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Screening of RIL mapping population derived from the rice variety- Rasi, for low soil phosphorous tolerance and bacterial leaf blight resistance

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ABSTRACT

A rice cultivar, Rasi showed greater tolerance to low soil phosphorous (P) when tested under acutely P-deficient plot at ICAR-Indian Institute of Rice Research (ICAR-IIRR), Hyderabad. Interestingly, Rasi was also devoid of the popular known QTL- Pup1 and is expected to be a novel source for low P tolerance gene(s)/QTLs. In the present study, an effort was made to develop breeding lines in the genetic background of an elite, bacterial blight (BLB) resistant, fine-grain type rice variety- Improved Samba Mahsuri (ISM) for tolerance to low soil phosphorus (P) by using Rasi, as the donor variety. Crosses were carried out and a set of 214 Recombinant inbred lines (RILs) were developed using single seed decent method. The developed RILs were then screened of which, 149 and 131 RILs showed excellent tolerance to low soil P and resistance to BLB respectively in Kharif 2019. Two RILs (RI-134 and RI-172) were identified and selected which showed tolerance to low soil P and BLB possessing medium slender grain type and were high yielding. These breeding lines identified can serve as potential donors, which can significantly reduce the application of phosphatic fertilizers, reducing cost of cultivation and increasing farmers' profits.

Keywords: *BLB, Improved Samba Mashuri, Low phosphorous tolerance, Rasi, Rice, RIL*

INTRODUCTION

Rice (Oryza sativa L.) is one of the world's most important principal cereal crops, which occupies the second most prominent position in global agriculture. Globally rice grows in 117 countries and is a chief source of staple food consumed by ~ 3 billion global population deriving one-fifth of the calories requirement from it. India is the largest producers of rice around the world and ranks first in the area (43.39 million hectares), and second in the production (104.32 million tonnes) with an average productivity of 2.40tonnes ha-1Agricultural Statistics at a glance 2016; http://eands.dacnet.nic.in/latest_2006.html. Rice production is adversely affected by various factors which include, rapidly changing climate, arable land, biotic and abiotic stresses, nutrient deficient soils, etc., and would present great challenges to the breeders and the scientists (Khush 2005). Among the various factors, abiotic stresses such as drought, salinity and nutrient deficiencies are most important. And among the nutrients, phosphorous (P) plays a vital role in the growth and development of the rice plant. It plays a pivotal role in numerous biochemical reactions and various structural components. Low P stress represents a major constraint on plant growth, development, productivity and yield worldwide (Zhang et al., 2014). P deficiency in rice causes stunted growth with reduced tiller number, spindly stem, narrow leaves and reduced grain number. The P deficient condition also causes delay in flowering and maturity by one week to 10 days and in severe conditions the plants may not flower at all (Dobermann and Fairhurst, 2000). It is a major limiting factor which causes great yield loss in rice crop (Fageria and Baligar, 1997).

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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 and Tiwari, 2001). Thus, to overcome this issue, Indian farmers are applying more and more P fertilizers, which in turn increase the cost of cultivation. India is the biggest importer of P fertilizer with 90% dependency on the world P reserves (Adavikolanu, 2014; Webeck et al., 2014) and by 2030 it is predicted that there is an increase up to 20 million tons P fertilizer consumption. Thus, there is need for genetic improvement of rice plants to adapt to P deficit conditions which helps to minimize the application of phosphatic fertilizers and to enhance sustainable rice production. Better P use efficiency in plants can also be achieved by improved uptake of phosphate from soil (P-acquisition efficiency) or by improved productivity per unit P taken up (P-use efficiency). Identification and transfer of the QTL/genes responsible for low phosphorous tolerance and its use efficiency can significantly enhance the rice yields in low P soils. Studies in rice showed that there is significant variability for both low P tolerance and P-use efficiency. This variability can be utilized for the development of rice lines, which will be tolerant to low soil P and have been use efficient of P. Low P tolerance is a complex quantitative trait and different studies has been carried out to know the genetic variation among different crops for tolerance to low soil P and have revealed that various phenotypic traits correlate 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). A rice cultivar, Rasi showed greater tolerance to low soil P when tested under acutely P-deficient plot at ICAR-Indian Institute of Rice Research (ICAR-IIRR), Hyderabad. Interestingly, Rasi was also observed to be devoid of the popular QTL associated with low P tolerance, Pup1, when checked with a set of functional markers specific for the QTL and hence the variety can be expected to be a novel source for low P tolerance gene(s)/QTLs. In the present study, an effort was made to develop breeding lines in the genetic background of an elite, bacterial blight (BB) resistant, fine-grain type rice variety- Improved Samba Mahsuri (ISM) for tolerance to low soil phosphorus (P).

MATERIALS AND METHODS

Experimental site

The site of experimentation, ICAR-Indian Institute of Rice Research (ICAR-IIRR), Rajendranagar, Hyderabad, is situated at an altitude of 542.30 meters above mean sea level (MSL), 17°19' North latitude and 78°23' East longitude and lies in the Southern Zone (i.e. Zone-3).

Plant material

Rasi (IET 1444), is a drought tolerant variety which was developed and released in 1977 by ICAR-IIRR, Hyderabad. It is a high yielding, early rice variety with medium slender grain type with high level of resistance to leaf blast, brown spot and can be grown in aerobic condition. Additionally, it showed high level of tolerance and was devoid of the known Pup1 QTL based on the analysis using functional markers reported by Chin et al., (2011). ISM is a high-yielding, fine-grain type rice variety, possessing low glycemic index (GI), developed through MABB strategy possessing three bacterial blight genes (Xa21, xa13and xa5) in the genetic background of the Indian mega-variety of rice, Samba Mahsuri and showed high sensitivity to low soil P (Anila et al., 2017). A recombinant inbred line (RIL) population consisting of 214 lines was developed by advancing the F2 plants derived from the cross Rasi and ISM through single seed descent (SSD) method till F9 generation (Figure-1). In Kharif 2019, RILs were further screened for low soil P tolerance and BLB resistance in the presence of tolerant checks (Rasi, Swarna and Vandana) and susceptible checks (ISM, MTU 1010 and IR-64).

Screening of RIL mapping population for low P tolerance and bacterial leaf blight resistance

Wet bed nursery was being raised and 21 days old seedlings of the mapping population were been transplanted into the low soil P plot (available P < 2 kg ha-1 tested using the Olsen P method (Olsen et al., 1954)) and the normal soil P plot (available Phosphorus >18 kg ha-1) of ICAR- IIRR, Hyderabad along with the donor and the recurrent parents for the screening and evaluation for the agro-morphological traits in two seasons i.e., Kharif 2019 and 2020. The experiment was conducted in RBD design consisting of three replications with spacing of 15 × 20 cm (in three rows, constituting of 10 hills per row in low soil P plot and in two rows, constituting of 16 hills per row in the normal soil P plot). All the recommended agronomic practices were followed, which included the basal application of N, K, Fe and Zn followed by top dressing of the N in two- three doses until the maximum tillering stage and no P fertilizer application was done during the entire crop season in the low soil P plot.

Visual phenotyping of the RILs were carried out for P use efficiency and evaluated for the various agro-morphological traits such as days to 50% flowering (DFF), plant height (PH), number of productive tillers (NPT), shoot length (SL), root length (RL) and root volume (RV). In addition to the above parameters the post- harvest agro-morphological traits such as grain yield per plant and thousand grain weight were also recorded. Three plants were selected randomly which represent the entire line and mean data was subjected to statistical analysis using open-source statistical software 'R' version 3.6.3 (R Core Team, 2016).

The RILs were simultaneously screened in the normal soil P plot for BLB resistance during Kharif 2019 using the virulent isolate of the bacterial blight pathogen, Xanthomonas oryzae pv. oryzae (Xoo) DX-020 (collected from Hyderabad, Telangana). Xoo strain was cultured and stored as described by Laha et al., (2009). The rice plants were clip inoculated with a bacterial suspension of 108-9cfu/ml at maximum tillering stage (45 –55 days after transplanting). Five to ten leaves of each plant were inoculated and the disease reaction was scored after 14 days of inoculation following the IRRI standard evaluation system (IRRI-SES) scale.

RESULTS AND DISCUSSION

P deficiency is considered as one of the major yield limiting factor in rice production (Wissuwa et al., 1998). Rice consumes about 1.07 M tonnes of P2O5 and the application of phosphorous fertilizer has become an essential need for better rice productivity. Significant phenotypic variation has been observed for various traits related to productivity under low soil P condition. The long term phosphorous issues is addressed by balancing the short term needs is critical and can be achieved by breeding P efficient genotypes, identification of novel sources, improving the crop residue management, adoption of integrated nutrient management, and development of low soil P tolerant rice varieties (Chin et al., 2011) shows a path in genetically improving the elite mega varieties which are very sensitive to low soil P condition.

ISM is an EDV of Samba Mahsuri (BPT 5204) which is steadily and effectively replacing Samba Mahsuri (~ 1.3 lakh ha) in the endemic areas of south India (Amarender, 2018). ISM is NIL of Samba Mahsuri and a popular mega variety with bacterial blight resistant, high yielding, fine-grain type with excellent cooking quality and low glycemic index (50.9) rice cultivar developed through marker assisted backcross breeding (MABB) by pyramiding the three BLB genes Xa21, xa13 and xa5. Despite all these desirable features, ISM is highly sensitive to low soil P condition resulting in low yield (Swamy et al., 2019). A major QTL Pup1 (PSTol kinase) was mapped and characterized on chr.12 which confers tolerance to low soil P condition (Gamuyao et al., 2012).

Rasi, when screened in the low soil P plot of ICAR- IIRR showed excellent tolerance to low soil phosphorous. In order to improve the performance of ISM for low soil P tolerance an attempt was made for the development of mapping population using Rasi as the donor (male parent) and ISM as the recurrent (female parent).

Rasi and Improved Samba Mahsuri showed a significant difference in performance in low soil P plot of ICAR- IIRR, Hyderabad (Figure- 2) w.r.t various agro-morphological traits related to low soil P tolerance (Table-1). The RILs developed from the above cross were phenotyped to identify the high tolerant, tolerant and the sensitive lines in the low soil P plot. The RILs showed a normal distribution with skewness more towards the tolerant side (Figure- 2) which indicates the trait is governed by major QTL responsible for the tolerance. Most of the lines fall in the intermediate range, while few RILs were showing a tolerance level equivalent to the donor, Rasi with better plant vigor and few RILs were as sensitive as the recurrent parent- ISM. Out of the 214 RIL lines, 68 RILs were highly tolerant, 79 RILs were tolerant, 52 RILs showed intermediate level of tolerance and nine RILs showed sensitive reaction to low soil P condition (Figure-2).

Similarly, the RIL population along with the parents, Rasi and ISM were inoculated with the DX-020 culture in the normal soil P plot to screen for BLB resistance. Rasi showed a susceptible reaction with a score of 9 with an affected lesion area of 51-100% whereas ISM showed high resistance with a score of 1 with an affected lesion area of 1-5% due to the presence of the BLB genes- Xa21, xa13 and xa5. RILs when screened showed a normal distribution with skewness more towards the tolerant side (Figure- 3). The majority of the RILs exhibited high resistance to BLB with a score of 1 and 3 and a lesion length of 1-12% based on the SES scale of IRRI (Figure-3). About 131 RILs showed high resistant reaction while the rest showed moderate to susceptible reaction.

Delay in flowering was observed w.r.t all the RILs in low soil P conditions, which could be a plant adoptive mechanism for effective/ increased phosphorus acquisition and utilization. Plant height was dramatically affected in low soil P condition which could be another adaptive mechanism that helps the plant to acquire more P for the growth and maintenance, thus reducing the cell growth. The tolerant plants under P stress will have shorten the life cycle and enters early into the reproductive stage which facilitates the effective utilization of the P available for the production of more productive tillers (Chin et al., 2011). The tolerant entries have great root system architecture for the better P acquisition and utilization, thus resulting in the increased root length and root volume in the tolerant genotypes in comparison to the parents in study (Gamuyao et al., 2012). Grain yield per plant greatly depends on the grain filling stage and many stresses such as drought, N and P deficiency have a significant reduction in the yield.

In the present study based on the various agro-morphological traits and screening reactions, two RILs- RI-134 and RI-172 were selected which showed better performance w.r.t. the parental lines used in the hybridization (Figure- 4). The selected RIL i.e., RI-134 showed a DFF of 100 days with a plant height of 70.1 cm possessing 12 productive tillers per plant with a root length and root volume of 23.22 cm and 25.14 ml respectively. It has a per plant yield of 12.32 gm and 15.23 gm of thousand seed weight with medium slender grain type in low soil P condition. RI-172 was identical to the above line which showed a DFF of 101 days with a plant height of 72.36 cm possessing 13 productive tillers per plant with a root length and root volume of 16.23 cm and 30.22 ml respectively. It has a per plant yield of 14.91 gm and 14.22 gm of thousand seed weight with medium slender grain type in low soil P condition. Both the RILs showed better root system and plant type and thus can be used as a donor for low soil P tolerance in future breeding programs and can be considered for varietal release after testing them in multiple locations.

CONCLUSION

In conclusion, the two RILs (RI-134 and RI-172) have high desirable characters such as, high yielding, medium slender grain type with better root system architecture, plant height along with resistance to BLB and low soil P tolerance in comparison to both the parents in P deficient conditions. Thus, cultivation of such lines can enhance the productivity of farmers, which in turn reduces the cost of production with the reduced application of P fertilizers.

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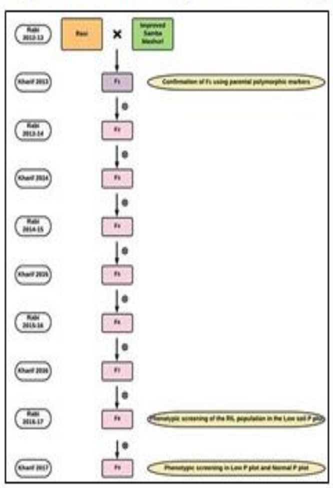
Table-1: Various agro- morphological traits in the study in low soil P plot and BLB screening in Normal P plot of ICAR-IIRR, Hyderabad

Agro- morphological traits- Low soil P condition								BLB screening in Normal soil P plot	
Trait	Days to 50% flowering	Plant Height (cm)	Number of productive tillers per plant (No.s)	Root length (cm)	Root volume (ml)	Grain yield per plant (g)	Thousand grain weight (g)	BLB score (SES Scale)	Description (affected lesion area)
Rasi	92	66.15	10	20.28	21.84	6.95	20.2	9	51-100%
ISM	127	41.29	4	21.56	10.57	2.59	10.47	1	1-5%
RI-134	100	70.1	12	23.22	25.14	12.32	15.23	1	1-5%
RI-172	101	72.36	13	21.83	30.22	14.91	14.22	1	1-5%
Min	84.17	38.93	2.59	16.23	2.68	0.85	10.47		
Max	132.67	80.35	12.92	36.81	50.79	9.69	28.03		
Mean	108.53	60.71	6.26	25.38	19.49	4	20.99		
S.E	0.83	0.62	0.14	0.26	0.67	0.14	0.28		
S.D	11.62	8.72	1.92	3.67	9.32	1.91	3.97		
CV%	2.2	5.39	17.09	9.79	7.27	7.77	1.89		

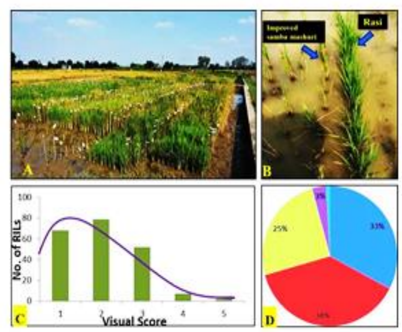
The RIL population showed various degrees of tolerance in the low soil phosphorous (P) plot (< 2 ppm) of ICAR-IIRR, Hyderabad, India. RILs (RI-134 and RI-172) showed high level of tolerance and BLB resistance in comparison to the donor parent- Rasi and recurrent parent-ISM. Skewness of the mapping population was more towards the tolerant end in most of the traits recorded.

Figure-1 : Crossing scheme for the development of RIL mapping population



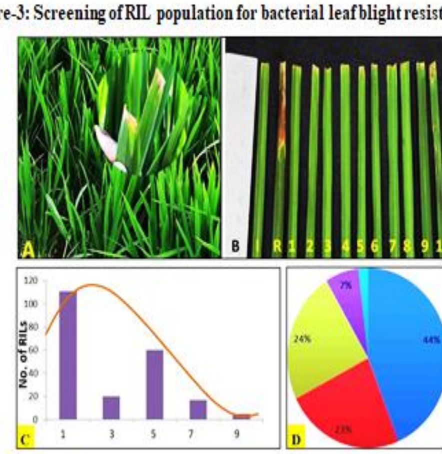
Schematic representation of the crossing program between the tolerant parent- Rasi and sensitive parent- Improved Samba Mahsuri (ISM) for the development of RIL population segregating for low soil phosphorous (P) tolerance and bacterial leaf blight.

Figure-2 : Screening of RIL population in the Low soil P plot of ICAR- IIRR, Hyderabad



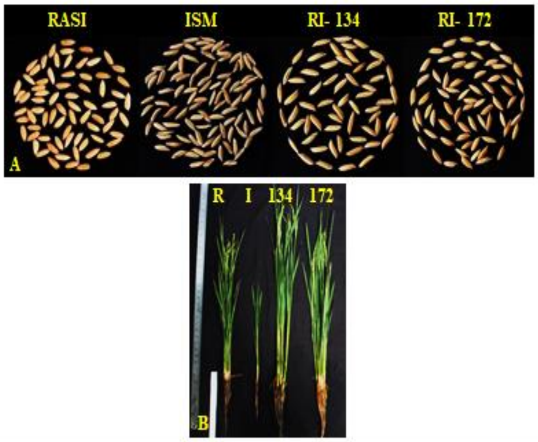
A. Screening of RIL population in the low soil phosphorus (P) plot of ICAR-IIRR, Hyderabad
B. Response of tolerant parent- Rasi and sensitive parent- Improved Samba Mahsuri in the Low soil P plot
C. Frequency distribution of RILs with the visual scoring in the low soil P plot
D. Plot representing percentage of RILs with various scores.

Figure-3: Screening of RIL population for bacterial leaf blight resistance



A. Inoculation of of RIL population for screening for BLB resistance using DX-O20 culture.
B. Disease reaction of parents- ISM and Rasi along with few RIL lines.
C. Frequency distribution of RILs with the visual scoring based on the SES scale for BLB
D. Plot representing percentage of RILs with various scores.

Figure 4-: Selected RIL lines in the low soil P plot of ICAR- IIRR



A- Grain type of RILs in comparison with the parents- Rasi and Improved Samba Mahsuri. RILs selected showed a medium slender grain type with low soil P tolerance and BLB resistance.
B- Comparision of RILs plant type with both the parents- Rasi and Improved Samba Mahsuri

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