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RESEARCH ARTICLE

Development of low-phosphorus tolerant rice culture WGL-1495 through protein kinase *OsPSTOL* (*Pup1*) introgression using restricted marker-assisted breeding

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Abstract

The study aimed to improve phosphorus use efficiency in rice through the introgression of the *Pup1* QTL from Swarna into MTU1121 using a marker-assisted backcross breeding strategy. Forty F_1 plants were generated and 25 true F_1 plants were identified using markers K20-2_{BspeI} and K46-1. These were backcrossed with MTU1121 to produce 180 BC_1F_1 plants, of which 86 were *Pup1* positive. Eight BC_1F_1 plants phenotypically resembling MTU1121 were selfed to produce 320 BC_1F_2 plants, with 25 homozygous plants advancing to BC_1F_3, BC_1F_4 and BC_1F_5 generations. Under normal phosphorus conditions, BC_1F_5 lines demonstrated a 24.68 % yield increase over MTU1121. Under low phosphorus conditions, WGL-1495 (IET 30233) showed superior performance, with traits such as higher grain yield, panicle length and 1000-grain weight compared to MTU1121. In AICRP trials (2021-2023), WGL-1495 consistently outperformed checks under stress-prone conditions both in 0 % and 50 % recommended dose of phosphorus conditions. It achieved 34 % higher yields than Swarna under zero phosphorus conditions in Zone VII during Kharif 2023. WGL-1495 also exhibited exceptional yields in Karnataka, Telangana and Jharkhand, demonstrating its adaptability and efficiency under phosphorus-stress environments. The potential of WGL-1495 as a promising phosphorus-efficient rice culture for sustainable cultivation in low-phosphorus soils.

Keywords: low phosphorus stress; marker-assisted backcross breeding; phosphorus use efficiency; *Pup1* QTL; rice improvement

Introduction

Rice (*Oryza sativa* L.) is a fundamental staple crop, sustaining more than half of the global population and serving as a cornerstone of global food security. India is the largest cultivator of rice, covering 46.5 million hectares and ranks second in production, with an output of 137.8 million metric tons (MMT) in 2023-2024 (1, 2). With the global population expected to reach 9.6 billion by 2050, a 70 % increase in cereal food supply will be required to meet demand (3). Despite the advancements of the Green Revolution, rice productivity has stagnated due to various biotic and abiotic stresses. Among these, low soil phosphorus (P) availability is a critical constraint, severely affecting rice growth at all phenological stages (4).

Phosphorus (P) is the second most vital inorganic nutrient for plants after nitrogen, playing a key role in energy transfer, root development and nitrogen uptake, which together contribute to increased grain protein content (5). However, approximately 5.7 billion hectares of land worldwide facing from insufficient plant-available P due to its limited mobility in soil (6). In India, many rice-growing regions experience moderate to severe P deficiency (7). Plants cultivated in P-deficient soils often exhibit stunted growth, dark green leaves, reduced root development and fewer tillers, ultimately causing significant yield losses (8). To address P deficiency, farmers typically resort to applying additional fertilizers. However, this is not a viable solution for low-input farming systems, where farmers may lack the financial resources or access to P fertilizers (9). The issue becomes more critical as the cost of fertilizers continues to rise. This is largely due to the finite availability of natural phosphorus reserves, which restricts access to sufficient fertilizer resources (10). Moreover, excessive use of P fertilizers can lead to groundwater contamination, algal blooms and eutrophication (11).

Rice shows substantial genetic diversity for adapting to low-phosphorus (P) soils (12). A major quantitative trait locus (QTL) named *Pup1* and several minor QTLs have been associated with tolerance to P deficiency (13). *Pup1*, discovered in the Indian upland rice genotype Kasalath, has been extensively studied for its contribution to improving phosphorus uptake efficiency. This QTL enhances root development and biomass production under conditions of low soil phosphorus making it a key focus in breeding programs aimed at developing P-efficient rice varieties. Over the years, *Pup1* has been fine-mapped and cloned (14-16). The *Pup1* QTL is common in upland rice, adapted to phosphorus-deficient soils, but rare in lowland varieties grown in nutrient-rich conditions (17). Closely and functional markers linked to *Pup1* have also been developed, enabling its use in marker-assisted selection to breed P-efficient rice varieties (18, 16).

Marker-Assisted Backcross Breeding (MABB) has emerged as an effective method for introgression *Pup1* By using molecular markers during backcrossing, this approach ensures precision in transferring target traits while maintaining the desirable genetic background of the recipient parent. Several rice breeders have utilized the *Pup1* and successfully introduced into elite rice varieties and hybrid rice parental lines (19-22).

MTU 1121 (Sri Druthi), a mega rice variety released by APRRS, ANGRAU, Maruteru, Andhra Pradesh, is highly valued for its medium-slender grain quality and adaptability to both wet and dry seasons. Known for its excellent grain quality, high yield potential, non-lodging nature, low shattering, and resistance to BPH (Brown Planthopper) and blast disease, MTU 1121 has gained significant popularity among farmers. However, MTU1121 is sensitive to low soil phosphorus condition. The present study aimed to improve the low phosphors tolerance of a high-yielding MTU1121 by deploying restricted marker-assisted selection combined with stringent phenotypic selection to introgression *Pup1* QTL.

Materials and Methods

Plant material

MTU 1121, is high-yielding variety (7.5 t/ha) developed from the cross BPT-5204 x MTU BB 8-24-1, was used as the recurrent parent. MTU-1121 possesses dark green foliage, semi-erect plant type with moderate tillering ability, medium slender grains, desirable cooking quality and minimal abiotic stress tolerance. However, MTU 1121 is highly sensitive to low soil phosphorus (P) conditions due to the absence of the *Pup1* locus. In a marker-assisted backcross breeding program, Swarna (MTU 7029), another high-yielding mega rice variety in India, was used as the donor parent. Swarna possesses the major *Pup1* QTL/gene associated with tolerance to low soil phosphorus, making it a suitable candidate for improving MTU 1121's phosphorus deficiency tolerance.

A restricted marker-assisted backcross breeding approach employed to introgress the Pup1 QTL

The cross was initiated between MTU 1121 and the donor parent Swarna and true F_1 plants were identified using the co-dominant CAPS marker K20-2 $_{Bspe1}$. These F_1 plants were then backcrossed with the recurrent parent MTU 1121 to develop the BC_1F_1. The heterozygosity of BC_1F_1 plants for *Pup1* was assessed using K20-2 $_{Bspe1}$ and further confirmed using K46-1, a dominant marker. Selected BC_1F_1 plants were self-pollinated to produce the BC_1F_2, which was subsequently screened using co-dominant and functional markers to identify homozygous *Pup1* plants. These homozygous BC_1F_2 plants were selfed to generate BC_1F_3. In the BC_1F_3 to BC_1F_5 generations, backcross-derived lines were selected for *Pup1*, medium-slender grain type, yield and other agro-morphological traits based on phenotype-based visual selection.

DNA extraction and PCR amplification

Leaves from 45-day-old seedlings were collected and genomic DNA was isolated from parents and backcross derived lines by using a mini prep protocol with few modifications (23). For foreground solution of *Pup1,* PCR conditions for markers K20-2 $_{Bspe1}$ and K46-1, followed the method (18). To identify recombinants flanking the Pup 1 locus, two SSR markers (RM28052 and RM28102)were amplified following the protocol described (24). PCR products were resolved on Seakem® LE agarose gels (Lonza, USA): 3.5 % for recombinant SSR markers, 2 % for K20-2 $_{Bspe1}$ and 1.2 % for K46-1.

Phenotypic screening for tolerance to low soil phosphorus.

The selected BC_1F_5 lines, along with their parents were evaluated in experimental plots with low and normal soil phosphorus levels. The low soil P plot, with available phosphorus below 2 kg/ha (tested prior to planting) and the normal soil P plot, with available phosphorus above 20 kg/ha, were established at the experimental farm of ICAR-Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, Telangana State, India. Standard agronomic practices were followed to ensure healthy crop growth in both conditions. Observations were recorded for key traits, including days to 50 % flowering (DFF), plant height (PH), number of productive tillers per plant (NPT), panicle length (PL), number of grains per panicle (NGPP) and grain yield per plant (GY/P).

Evaluation of agro-morphological traits in improved MTU-1121 breeding lines.

The selected BC_1F_5 lines carrying the *Pup1* QTL, along with their parents Swarna and MTU-1121, were evaluated in the experimental farm of RARS-Warangal, Telangana State, India. The lines were conducted in 2 m^2 plots following a randomized block design with a planting spacing of 15 × 20 cm. The soil in the experimental plots was enriched with optimal levels of macro- and micronutrients. Data were collected for key agro-morphological traits, including days to 50 % flowering (DFF), plant height (PH), number of productive tillers per plant (NPT/P), panicle length (PL), number of grains per panicle (NGPP) and grain yield per plant (GY/P).

Statistical analysis

Data for all parameters was recorded from both normal and low soil P plots. Statistical analyses were performed using SAS version 9.2 (SAS Institute, Inc., Cary, NC). The analysis included the calculation of the coefficient of variation (CV), critical difference (CD), standard error (SE) and analysis of variance (ANOVA) to assess the differences among the lines.

Results

A cross was made between MTU1121 (the recurrent parent) and Swarna (the donor parent for *Pup1* QTL) and obtained 40 F_1 plants. These 40 F_1 plants were genotyped using the co-dominant marker K20-2_{BspeI} and the *Pup1*-specific functional marker K46-1 and obtained 25 true F_1 plants. The 25 true F_1s were backcrossed to the recurrent parent MTU1121 to generate 180 BC_1F_1 plants. All these 180 BC_1F_1 plants were subjected to foreground selection using the markers K20-2_{BspeI} and K46-1. Out of 180 BC_1F_1 plants, 86 plants were observed to be positive for *Pup1* QTL. (Fig. 1). Out of these, 8 BC_1F_1 plants phenotypically similar to MTU1121 (in plant type, grain type and other agro-morphological traits) were selected and selfed to generate 320 BC_1F_2 plants. Out of 320 BC_1F_2 plants, 25 plants were observed to be homozygous for *Pup1* QTL (foreground selection) and these 25 homozygous BC_1F_2 plants were selfed to generate BC_1F_3, generation. From BC_1F_3 generation plants were advanced to BC_1F_4 and BC_1F_5 generations through Restricted Marker-assisted backcross breeding strategy combined with phenotype-based visual selection for plant stature, medium-slender grain type, high yield and other agro-morphological characters similar to recurrent parent (Fig. 2).

Performance of BC_1F_5 lines with Pup1 under normal soil phosphorus conditions

Under normal soil phosphorus levels (>25 kg P ha^{-1}), the BC_1F_5 lines possessing *Pup1* QTL exhibited equivalent or superior performance compared to MTU-1121 in terms of days to fifty percent flowering, plant height, number of productive tillers and other agro-morphological traits (Table 1).

Performance of Pup1-introgressed BC_1F_5 lines under low phosphorus conditions

The phenotypic performance of six introgressed BC_1F_5 lines along with the parents and checks were evaluated under low P (<2 kg P ha^{-1}) conditions in Table 2. The grain yield of improved lines ranged between 20.1 to 27.4 g per plant, the improved BC_1F_5 lines possessing *Pup1* lines were recorded equivalent or higher yield than the recurrent parent MTU1121.Days to 50 % flowering (DFF) ranged between the 121 days to 128 days, indicating a mean value range of flowering durations suitable

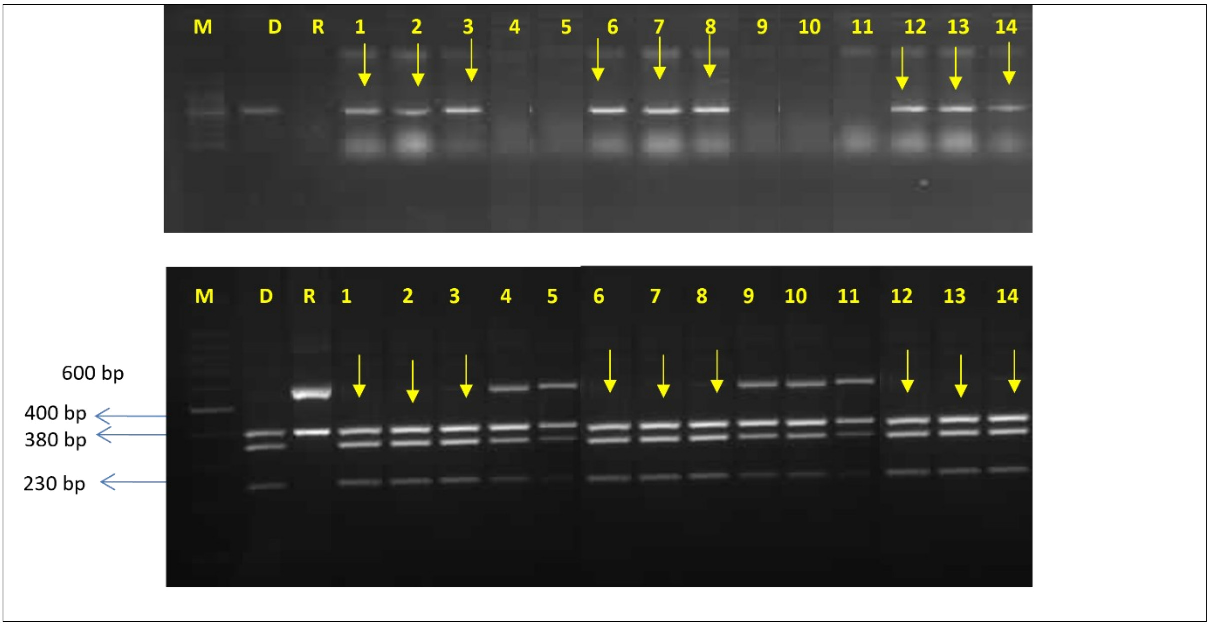

Fig. 1. Molecular marker-based foreground selections for *Pup1* QTL in BC_1F_1, generation. L- indicates100-bp molecular weight marker/ladder, D-Swarna, R MTU 1121, Lanes 1 to 24 represent BC_1F_1, plants derived from the cross MTU 1121/Swarna. Arrow indicates positive plants for K 46-1 and K 20-2_{BspeI}. K46-1, a functional marker of *Pupl* and K 20-2 $_{BspeI}$ a co-dominant marker linked to *Pupl* were used for foreground selection.

Fig. 2. Field view, plant type: paddy and grains of low soil phosphorus tolerant rice culture i.e. WGL 1495 (IET 30233).

Table 1. Phenotypic evaluation of BC1F5 lines possessing Pup1 QTL in a plot with optimum soil phosphorus

S.No	Entry Name	Days to 50% flowering	Plant Height (cm)	Panicle length (cm)	Test Weight (g)	No. of productive tillers /plant	Grain yield/ plant (g)	Grain type
1	SWARNA[A]	120	99.2	24	18.7	10	27.01	MB
2	MTU-1121[B]	116	127.8	29.4	19.85	10	30.72	MS
1	WGL-1482	111	117.4	26.4	19.65	11	30.05	MS
2	WGL-1483	108	121.2	27.9	19.4	11	33.32	MS
3	WGL-1484	108	111.2	25.8	18.1	11	32.50	MS
4	WGL-1485	115	123.4	26.7	19.9	10	29.85	MS
5	WGL-1486	106	118.6	25.9	18.65	10	28.15	MS
6	WGL-1495	114	121.8	27.7	21.05	11	35.21	MS
	C.D.		0.178	0.086	0.009	0.006	0.096	
	SE(m)		0.058	0.028	0.003	0.002	0.031	
	C.V.		0.086	0.183	0.026	0.033	0.151	

A: Donor parent **B**: Recipient parent

Table 2. Phenotypic evaluation of BC_1F_5 lines possessing *Pup1* QTL in low soil P plot of IIRR, Hyderabad

S.No	Entry Name	Days to 50% flowering	Plant Height (cm)	Panicle length (cm)	Test Weight (g)	No. of productive tillers /plant	Grain yield/ plant (g)	Grain type
1	SWARNA[A]	128	163.6	10	23.8	16.8	22.0	MB
2	MTU-1121[B]	114	90.8	5	22.4	14.5	11.2	MS
1	WGL-1482	128	163.6	10	23.8	16.8	26.0	MS
2	WGL-1483	125	144.5	8	23.4	16.1	23.2	MS
3	WGL-1484	125	144.5	8	23.4	16.1	23.0	MS
4	WGL-1485	121	125.4	7	23.1	15.4	20.1	MS
5	WGL-1486	125	144.5	8	23.4	16.2	23.2	MS
6	WGL-1495	128	163.6	10	23.8	16.8	27.4	MS
	C.D.		0.454	0.177	0.131	0.137	0.387	
	SE(m)		0.148	0.058	0.043	0.045	0.126	
	C.V.		0.180	1.196	0.317	0.481	0.994	

A: Donor parent **B**: Recipient parent

for different growing conditions. Plant height showed considerable variation with the mean value ranged (163.6 cm) and (125.4 cm). The selected lines showed number of productive tillers per plant mean value ranged from 7 to 10 with the highest tillering observed in these high-yielding lines, While Panicle length ranged between from 23.1 cm to 23.8 cm. Test weight (thousand-grain weight) mean value ranged from 15.4 g to 16.8 g indicating lines possessing desirable medium slender and good grain quality. The improved line WGL-1495 demonstrated the best phenotypic performance, combining higher grain yield, optimal panicle length and productive tillers, making it a promising candidate for low soil phosphorus condition.

Performance of WGL-1495 (IET 30233) in AICRP trials for zone VII and zone III

WGL-1495 (IET 30233) has demonstrated exceptional performance across three years of evaluation under the All India Coordinated Rice Improvement Project (AICRP) trials. In its first year of evaluation during the *Kharif* 2021, under stress conditions (0 % recommended dosage of P), WGL-1495 achieved an average yield of 5260 kg/ha in Zone VII, which was 31.17% higher than the best check variety, Swarna 4010 kg/ha, but under stress conditions (50 % recommended dosage of P), Swarna performed better than all other test entries, including WGL-1495 (IET 30233). However, based on yield potential under P_0 conditions, WGL-1495 (IET 30233) was promoted to Advanced Varietal Trial-I (AVT-I). years (Table 3).

Table 3. Summary of average yield (kg/ha) from IVT -LPT, AVT-I-LPT and AVT-II-LPT trials conducted under stress-prone conditions in Zone VII (0 % recommended dose of phosphorus)

	Year of Testing	Season of testing	No. of trials	Proposed entry (IET 30233 (WGL 1495))	Positive check-Swarna
Mean yield (kg/ha)	I Year (IVT- LPT)	*Kharif* 2021	2*	5260	4010
	II Year (AVT- 1-LPT)	*Kharif* 2022	4**	4731	4344
	III Year AVT-2 LPT	*Kharif* 2023	4***	5222	3896
	Wt. Avg.		10	5071	4083.33
Percent increase or decrease over the Positive check	I Year (IVT- LPT)	*Kharif* 2021	2*	-	+31.17
	II Year (AVT- 1-LPT)	*Kharif* 2022	4**	-	+8.90
	III Year AVT-2 LPT	*Kharif* 2023	4***	-	+34.03
	Wt. Avg.		10	-	+24.35

Source: ICAR-Indian Institute of Rice Research, *Progress Report Vol. 1*: *Varietal Improvement* (IVT-LPT) 2021, (AVT1-LPT) 2022 & (AVT2- LPT) 2023*During I Year (IVT- LPT) evaluation was done in 2 locations: Karnataka: Gangavati and Mandya in Zone VII *Kharif* 2021.

**During II Year (AVT 1-LPT) evaluation was done in 4 locations: Andhra Pradesh (1), Telangana (1), Karnataka (2) in Zone VII *Kharif* 2022.

**During III Year (AVT 2-LPT) evaluation was done in 4 locations: Andhra Pradesh (1), Telangana (1), Karnataka (2) in Zone VII *Kharif* 2023.

In its second year of evaluation during *Kharif* 2022, WGL -1495 demonstrated consistent yield advantages in the AVT-I LPT trial. under stress conditions (0 % recommended dosage of P), it achieved an average yield of 4731 kg/ha, showing an 8.90 % improvement over Swarna (4344 kg/ha) in Zone VII. The performance further underscored its potential with yields of 3665 kg/ha in Odisha (8.4 % over Swarna) in Zone III, 4953 kg/ ha in Karnataka (17 % over Swarna) and 4767 kg/ha in Telangana (21 % over Swarna) in Zone VII (Table 3 & 5). under stress conditions (50 % recommended dosage of P), WGL-1495 exhibited a remarkable average yield of 6036 kg/ha, which was 14 % higher than Swarna average yield of 5303 kg/ha in Zone VII. Notably, it outperformed the best check by 2867 kg/ha in Jharkhand state (Zone III) and by 7140 kg/ha in Telangana state (Zone VII) (Table 4 & 6).

In its third year of evaluation during *Kharif* 2023 (AVT-II) LPT, WGL-1495 continued to outperform the check variety under both P_0 and P_{50} conditions. under stress conditions (0 % recommended dosage of P), it achieved average yield of 5222 kg/ha, which was 34 % higher than Swarna average yield 3896 kg/ha in zone VII. it recorded yields of 4822 kg/ha in Telangana (28.35 % over Swarna) and 5350 kg/ha in Karnataka (45.97 % over Swarna). Similarly, under stress conditions (50 % recommended dosage of P), it demonstrated average yield of 5601 kg/ha, which was 29.50 % higher than Swarna (Table 3 & 5). In Telangana and Karnataka, the IET 30233 achieved yields of 5289 kg/ha (19.01 % over Swarna) and 5570 kg/ha (28.78 % over Swarna), respectively (Table 4 & 6).

Based on its performance under both P_0 % and P_{50} % conditions, IET 30233 was observed to be promising in Zone VII in Telangana and Karnataka states across the three testing years (2021, 2022 and 2023). Additionally, the entry outperformed the best check in Zone III (Jharkand) during 2022.

Table 4. Summary of average yield (kg/ha) from IVT -LPT, AVT-I-LPT and AVT-II-LPT trials conducted under stress-prone conditions in Zone VII (50 % Recommended Dose of Phosphorus)

	Year of Testing	Season of testing	No. of trials	Proposed variety (IET 30233)	Positive check- Swarna
Mean yield (kg/ha)	I Year (IVT- LPT)	*Kharif* 2021	2*	3760	4640
	II Year (AVT- 1-LPT)	*Kharif* 2022	4**	6036	5303
	III Year AVT-2 LPT	*Kharif* 2023	4***	5601	4325
	Wt. Avg.		10	5132.33	4756
Percent increase or decrease over the Positive check	I Year (IVT- LPT)	*Kharif* 2021	2*	-	-18.96
	II Year (AVT- 1-LPT)	*Kharif* 2022	4**	-	+13.82
	III Year AVT-2 LPT	*Kharif* 2023	4***	-	+29.50
	Wt. Avg.		10	-	+8.12

Source: ICAR-Indian Institute of Rice Research, *Progress Report Vol. 1*: *Varietal Improvement* (IVT-LPT) 2021, (AVT1-LPT) 2022 & (AVT2- LPT) 2023

*During I Year (IVT- LPT) evaluation was done in 2 locations: Karnataka: Gangavati and Mandya in Zone VII *Kharif* 2021.

**During II Year (AVT 1-LPT) evaluation was done in 4 locations: Andhra Pradesh (1), Telangana (1), Karnataka (2) in Zone VII *Kharif* 2022.

**During III Year (AVT 2-LPT) evaluation was done in 4 locations: Andhra Pradesh (1), Telangana (1), Karnataka (2) in Zone VII *Kharif* 2023.

Table 5. Summary of average yield (kg/ha) from IVT -LPT, AVT-I-LPT and AVT-II-LPT trials conducted state wise under stress-prone conditions VII (0 % recommended dose of phosphorus)

Seasons	Trial	Zones	States	Proposed variety (IET 30233 kg/ha)	Best check kg/ha	Percent increase or decrease over the check
Kharif 2021	I Year (IVT- LPT)	VII	Karnataka	5260	4010 (Swarna)	+31.17%
Kharif 2022	II Year (AVT- 1-LPT)	III	Odisha	3665	3381 (Swarna)	+8.4%
		VII	Telangana	4767	3939 (Rasi)	+21%
		VII	Karnataka	4953	4235 (Rasi)	+17%
		III	Jharkhand	3333	3011 (Vandana)	+11%
Kharif 2023	III Year (AVT- 2-LPT)	VII	Telangana	4822	3756 (Swarna)	+28.35%
		VII	Karnataka	5350	3665 (Rasi)	+45.97%

Table 6. Summary of average yield (kg/ha) from IVT -LPT, AVT-I-LPT and AVT-II-LPT trials conducted state wise under stress-prone conditions (50 % recommended dose of phosphorus)

Seasons	Trial	Zones	States	Proposed variety (IET 30233 kg/ha)	Best check kg/ha	Percent increase or decrease over the check
Kharif 2022	II Year (AVT- 1-LPT)	III	Bihar	4762	4107 (Rasi)	+13%
		III	Jharkhand	2867	2267 (Rasi)	+26%
		VII	Telangana	7140	3615 (Rasi)	+98%
Kharif 2023	III Year (AVT- 2-LPT)	VII	Telangana	5289	4444 (Swarna)	+19.01%
		VII	Karnataka	5570	4325 (Swarna)	+28.78%

Discussion

India leads the world in rice cultivation, with an impressive 46.5 million hectares under cultivation. However, a significant portion of this area, particularly uplands and acid soil, exhibits high phosphorus (P)-fixing capacity. This phenomenon reduces the availability of phosphorus in soils, consequently limiting rice yields (25). Furthermore, in irrigated conditions, the rising cost of phosphorus fertilizers in recent years has led to reduced fertilizer application, resulting in low to moderate deficiency of the nutrient and lesser crop yields (21). The development and cultivation of phosphorus-use-efficient rice varieties emerge as a cost-effective and environmentally sustainable strategy to address this issue (26). Such varieties can enhance phosphorus utilization from the soil, reduce dependence on external fertilizers and maintain or improve rice yields in phosphorus-deficient soils

Phosphorus (P) uptake and use efficiency exhibit significant genetic variability in rice, as demonstrated by studies (27, 28, 12). This variability offers opportunities to improve rice yields in phosphorus-deficient conditions through MABB. Research has consistently shown that rice genotypes experience considerable yield reductions under low P conditions, with tolerance levels varying widely among varieties. Recent studies have focused on screening rice genotypes for phosphorus deficiency tolerance. These findings highlight the importance of identifying and utilizing phosphorus-efficient genotypes in rice breeding (29-31).

Swarna, a highly popular and high-yielding lowland rice variety in India, has demonstrated exceptional tolerance to low phosphorus (P) soils. This attribute is primarily attributed to the presence of the *Pup1* QTL, which enhances phosphorus uptake efficiency. Analysis using *Pup1*-specific markers confirmed that Swarna possesses the tolerant allele for *Pup1* (32). For the present study, Swarna was selected as the *Pup1* donor due to its compatibility as a general combiner (21). Earlier, the integration of *Pup1* from Swarna into elite rice varieties through marker-assisted backcross breeding (MABB) has been documented in several successful efforts. Notably, utilized Swarna as the donor to enhance phosphorus-use efficiency in various rice genotypes. MTU 1121, commonly known as Sri Druthi, is a highly popular and widely cultivated rice variety in India (21, 26, 33). Released by APRRS, ANGRAU, Maruteru, Andhra Pradesh, it has gained prominence due to its medium-duration maturity (125-130 days), excellent grain quality and high yielding potential of 6-7 tons per hectare. Its adaptability across both wet and dry seasons, combined with resistance to blast and brown planthopper (BPH), has made it a staple variety, occupying over 12 % of the rice-growing area in India. However, despite its agronomic and market advantages, MTU 1121 is sensitive to low phosphorus (P) conditions, a common limitation in Indian rice-growing regions. To address this limitation, we targeted the introgression of *Pup1*, a major quantitative trait locus (QTL) associated with enhanced phosphorus uptake, into the genetic background of MTU 1121 through marker-assisted backcross breeding (MABB).

The *Pup1* locus represents a significant breakthrough in improving phosphorus (P) acquisition in rice, especially under P-deficient conditions. Originally identified in the Indian Aus rice variety *Kasalath*, *Pup1* was fine-mapped to a 90-kb region on chromosome 12 using molecular markers within a recombinant inbred line (RIL) population derived from the cross between *Kasalath* and *Nipponbare* (13-15). This discovery underscores the power of molecular breeding approaches in elucidating traits of agronomic importance. Subsequent cloning efforts identified *OsPSTOL1* (phosphorus starvation tolerance gene 1) as the candidate gene for *Pup1* (16). The protein kinase encoded by *OsPSTOL1* plays a critical role in enhancing early root growth, enabling plants to acquire more phosphorus and other nutrients from the soil. Its activation under nutrient-limited conditions illustrates its adaptive significance, making it a valuable genetic resource for sustainable rice cultivation in P-deficient soils.

Two molecular markers, K20-2$_{BspeI}$ and K46-1, were utilized for precise selection during the MABB. These markers, previously validated in other studies, are closely linked to the *Pup1* locus, ensuring the accuracy of introgression (18, 21). Foreground selection in the BC_1F_1 generation utilized the co-dominant marker K20-2$_{BspeI}$, located approximately 106 kb (~1 cm) from the *Pup1* locus, to identify plants carrying the desired allele. The functional marker K46-1 was then used to validate the presence of *Pup1* in selected lines. Subsequent generations (up to BC_1F_5) underwent selfing and pedigree-based selection to recover the desirable agronomic traits of MTU 1121 while retaining the *Pup1* QTL. This strategy aligns with previous reports that employed similar markers for *Pup1* introgression. Similar study reported by used a combination of K20-1, K20-1-1 and K46-1 markers for *Pup1* transfer, while utilized K20-2, applied functional dominant markers K46-1 and K46-2 (22, 26, 33).

Marker-assisted backcross breeding (MABB) has been a transformative approach for intrgression the *Pup1* into elite rice varieties. This strategy has been successfully employed in multiple studies to address low phosphorus (P) soil tolerance. Similar studies introgressed the *Pup1* QTL into varieties such as Improved Samba Mahsuri, MTU1010 and IR64 (19). Their work also identified novel, non-*Pup1* type donors for enhancing P-use efficiency, broadening the genetic base for low P tolerance. Study utilized MABB to integrate *Pup1* QTL into bacterial blight-resistant Improved Samba Mahsuri, while a study incorporated *Pup1* into ASD 16 and ADT 43, which already possessed resistance to bacterial blight and blast (20, 26). A study adopted marker-assisted pedigree breeding (MAPB) rather than MABB for transferring *Pup1* into MTU1010, indicating the flexibility of molecular breeding techniques (33). Similarly, employed MABB to introgress *Pup1* from *Kasalath* into the rice maintainer line APMS6B, further demonstrating its utility across different genetic backgrounds (22). A study introgressed the *Pup1* QTL into three temperate rice varieties-MS11, TR22183 and Dasanbyeo (33).

In this study, *Pup1* QTL was introgressed from Swarna into MTU1121 using a restricted MABB strategy coupled with phenotype based visual selection for grain type, plant stature and other agro-morphological traits. The restricted approach minimized the number of backcross generations while ensuring precise transfer of the desired QTL, linked molecular markers such as K20-2$_{BspeI}$ and K46-1 for efficient selection. The use of restricted MABB mirrors strategies employed for other traits. Similar reports enhanced Pusa Basmati 1 with bacterial blight resistance using a similar approach, for Pusa RH10, used restricted MABB to incorporate bacterial leaf blight resistance into KMR-3R (35-37).

The introgression of the *Pup1* QTL into MTU 1121 significantly enhanced its performance under low phosphorus (P) soil conditions. *Pup1*-introgressed lines exhibited notable improvements in key agronomic traits, including plant height, the number of productive tillers, panicle length and grain yield per plant, compared to the recipient parent MTU 1121. These enhancements are directly attributable to *Pup1*'s role in promoting root growth under phosphorus-deficient conditions, as previously reported by (16). Under normal soil P conditions, there was no significant difference in performance between the *Pup1*-introgressed lines and MTU 1121, indicating that the introgression process successfully preserved the recipient parent's desirable traits. Interestingly, even under normal conditions.

WGL-1495 (IET 30233) has emerged as a highly promising rice culture, demonstrating exceptional performance during multi-year evaluations in the All India Coordinated Rice Improvement Project (AICRP) trials. Its consistent superiority under low phosphorus (P) and moderate P input conditions highlights its adaptability and potential for diverse agro-ecological zones, particularly in Zones VII and III. The high-yield potential of WGL-1495 in low-phosphorus soils is due to its special genetic traits that help it absorb and use phosphorus more effectively. It is also well-suited for farming systems that use high inputs, making it useful for both low-resource and intensive farming. This flexibility makes WGL-1495 an important option for tackling low-phosphorus soils while maintaining good yields in better farming conditions. Promoting WGL-1495 could play a big role in achieving sustainable rice production and improving food security, especially in areas where phosphorus deficiency is a common problem.

Conclusion

The study successfully introgressed the *Pup1* QTL into MTU1121 through restricted marker-assisted backcross breeding, resulting in phosphorus-efficient rice lines. WGL-1495 (IET 30233) demonstrated superior performance under both normal and low phosphorus soil conditions, with significant yield advantages and improved agro-morphological traits compared to MTU1121 and Swarna. Its consistent performance across multiple environments and years in AICRP trials highlights its potential as a promising culture for low-phosphorus soils, contributing to sustainable rice production.

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Authors' contributions

This study was conducted through the collaborative efforts of all authors. YH led the research work, developed the methodology and drafted the final manuscript. YH, BE, VV, BSC, KRD, MA, GN, KRP, RSK, MSA and DA contributed to field trials and laboratory experiments. CR and RUR conducted agronomic trials. YH supervised the overall research work. YH and BE analyzed the data, prepared figures and tables, interpreted the results and finalized the manuscript draft. All authors have reviewed and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

References

1. Statista Research Department. Area under rice cultivation worldwide and production volume for India in 2023–2024 [Internet]. Available from: https://www.statista.com/
2. Directorate of Economics and Statistics (DES). Agricultural statistics at a glance - Rice area and production estimates for India 2023-2024. Government of India [Internet]. Available from: https://desagri.gov.in/en
3. Godfray HCJ, Garnett T. Food security and sustainable intensification. Philo Trans Royal Soc B: Bio Sci. 2014;369 (1639):20120273. https://doi.org/10.1098/rstb.2012.0273
4. Ismail AM, Heuer S, Thomson MJ, Wissuwa M. Genetic and genomic approaches to develop rice germplasm for problem soils. Plant Mol Bio. 2007;65:547-70. https://doi.org/10.1007/s11103-007-9215-2
5. Huang CY, Roessner U, Eickmeier I, Genc Y, Callahan DL, Shirley N, et al. Metabolite profiling reveals distinct changes in carbon and nitrogen metabolism in phosphate-deficiency barley plants (*Hordeum vulgare L.*). Plant Cell Phy. 2008;49:691–703. https://doi.org/10.1093/pcp/pcn044
6. Batjes NH. A world data set of derived soil properties by FAO-UNESCO soil unit for global modeling. Soil Use Manage. 1997;13:9–16. https://doi.org/10.1111/j.1475-2743.1997.tb00550.x
7. Dobermann A, Fairhurst T. Phosphorus deficiency. In: Rice: Nutrient Disorders and Nutrient Management. International Rice Research Institute: Los Banos, Philippines; 2000. p. 60–71
8. Dobermann A, Dawe D, Roetter RP, Cassman KG. Reversal of rice yield decline in a long-term continuous cropping experiment. Agro J. 2000;92(4):633–643. https://doi.org/10.2134/agronj2000.924633x
9. Kirk GJD, George T, Courtois B, Senadhira D. Opportunities to improve phosphorus efficiency and soil fertility in rainfed lowland and upland rice ecosystems. Field Crops Res. 1998;56:73–92. https://doi.org/10.1016/S0378-4290(97)00141-X
10. Van Kauwenbergh S, Steward M, Mikkelsen R. World reserves of phosphate rock: A dynamic and unfolding story. Better Crops. 2013;97:18–20.
11. Carpenter SR. Phosphorus control is critical to mitigating eutrophication. Proceed Nat Acad Sci USA. 2008;105:11039–40. https://doi.org/10.1073/pnas.0806112105
12. Fageria NK, Wright RJ, Baligar VC. Rice cultivar evaluation for phosphorus use efficiency. Plant Soil. 1998;111:105–09. https://doi.org/10.1007/BF02182043
13. Wissuwa M, Yano M, Ae N. Mapping of QTLs for phosphorus deficiency tolerance in rice (*Oryza sativa* L.). Theor Appli Genet. 1998;97:777–83. https://doi.org/10.1007/s001220050955

14. Heuer S, Lu X, Chin JH, Tanaka JP, Kanamori H, Matsumoto T, et al. Comparative sequence analyses of the major quantitative trait locus phosphorus uptake 1 (Pup1) reveal a complex genetic structure. Plant Biotech J. 2009;7:456–57. https://doi.org/10.1111/j.1467-7652.2009.00415.x
15. Wissuwa M, Wegner J, Ae N, Yano M. Substitution mapping of Pup1: a major QTL increasing phosphorus uptake from a phosphorus-deficient soil. Theor Appli Genet. 2002;105:890–97. https://doi.org/10.1007/s00122-002-1051-9
16. Gamuyao R, Chin HJ, Tanaka JP, Pesaresi P, Catausan S, Dalid C, et al. The protein kinase Pstol1 from traditional rice confers tolerance of phosphorus deficiency. Nature. 2012;88:535–541. https://doi.org/10.1038/nature11346
17. Chin JH, Kim JH, Jiang WZ, Chu SH, Woo MO, Han LZ, et al. Identification of subspecies-specific STS markers and their association with segregation distortion in rice (*Oryza sativa L.*). J Crop Sci Biotech. 2007;10:175–84.
18. Chin JH, Gamuyao R, Dalid C, Bustamam M, Prasetiyono J, Moeljopawiro S, et al. Developing rice with high yield under phosphorus deficiency: Pup1 sequence to application. Plant Phy. 2011;156(3):1202–16. https://doi.org/10.1104/pp.111.175471
19. Sundaram RM, Madhav MS, Neeraja CN, Balachandran SM, Mangrauthia SK, Barbadikar KB, et al. Multi-trait improvement in rice through marker-assisted breeding. Oryza. 2018;55(spl):24–37. http://dx.doi.org/10.5958/2249-5266.2018.00003.6
20. Chithrameenal K, Alagarasan G, Raveendran M, Robin S, Meena S, Ramanathan A, et al. Genetic enhancement of phosphorus starvation tolerance through marker-assisted introgression of *OsPSTOL1* gene in rice genotypes harboring bacterial blight and blast resistance. PLoS One. 2018;13(9):e0204144. https://doi.org/10.1371/journal.pone.0204144
21. Anila M, Swamy MHK, Kale RR, Bhadana VP, Anantha MS, Brajendra, et al. 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. Mol Breed. 2018;38:1–9. https://doi.org/10.1007/s11032-018-0903-1
22. Madhusudan N, Beulah P, Veerendra J, Nagaraju P, Manasa Y, Sundaram RM, et al. Stacking of Pup1 QTL for low soil phosphorus tolerance and bacterial blight resistance genes in the background of APMS6B, the maintainer line of rice hybrid DRRH-3. Euphytica. 2022;218(4):37. https://doi.org/10.1007/s10681-022-02987-0
23. Zheng K, Subudhi PK, Domingo J, Maopanty G, Huang N. Rapid DNA isolation for marker-assisted selection in rice breeding. Rice Genetics Newsletter. 1995;12:255–58.
24. Sundaram RM, Vishnupriya MR, Biradar SK. Marker-assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. Euphytica. 2008;160:411–22. https://doi.org/10.1007/s10681-007-9564-6
25. Vance CP, Uhde-Stone C, Allan DL. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytologist. 2003;157:423–47. https://doi.org/10.1046/j.1469-8137.2003.00695.x
26. Swamy HM, Anila M, Kale RR, Rekha G, Bhadana VP, Anantha MS, et al. Marker-assisted improvement of low soil phosphorus tolerance in the bacterial blight-resistant, fine-grain type rice variety, improved Samba Mahsuri. Sci Reports. 2020;10(1):21143. https://doi.org/10.1038/s41598-020-78186-5
27. Fageria NK, Baligar VC. Upland rice genotypes evaluation for phosphorus use efficiency. J Plant Nutr. 1997;20:499–509. https://doi.org/10.1080/01904169709365270
28. Akinrinde EA, Gaizer T. Differences in the performance and phosphorus-use efficiency of some tropical rice (*Oryza sativa L.*) varieties. Pak J Nutr. 2006;5(3):206–11. http://dx.doi.org/10.3923/pjn.2006.206.211
29. Harisha T, Shruti, Anantha MS, Gireesh C, Sundaram RM, Senguttuvel P, et al. Exploring genetic variability for yield and its attributing traits in rice (*Oryza sativa L.*) under low soil phosphorus conditions. Asian J Soil Sci Plant Nutr. 2024;10(3):297–303. https://doi.org/10.9734/ajsspn/2024/v10i3340
30. Manoj CA, Muralidhara B, Basavaraj PS, Gireesh C, Sundaram RM, Senguttuvel P, et al. Evaluation of rice genotypes for low phosphorus stress and identification of tolerant genotypes using stress tolerance indices. Indian J Genet Plant Breed. 2023;83(1):24–31.
31. Roy M, Khatun SM, Hassan L, Hossain MA. Evaluation of rice (*Oryza sativa L.*) genotypes for low phosphorus stress tolerance. J Plant Stress Phys. 2023;9:27–35. https://doi.org/10.25081/jpsp.2023.v9.8598
32. Sarkar S, Yelne R, Chatterjee M, Das P, Debnath S, Chakraborty A, et al. Screening of phosphorus tolerance and validation of Pup1-linked markers in indica rice. Indian J Genet Plant Breed. 2011;71:20913.
33. Laxmi PB, Dangi KS, Damodar RC, Jagadeeshwar R, Rekha G, Pragya S, et al. Marker-assisted pedigree breeding-based improvement of the Indian mega variety of rice MTU1010 for resistance against bacterial blight and blast and tolerance to low soil phosphorus. PLoS One. 2022;17(1):e0260535. https://doi.org/10.1371/journal.pone.0260535
34. Navea IP, Yang S, Tolangi P, Sumabat RM, Zhang W, Chin JH. Enhancement of rice traits for the maintenance of the phosphorus balance between rice plants and the soil. Current Plant Bio. 2024;38:100332. https://doi.org/10.1016/j.cpb.2024.100332
35. Gopalakrishnan S, Sharma RK, Rajkumar KA, Joseph M, Singh VP, Singh AK, et al. Integrating marker-assisted background analysis with foreground selection for identification of superior bacterial blight-resistant recombinants in basmati rice. Plant Breed. 2008;127(2):131–39. https://doi.org/10.1111/j.1439-0523.2007.01458.x
36. Basavaraj SH, Singh VK, Singh A, Singh A, Singh A, Anand D, et al. Marker-assisted improvement of bacterial blight resistance in parental lines of Pusa RH10, a superfine-grain aromatic rice hybrid. Molecular Breed. 2010;26:293–305. https://doi.org/10.1007/s11032-010-9407-3
37. Hari Y, Srinivasarao K, Viraktamath BC, Hariprasad AS, Laha GS, Ahmed MI, et al. Marker-assisted improvement of a stable restorer line, KMR-3R and its derived hybrid KRH2 for bacterial blight resistance and grain quality. Plant Breed. 2011;130(6):608–16. https://doi.org/10.1111/j.1439-0523.2011.01881.x

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