices like stress tolerance index (STI), tolerance index (TOL), yield reduction (YR), stress susceptibility index (SSI), yield stability index (YSI), yield index (YI) and per cent yield reduction (PYR) were calculated on the basis of grain yield under normal (under recommended dose of P) condition and grain yield under low soil P (i.e.< 2 Kg ha^{-1}; low P stress) condition (**Table 2**). The RILs were then clustered into highly tolerant, tolerant and sensitive genotypes (**Fig. 2**), based on the results obtained. Many of the studies carried out earlier for various abiotic stresses also carried out a similar strategy, were the stress indices were calculated for the clustering/ grouping of the genotypes. For drought tolerance studies carried out by Mollasadeghi *et al.* (2011) and Ashraf *et al.* (2015), for salinity tolerance- Singh *et al.* (2015), for nitrogen deficiency tolerance- Rameeh (2015) in rapeseed, Khan and Mohammed (2016) in wheat and for low soil P tolerance (Swamy *et al.,* 2019; Kale *et al.,* 2020) have calculated the stress indices to cluster the genotypes in a similar way.

In the present study, RIL- 106 (0.56) showed the highest and RIL-28 (0.03) lowest STI value among the RILs while the tolerant checks Swarna and Rasi recorded a value of 1.48 and 1.38, respectively and sensitive checks- MTU 1010 and ISM showed a value of 0.35 and 0.14, respectively. The lines with higher STI values are considered tolerant in low soil P conditions (Fernandez 1992; Ashraf *et al.,* 2015; Swamy *et al,*. 2019; Kale *et al.,* 2020). The highest value for tolerance index (TOL) was recorded for the RIL- 83 (23.93) and RIL-28 (0.26) the lowest, while the tolerant checks Swarna and Rasi recorded a value of 8.92 and 8.98, respectively and sensitive checks MTU 1010 and ISM recorded a value of 20.83 and 16.12, respectively. The highest value for yield reduction (YR) was recorded for the RIL- 83 (0.96) and the lowest for RIL-28 (0.08), while the tolerant checks Swarna and Rasi recorded a value of 0.34 and 0.35, respectively and sensitive checks MTU 1010 and ISM recorded a value of 0.83 and 0.87, respectively. The lower TOL and YR value indicate a higher tolerance

Table 2. Stress indices calculated for RILs based on the single plant yield under low and normal soil P conditions of ICAR- IIRR, Hyderabad

S.No.	Stress indices	Range		Swarna	Rasi	MTU 1010	ISM
		Highest	Lowest				
1.	Stress Tolerance Index (STI)	0.56 (RIL-106)	0.03 (RIL-83)	1.48	1.38	0.35	0.14
2.	Tolerance Index (TOL)	23.93 (RIL-83)	0.26 (RIL-28)	8.92	8.98	20.83	16.12
3.	Yield Reduction ratio (YR)	0.96 (RIL-83)	0.08 (RIL-28)	0.34	0.35	0.83	0.87
4.	Stress Susceptibility Index (SSI)	1.36 (RIL-83)	0.11 (RIL-28)	0.46	0.48	1.13	1.18
5.	Yield Stability Index (YSI)	0.91 (RIL-28)	0.03 (RIL-83)	0.66	0.65	0.17	0.13
6.	Yield Index (YI)	2.41 (RIL-60)	0.21 (RIL-79)	3.71	3.55	0.91	0.51
7.	Per cent Yield Reduction (% YR)	96.18 (RIL-83)	8.22 (RIL-28)	34.15	35.27	83.19	87.23

Note**:** Stress indices were calculated for the RILs based on the single plant yield under low and normal soil P conditions of ICAR- IIRR. Swarna was used as a tolerant check along with Rasi and MTU 1010 as the sensitive check along with Improved Samba Mashuri (ISM).

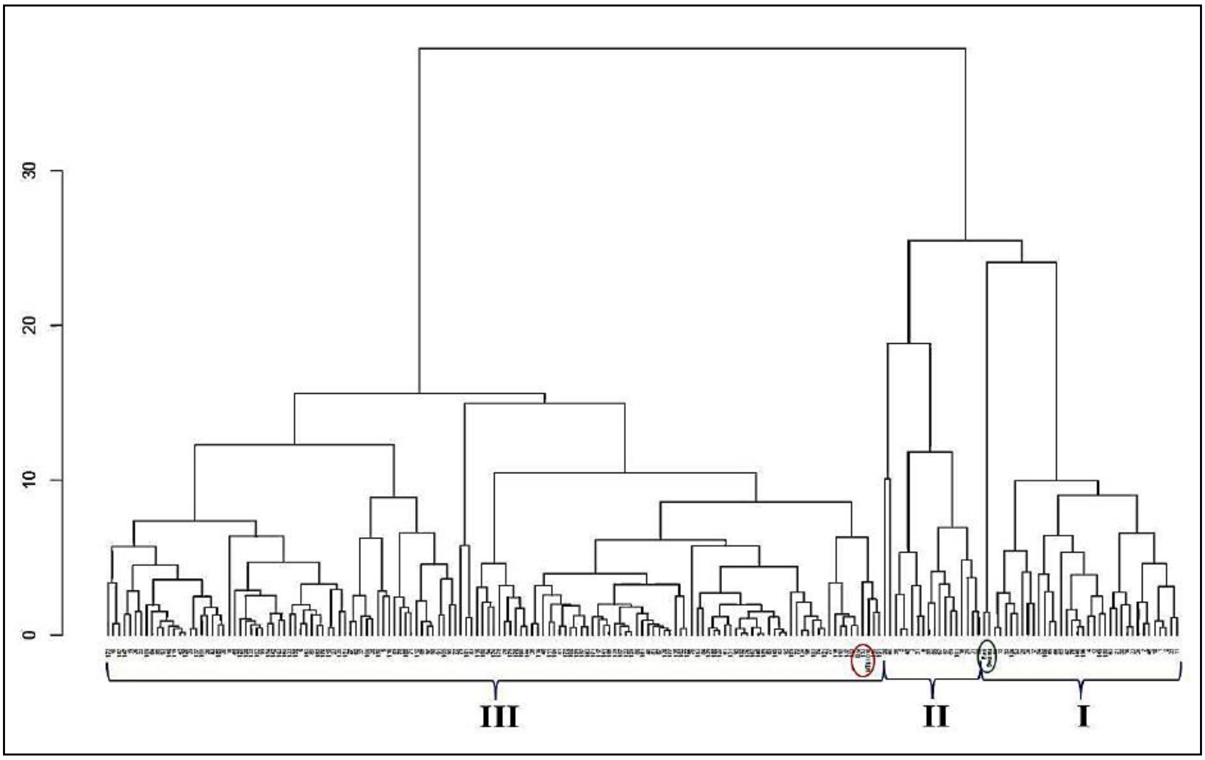

Fig. 2. Clustering based upon the stress indices and yield reduction under low soil P in comparison with normal soil P condition

Note: The RILs were clustered into three clusters. First cluster constituted of RILs which showed >40 % yield reduction (highly tolerant) along with the tolerant checks-Rasi and Swarna, second cluster constituted of RILs with an yield reduction of 40-70% (tolerant) and third cluster constituted of RILs with < 70% yield reduction (sensitive) along with the sensitive checks- MTU 1010 and ISM under low soil P condition.

level of the individuals to low soil P tolerance (Rosielle and Hamblin, 1981; Singh *et al*., 2015; Golestani-Araghi and Assad, 1998; Ashraf *et al*., 2015). The highest value for stress susceptibility index (SSI) was recorded for the RIL- 83 (1.36) and the lowest for RIL-28 (0.11), while the tolerant checks Swarna and Rasi recorded a value of 0.46 and 0.48, respectively and sensitive checks MTU 1010 and ISM recorded a value of 1.13 and 1.18, respectively. The presence of an SSI value less than one indicates a higher tolerance level (Fisher and Maurer, 1978; Ashraf *et al.,* 2015), most of the RILs in the mapping population from the present study showed less than one SSI value whereas the sensitive checks MTU 1010 and ISM showed SSI value of more than one. The highest value for yield stability index (YSI) was recorded for the RIL- 28 (0.91) and the lowest for RIL-83 (0.03), while the tolerant checks Swarna and Rasi recorded a value of 0.66 and 0.65, respectively and sensitive checks MTU 1010 and ISM recorded a value of 0.17 and 0.13, respectively. The highest value for yield index (YI) was recorded for the RIL- 60 (2.41) and the lowest for RIL-79 (0.21), while the tolerant checks Swarna and Rasi recorded a value of 3.71 and 3.55, respectively and sensitive checks MTU 1010 and ISM recorded a value of 0.91 and 0.51, respectively. The higher YSI and YI value indicate the stability of the individual in stress and non-stress conditions (Bouslama and Schapaug, 1984; Gavuzzi *et al.,* 1997; Ashraf *et al.,* 2015; Singh *et al.,* 2015; Swamy *et al*., 2019; Kale *et al.,* 2020). Many RILs showed higher YSI values in comparison to the sensitive parents- MTU 1010 and ISM. The highest value for percentage yield reduction (% YR) was recorded for RIL 83 (96.18) and the lowest for RIL28 (8.22), while the tolerant checks Swarna and Rasi recorded a value of 34.15 and 35.27 % reduction, respectively and sensitive checks MTU 1010 and ISM recorded a value of 83.19 and 87.23 % reduction in the yield, respectively. Thus, the genotypes with more yield reduction are considered sensitive while the genotypes with less YR are tolerant for low P.

In order to better understand and interpret the results obtained from stress and non-stress conditions, cluster analysis was carried out based on the percentage yield reduction in stress and non-stress conditions using the Euclidean distance, with UPGMA (Unweighted Pair Group Method using Arithmetic means) in DARwin 6.0 to group the RILs into 3 major groups, *i.e.,* highly tolerant, tolerant and sensitive (Swamy *et al.,* 2019; Kale *et al.,* 2020). The first cluster constituted of 36 RILs which showed < 40 % yield reduction (highly tolerant) and the tolerant checks- Swarna and Rasi were part of cluster I which can serve as donors in various future breeding programs which involve the transfer of *non- Pup1* type QTL/ genes. The second cluster constituted 18 RILs with a yield reduction of 40-70% (tolerant) but still can be used as donors and can yield well under normal P conditions. The third cluster was the largest identified in the study, which constituted 141 RILs with > 70% yield reduction (sensitive) under low soil P conditions along with the sensitive checks MTU 1010 and ISM (**Fig. 2**).

Rasi, might possess a different (*i.e.,* novel) mechanism for low soil P tolerance and the RILs developed from the population facilitie the identification of the *non- Pup1* type tolerance mechanism. The RILs showed desirable traits such as, high yield, medium slender grain type with better root system architecture, plant height along with low soil P tolerance. The cultivation of such lines will enhance the productivity for farmers and help reduce the cost of production owing to the reduced application of P fertilizers. The 36 RILs identified from the population can also serve as additional potent donors for the transfer of *non- Pup1* type tolerance under low soil P conditions in future breeding programs.

ACKNOWLEDGEMENT

The authors thank friends and colleagues of the RMS group, ICAR- Indian Institute of Rice Research for providing support and care at all levels along with the Director, ICAR-Indian Institute of Rice Research, for providing all the necessary facilities to carry out the research.

REFERENCES

Adavikolanu, A.V. 2014. Opinion: Peak phosphorus and implications for India. *Sciencenordic.* September 2014. 1-2. http://sciencenordic.com/opinion-peak-phosphorus-and-implicatio ns-india.

Akinrinde, E.A. and Gaizer, T. 2006. Differences in the performance and phosphorus-use efficiency of some tropical rice (*Oryza sativa* L.) varieties. *Pakistan Journal of Nutrition*. **5**(3): 206-211. [Cross Ref]

Aluwihare, C., Ishan, M.,Chamikara, M.D.M.,Weebadde, C.K., Sirisena, D.N., Samarasinghe, W.L.G. and Sooriyapathirana, S.D.S.S. 2016. Characterization and selection of phosphorus deficiency tolerant rice genotypes in Sri Lanka. *Rice Science*, **23**(4):184-195. [Cross Ref]

Anila, M., Mahadeva Swamy, H.K., Kale, R.R., Bhadana, V.P., Anantha, M.S., Brajendra Hajira, S.K., Balachiranjeevi, C.H., Ayyappa Dass, M., Bhaskar. S., Dilip, T., Pranathi, K., Kousik, MBVN., Harika, G., Swapnil, K., Chaitra, K., Laxmi Prasanna, B., Punniakotti, E., Sinha, P., Rekha, G., Abhilash Kumar, V., Balachandran, S.M., Madhav, M.S., Giri, A., Viraktamath, B.C., Sundaram, R.M. 2018. Breeding lines of the Indian mega-rice variety, MTU 1010, possessing protein kinase OsPSTOL (Pup1), show better root system architecture and higher yield in soils with low phosphorus. *Molecular Breeding,* **38**:147

Ashraf, A., El-Mohsen, A., Abd El-Shafi, M.A., Gheith, E.M.S.

and Suleiman, H.S. 2015. Using different statistical procedures for evaluating drought tolerance indices of bread wheat genotypes. *Advance in Agriculture and Biology*, **3**(1): 19-30.

Bouslama, M. and Schapaugh, W.T. 1984. Stress tolerance in soybean. Part 1: evaluation of three screening techniques for heat and drought tolerance. *Crop Science*, **24**: 933- 937.

Cancellier, E.L., Brandao, D.R., Silva, J., Santos, M.M. and Fidelis, R.R. 2012. Phosphorus use efficiency of upland rice cultivars on Cerrado soil. *Ambience,* **8**(2): 307-318.

Chankaew, S., Monkham, T., Pinta, W., Sanitchon, J., Kaewpradit, W. and Srinives, P. 2019. Screening tolerance to phosphorus deficiency and validation of *phosphorus uptake 1*(*Pup1*) gene-linked markers in Thai indigenous upland rice germplasm. *Agron*., **9**(81): 1-12. [Cross Ref]

Chauhan, Y.S., Johansen. C. and Venkataratnam, N. 1992. Effects of phosphorus deficiency on phenology and yield components of short-duration pigeonpea. *Tropical Agriculture*., **69**(3): 235-238.

Chin, J.H., Lu, X., Haefele, S.M., Gamuyao, R., Ismail, A., Wissuwa, M. and Heuer, S. 2010. Development and application of gene-based markers for the major rice QTL Phosphorus uptake 1. *Theoretical and Applied Genetics*, **120**(6):1073-1086. [Cross Ref]

Chithrameenal, K., Alagarasan, G., Raveendran, M., Robin, S., Meena, S., Ramanathan, A. and Ramalingam, J. 2018. Genetic enhancement of phosphorus starvation tolerance through marker assisted introgression of *OsPSTOL1* gene in rice genotypes harboring bacterial blight and blast resistance. *PLoS ONE*, **13**(9): 1-20. [Cross Ref]

Choudhury, A.T.M.A., Kennedy, I.R., Ahmed, M.F. and Kecskés, M.L. 2007. Phosphorus fertilization for rice and control of environmental pollution problems. *Pakistan Journal of Biological Sciences*, **10**(13): 2098-2105. [Cross Ref]

Deng, Y Men, C., Qiao, S., Wang, W., Gu, J., Liu, L., Zhang, Z., Zhang, H., Wang, Z. and Yang, J. 2020. Tolerance to low phosphorus in rice varieties is conferred by regulation of root growth. *The Crop Journal*, **8**(4): 534-547. [Cross Ref]

Dobermann, A. and Fairhurst, T. 2000. Rice: Nutrient disorders & nutrient management. Handbook series. *Potash& Phosphate Institute (PPI)*, Potash & Phosphate Institute of Canada (PPIC) and International Rice Research Institute. 191.

Du, J., Zeng, Y., Pu, X.Y., Yang, S.M., Zhu, G.B. and Wang, Y.C. 2008. Evaluation method and primary screening of high-phosphorus efficiency germplasm of Yunnan rice landraces. *Southwest China Journal of Agricultural Sciences,* **2**: 10-35.

Fageria, N. K., Baligar,V. C. and Jones, C. A. 2011. Growth and mineral nutrition of field crops. Boca Raton, FL: CRC Press.

Fageria, N.K. and Knupp, A.M. 2013. Upland rice phenology and nutrient uptake in tropical climate. *Journal of Plant Nutrition*., **36**: 1-14.

Fageria, N.K. and Baligar, V.C. 1997. Upland rice genotypes evaluation for phosphorus use efficiency. *Journal of Plant Nutrition*, **20**(4-5):499-509.

Fageria, N.K., Wright, R.J. and Baligar, V.C. 1988. Rice cultivar evaluation for phosphorus use efficiency. *Plant and Soil*, **111**(1):105-109. [Cross Ref]

Fernandez, G.C.J. 1992. Effective selection criteria for assessing plant stress tolerance. In: Proceeding of the *International. Symposium, On adaptation of vegetables and other food crops in temperature and water stress.* Taiwan: 257-270.

Fisher, R. A. and Maurer, R.1978. Drought resistance in spring wheat cultivars. I. Grain yield responses. *Australian Journal of Agricultural Research,* **29**: 897-912.

Gavuzzi, P., Rizza, F., Palumbo, M., Campaline, R.G., Ricciardi, G.L. and Borghi, B. 1997. Evaluation of field and laboratory predictors of drought and heat tolerance in winter cereals. *Canadian Journal of Plant Science,* **77**:523-531. [Cross Ref]

Golestani-Araghi, S. and Assad, M.T. 1998. Evaluation of four screening techniques for drought resistance and their relationship to yield reduction ratio in wheat. *Euphytica,* **103**: 293-299.

Grimoldi, A.A., Kavanova, M., Lattanzi, F.A. and Schnyder, H. 2005. Phosphorus nutrition-mediated effects of arbuscular mycorrhiza on leaf morphology and carbon allocation in perennial ryegrass. *New Phytologist* .00: 000–000. 10.1111/j.1469-8137.2005.01500.x

Hasan, R.1996. Phosphorus status of soils in India. *Better Crops International*, **10**(2): 4-5.

Islam, M.A., Islam, M.R. and Sarker, A.B.S. 2008. Effect of phosphorus on nutrient uptake of Japonica and Indica rice. *Journal of Agriculture & Rural Development*, **6**(1):7-12. [Cross Ref]

Jackson, M.L.1964.Soil chemical analysis. Prentice Hall ofIndia. Pvt. Ltd. New Delhi, p 498

Jackson, M.L.1967. Soil chemical analysis. Prentice Hall of India Pvt. Ltd. New Delhi

Kale, R. R., Anila, M., Mahadeva Swamy, H. K., Bhadana, V. P., Durga Rani, C. V., Senguttuvel, P., Subrahmanyam, D, Hajira, S. K., Rekha, G, Ayyappadass, M, Laxmiprasanna, B, Punniakotti, E, Kousik, M. B. V. N, Swapnil. K, Dilip, T, Sinha. P, Harika, G, Pranathi. K, Chaitra. K, Anantha, M. S, Brajendra. P, Subbarao, L. V, Balachandran, S. M, Mangrauhuia, S. K, & Sundaram, R. M. 2020. Morphological and molecular screening of rice germplasm lines for low soil P tolerance. *Journal of Plant Biochemistry and Biotechnology*, **30**(2): 275-286. [Cross Ref]

Kale, R. R., Durga Rani, C. V., Anila, M., Mahadeva Swamy, H. K., Bhadana, V. P., Senguttuvel, P., Subrahmanyam, D., Ayyappa Dass, M, Swapnil, K., Anantha, M.S., Punniakotti, E., Laxmi Prasanna, B., Rekha, G., Sinha, P., Kousik, M.B.V.N., Hajira, S.K., Brajendra, P., Mangrauthia, S.K., Gireesh, C., Mangaldeep Tuti, Mahendra Kumar, R., Jitendra Giri., Pawandeep Singh and Sundaram, R. M. 2021. Novel major QTLs associated with low soil phosphorus tolerance identified from the Indian rice landrace, Wazuhophek. *PloS one*, **16**(7), e0254526.

Kavanova, M., Grimoldi, A.A., Lattanzi, F.A., Schnyder, H. 2006. Phosphorus nutrition and mycorrhiza effects on grass leaf growth. P status- and size mediated effects on growth zonekinematics. Plant Cell Environ, **29**:511–520

Khan, U. and Mohammad F. 2016. Application of stress selection indices for assessment of nitrogen tolerance in wheat (*Triticum aestivum* L.). *The Journal of Animal & Plant Sciences*. **26**(1): 201-210.

Krishnamurthy, P., Sreedevi, B., Ram, T. and Latha, P.C. 2014. Evaluation of rice genotypes for phosphorus use efficiency under soil mineral stress conditions. *Journal of Rice Research*. **7** (1&2): 53-61.

Li, Z., Xie, Y., Dai, A. and Liu, L. 2009. Root and shoot traits responses to phosphorus deficiency and QTL analysis at seedling stage using introgression lines of rice. *Journal of Genetics and Genomics,* **36**:173-183. [Cross Ref]

Mollasadeghi, V., Valizadeh, M., Shahryariand, R.A. and Imani, A. 2011. Evaluation of end drought tolerance of 12 wheat genotypes by stress in dices. *Middle-East Journal of Scientific Research*, **7**(2):241-247.

Muralidharudu, Y., Mandal, B.N., Sammi Reddy, K. and Subba Rao, A. 2011. In : Progress report of the *All India Coordinated Research Project for Investigation on Soil Test Crop Response Correlation*. Indian Institute of Soil Science, Bhopal. pp. 11-61.

Nord, E. and Lynch, J. 2008. Delayed Reproduction in Arabidopsis thaliana Improves Fitness in Soil with Suboptimal P Availability. *Plant, Cell and Environment,* **31**:1432-1441. [Cross Ref]

Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A.1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate, Circular U.S. Department of Agriculture, p 939

Panigrahy, M., Nageswara Rao, D., Yugandhar, P., Sravan Raju, N., Krishnamurthy, P., Voleti, S.R. and Sarla, N. 2014. Hydroponic experiment for identification of tolerance traits developed by rice Nagina 22 mutants to low-phosphorus in field condition. *Archives of Agronomy and Soil Science*, **60**(4): 565-576. [Cross Ref]

Perrier, X. and Jacquemoud-Collet, J.P. 2006 Darwin software.http://darwin.cirad.fr/darwin. Accessed 22 March 2022

Rameeh, V. 2015. Nitrogen deficiency stress indices of seed yield in rapeseed (*Brassica napus* L.) genotypes. *Cercetari Agronomice in Moldova*, **158** (1/161): 89-96.

Rodriguez, D., Pomar, M.C. and Goudriaan, J. 1998. Leaf primordia initiation, leaf emergence and tillering in wheat (*Triticum aestivum* L.) grown under low-phosphorus conditions. *Plant and soil*, **202**(1):149-157. [Cross Ref]

Rosielle, A.A. and Hamblin, J. 1981. Theoretical aspect of selection for yield in stress and non-stress environment. *Crop Science*, **21**: 943-946.

Shepherd, K.D., Cooper, P.J.M., Allan, A.Y., Drennan, D.S.H. and Keatinge, J.D.H. 1987. Growth, water-use and yield of barley in Mediterranean-type environments. *Journal of Agricultural Science*, **108**:365-378. [Cross Ref]

Singh, S., Sengar, R.S., Kulshreshtha, N., Datta, D., Tomar, R.S. Rao, V.P., Garg, D. and Ojha, A. 2015. Assessment of multiple tolerance indices for salinity stress in bread wheat (*Triticum aestivum* L.). *Journal of Agricultural Science*, **7**(3):49-57. [Cross Ref]

Subbaiah, B.V. and Asija, G.L. 1956. A rapid procedure for the determination of available nitrogen in soils. *Current Science,* **25**:259–260

Mahadeva swamy, H.K., Anila, M., Ravindra, R.K., Bhadana, V.P., Anantha, M.S., Brajendra, P., Hajira, S.K., Balachiranjeevi, C.H., Laxmi Prasanna, B., Pranathi, K., Dilip, T., Bhaskar, S., Abhilash Kumar, V., Kousik, M.B.V.N., Harika, G., Swapnil, K., Rekha, G., Cheralu, C., Gouri Shankar, V.S., Reddy, N., Kumar, S., Balachandran, S.M., Madhav, M.S., Mahendra Kumar, R. and Sundaram, R.M. 2019.

Phenotypic and molecular characterization of rice germplasm lines and identification of novel source for low soil phosphorus tolerance in rice. *Euphytica,* **215**: 118. [Cross Ref]

Tandon, H. L. S. 1987. Phosphorus Research in India. Fertilizer Development Consultancy Organization: New Delhi, India.

Tian, Z., Li, J., He, X., Jia, X., Yang, F. and Wang, Z. 2017. Grain yield, dry weight and phosphorus accumulation and translocation in two rice (*Oryza sativa* L.) varieties as affected by salt-alkali and phosphorus. *Sustainability*, **9**:1461. [Cross Ref]

Tiwari, K.N. 2001. Phosphorus Needs of Indian Soils and Crops. *Better Crops International*. **15**(2): 23-2.

Wang, K., Cui, K., Liu, G., Luo, X., Huanga, J., Niea, L., Weia, D. and Peng, S. 2017. Low straw phosphorus concentration is beneficial for high phosphorus use efficiency for grain production in rice recombinant in bred lines. *Field Crops Research,***203**:65-73. [Cross Ref]

Webeck, E., Matsubae, K., Nakajima, K., Nansai, K. and Nagasaka, T. 2014. Analysis of phosphorus dependency in asia. *Sociotechnica,* **11**: 119-126

Wissuwa, M. and Ae, N. 2001. Genotypic variation for tolerance to phosphorus deficiency in rice and the potential for its exploitation in rice improvement. *Plant Breeding*, **120**: 43–48.

Wissuwa, M. 2005. Combining a modelling with a genetic approach in establishing associations between genetic and physiological effects in relation to phosphorus uptake. *Plant and Soil*. **269**: 57-68.

Wissuwa, M., Mazzola, M. and Picard, C. 2009. Novel approaches in plant breeding for rhizosphere-related traits. *Plant and Soil*, **321**:409-430. [Cross Ref]

Wissuwa, M., Yano, M. and Ae, N. 1998. Mapping of QTLs for phosphorus-deficiency tolerance in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, **97**(5):777-783. [Cross Ref]

Yoshida, S. 1981. *Fundamentals of Rice Crop Science. Los Banos: IRRI, 1981*. IRRI.

Zhang, D., Song, H., Cheng, H., Hao, D., Wang, H., Kan, G., Jin, H. and Yu, D. 2014. The acid phosphatase-encoding gene GmACP1 contributes to soybean tolerance to low-phosphorus stress. *PLoS genetics*, **10**(1): p.e1004061