The Pharma Innovation Journal 2022; SP-11(6): 1983-1991

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(6): 1983-1991

www.thepharmajournal.com
Received: 25-03-2022
Accepted: 30-04-2022

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Marker assisted backcrossing to develop the low phosphorus tolerant version of KMR-3R, a popular restorer line of hybrid rice

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Abstract

Low phosphorus stress has become more important abiotic factor affecting rice yield under present changing climate conditions. KMR-3R is one of the popular restorer line used in commercial rice hybrids production in India. Most of the rice hybrids released for the diverse ecosystem conditions using three-line system for normal phosphorus conditions. Consequently, these derived hybrids suffer drastic yield decline under low phosphorus conditions. To facilitate the efficient use of complex polygenic traits in hybrid rice molecular breeding research, we undertook development of introgression lines in background of KMR-3R with tolerance to low phosphorus stress by introgression of major QTL *Pup1* controlling grain yield under low phosphorus stress. The present study reports the development and evaluation of 250 introgression lines (BC_2F_2) lines in KMR-3R background for improvement of grain yield under low phosphorus stress condition. Among 250 ILs, 26 introgression lines were found positive both for *Pup1* and *Rf4*. Promising BILs exhibited earliness of 15 days under low phosphorus stress in comparison to the recurrent parent. Grain yield advantage of 7.72 g to 10.05 g was recorded among selected BILs compared to KMR-3R under low phosphorus stress condition. This suggested that the efficacy of introgressed low phosphorus stress tolerant QTL *Pup1* in enhancing grain yield under low phosphorus stress conditions. Further, breeding lines of KMR-3R possessing *Pup1* could also serve as good donors for development of tolerant rice hybrids under low phosphorus stress.

Keywords: Rice, KMR-3R, low phosphorus stress, yield, BILs and marker assisted backcross breeding

Introduction

Rice is one of the world's major staple food crops. The revolutionary changes occurred by the advent of green revolution in the past six decades and the rice production are equivalent to the growth of the population in India. Considering the annual average population growth rate of 1.5% and current per capita consumption of about 250g of rice per day, rice demand is expected to be atleast 140 M T by 2050. India needs to increase the production of rice by 2.5 M T per year to meet its population demand by 2050 (http:/www.fao.org/rice2004/en/pdf/khush.pdf). The Hybrid rice technology is indispensable to raise the productivity of rice in the country. A total of 127 rice hybrids were released so far for commercial cultivation in India (AICRIP progress report 2021). Hybrid rice is grown in 3 M ha with a production of 3.5 M t during *Kharif* 2017 (Hari Prasad *et al.*, 2018) [12]. Majority of the rice hybrids were developed for irrigated conditions. Currently, three line CMS system was widely used for exploitation of hybrid vigour in rice. Yields of the rice crop are being adversely affected by both biotic and abiotic stress factors. Phosphorus (P), one of the most important macronutrients necessary for healthy rice plant growth and development, is one of the most deficient elements in rice soils, and its low availability in the soil is one of the major yield-limiting factor in rice production (Wissuwa *et al.*, 1998) [21]. Global demand for P fertiliser is steadily rising, despite the fact that commercial phosphate supplies are expected to run out in a few decades. In India, soils are either low (49.3% of soils) or medium (48.8% of soils) in terms of available P (Tiwari *et al.*, 2001) [20] necessitating the country's importation of phosphorus-based fertilisers on a massive scale. As a result, it is critical to be prepared with alternate solutions to this problem, such as improved crop residue management, adoption of integrated nutrient management, and creation of low soil P tolerant rice cultivars to handle low soil P levels. Genetic enhancement of rice tolerance to P-limiting soils should be one of the focus areas of rice research and development in order to reduce the use of phosphatic fertilisers, which is required not only to increase farmers' income by lowering fertiliser costs, but also to maintain rice production.

There is a lot of variation in P usage efficiency/low soil P tolerance across rice genotypes (Akirinde *et al.*, 2006) [1]. The discovery of the *Pup1* QTL, which imparts tolerance to low soil phosphorus conditions and superior performance of *Pup1*-containing rice lines in diverse genetic backgrounds in both upland and irrigated conditions provides an opportunity to develop rice cultivars tolerant to low phosphorus conditions (Chin *et al.*, 2011) [5]. The *Pup1* QTL has been fine-mapped (Wissuwa *et al.*, 2002) [23] and markers that are closely related have been developed (Chin *et al.*, 2010) [6]. *Pup1* has been introduced into the rice types IR-64, IR-72, Dodokan, Batur, and Situ14 (Chin *et al.*, 2011) [5], Nerica1, Nerica4, Nerica 10, Dourado-Perecoce, WAB-515, WAB-96-1-1, WAB-189 (Darme *et al.*, 2013) [7], MTU1010, IR64, Improved Samba mahsuri (Sundaram *et al.*, 2018) [18] using these markers using marker-assisted backcross breeding. *Pup1* has also been cloned, and *OsPSTOL1* (Gamuyao *et al.*, 2012) [11] has been identified as the candidate gene underlying the QTL.

KMR-3R is the restorer line of widely used hybrid KRH-2 in commercial rice hybrids production. KMR-3R is commonly used in the production of superior hybrids in rice due to its desirable features like good combining ability, plant stature, high pollen load, synchronized tillering, dark green and thick leaves with long and heavy panicles. This study reports the introgression of *Pup1* QTLinto KMR-3R to develop low phosphorus tolerant BILs with high grain yield under low phosphorus stress condition using marker assisted backcross breeding. Kasalath is a low P tolerant indica genotype harboring major effect QTL *Pup1* controlling grain yield under low phosphorus stress.

Materials and Methods

This study was carried out from *Kharif* 2016 to *Rabi* 2018 at Indian Institute of Rice Research, Rajendranagar, Hyderabad with the intention of developing backcross inbred lines of KMR-3R with *Pup1* using Kasalath as low phosphorus tolerance QTL donor and KMR-3R as recurrent parent. Molecular and hybridization works were conducted in the MAS laboratory and greenhouse facility available at Department of Hybrid Rice, Indian Institute of Rice Research, Rajendranagar, Hyderabad respectively. Phenotypic evaluation of backcross inbred lines under normal phosphorus and low phosphorus stress conditions were carried out during *Rabi* 2018 at the farm of Indian Institute of Rice Research, Hyderabad.

QTL introgression

Introgressive backcross inbred lines of KMR-3R with low phosphorus tolerance QTL *Pup1* were developed by crossing KMR-3R (female parent) with Kasalath (male parent) deploying marker assisted backcross breeding. Parental polymorphism was done betweenKMR-3R and Kasalath using reported SSR makers linked to low phosphorus tolerance QTL *Pup1* (Chin *et al.*, 2011) [5] (Table 1). Among them, K-46-1 and K-46-2 were identified as foreground selection markers for QTL *Pup1*. Parental polymorphism between KMR-3R and Kasalath was carried out with 300 SSR markers covering the entire genome. Out of 300 markers, 85 markers were found to be polymorphic between KMR-3R and Kasalath. These 85 markers were used for background selection.

Table 1: Details of polymorphic primers used for foreground selection of *Pup1*

QTL	Chromosome	Position	Primer	Sequence
Pup1	12	523 bp	K-46-1	F: TGAGATAGCCGTCAAGATGCT R: AAGGACCACCATTCCATAGC3
Pup1	12	227 bp	K-46-2	F: AGGAAGATGGTTGTCGTTGG R: TTCACACCAAACAGTGTTGTC

Table 2: Details of the fertility restorer genes used for identification of KMR-3R

Fertility restorer gene	Chromosome	Position	Primer	Sequence
Rf4	10	185 bp	RM6100	TTCCCTGCAAGATTCTAGCTACACC TGTTCGTCGACCAAGAACTCAGG

Genotyping

Fresh leaves from 25 days old young seedlings were collected freeze-dried and the DNA was extracted using the CTAB method (Murray and Thompson, 1980) [15]. Amplification was carried out using Polymerase Chain Reaction (PCR).

The cocktail for PCR amplification was prepared in a total reaction volume of 10 µlconsisting of 2 µl DNA, 0.3 µl forward primer, 0.3 µl reverse primer, 6.0 µl distilled water, 0.3 µl dNTP's, 1 µl 10X Taq buffer and 0.1 µl Taq polymerase. The reaction mixture was given a momentary spin for thorough mixing of the cocktail components. Then 0.20 ml PCR tubes were loaded in a thermal cycler. PCR amplifications were performed in thermal cycler (Eppendorf Mastercycler®). The cycling conditions included initial denaturation at 94°C for 5 minutes, followed by 30 seconds at 94°C, 1 minute at 55°C and 1 minute at 72°C for 35 cycles, followed by final extension at 72°C for 10 minutes. The PCR products were kept in storage at 4°C until further use. The PCR amplified products (10 µl) along with 100 bp ladder were subjected to electrophoresis in a 3.0 percent agarose gel in 1X TBE buffer at 120 Volts for 2 hours. The ethidium bromide stained gels were documented in UV gel documentation system (Syngene) and later banding pattern was scored. Stepwise selection involving phenotyping and genotyping was used to select and advance the desirable plants in every generation.

Graphical representation of the genome

Graphical representation of molecular marker data was performed using the software Graphical Genotype (GGT2.0).

Field experiment for evaluation of backcross inbred lines of KMR-3R introgressed with phosphorus uptake QTL *Pup1*

The experiment was carried out during *Rabi* 2018 at farm of Indian Institute of Rice Research, Hyderabad. The experiment was laid out in randomized block design with two replications for both low phosphorus stress (soil with $P < 2$ ppm) and normal phosphorus experiment (soil with P > 18.3 ppm).

The seeds of BC_2F_2 of KMR-3R x Kasalath were sown in nursery bed along with parents and seedlings were raised. 17 days old seedlings were transplanted in field with a row length of 2.5 m. Inter and intra row spacing of 30 cm and 10 cm respectively. Standard agronomic practices were followed to raise a healthy crop. Apart from these, need based plant protection practices were taken during the crop season for the control of pests and diseases. The crop was harvested at physiological maturity. Low 'P' level in the field was maintained without external application of P fertilizers. The levels of phosphorus in the field were estimated from the collected soil sample in each season through Olsen P method before taking up of planting of experimental breeding material in the field plot.

Observations recorded

Plant height, days to 50% flowering, number of panicles per plant, panicle length, 1000 grain weight, spikelet fertility and grain yield per plant were recorded for five competitive BIL progenies.

Statistical Analysis

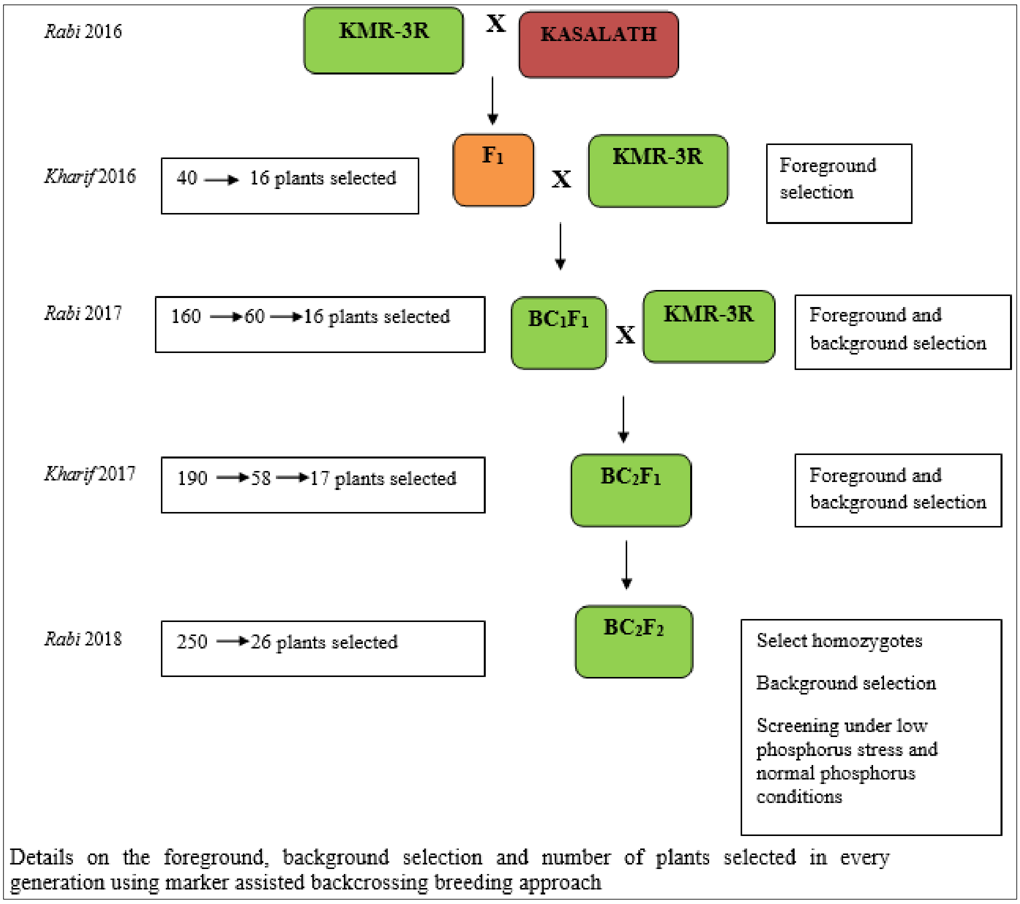
WINDOWSTAT software version 9.1 was used to analyze the data of the present experiment for the computation of means and standard error of difference (SED).

Results

Marker assisted backcross breeding and BILs development

Figure 1 represents the marker assisted backcross breeding scheme used for the development of *Pup1* positive BILs of KMR-3R. A total of 40 F_1 plants were obtained by crossing KMR-3R with Kasalath. Among these, 16 true F_1 plants were identified and forwarded to next backcross generation. True F_1s plants were backcrossed with the recurrent parent KMR-3R and 160 BC_1F_1 seeds were produced. Individual plant hills of BC_1F_1 seeds were sown in field and evaluated during *rabi* 2017. The foreground selection was carried out for 160 BC_1F_1 plants and 60 true BC_1F_1were obtained as positive for *Pup1*. Among 60 plants, 16 BILs were chosen based on vigorous phenotype and genotypic selection. The introgressed BILs exhibited a maximum recurrent parent genome recovery ranged between76% to 85%. The selected 16 plants were advanced to further backcross generation *i.e.*, BC_2F_1. 16 BILs from BC_1F_1 were individually backcrossed with KMR-3R to produce 190 BC_2F_1 seeds. All 190 BC_2F_1 plants were evaluated in the field by growing in individual plant hills during *kharif* 2017.

Foreground selection was carried out in 190 BC_2F_1 plants with K-46-1 and K-46-2 markers linked to low phosphorus tolerance QTL *Pup1*. On scoring, 58 plants were positive for *Pup1* (heterozygous condition) and were subjected to background selection by using 85 SSR markers. A total of 17 genotypes with target QTL were selected. Recurrent parent genome recovery ranged from 90% to 92% among the best BILs. Seventeen positive plants of BC_2F_1 generation were selfed to produce BC_2F_2 generation. Seeds of BC_2F_2 were raised as single plant hills during *rabi* 2018 under field conditions. A total of 250 BC_2F_2 plants were screened for target QTL linked SSR markers *i.e.*, using K-46-1 and K-46-2. On scoring, 26 BC_2F_2 plants were found positive for target QTL *Pup1*.



Details on the foreground, background selection and number of plants selected in every generation using marker assisted backcrossing breeding approach

Fig 1: A detailed scheme of marker assisted backcross breeding adopted for introgression of low phosphorus stress tolerant QTL *Pup1* from Kasalath into KMR-3R

Validation of marker assisted breeding for low phosphorus tolerance by phenotyping

The overall mean performance of the KMR-3R introgressed BILs and their parents (Kasalath, KMR-3R) is shown in Table 3. Plant height ranged from 90.58 cm to 115.50 cm under normal P and 80.58 cm to 105.50 cm under low P stress conditions. The mean days to 50% flowering in the KMR-3R BILs varied from 85 to 94 days under normal P and 88 to 96 days under low P stress conditions. Late flowering of BILs by 3 days was observed in low P stress conditions. Number of panicles per plant of BILs ranged from 9 to 13 and 7 to 10 for under normal P and low P stress conditions. The 22.2% to 23.07% reduction in panicles per plant in BILs was recorded under low P stress condition. Panicle length ranged from 20.11 cm to 24.59 cm under normal P and 17.49 cm to 25.38 cm under low P stress condition. Low P stress reduced panicle length by 2.62 cm. Mean 1000 grain weight in the KMR-3R BILs varied from 20.14 g to 25.60 g under normal P and 19.18 g to 24.47 g under low P stress conditions. A reduction of 0.96 g in 1000 grain weight was recorded under low P stress conditions. Spikelet fertility ranged from 82.82% to 95.23% under normal P control and 68.94% to 80.54% under low P stress conditions. Low P stress reduced spikelet fertility by 13.88%. The mean grain yield of BILs ranged from 20.10 g to 24.81 g under normal P and 14.05 g to 22.46 g under low P stress conditions. The 9.47% to 30.09% reduction in yield in BILs was recorded under low P stress conditions. The analysis of variance revealed significant difference among 26 BILs (BC_2F_2 progenies) for all the traits except for number of panicles per plant in low P stress conditions showing the presence of sufficient genetic variability in 26 BILs (Table 4).

Table 3: Trait mean values of parents and backcross inbred lines along with range and coefficient of variation for yield and its contributing traits under normal P (C) and low P stress (S) conditions

S. No.	Character	Environment	Parents		Mean	Range	C.V
1.	Plant height (cm)	C	115.12	94.24	102.16	90.58-115.50	7.54
		S	105.12	83.19	92.66	80.58-105.54	8.40
2.	Days to 50% flowering	C	103.00	85.00	90.33	85-94	4.37
		S	110.00	90.00	87.31	88-96	3.97
3.	No. of panicles per plant	C	12.00	14.00	10.46	9.00-13.00	10.89
		S	7.00	8.00	8.38	7.00-10.00	7.60
4.	Panicle length (cm)	C	25.10	23.62	22.43	20.11-24.59	6.28
		S	19.92	21.24	19.79	17.49-25.38	9.55
5.	1000 grain weight (g)	C	24.12	30.56	22.84	20.14-25.60	7.49
		S	20.21	21.90	20.54	19.18-24.47	5.45
6.	Spikelet fertility (%)	C	94.93	79.41	89.78	82.82-95.23	3.84
		S	56.06	65.77	74.74	68.94-80.54	4.81
7.	Grain yield per plant (g)	C	24.14	19.12	22.47	20.10-24.81	6.63
		S	12.41	18.04	18.04	14.05-22.46	12.03

C: Normal Phosphorus conditions; S: Low Phosphorus conditions

Table 4: ANOVA of BILs for yield and its component traits under normal P and low P conditions

S. No.	Traits	Treatment mean sum of squares (df: 25)	
		Normal P	Low P
1	Plant height (cm)	170.93**	229.48**
2	Days to 50% flowering	5.27**	6.16**
3	No. of panicles per plant	2.68**	0.77
4	Panicle length (cm)	3.98**	4.32**
5	1000 grain weight (g)	6.63**	3.38*
6	Spikelet fertility (%)	1.96*	8.59**
7	Grain yield per plant (g)	4.60*	4.37*

* Significant at 5% level;** Significant at 1% level

Performance of the promising BILs of KMR-3R with target QTL *Pup1* for grain yield and its component traits

In the present study, seven BILs of KMR-3R *viz.*, GSP-2-6, GSP-4-5, GSP-12-8, GSP-16-7, GSP-20-2, GSP-23-1 and GSP-26-4 were selected based on their higher grain yield under low P stress (Table 3). All the BILs out-yielded KMR-3R under low P stress condition. Among the BILs, GSP-16 had recorded the highest grain yield followed by GSP-26-4, GSP-4-5, GSP-20-2, GSP-23-1, GSP-12-8 and GSP-2-6 under low P stress condition. Grain yield advantage of 7.72g to 10.05g was recorded among the selected BILs compared to KMR-3R under low P stress conditions and is accordance with findings of Chin *et al.* (2011) [5], Darme *et al.* (2013) [7], Sundaram *et al.* (2018) [18], Anila *et al.* (2018) [2] and Swamy *et al.* (2020) [19]. The promising lines with *Pup1* exhibited earliness of 15 days under low P conditions in comparison to the recurrent parent respectively.

In the present study, three BILs of KMR-3R *viz.*, GSP-2, GSP-23 and GSP-26 were selected based on their higher grain yield under normal phosphorus condition (Table 5). All the BILs out-yielded KMR-3R and Kasalath under normal phosphorus condition. Among the BILs, GSP-26 had recorded the highest grain yield followed by GSP-2 and GSP-23 under normal phosphorus condition. Grain yield advantage of 5.39g to 5.75g was recorded among the selected BILs compared to Kasalath under normal phosphorus condition and is accordance with results of Chin *et al.* (2011) [5], Darme *et al.* (2013) [7], Sundaram *et al.* (2018) [18], Anila *et al.* (2018) [2] and Swamy *et al.* (2020) [19]. The promising lines with *Pup1* exhibited earliness of 11 days under normal phosphorus conditions in comparison to the recurrent parent. The BILs of KMR-3R with *Pup1* has to be further validated under preliminary and multi-location trials for confirming their stability of performance under target environments and finally could be released as improved version of KMR-3R tolerance to low P stress condition. Further, breeding lines of KMR-3R possessing *Pup1* could also serve as good donors for development of rice hybrids with tolerance to low P stress and finally could be released as improved version of KMR-3R tolerance to low phosphorus stress condition.

Background screening was done with 85 polymorphic SSR markers for the promising BILs of KMR-3R using GGT2.0 software (fig 4). The background genome recovery of the selected BILs was ranged from 88.37% to 95.34 (Table 6). Maximum recovery of 95.29% was observed in the line GSP-26 and could be further utilized in breeding programme as donor since it had desirable agronomic characters in addition to introgressed QTL *Pup1* in it.These lines could be further utilized in breeding programme as donor since it had desirable agronomic characters in addition to introgressed *Pup1* in it.

Table 5: Agronomic performance of promising BILs of KMR-3R with target QTL *Pup1* for grain yield and its component traits

Agronomic trait	Environment	GSP-2-6	GSP-4-5	GSP-12-8	GSP-16-7	GSP-20-2	GSP-23-1	GSP-26-4
Plant height	C	111.50	107.30	109.35	113.00	113.50	113.80	110.50
	S	102.50	106.30	104.35	101.21	109.50	105.80	102.50
Days to 50% flowering	C	94.00	93.00	92.00	91.00	92.00	92.00	94.00
	S	96.00	95.00	94.00	93.00	94.00	94.00	96.00
Number of panicles per plant	C	11.00	13.00	12.00	9.00	10.00	13.00	10.00
	S	8.00	9.00	9.00	8.00	8.00	10.00	9.00
Panicle length	C	24.59	21.42	23.82	23.71	23.22	22.49	23.09
	S	21.57	18.50	20.40	19.76	21.30	18.73	20.57
1000 grain weight	C	25.35	24.94	24.06	24.97	24.27	23.80	20.85
	S	19.35	19.44	19.18	24.47	21.77	19.80	20.35
Spikelet fertility	C	92.39	94.40	91.84	91.32	90.80	91.79	90.74
	S	69.94	73.64	73.70	72.97	72.74	73.16	72.29
Grain yield per plant	C	24.80	23.83	24.19	24.20	23.59	24.51	24.87
	S	20.13	21.09	20.45	22.46	20.85	20.77	21.13

C: Normal Phosphorus conditions;S: Low Phosphorus conditions

Table 6: Grain yield advantage and recurrent parent genome recovery of the promising BILs of KMR-3R x Kasalath under low P stress condition

S. No.	Genotypes	*Pup1*	Grain yield per plant(g)	Grain yield advantage over KMR-3R	Recurrent parent genome recovery
1	GSP-16-7	BB	22.46	10.05	93.02%
2	GSP-26-4	BB	21.13	8.72	95.34%
3	GSP-4-5	BB	21.09	8.68	93.02%
4	GSP-20-2	BB	20.85	8.44	90.69%
5	GSP-23-1	BB	20.77	8.36	89.53%
6	GSP-12-8	BB	20.45	8.04	94.18%
7	GSP-2-6	BB	20.13	7.72	88.37%
8	KMR-3R	AA	12.41	-	-

Discussion

Phosphorus is one of the major plant nutrients and plays a vital role in productivity of rice. It is one of the least abundant of all essential nutrients in soil. Even when present in soil, a high content of free ferric oxides and high aluminium restrict the availability of phosphorus to plants and has become a wide spread problem. The present situation in India in both high and low input farming systems is unsustainable because P is a finite resource. Several recent studies have concluded that sustainable stocks of mineral P are expected to decline in the near future. Approximately 80% of India's arable land had low to medium phosphorus deficiency (Elser, 2012) [9].

It is difficult to recognize the deficiency of mild to moderate P in the field. As a result, phosphorus deficiency in rice is known as hidden hunger. Severe phosphorus deficiency leads to formation of large proportion of empty grains. Application of phosphorus fertilizer can solve this problem. But it is difficult for resource poor farmers in India to apply phosphorus fertilizer every year. Therefore selection of rice cultivars capable of extracting phosphorus from phosphorus limiting soils with higher phosphorus use efficiency is considered as a significant cost-effective solution.

Fortunately, significant genetic variation has been reported in rice for tolerance to soil phosphorus deficiency (Fageria and Baligar, 1997) [9]. A large effect QTL known as *Pup1* controlling grain yield under phosphorus deficiency has been mapped on chromosome12 of Kasalath rice cultivar (Wissuwa *et al.*, 1998) [21]. The successful introgression of *Pup1* QTL linked to grain yield under phosphorus deficiency into distinct rice cultivars was carried out earlier by Chin *et al.* (2011) [5], Darme *et al.* (2013) [7], Sundaram *et al.* (2018) [18], Anila *et al.* (2018) [2] and Swamy *et al.* (2020) [19]. It indicates the scope for genetically improving the elite rice varieties, which are sensitive to low soil P.

Selection of parents

The most important step in hybridization programme is selection of parents. The present study was undertaken with the intention of developing backcross inbred lines of KMR-3R with *Pup1* by introgressing low P tolerant QTL *Pup1* from Kasalath into KMR-3R.Kasalath is a low P tolerant indica genotype harboring major effect QTL *Pup1* controlling grain yield under phosphorus stress. KMR-3R is the restorer line of popular hybrid KRH2 which is used in hybrid rice production. This line is commonly used in the production of superior hybrids in rice due to its desirable features like good combining ability, plant stature, high pollen load, synchronized tillering, dark green and thick leaves with long and heavy panicles. Hence it obviates genetic enhancement of parental lines for moisture stress in order to develop superior rice hybrids.

Marker assisted backcross breeding for low phosphorus tolerance in rice

Marker-assisted breeding, particularly using the backcross method, has been extensively used for introgressing genes governing resistance/tolerance biotic as well as abiotic stresses in rice (Muthu *et al.* 2020) [16]. In rice, phosphorus is very important in the early vegetative growth and reproductive stages as it affects tillering, root development, early flowering, and ripening. Often P deficiency in rice is referred to as a "hidden hunger" because the symptoms are not apparent unless P-deficient plants are directly compared with plants that have sufficient P. When compared with healthy rice of the same age, P-deficient rice is characterized by an abnormal bluish green color of the foliage with poor tillering and plants that are slow to canopy and slow to mature (Dobermann and Fairhust, 2000) [8]. Rice, like all plants, needs phosphorus to survive and thrive. It is a key element in plant metabolism, root growth, maturity and yield.

Major QTL for P uptake in P deficiency was identified on the long arm of chromosome 12 with 13.2 cM marker interval in rice using back cross inbred lines derived from Japonica x Indica cross. The QTL fine mapped from this region with 3 cM distance from marker C443 was named as phosphorus uptake1 (*Pup1*) (Wissuwa *et al*., 2002; Wissuwa and Ae, 2001) [23, 22]. Four more minor QTLs were detected for P uptake on chromosomes 2, 6, 9, and 10 (Wissuwa *et al*., 1998; Ni *et al*., 1998) [17]. During the recent years, progress has been made in introgression of identified grain yield QTLs under low phosphorus stress in different genetic backgrounds of rice using marker assisted backcrossing with grain yield advantage under low phosphorus stress conditions Chin *et al.* (2011) [5], Darme *et al.* (2013) [7], Sundaram *et al.* (2018) [18], Anila *et al.* (2018) [2] and Swamy *et al.* (2020) [19]. However, the introgression of yield QTLs into elite Indian cultivars not yet accomplished. Hence, there is a dire need to further introgress the identified grain yield QTLs into the genetic background of elite cultivars of rice to further improve its genetic level. Hence the present investigation was undertaken to develop low phosphorus tolerant version of KMR-3R using Kasalath as *Pup1* QTL donor through marker assisted backcrossing.

Development of low phosphorus tolerant KMR-3R BILs using stepwise marker assisted backcross breeding approach

In the present study two peak markers *i.e.*, K-46-1 and K-46-2 tightly linked to low phosphorus tolerant QTL *Pup1* was taken for foreground selection (fig 3). 85 background selection markers were used for fast recovery of the recurrent parent. A total of 40, 160, 190 and 250 plants were genotyped with K-46-1 and K-46-2 markers linked to low phosphorus tolerant QTL *Pup1* in F_1 (*kharif*, 2016), BC_1F_1 (*rabi*, 2017), BC_2F_1 (*kharif*, 2017) and BC_2F_2 (*rabi*, 2018) generations respectively resulted in the development of 26 *Pup1* positive lines BILs (BC_2F_2) of KMR-3R.

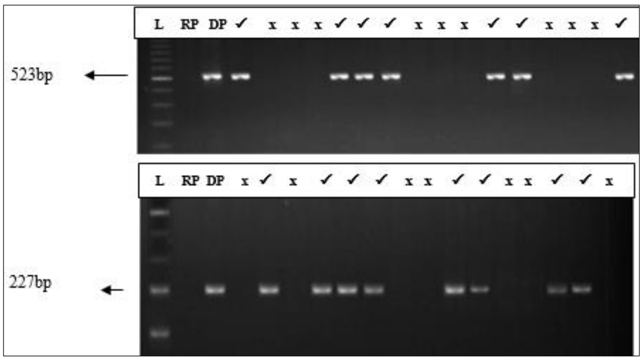


Fig 2: Foreground selection for *Pup1* among BILs (BC_2F_2)of KMR-3R using K-46-1 and K-46-2 SSR marker

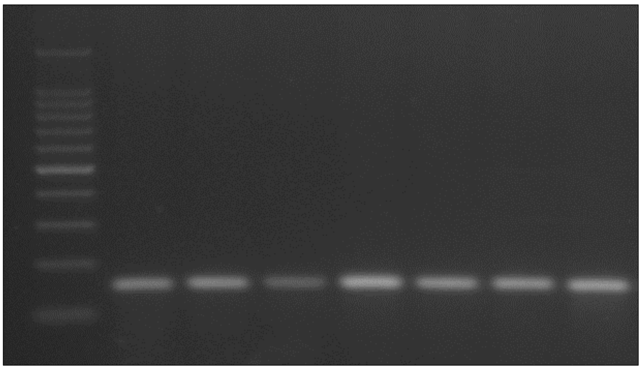


Fig 3: Screening for *Rf4* gene using RM 6100 SSR marker among the promising BILs ofKMR-3R

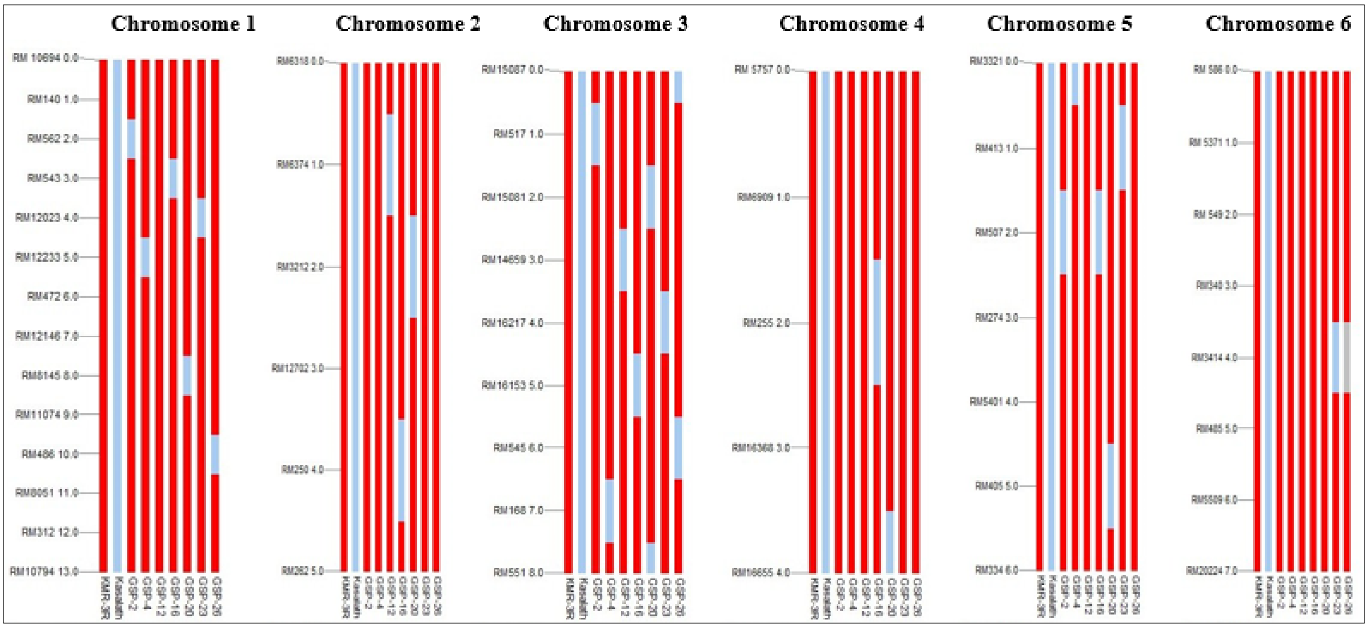
Agronomic performance of KMR-3R BILs under low phosphorus stress and normal phosphorus conditions

A significant disparity found in expression of traits by BILs of *Pup1* under low phosphorus stress and normal phosphorus environments. The BIL progenies as well as parents *viz.*, KMR-3R and Kasalath grown under low phosphorus stress condition, in general, produced significantly lower grain yield than normal phosphorus conditions this might be due to effect of phosphorus on tillers production. Yughandar *et al.* (2017) [24] also observed yield reduction of 38.24% under low phosphorus conditions in 67 mutant lines of Nagina 22. Delayed flowering was hastened in all the BILs grown under low phosphorus in comparison to normal phosphorus conditions and corroborates with the findings of Atakora *et al.* (2015) [3], Anila *et al.* (2018) [2], Swamy *et al.* (2020) [19]. Significant decrease in plant height was observed among the BILs grown under low P stress condition. Similar findings were reported by Wissuwa and Ae, (2001) [22], Li *et al.* (2009) [14], Heuer *et al.* (2009) [13] Swamy *et al.* (2020) [19] in plant height under phosphorus stress. The number of tillers and panicles per plant were significantly reduced under phosphorus deficiency conditions. Rice plants respond differently to phosphorus deficiency by reducing number of tillers and leaves as adaptive features to cope with induced phosphorus stress. The phosphorus deficiency had lowered 1000 grain weight and spikelet fertility than control as evident from the present study. This may be due to lack of sufficient phosphorus for grain development and corrobates with the findings of Swamy *et al.* (2020) [19].

Performance of identified improved low phosphorus tolerant KMR-3R BILs

Delayed flowering was observed in all the BILs grown under low P condition in comparison to normal condition. The phenological delays have been reported for crop plants when exposed to low P condition (Chauhan *et al.* 1992) [4]. It is predicted to be an adaptive mechanism of plants which leads to increased phosphorus acquisition and utilization helping crop plants to attain maximum reproductive biomass. Under both low phosphorus stress and normal phosphorus conditions, all of the *Pup1* introgressed KMR-3R BILs flowered earlier as compared to KMR-3R (Table 3). This could be because of better uptake of P by *Pup1* introgressed KMR-3R BILs in the presence of *Pup1* increased phosphorus acquisition, and utilization of P, which could help to overcome the phenological delay noticed under low P condition (Chin *et al.* 2011) [5]. The height of the KMR-3R BILs was almost similar to KMR-3R under both low phosphorus stress and normal phosphorus conditions. Availability of P significantly affects plant height and the presence of *Pup1* in the introgression lines helped them to acquire more P and maintain tissue growth in comparison to KMR-3R. Number of panicles per plant and panicle length of *Pup1* introgressed KMR-3R BILs was significantly higher as compared to KMR-3R under the low soil P condition. Once the plants sense the limited availability of P, they try to shorten their life cycle through early entry into the reproductive phase to acquire the required threshold P to complete the life cycle. Some plants also change the metabolic machinery to utilize the available P efficiently to complete their life cycle, with few healthy seeds produced for perpetuation, thus resulting in lower panicles per plant (Chin *et al.* 2011) [5]. In the present study, the presence of *Pup1* in the introgression lines might have helped them in acquiring more and more P from the soil due to better foraging ability resulting in more panicles and panicle length and better yield under low P condition, as compared to KMR-3R. The 1000 grain weight of most of the *Pup1* introgressed BILs of KMR-3R was observed to be similar to KMR-3R in normal phosphorus condition and higher than KMR-3R under low phosphorus stress condition. The spikelet fertility of most of the *Pup1* introgressed BILs of KMR-3R was observed to be similar to KMR-3R in normal phosphorus condition and higher than KMR-3R under low phosphorus stress condition. The grain yield of *Pup1* introgressed BILs of KMR-3R was significantly higher as compared to KMR-3R under low phosphorus stress conditions. This improvement of yield under low phosphorus stress condition was mainly due to the presence of *Pup1* in the introgressed lines as reported earlier (Chin *et al.* (2011) [5], Darme *et al.* (2013) [7], Sundaram *et al.* (2018) [18], Anila *et al.* (2018) [2] and Swamy *et al.* (2020) [19]. Under normal conditions, these BILs of KMR-3R performed equivalent to or better than the recipient parent KMR-3R.

The BILs of KMR-3R with *Pup1* has to be further validated under preliminary and multi-location trials for confirming their stability of performance under target environments and finally could be released as improved version of KMR-3R tolerance to low phosphorus stress condition. Further, breeding lines of KMR-3R possessing *Pup1* could also serve as good donors for development of rice hybrids with tolerance to low phosphorus stress. Background screening was done with 85 polymorphic SSR markers for the promising BILs of KMR-3R using GGT 2.0 software (fig 4). The background genome recovery of the selected BILs ranged from 88.37% to 95.34%. Maximum recovery of 95.34% was observed in the line GSP-26-4 and could be further utilized in breeding programme as donor since it had desirable agronomic characters in addition to introgressed*Pup1* in it. Further, the obtained best lines of KMR-3R were screened for the presence of fertility restorer gene (fig 3) *i.e., Rf4* (Table 2). The best lines of KMR-3R showed positive for *Rf4* gene indicating that these best lines were good restorers as that of KMR-3R.



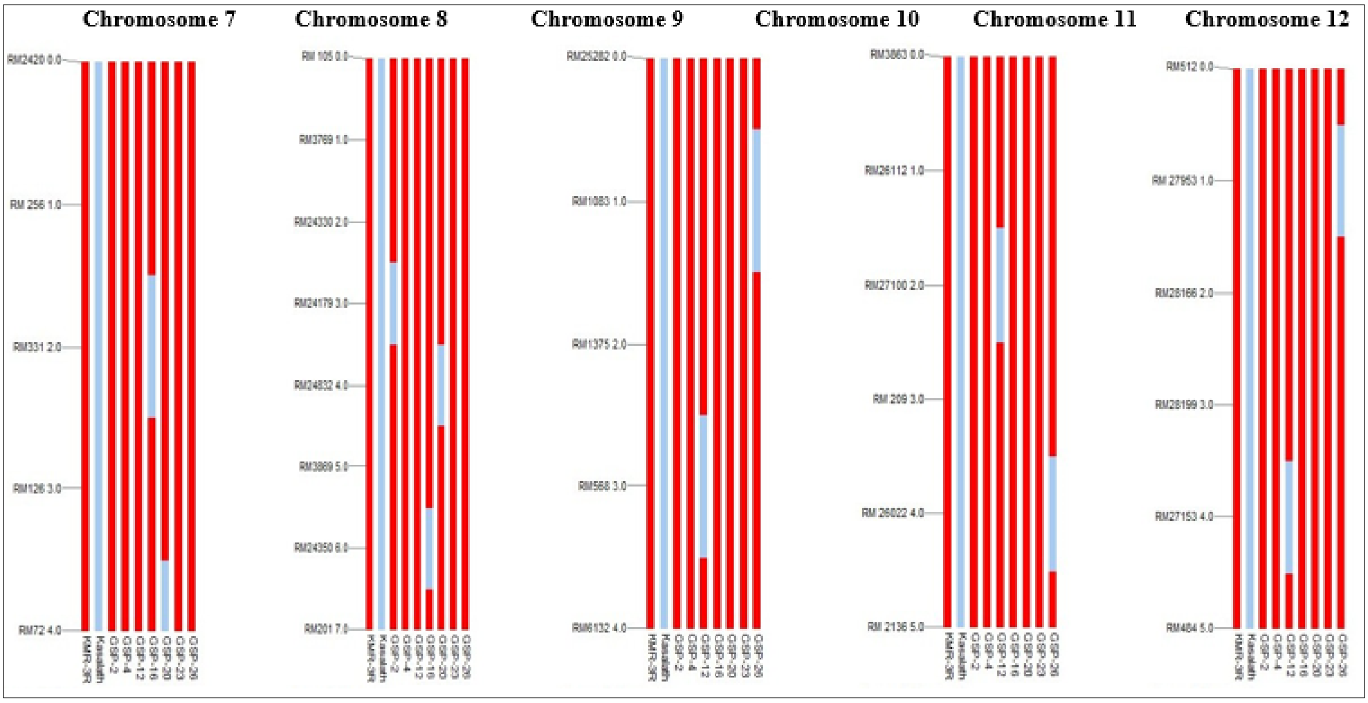


Fig 4: Graphical representation of background genome recovery in seven selected BC$_2$F$_2$ lines of KMR-3R x Kasalath

Conclusion

Low phosphorus stress is one of the major constraints in rainfed areas for rice productivity and has the greatest impact during tillering stage where the formation of grain is suppressed. This results in significant yield losses. The identification and introgression of QTL regions with a large and consistent effect on grain yield under low phosphorus stress represents an opportunity to improve high yielding low phosphorus susceptible varieties through marker assisted backcross breeding The Hybrid rice technology is indispensable to rise the productivity of rice in the country. Majority of the rice hybrids were developed for irrigated conditions. These were severely affected by both biotic and abiotic stresses. Currently, CMS system utilizing A line, B line and R line was widely used for exploitation of hybrid vigour in rice. Therefore, developing hybrids tolerant to low phosphorus stresses will help in avoiding the quantum yield losses. This goal can be accomplished by genetic enhancement of parental lines of hybrid rice to these low phosphorus stresses. In our study we have developed *Pup1* positive BILs of KMR-3R. The selected BILs conferred a grain yield advantage of 7.72 g to 10.05 g compared to KMR-3R under low phosphorus stress condition. This suggested that the effectiveness of introgressed low phosphorus tolerant QTL *Pup1* in increasing grain yield under low phosphorus stress conditions. Further, backcross inbred lines of KMR-3R possessing *Pup1* could also serve as good donors for development of rice hybrids with tolerance to phosphorus stress. This suggested that phosphorus deficit tolerance rice hybrids can be successfully developed through marker assisted backcross breeding by introgression of QTL regions with a large and consistent effect on grain yield under phosphorus stress into elite parental lines of hybrid rice.

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