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Appraisal of Gobindabhog type PHO_2 allele for phosphorous deficiency tolerance in rice under West Bengal condition

[1]Poulomi Sen, [2]Amrita Sankar Chakraborty, [3]Sutanu Sarkar, [4]Ananti Pathak, [5]Arijit Mukherjee, [6]Somnath Bhattacharyya and [7]Avishek Chatterjee

[1]Genetics and Plant Breeding, School of Agricultural Sciences, Sister Nivedita University, West Bengal, India

[2]Seed testing Officer, Zonal Adaptive Research Station, Krishnanagar, West Bengal, India

[3]Genetics and Plant Breeding, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India

[4]Assistant Botanist, Field Crop Research Station, Burdwan, West Bengal, India

[5]Senior Breeder, Field Crop and Oilseed, Ramnagar Seed Farm Pvt. Ltd., Burdwan, West Bengal, India

[6]Genetics and Plant Breeding, Crop Research Unit, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India

[7]School of Agriculture, Swami Vivekananda University, West Bengal, India

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Corresponding Author: Avishek Chatterjee

Abstract

An aromatic Bengal landrace, Gobindabhog is a registered phosphorous (P) deficiency tolerant genotype which is able to accumulate P nearly 27 mg/plant even if available P in soil is low. On the contrary a popular cultivar of Bengal, Shatabdi accumulates only 6 mg/plant when grown in a P deficient soil. Phosphate Over accumulator 2 (PHO_2) gene of rice is a key determinant for maintaining the P homeostasis in the plant body in P fluctuating condition. Relative abundance of PHO_2 transcript in roots of 15 days old seeding of Gobindabhog was significantly higher than that of Shatabdi. In order to assess the efficiency of PHO_2 allele of Gobindabhog, Recombinant inbred lines (RILs) were developed by crossing between Gobindabhog, a P deficiency tolerant genotype with a P deficiency susceptible variety, Shatabdi. RM19080, a SSR marker linked to PHO_2, has been used for genotyping of the RIL population. The two sets of RILs carrying Gobindabhog type and Shatabdi type PHO_2 allele grown in P-deficient soil were used to quantify P-accumulation in the shoot at the pre-flowering stage and yield attributing parameters during maturity. Single Point ANOVA suggests that PHO_2 allele of Gobindabhog and its linked marker SSR RM19080 have no association with P-deficiency tolerance in rice. Therefore, it is necessary to determine the role of the PHO_2 allele from the donor parent before considering it for MAS.

Keywords: PHO_2, RILs, MAS, SSR, phosphorous

Introduction

In addition to being a crucial part of fundamental macromolecules like phospholipids and nucleic acids, phosphorous (P) is also crucial for energy transfer, the regulation of enzyme activities, and the control of metabolic pathways (Bieleski and Ferguson 1983; Theodorou and Plaxton 1993) [5, 17]. Phosphorus is primarily absorbed by plant roots directly in the form of inorganic phosphate (Pi), which is also a significant form of P transport inside plant system (Marschner 1995; Schachtman *et al*. 1998) [11, 15]. Pi uptake by roots and subsequent distribution among different tissues are strongly believed to be supervised by a complex regulatory mechanism involving different genes like Pi Transporters; OsPT1, OsPT2, OsPT4, OsPT6, and OsPT8 of rice high-affinity phosphate transporters (PHTs), microRNA (miRNAs) etc. (Chiou *et al*. 2006; Poirier and Bucher 2002; Ticconi and Abel 2004) [6, 13, 18]. Phosphate over accumulator 2 (PHO_2) gene encodes a potential ubiquitinconjugating (UBC) E2 enzyme.

A single nucleotide alteration results in the mutation of PHO_2 gene leads to an early termination in UBC24 (Aung *et al*. 2006; Bari *et al*. 2006) [1, 2]. When the supply of Pi is abundant, the PHO_2 mutant displays high shoot P levels and signs of Pi toxicity (Delhaize and Randall 1995; Dong *et al*. 1998) [8, 9]. During P deficiency, miR399 enhances Pi acquisition, translocation and xylem Pi loading because miR399 is specifically induced by P deficiency and inhibits the degradation of the Pi transporter protein PHO1 and the PHT1 family by mediating the cleavage of PHO_2. This helps plants accumulate enough Pi (Wang *et al*. 2012; de Souza *et al*. 2019; Xu *et al*. 2013) [19, 7, 20]. Thus, the transgenic plants overexpressing miR399 photocopied PHO_2 mutants. A decrease in miR399 under P repletion conditions causes an increase in PHO_2 transcripts and further Pi absorption from the soil to the root (Hu *et al*. 2011) [10].

In comparison to many micronutrients, P content is frequently lower in soil. Due to its propensity to form insoluble compounds with aluminum, iron, calcium, and magnesium, phosphorus is only partially available in soils with either low or high pH levels (Bar-Yosef 1991) [3]. Development of rice varieties that can effectively absorb more phosphorus, especially from phosphorus-deficient soils, and make better use of phosphate fertilizer is therefore necessary. This method is regarded as a beneficial and economical management solution.

Many P-deficiency tolerant rice genotypes, such as the aromatic Bengal landrace Gobindabhog, have already been identified based on their capacity to accumulate high P from the soil even though the plant available P is lower. Gobindabhog's P deficiency tolerance capacity is almost identical to Kasalath, a well-known tolerant accession of Assam, India. However, Shatabdi lacks the capacity to tolerate a P shortage, much as the majority of high producing cultivars (Sarkar *et al*. 2011) [14]. This suggests Gobindabhog as a source of favorable alleles of different P deficiency tolerant genes. Therefore, any effort to assess the efficiency of the Gobindabhog type alleles of these genes and identification of their functional markers will be highly profitable for P deficiency tolerance improvement in rice under West Bengal conditions (Mukherjee *et al*. 2014; Sen *et al*. 2023) [12, 16]. In this study, efficiency of PHO_2 allele of Gobindabhog has been assessed by using functional molecular marker in P deficit conditions.

Materials and Methods

Plant Materials

Recombinant Inbred Lines (RILs) population was generated by crossing between Gobindabhog (which accumulates almost 27 mg of phosphorus per plant when planted in a field poor in phosphorus) and Shatabdi (which accumulates roughly 6 mg of phosphorus per plant when grown in phosphorus-deficient soil). From F_2 generation the cross population was maintained by following single seed descent method up to F_{10} generation. In Sekhampur's red-lateritic P-deficient soil, where P availability was low (3.36 mg/kg), two sets of 160 RILs carrying both Gobindabhog (G) and Shatabdi (S) type PHO_2 allele in homozygous condition along with two parental lines were grown in 4 m rows with three lines of each RIL. Heterozygote RILs for PHO_2 locus were excluded from the experiment.

P estimation

A tri-acid digestion method has been used for estimating P content (mg) in the shoot samples of the RILs and two parental lines (two replications of each line) during pre-flowering stage. An Agilent 8453 spectrophotometer has been employed for final analysis (Sarkar *et al*. 2011) [14].

Yield assessment

Filled grain number/plant, tiller number/plant and total grain weight/plant (g) from 10 plants of each RIL have been analyzed during maturity.

Identification of PHO_2 linked SSR marker and genotyping of RILs

As described by Sarkar *et al*. 2011 [14], DNA extraction from 40 mg fresh leaf tissue of RILs and parental lines has been done. A standard PCR with a reaction volume of 25μL comprising of 16.5 μL of deionized sterile water, 20ng of diluted DNA sample, 1.5 mM $MgCl_2$, 1μL of 2.5 mM dNTPs, 2.5 μL of 10× buffer, 0.5 U of Taq DNA polymerase enzyme and 100ng of each forward and reverse primers (Invitrogen, California, USA) was carried out. GeneAmp PCR System 9700, California, USA has been used as a thermal cycler.

A PCR amplification process included a five-minute initial template DNA denaturation at 94 degrees Celsius followed by 35 cycles of denaturation at 94 degrees Celsius for 45 seconds, annealing at 55 degrees Celsius for 45 seconds, and polymerization at 72 degrees Celsius for one minute with a final extension of 7minutes at 72 degrees Celsius. Ethidium bromide staining was used to capture images after the PCR products were sorted by size using a 3% agarose gel.

For the examination of variations in each category of each parameter, such as P accumulation (mg/plant), filled grain number/plant, tiller number/plant and total grain weight/plant (g) data, a single factor analysis (ANOVA) has been conducted.

Relative expression analysis of PHO_2

Seedlings of Gobindabhog and Shatabdi were raised in a glass plate containing Yoshida solution (Yoshida *et al*. 1976) [21]. In order to make a P-sufficient solution, the Yoshida solution was supplemented with 10 mg/L of inorganic P, and a P-depleted solution was created using 0.05 mg/L of inorganic P. After 15 days, total RNA was extracted from 50 ng of the seedling roots of three independent plants of each genotype using the RNeasy plant mini kit from Qiagen Inc. in the United States. The RNA was then treated with RNase-free DNase, and first strand cDNA was produced using the High Capacity cDNA Reverse Transcription Kit from Invitrogen, United States with an oligo d(T)18 primer. As previously mentioned, quantitative real-time PCR analysis was performed using a StepOne Plus Real-Time PCR System (Applied Biosystems, USA) and a SYBR green PCR master mix (Invitrogen, USA) (Bhattacharyya *et al*. 2003) [4]. A melting curve study was done to verify the specificity of amplification. The housekeeping gene β -tubulin was used to normalize the target gene expression, allowing for sample-to-sample fluctuations and ensuring experimental recurrence. $^{\Delta\Delta}CT$ method was employed for fold changes determination.

Results

Parent selection for RIL development

In our earlier research, it was demonstrated that Gobindabhog, a well-known short-grain aromatic landrace from a significant rice-producing state in India, West Bengal, displayed the highest P content just before the onset of the flowering initiation stage out of 108 rice genotypes in a P-deficient soil at the Sekhampur regional research substation. It builds up nearly the same amount of P as soil in the Gangetic alluvial zone where P availability is greater than 30 mg/kg (Instructional farm, Jaguli). As a result, Gobindabhog could be a prospective donor parent for the ability to tolerate P-deficiency (Fig. 1)

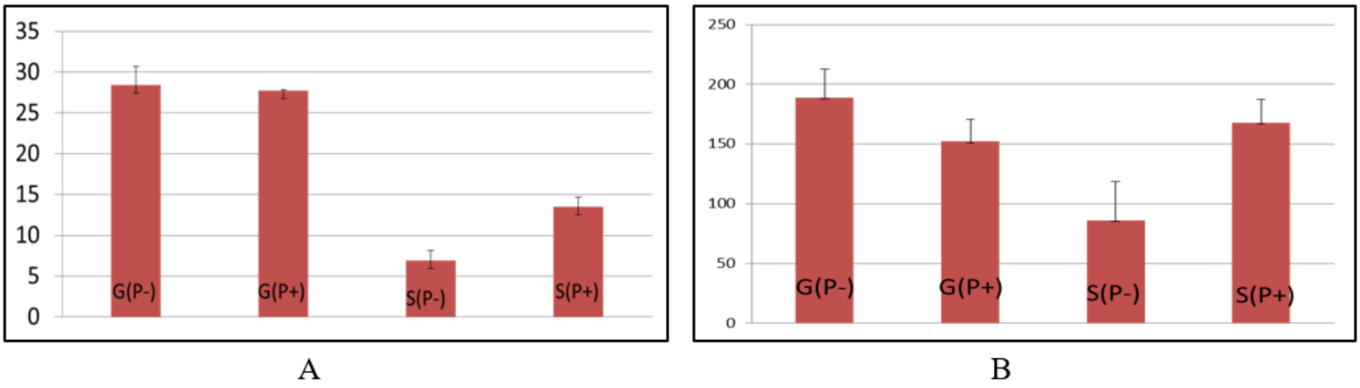

Fig 1: (A) P accumulation in the shoot during pre-flowering stage (mg/plant) (B) Filled grain number/ panicle during maturity. Biological replicated samples are indicated with ±SD. G(P-), Gobindabhog in Sekhampur; G(P+), Gobindabhog in Jaguli; S(P-), Shatabdi in Sekhampur; S(P+), Shatabdi in Jaguli.

Relative quantification of PHO_2 gene

Relative expressions of candidate gene PHO_2 in Gobindabhog roots were taken into consideration for comparison with the vulnerable yet well-known Bengali cultivar Shatabdi in order to identify the process causing increased P-accumulation in Gobindabhog. Three plants of each genotype were grown in both P inadequate (0.05 mg/l) and P sufficient (10 mg/l) nutritional media for isolation of RNA during seedling stage for relative measurement of transcripts. In comparison to a sufficient solution, Gobindabhog demonstrated 2.5 folds up regulation in a deficient environment. On the other hand Shatabdi showed 3.5 fold down regulation of PHO_2 expression in P-deficient solution than P-adequate solution (Fig. 2).

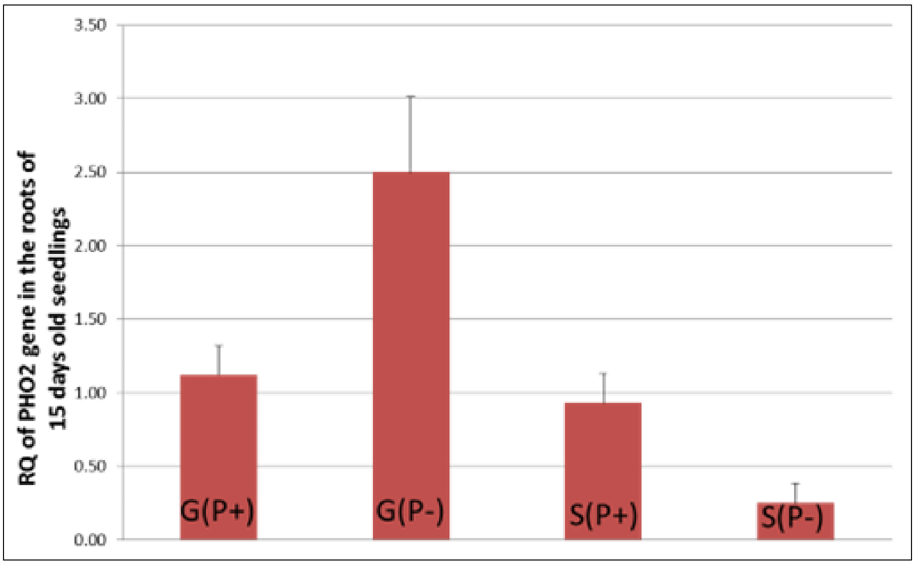

Fig 2: Quantified information on the relative abundance of PHO_2 transcript in the roots of 15-day-old seedlings of Shatabdi and Gobindabhog cultivated in nutritional media with sufficient P+ (10 mg/l) and insufficient P- (0.05 mg/l) levels. Biological duplicated samples from three different sources are denoted by ±SD. G(P+), Gobindabhog in P sufficient solution; G(P-), Gobindabhog in P deficient solution; S(P+), Shatabdi in P sufficient solution; S(P-), Shatabdi in P deficient solution.

Polymorphic SSR marker linked to PHO_2 identification

The position of PHO_2 (AK241747) was confirmed on chromosome number 5 in RAB-DB [http://rapdb.dna.affrc.go.jp/] data base. Total nine pairs of SSR markers nearer to the gene PHO_2 (within 100kb zone) in each side were used for identifying the polymorphic SSR marker between Gobindabhog and Shatabdi (Table 1).

Table 1: Marker name and primer sequences used for identification of polymorphic marker between Gobindabhog and Shatabdi

SSR Marker name	Primer type	Primer Sequence used in this study(5'→3')
RM19086	F	GCCCTCAGATGTTTGTTACACC
	R	CACAAGTTCTTCTGAGGATTGG
RM19085	F	CTCCTGCATCCATCCTCTCTCC
	R	AGTGGTGTCACAGCAGCTAAAGG
RM19080	F	AAAGCCGTCTCTGAGAAGAAGAGAGG
	R	GGGAATCGTTTCGAGAGCAGAGG
RM19077	F	CTCGAGTTCACTCACCAGTCTGG

	R	CCCGGCCCAGTATATCTATCAGC
RM19075	F	CCTTCTCGTCATTCTTGCTCTC
	R	ACGAGTCTCAGTAGCACATCAGC
RM19091	F	GCGAACGAGATGTTGCTGTAGCC
	R	TTGGTGGTGGGCAGTTACAACG
RM19090	F	CCTGGACTCACAAGTTATTGAGAACG
	R	GGAGGAGGTGTGTGGTTGTTGG
RM19089	F	AGGAACTGCAGGTGTATTCTTGG
	R	CCGTTCTCAATAACTTGTGAGTCC
RM19087	F	CTCTTGACGGAGAGCTTCATGG
	R	CTCGACCCAAGACGAGACACC
q β-tubulin (For RT-PCR)	F	GCTGACCACACCTAGCTTTGG
	R	AGGGAACCTTAGGCAGCATGT
q PHO_2 (For RT-PCR)	F	CCAAACTTTCAGGACCCAAA
	R	GAGTGTCGGAGCTGGACTTC

PCR products of these nine SSR markers were analyzed in 3% agarose gel electrophoresis and only RM19080 showed polymorphism between Gobindabhog and Shatabdi, amplifying 122 bp band size in Gobindabhog and 133 bp band size in Shatbdi (Fig. 3). The 'G' and 'S' alleles, which correspond to the Gobindabhog and Shatabdi, respectively, were scored using RM19080 amplified fragments from 160 RILs (Fig. 4).

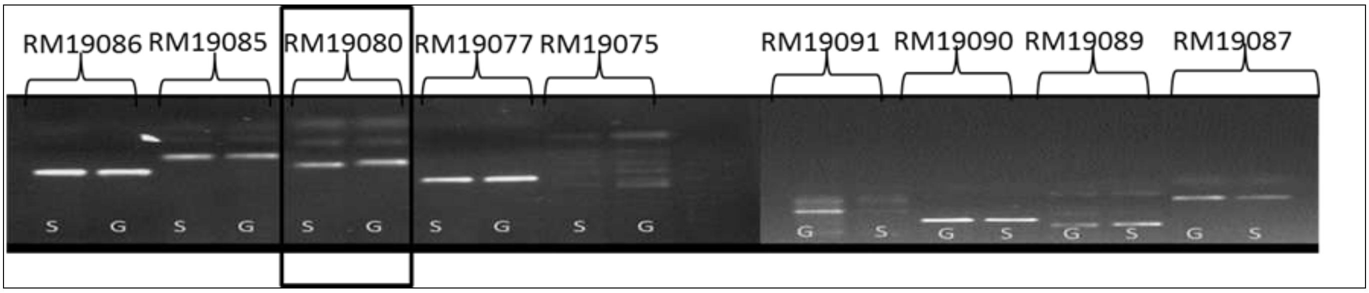

Fig 3: PCR amplification of Shatabdi and Gobindabhog by nine pairs of SSR. S. Shatabdi, G. Gobindabhog; Name of primers are shown on the top and polymorphic SSR RM19080 is shown in black box.

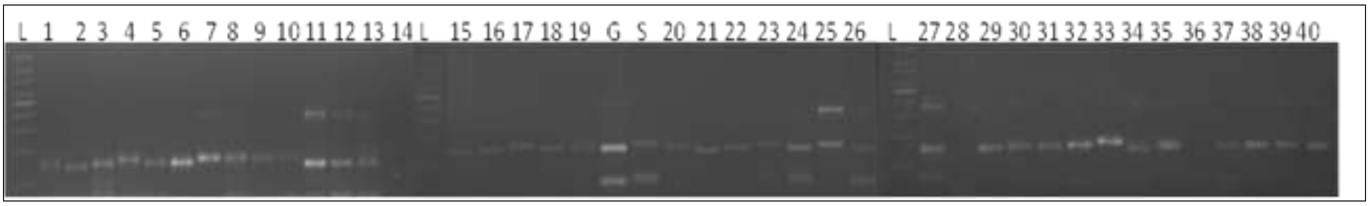

Fig 4: Genotyping of 40 RILs including two parental lines using RM19080. Serial number denotes individual RIL and L denotes 100 kb ladder.

Functional validation of Gobindabhog type PHO_2 allele

A recombinant inbred line population (RILs) consisting of 160 RILs and two parents were grown in P deficient soils of Sekhampur for the assessment of P accumulation ability and yield attributing parameters like filled grain number/plant, tiller number/plant and total grain weight/plant (g). This was done in order to understand the effect PHO_2 allele of Gobindabhog towards P-accumulation vis-à-vis grain yield in P-deficient condition. During cultivation, no external P fertilizer was applied to the plants. A Single Factor analysis (ANOVA) was performed using marker RM19080 as the classifying variable to find probable markers that influence the P accumulation (mg/plant), filled grain number/plant, tiller number/plant and total grain weight/plant (g) data. Results have been furnished in Table 2.

Table 2: Mean performance of 84 G type & 76 S type PHO_2 allele carrying RILs using Single Factor analysis (ANOVA)

Characters	RILs carrying G allele	RILs carrying S allele	Mean difference	p value*
P-accumulation (mg/Plant)	13.72±3.91	14.64±4.4	9.91	0.16
Total grain Weight/Plant (g)	23.52±8.1	25.26±9.45	1.73	0.21
Filled grain no/Panicle	109.58±25.52	113.61±29.55	4.03	0.35
Tiller no/plant	9.29±2.13	9.52±2.17	0.23	0.49

Significance level at 5% is denoted by *

Out of 160 RILs (F_{10} generation), 84 RILs were carrying Gobindabhog type PHO_2 allele and rest 76 RILs were carrying Shatabdi type PHO_2 allele (Supplementary Table S1). RILs carrying the G and S alleles had an average P-accumulation of 13.72±3.91 and 14.64±4.4 mg/plant, respectively. According to Single Factor analysis ANOVA analysis these two means do not differ significantly at 5% level of significance. Average filled grain number/plant, total grain weight/ plant and tiller number/plant of G allele carrying RILs were 109.58±25.52, 23.52±8.1(g) and 9.29±2.13 respectively whereas, S allele carrying RILs showed 113.61±29.55, 25.26±9.45(g) and 9.52±2.17 respectively. Subsequent Single Factor Analysis suggests no significant differences between them. The presence of 84 and 76 RILs with alternate alleles (G or S) that followed a 1:1 ratio ($p < 0.05$) demonstrated the RIL population's impartiality (data not provided).

Discussion

Gobindabhog, a fragrant Bengal landrace, emerged as the optimal parent for donation, demonstrating superior phosphorus (P) accumulation and yield under both P-deficient and sufficient conditions. Tulaipanji, Radhunipagal, and Raghusail are widely embraced landraces in the red and lateritic zones, prevalent among farmers prior to the widespread adoption of semi-dwarf cultivars. This preference may be attributed to their effectiveness in P-deficient environments. High-yielding varieties (HYV) such as IR36 and IR64 exhibited moderate P levels even in soils with sufficient phosphorus, possibly indicative of elevated internal P use efficiency or efficient remobilization capabilities. Tolerance to phosphorus deficiency is a multifaceted trait regulated by P-starvation-induced transcription factors, which subsequently lead to the activation of high-affinity transporter genes and/or alterations in root architecture. A balance of P uptake and its transport in plant body is maintained by PHO_2 and miR399 equilibrium.

In the analysis of single-point ANOVA among the RILs harboring both G and S type PHO_2 alleles, there was no significant difference in mean phosphorus (P) accumulation between the two groups. Consequently, within the examined RILs, the amplification of PHO_2 was not found to be linked with tolerance to phosphorus deficiency. Thus, PHO_2 allele of Gobindabhog does not impart P deficiency tolerance in rice efficiently. Allele of Gobindabhog and Shatabdi should be considered as equally effective if they have any contribution towards increasing P-accumulation. Actually in P-deficient situation, plant upregulates number of miRNA including miR399. miR399 helps in transfer of P from root to shoot by degrading PHO_2 during P-depleted field. As expression of PHO_2 is upregulated in Gobindabhog in P-deficient solution, probably expression of miR399 was not sufficient to degrade the transcript in this genotype. Thus, allele of Gobindabhog-PHO_2 was not suitable for targeting Marker Assisted Selection (MAS). Therefore, when employing marker-assisted selection and focusing on the PHO_2 allele, it is crucial to ensure its confirmed contribution to the P-deficiency tolerance ability in the recipient parents.

Conclusion

As many P deficient tolerance genes of rice become active during P starvation, to investigate the impact of the PHO_2 of the tolerant clandrace Gobindabhog, the performance of RILs developed by a cross between Gobindabhog and Shatabdi was analyzed. Single point ANOVA concludes that the presence of Gobindabhog type PHO_2 allele does not confer to P deficiency tolerance and yield attributing parameters under West Bengal condition. Thus, before considering PHO_2 gene in MAS its role in P-accumulation ability must have to be ascertained in the recipient parents. Taking miR399 under consideration is also required.

Future scope

Based on its effectiveness at acquiring P in P-deficient soil, Gobindabhog has been proven to be the tolerant genotype of West Bengal. Inclusion of remaining P-deficiency tolerance genes other than PHO_2 must have to be considered for identification of superior alleles from Gobindabhog. Instead of SSR, gene based markers can be utilized for precision breeding. Multiple high-yielding rice backgrounds can be used to assess the superior alleles of P-deficiency tolerance genes found in Gobindabhog.

Conflict of Interest: No competing interests have been stated by the authors.

Author contributions

Somnath Bhattacharyya conceived and designed the analysis; Poulomi Sen and Amrita Sankar Chakraborty collected the data, analysed and wrote the paper; Ananti Pathak and Arijit Mukherjee helped in the analysis; Sutanu Sarkar and Avishek Chatterjee helped in preparing the manuscript.

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Supplementary materials

Supplementary Table S1 RIL Yield attributing parameters and PHO_2 scoring data with SSR RM19080 have been furnished.

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Supplementary Table: S1

RIL	PHO_2 RM19080	Grain Weight/Plant (g)		Filled grain/Panicle		Tiller no/Plant		P (mg/Plant)			
		Avg	SD	Avg	SD	Avg	SD	R1	R2	Avg	SD
1.	G	26.8	2.89	121.9	18	7.8	2.14	15.06	13.42	14.24	0.82
2.	G	41.2	2.89	111.8	43.9	7.2	1.47	17.82	15.62	16.72	1.1
3.	S	10	5	62.8	15.7	7.6	1.36	14.04	9.35	11.695	2.345
4.	S	18.3	7.64	106	44.5	9.4	2.73	8.08	5.25	6.665	1.415
5.	S	17.9	4.6	132	23.1	6	1.1	17.3	18.8	18.05	0.75
6.	S	12.7	3.2	108	21	7	0.89	6.9	7.8	7.35	0.45
7.	G	6.7	2.89	42.7	16.5	9.6	1.36	5.51	10.1	7.805	2.295
8.	G	15.3	5.2	126.3	16.4	6.4	1.36	17.9	21.2	19.55	1.65
9.	S	14.3	1.58	35	22.3	9.6	1.62	15.95	16.11	16.03	0.08
10.	G	22.1	5.2	137	34.2	10.3	1.6	17.3	15.9	16.6	0.7
11.	S	6.7	2.89	61	5.9	7.2	4.53	7.96	3.25	5.605	2.355
12.	S	7.3	1.15	57.7	20.7	9.4	3.2	8.21	10.71	9.46	1.25
13.	G	10	2.1	74.3	23.5	7.6	1.5	11.09	9.62	10.355	0.735
14.	S	8.3	2.4	84.7	11.3	7.1	1.72	8.5	7.3	7.9	0.6
15.	S	12.1	3.2	109.2	12.1	9.4	2.87	6.7	5.9	6.3	0.4
16.	G	22.7	6.2	141	25.3	8.8	1.6	21.8	17.9	19.85	1.95
17.	S	10.1	2.8	87.9	12.2	8.4	1.85	11.2	8.7	9.95	1.25
18.	G	8.6	3.2	92.2	13.2	9.6	3.88	8.2	7.3	7.75	0.45
19.	G	15.2	11.55	109.8	18.8	8.4	2.58	20.88	18.01	19.445	1.435
20.	G	28.1	5.6	128	14.2	13.2	2.32	18.3	16.9	17.6	0.7
21.	S	22.7	11.2	144	21.4	10.8	2.64	19.2	21.2	20.2	1
22.	G	30.3	10.4	137	32.1	11.3	1.36	17.9	22.8	20.35	2.45
23.	S	26.2	8.3	141	25.1	8.4	2.15	16.9	20.3	18.6	1.7
24.	G	13.8	6.9	121	23.4	12.6	3.14	11	8.2	9.6	1.4
25.	S	11.8	4.9	112	14.4	10.6	1.85	7.8	9.2	8.5	0.7
26.	S	22.2	8.3	131	27.1	8.8	2.4	17.9	15.3	16.6	1.3
27.	S	26.2	11.2	133	25.1	8.3	2.42	14.9	20.3	17.6	2.7
28.	G	28.9	12.1	144	14.3	11.6	2.06	16.5	18.2	17.35	0.85
29.	G	28.3	12.58	112.1	31.2	8.2	2.32	8.75	11.52	10.135	1.385
30.	S	39.8	7.64	158.8	109.1	12.6	3.01	14.3	13.7	14	0.3
31.	S	26.7	12.58	108.9	31.9	10	1.6	10.97	8.85	9.91	1.06

32.	G	12.1	4.2	111.3	21.3	7.8	1.72	12.1	7.5	9.8	2.3
33.	S	38.3	13.9	117.2	15.9	11.2	1.94	17.6	16.2	16.9	0.7
34.	S	43.3	11.9	152.2	25.3	10	2.53	18.2	19.2	18.7	0.5
35.	S	36.9	9.2	122	17.3	7.8	1.47	15.3	14.8	15.05	0.25
36.	S	29.8	7.64	118.3	11.5	9.4	2.06	12	12.86	12.43	0.43
37.	G	28.4	17.64	108.5	14.5	5	3.83	13.7	9.53	11.615	2.085
38.	S	25.2	8.1	112.5	20.1	10.4	1.62	15.7	20.1	17.9	2.2
39.	G	30.2	7.9	122	16.9	7	5.78	17.9	14.9	16.4	1.5
40.	S	27.2	11.2	133.2	21.4	6.4	1.5	18.2	16.5	17.35	0.85
41.	G	35.2	11.9	141	22.3	15	3.46	15.9	19.2	17.55	1.65
42.	S	37.2	9.1	137.3	28.3	5.4	1.36	21.3	18.5	19.9	1.4
43.	G	19.5	5.6	121.4	22.2	12.8	5.15	11.2	9.6	10.4	0.8
44.	S	31.2	9.1	120.3	20.8	6.8	1.47	15.8	17.5	16.65	0.85
45.	G	40.2	12.4	131.4	32.2	5.8	1.72	17.4	14.3	15.85	1.55
46.	G	30.2	14.1	127.4	22.4	7	2.1	18.1	15.9	17	1.1
47.	S	31.6	8.39	119.8	13.2	9.6	3.2	17.5	19.2	18.35	0.85
48.	G	22.7	9.1	107.3	14.6	10	1.72	12.5	9.5	11	1.5
49.	G	16.3	7.6	94.6	13.2	11.1	3.83	8.8	10.3	9.55	0.75
50.	G	33.2	13.1	131.6	27.8	9.4	1.74	16.1	16.9	16.5	0.4
51.	G	19.2	11.5	102.6	15.3	9.6	1.85	13.1	11.9	12.5	0.6
52.	S	37.2	15.6	130.7	20.4	7.4	1.2	19.1	16.2	17.65	1.45
53.	G	41.2	9.6	128.7	14.4	7.4	2.06	16.1	15.2	15.65	0.45
54.	S	35.2	13.5	119.2	19.5	9.6	2.94	17.2	19.1	18.15	0.95
55.	G	29.2	8.5	141.2	32.5	8.6	2.24	11.2	13.1	12.15	0.95
56.	S	14.7	8.58	80.2	8.7	12	2.9	11.25	14.06	12.655	1.405
57.	G	18.3	6.2	121.6	19.2	8.8	2.99	16.9	20.4	18.65	1.75
58.	G	32.9	4.9	138.4	20.3	10.4	1.62	16.4	19.3	17.85	1.45
59.	G	27.3	8.2	119	12.4	9.6	3.01	15.1	16.2	15.65	0.55
60.	S	31.2	13.9	142.7	23.9	9.8	2.48	19.2	14.8	17	2.2
61.	G	38.6	16.2	151.6	22.2	10	3.35	16.9	18.4	17.65	0.75
62.	G	42.1	14.2	132.5	25.3	9.2	3.31	18.2	21.2	19.7	1.5
63.	G	22.6	6.2	119.3	17.3	7.6	2.87	15.2	18.1	16.65	1.45
64.	G	12.7	4.3	76.5	9.8	6.2	2.04	9.3	8.2	8.75	0.55
65.	G	23.3	8.5	118.1	14.5	10.6	2.8	16.3	15.2	15.75	0.55
66.	G	16.8	6.1	89.7	11.3	8.6	1.94	12.1	11	11.55	0.55
67.	S	25.3	8.3	111.3	15.5	7	0.63	18.3	19.1	18.7	0.4
68.	S	28.3	11.7	121.5	31.3	5	0.89	16.3	17.8	17.05	0.75
70.	G	19.5	3.9	103.2	17.5	7.8	1.47	15.8	18.2	17	1.2
71.	S	26.5	9.2	122.3	14.5	8.8	1.6	19.3	16.3	17.8	1.5
72.	S	19.5	7.2	101.1	12.3	10	3.03	12.5	10.3	11.4	1.1
73.	G	31.5	9.2	132	19.3	6.6	1.62	21.2	17.5	19.35	1.85
74.	S	17.5	4.8	82.1	10.2	9	2.9	11.5	12.3	11.9	0.4
75.	G	28.2	10.1	125.1	18.2	7.4	1.85	18.3	15.7	17	1.3
76.	G	17.5	6.3	95.7	13.2	8.6	2.33	14.8	13.7	14.25	0.55
77.	S	31.2	9.1	131.5	23.3	7.8	2.23	17.5	16.2	16.85	0.65
78.	G	10	5	99.8	15.2	10.3	2.1	8.29	10.08	9.185	0.895
79.	S	5	1.22	48.2	12.5	10.4	4.08	6.22	8.4	7.31	1.09
80.	S	15	2.37	69.1	4.5	9.2	3.12	4.93	10.31	7.62	2.69
81.	G	25	0	96	18.3	10.8	2.14	8.9	14.69	11.795	2.895
82.	S	15	3.21	96.8	23.3	7.1	3.31	5.83	2.58	4.205	1.625
83.	G	21.7	5.77	92.2	17.7	9	1.79	10.02	12.88	11.45	1.43
84.	S	32.1	11.2	133.5	20.2	8.8	2.48	13.3	15.2	14.25	0.95
85.	S	28.4	5.3	124.5	13.6	10.2	2.56	19.2	15.6	17.4	1.8
86.	G	21.3	7.1	120.4	21.5	5.8	2.14	12.3	11.1	11.7	0.6
87.	S	18.3	4.2	92.4	10.4	10.8	2.56	8.2	12.1	10.15	1.95
88.	G	15	5	64.9	8.9	6.4	1.85	10.92	12.38	11.65	0.73
89.	S	29.6	9.1	123.8	18.3	9.2	1.72	14.7	13.9	14.3	0.4
90.	S	32.1	12.3	142	31.3	11	4.24	15.2	16.1	15.65	0.45
91.	G	27.2	11.4	135.2	23.4	8.1	1.85	16.1	14.6	15.35	0.75
92.	G	12.1	3.2	106.6	13.2	10	2.19	10.3	7.5	8.9	1.4
93.	G	34.2	10.2	98.3	12.4	9.3	2.79	9.2	7.8	8.5	0.7
94.	G	16.9	6.6	119.5	18.3	10	1.41	14.5	11.2	12.85	1.65
95.	S	28.2	8.3	134.2	19.1	7	3.03	15.4	16.8	16.1	0.7
96.	G	21.9	10.6	122.5	20.1	6.6	1.5	13.5	12.2	12.85	0.65
97.	S	32.1	6.9	99.8	13.2	14.2	4.66	16.4	18.1	17.25	0.85
98.	G	18.9	7.6	104.2	13.5	7.6	2.42	12.6	9.3	10.95	1.65

99.	S	28.4	11.1	142.2	22.1	11.2	1.17	18.2	15.8	17	1.2
100.	S	11.9	3.7	103.7	14.5	11.2	4.07	8.2	9.1	8.65	0.45
101.	G	20.9	12.1	116.7	18.2	9.3	3.38	11.2	9.1	10.15	1.05
102.	S	22.5	8.3	127.2	9.8	12	1.26	15.3	16.1	15.7	0.4
103.	G	25.2	7.3	115.2	7.6	8.8	4.17	18.2	15.6	16.9	1.3
104.	S	23.2	8.1	109.2	13.5	8.6	3.5	16.3	17.1	16.7	0.4
105.	S	19.2	26.46	114.9	53.4	8.2	2.04	9.87	8.2	9.035	0.835
106.	G	28.2	10.5	132.5	17.3	12.8	6.05	18.9	19.3	19.1	0.2
107.	S	31.7	7.2	128.5	32.5	10.8	1.94	16.3	17.1	16.7	0.4
108.	G	26.5	8.3	119	18.5	11.8	3.31	17.2	14.3	15.75	1.45
109.	G	19.3	7.2	121	17.5	6.2	0.75	11.3	9.2	10.25	1.05
110.	S	28.5	10.3	131	19.3	13.6	2.94	16.5	14.3	15.4	1.1
111.	S	21.2	7.3	82	11.3	13	2.83	8.7	6.9	7.8	0.9
112.	G	20	0	75.4	5.2	11.6	1.85	5.65	8.17	6.91	1.26
113.	G	23.2	7.9	82	9.3	10.6	2.33	14.3	16.2	15.25	0.95
114.	G	18.3	7.5	83	14.2	8.8	7.47	11.3	9.8	10.55	0.75
115.	S	28.2	11.1	92	13.8	12.6	2.58	16.5	17.8	17.15	0.65
116.	S	20.3	6.9	87	11.5	11.6	0.8	9.3	8.7	9	0.3
117.	G	31.2	10.3	133	21.2	11.3	2.04	17.6	25.3	21.45	3.85
118.	G	11.7	5.77	71.6	16.3	11.2	2.48	10.49	8.76	9.625	0.865
119.	G	21.2	9.7	83.1	15.2	8.6	1.85	9.4	7.3	8.35	1.05
120.	G	19.2	6.5	81.1	10.2	10.4	0.75	11.3	8.1	9.7	1.6
121.	S	28.2	3.2	121	21.2	12	0.63	19.2	15.4	17.3	1.9
122.	S	18.3	10.41	95.3	17.2	8	3.29	7.43	8.35	7.89	0.46
123.	G	22.3	9.3	117.2	19.1	10.6	2.73	13.3	15.2	14.25	0.95
124.	S	27.1	12.3	132.1	29.1	7.3	3.71	16.2	15.1	15.65	0.55
125.	S	31.2	10.3	121.1	30.7	10.2	3.54	19.2	16.9	18.05	1.15
126.	G	20.1	7.2	81.2	16.5	9.8	4.62	12.9	14.2	13.55	0.65
127.	G	32.2	11.1	121.3	28.7	12.6	5.54	19.3	22.1	20.7	1.4
128.	S	28.3	7.3	109.1	21.1	9	4.15	16.2	17.2	16.7	0.5
129.	G	16.5	4.9	80.3	15.2	9.2	2.48	12.1	9.3	10.7	1.4
130.	G	29.1	7.2	121.1	19.7	11.6	1.85	16.3	16.1	16.2	0.1
131.	S	32.7	10.3	132	22.3	11.6	2.73	19.1	20.7	19.9	0.8
132.	G	23.1	9.3	110	18.2	9.6	2.8	12.3	13.4	12.85	0.55
133.	S	22.1	7.3	121	16.7	9.2	1.33	15.3	13.9	14.6	0.7
134.	G	28.2	6.9	131	18.2	10.4	3.01	16.4	17.8	17.1	0.7
135.	G	27.3	7.8	84	11.1	6.8	0.75	24.2	18.1	21.15	3.05
136.	G	6.7	2.89	35.6	11.4	7	2.5	8.14	11.57	9.855	1.715
137.	G	21.3	7.2	82	11.7	8	1.41	10.2	6.7	8.45	1.75
138.	S	23.2	6.5	90.2	10.8	8.4	0.49	12.3	19.7	16	3.7
139.	G	18.3	6.2	72.1	9.5	4.4	1.5	9.8	8.3	9.05	0.75
140.	S	28.3	12.2	122	18.7	8	3.19	18.3	20.2	19.25	0.95
141.	S	38	2.89	151.3	7	8	3.5	30.51	19.87	25.19	5.32
142.	S	35.7	7.64	117.2	15.5	9.8	1.6	15.34	14.47	14.905	0.435
143.	G	26.2	6.5	118.2	16.5	12.8	4.26	14.3	18.2	16.25	1.95
144.	S	32.1	12.3	122	10.5	9	2	19.3	20.2	19.75	0.45
145.	S	35.2	11.5	121	10.5	8	0.89	20.2	16.4	18.3	1.9
146.	S	20.2	6.5	75	10.5	8.8	2.99	16.3	15.2	15.75	0.55
147.	G	22.1	7.2	82.1	10.5	10	3.66	11.5	12.3	11.9	0.4
148.	G	18.7	7.6	82.3	10.5	12.6	2.65	8.9	9.2	9.05	0.15
149.	G	22.1	9.5	76.7	10.5	7.8	2.14	10.2	7.7	8.95	1.25
150.	G	31.8	13.23	118.1	10.5	8	3.79	9.78	11.47	10.625	0.845
151.	S	29.5	10.3	122	10.5	16	5.69	18.7	16.9	17.8	0.9
152.	S	35.3	11.5	126	10.5	12	2.76	17.3	19.2	18.25	0.95
153.	G	19.5	8.3	101.1	10.5	10.8	2.93	15.3	16.1	15.7	0.4
161.	S	27.2	8.66	126.2	10.5	10.8	2.79	8.66	11.3	9.98	1.32
163.	G	15	5	69.2	10.5	8.8	4.26	10.08	13.46	11.77	1.69
164.	G	33.3	12.58	137.3	10.5	14.2	3.87	11.11	9.47	10.29	0.82
165.	G	32.8	8.66	132.8	10.5	8.8	3.31	14.52	10.72	12.62	1.9
166.	S	29.7	7.3	129	10.5	16	2.37	18.7	21.3	20	1.3
167.	S	53.7	12.66	232	10.5	9.2	3.76	18.93	14.87	16.9	2.03
168.	G	33.5	16.5	187.3	10.5	11	4.22	21.5	18.5	20	1.5
169.	S	12.5	2.41	38.7	10.5	8	3.85	15.23	18.49	16.86	1.63