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# 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

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**Abstract** MTU 1010 is a high-yielding mega-variety of rice grown extensively in India. However, it does not perform well in soils with low phosphorus (P) levels. With an objective to improve MTU 1010 for tolerance to low soil P, we have transferred *Pup1*, a major quantitative trait locus (QTL) associated with tolerance from another mega-variety, Swarna, through marker-assisted backcross breeding (MABB). Foreground selection of the $F_1$ and backcross plants was performed with the co-dominant, closely linked CAPS marker, K20-2, while two flanking markers RM28011 and RM28157 were utilized for recombinant selection. At each backcross generation, positive plants were also analyzed with a set of 85 parental polymorphic SSR markers to identify the QTL-positive plants possessing maximum introgression of MTU 1010 genome. At $BC_2F_1$, the best backcross plant was selfed to generate $BC_2F_2$s. Among them, the plants homozygous for *Pup1* ($n = 22$) were reconfirmed using the functional marker for *Pup1*, viz., K46-1, and they were advanced through pedigree method of selection until $BC_2F_6$ generation. A total of five elite $BC_2F_6$ lines, possessing *Pup1* and phenotypically similar to MTU 1010, were screened in the low soil P plot and normal plot (with optimum soil P levels) during wet season, 2016. All the selected lines showed better performance under low P soil with more number of productive tillers, better root system architecture, and significantly higher yield (> 390%) as compared to MTU 1010. Further, under normal soil, the lines were

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observed to be similar to or better than MTU 1010 for most of the agro-morphological traits and yield. This study represents the successful application of marker-assisted selection for improvement of tolerance to low soil P in a high-yielding Indian rice variety.

**Keywords** Low soil phosphorus tolerance · *OsPSTOL* · *Pup1* · Marker-assisted backcross breeding (MABB) · Introgression · MTU 1010 · Root architecture

## Introduction

Phosphorus (P) is a vital element required for the growth and development of rice crop. It is an important component of nutrient supply chain and plays a crucial role in energy storage and transfer within cells, speeds up root development, facilitates greater N uptake, and results in higher grain protein yields (Huang et al. 2008). The current situation in both high- and low-input farming systems is unsustainable because P (unlike other plant nutrients) is a finite resource, with economically viable sources of rock phosphate likely to be depleted within a century (Steen 1998). This coupled with reduced subsidy and increasing cost of phosphatic fertilizers in major rice growing countries like India is resulting in significant reduction in application of P fertilizers in the recent years. A major part of rice-growing soils in India have moderate to severe P deficiency (Dobermann and Fairhurst 2000). This is accentuated by the fact that a significant area under rice, viz., uplands and acid soils, have a high P–fixing capacity, resulting in reduced availability of P in soils and hence reduced yields (Vance et al. 2003). Even, in irrigated conditions, in the recent years, due to increasing cost of P fertilizers, their application has reduced significantly, resulting in low to moderate deficiency of the nutrient and lesser crop yields.

Fortunately, in rice crop, there is significant genetic variability for low soil P tolerance (Fageria et al. 1998) and a major quantitative trait locus (QTL) named *Pup1* and several minor QTLs, associated with tolerance have been identified (Wissuwa et al. 1998). *Pup1*, originally identified from the Indian upland rice genotype, Kasalath, is associated with P uptake efficiency and it was characterized to increase the root growth and biomass under low soil P conditions significantly (Heuer et al. 2009; Wissuwa et al. 2002). The QTL has since then been fine-mapped and cloned (Gamuyao et al. 2012) and closely linked and functional markers are available for marker-assisted selection (Chin et al. 2011; Gamuyao et al. 2012).

MTU 1010 (also known as Cotton Dora Sannalu) is one of the very popular mega-rice varieties of India, which is extensively cultivated in many rice-growing states during both wet and dry seasons (http://www.rkmp.co.in/content/mtu-1010) due to its high yield, short duration, and desirable long slender grain type. However, despite these features, the variety is highly susceptible to low soil P (Veronica et al. 2017), which is prevalent in many upland and irrigated rice growing areas in India (http://www.rarstpt.org/files/rars/package%20of%20practices/Rice.pdf). Considering the above mentioned points, in the present study, we attempted to improve MTU 1010 for tolerance to low soil P, by targeted introgression of *Pup1* into the mega-variety through marker-assisted backcross breeding.

## Materials and methods

### Plant material

Swarna (also known as MTU 7029), a mega-variety of India, developed and released from the cross Vasishta/Mahsuri by Andhra Pradesh Rice Research, Station of Acharya N.G. Ranga Agricultural University (ANGRA), Maruteru, Andhra Pradesh State, India, was used as the donor parent for *Pup1* in the present study. Swarna has dark green foliage, high tillering ability, semi-dwarf plant stature, and high-yield, and has tolerance to many biotic and abiotic stresses. It has shown good tolerance to low soil P, when screened in low P plot of ICAR-IIRR (ICAR-Indian Institute of Rice Research), Hyderabad, India, and later we confirmed that it possesses the major QTL associated with tolerance, i.e., *Pup1* based on analysis with functional markers reported earlier for *Pup1* (Chin et al. 2011, Plant Physiology 156: 1202–1216). In specific, Swarna possessed the Kasalath allele of the PsTOL kinase gene of *Pup1* (Pariasca-Tanaka et al. 2014). MTU 1010 (also known as Cotton Dora Sannalu), an elite, high-yielding, short duration, widely cultivated mega-variety possessing long slender grain type, was developed and released by Acharya NG Ranga Agricultural University (ANGRAU), India. MTU1010, which is highly

sensitive (i.e., susceptible) to low soil P, was used as the recurrent parent.

### Marker-assisted backcross breeding strategy for transfer of *Pup1*

MTU 1010 was crossed with the donor parent, Swarna, and true $F_1$s were identified with the help of the co-dominant CAPS marker, K20-2 (Chin et al. 2011). They were crossed with the recurrent parent MTU 1010 to develop $BC_1F_1$s. The heterozygosity of $BC_1F_1$ plants with respect to *Pup1* was analyzed with K20-2 and reconfirmed with K46-1, a dominant marker reported by Chin et al. (2011) (i.e., foreground selection). The positive $BC_1F_1$ plants, thus identified, were then screened with two parental polymorphic SSR markers, RM28011 and RM28157, which are located at a physical distance of ~ 1.5–2.0 Mb from *Pup1* locus on either side to identify the backcross plants possessing recombination on one side and on both sides of *Pup1* locus (i.e., recombinant selection). Such plants were then screened with parental polymorphic SSR markers ($n = 85$) which were distributed covering almost the entire chromosome length of all the 12 chromosomes (Supplementary figure-1) and are listed in Supplementary table-3 through background selection to identify those positive plants, which have maximum recovery of the recurrent parent genome as described in Sundaram et al. (2008). The process of marker-assisted backcross breeding (MABB), involving foreground, recombinant, and background selections, was repeated until $BC_2F_1$ generation, wherein a plant possessing *Pup1* in heterozygous condition, with recombination happening on both side of *Pup1* and possessing maximum recovery of MTU 1010 genome, was identified and selfed to obtain $BC_2F_2$s. They were then analyzed with the co-dominant CAPS marker K20-2 and dominant marker 46-1 to identify plants homozygous for *Pup1*. The homozygous plants were then advanced through pedigree method until $BC_2F_6$ generation. DNA isolation from the parents, $F_1$s, and backcross-derived plants was done using a Miniprep protocol (Zheng et al. 1995). PCR protocol described in Chin et al. (2011) was adopted for the markers K20-2 and K46-1, while for the amplification of rice SSR markers, the protocol described in Sundaram et al. (2008), was followed. The amplified products of K20-2 and K46-1 were electrophoretically resolved in 2% and 1.2% Seakem LE agarose gel (Lonza, USA), while the SSR markers were resolved on 3.5% agarose gels. Bsp1286I restriction enzyme (New England Biolabs, UK) was used for digestion of K20-2 PCR product (as described in Chin et al. 2011). The details of the markers used in the study for foreground and recombinant selection are given in Supplementary table-2.

### Phenotypic screening of backcross-derived lines of MTU1010 in low soil P and normal soil P plots

Five $BC_2F_6$ selected lines were grown in normal soils and transplanted at 30-day-old seedling stage into low P (low soil phosphorus <3 ppm tested using Olsen P method (Olsen et al. 1954)) plot of ICAR-IIRR, Hyderabad, India, at a spacing of 15 × 20 cm (in three rows, 10 hills per row) along with the donor and recurrent parents and grown until maturity. The plants were also grown in plots (in two rows, 20 hills per row, i.e., 40 hills per entry) with normal soil P (~ 18.3 ppm) for comparison of performance.

Standard agronomic practices were followed to raise a healthy crop in both normal and low P plots. Pre-harvest agromorphological character days to 50% flowering (DFF), plant height (PH), number of tillers per plant (NT), number of productive tillers (NPT), flag leaf length (FLL), flag leaf width (FLW), panicle length (PL), shoot length (SL), root length(RL), and root volume (RV) constitute the root architecture. To measure the root length (cm), plants were uprooted carefully, without causing any damage to the root system and the roots were washed in running water to remove the soil particles. The average root length was measured from crown of the root to tip of the root, while the root volume was measured by using water displacement method, wherein the cleaned roots were placed in a measuring cylinder containing water and the rise in the level of water in the measuring cylinder was calculated by subtracting the final volume (level of water after placing the root in the measuring cylinder) from the initial volume (level of water before placing the root in the measuring cylinder) and expressed as root volume in milliliters. Post-harvest agro-morphological characters such as yield per plant, number of grains per panicle, grain type, 1000-seed weight, dry straw weight, and dry root weight were recorded.

## Results

A total of 32 true $F_1$ plants were identified and used as male parent for backcrossing with MTU1010 to obtain $BC_1F_1$ plants (Supplementary Table 1). A total of 145 $BC_1F_1$ plants were subjected to foreground selection and 52 of them were identified to be heterozygous for *Pup1* locus (Supplementary figure 3a). They were later subjected for recombinant selection using two rice SSR markers flanking to the *Pup1* QTL, viz., RM28011 and RM28157. A total of 12 plants showed recombination at one end and none of the plants displayed recombination on both the sides of *Pup1*. Among them, a single $BC_1F_1$ plant, viz., RP5972, possessing maximum recovery of the recurrent parent genome for MTU 1010 parent (~ 74%; Supplementary fig. 2a) was identified through background selection using 85 parental polymorphic SSR markers and backcrossed with MTU 1010 to produce $BC_2F_1$s. Details of foreground, recombinant, and background selection at $BC_1F_1$ and $BC_2F_1$ generation are given in Supplementary table 1. A single $BC_2F_1$ plant, viz., RP5972-13, possessing *Pup1* along with recombination at both ends (Supplementary figure 3b) and maximum recurrent parent genome recovery of 89.4% (Supplementary figure 2b) was identified and selfed to produce $BC_2F_2$s. When they were analyzed with the marker K20-2, a total of 237 $BC_2F_2$ plants were identified to be homozygous for *Pup1* locus and these plants were reconfirmed for presence of *Pup1* using the functional marker, K 46-1 (Fig. 1). A total of 22 homozygous $BC_2F_2$ plants, which were similar to MTU 1010, were analyzed for background genome recovery; it was observed that they possessed a recovery of MTU1010 in the range of 91.6–92.4% and the best homozygous $BC_2F_2$ plant had a recovery of 92.4% (RP 5972-13-1). Among them, a total of ten, observed to be quite similar to MTU 1010 in terms of phenotypic traits (including RP 5972-13-1), were advanced through pedigree method of selection through selfing.

At $BC_2F_6$, a set of five breeding lines of MTU 1010, possessing *Pup1* (viz., RP5972-13-1-6-67-129-21, RP5972-13-1-6-67-129-36, RP5972-13-1-6-67-129-57, RP5972-13-1-6-67-129-266, and RP5972-13-1-6-67-129-268), which were identical to MTU 1010 in terms of agro-morphological traits and grain quality, and possessing recurrent parent genome recovery ranging from 92.2 to 95.2% (Fig. 2), were evaluated in a plot having optimum soil P level and also in the low soil P plot of ICAR-IIRR, Hyderabad, during Kharif 2016 (Tables 1 and 2, Fig. 3). A majority of the breeding lines of MTU 1010, containing *Pup1*, showed better performance as compared to the original parent in low P soil. The root architecture of the $BC_2F_6$ selected lines showed considerable positive difference in comparison with that of MTU 1010 under low soil P. The five selected $BC_2F_6$ lines were observed to have significant increase in root length and root volume as compared to the original parent, MTU1010, in low soil P plot, while the lines were identical or slightly better than the recurrent parent in normal P plot. The yield of the lines showed an increase of > 390% as compared to MTU 1010 with better performance in most of the agro-morphological traits in low soil P plot with the line # RP5972-13-1-6-67-129-266 showing significantly increased yield in

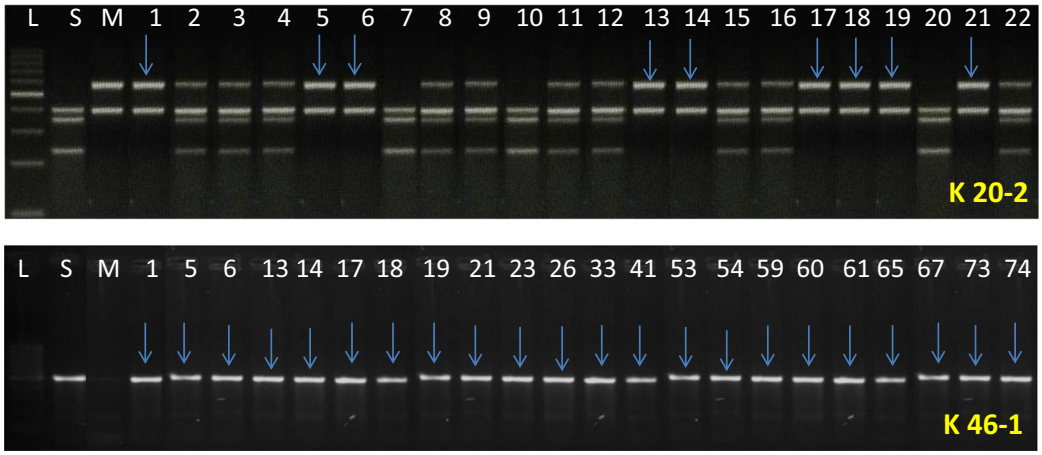


**Fig. 1** Foreground selection of $BC_2F_2$ individuals derived from the cross MTU1010 × Swarna with co-dominant marker K20-2 and reconformation with K46-1 dominant marker

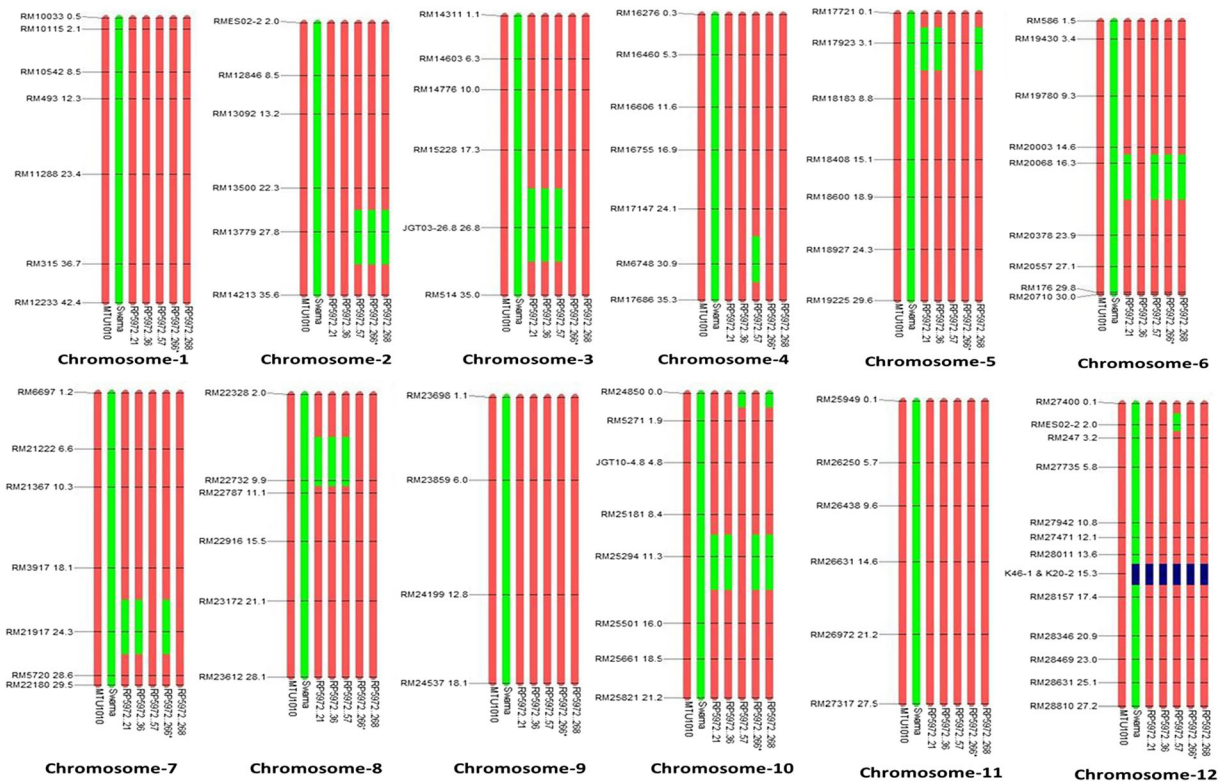


**Fig. 2** Graphical representation of background genome recovery in five selected $BC_2F_6$ lines

both the plots as compared to MTU 1010 with the line showing better root- and shoot-related parameters. Further, it was observed to have the highest recurrent parent genome recovery of ~ 95.2% (Supplementary fig. 2c).

## Discussion

MTU 1010 is a very popular mega-rice variety in India and is estimated to be cultivated in an area of > 3 Mha. Even though the area under MTU 1010 has significantly increased over the last few years, principally due to its high yield and shorter duration, its spread to upland conditions has been limited as it is highly vulnerable to low soil P stress in the vegetative stage. Under low soil P, upland conditions, MTU1010, has reduced production of root hairs, thus affecting the uptake of plant nutrients and this has an effect on the plant height, tillering capacity, flowering, and grain filling capacity (Aaker et al. 1980 book chapter-8 of Rice production by peace corps). We therefore attempted to improve MTU 1010 for its tolerance to low soil P by targeted introgression of *Pup1*, a major QTL for enhanced phosphorous uptake from soil. *Pup1* was originally identified from the Indian Aus rice variety, Kasalath, (Wissuwa et al. 1998, 2002) and fine-mapped to a 90-kb region on Chr. 12 with the help of molecular markers using a RIL population derived from the cross Kasalath/Nipponbare (Heuer et al. 2009). After fine-mapping, *Pup1* was later cloned and a gene encoding a protein kinase, which has been named phosphorus starvation tolerance gene 1(*OsPSTOL1*), was identified to be the candidate for *Pup1* (Gamuyao et al. 2012). *PSTOL1* acts as an enhancer of early root growth (Gamuyao et al. 2012), thereby enabling plants to acquire more phosphorus and other nutrient and the gene gets activated when soil nutrients are limiting. Interestingly, closely linked co-dominant markers and dominant functional markers have been developed for *Pup1*, after it was cloned (Pandit et al. 2016). Two such markers, viz., K20-2 and K46-1 (Chin et al. 2011), were used in the present study for targeted transfer of *Pup1* into the genetic background of MTU 1010 through the strategy of MABB.

Using tightly linked flanking *Pup1* locus, Chin et al. (2011) screened a considerable number of diverse rice

**Table 1** Agromorphological characters of selected $BC_2F_6$ plants recorded in a plot with optimum soil phosphorus.

| S. no | Genotype | Days to 50% flowering | Plant height (cm) | Number of tillers per plant | Number of productive tillers | Root length (cm) | Root volume (ml) | Panicle length (cm) | 1000 seed weight (g) | Grain yield per plant (g) | Grain type |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Swarna (donor) | 117 | 66.1 ± 1.37 | 15.0 ± 0.33 | 15.0 ± 0.33 | 29.1 ± 0.69 | 68.6 ± 1.13 | 21.4 ± 0.06 | 19.9 ± 0.27 | 30.6 ± 0.53 | MB |
| 2 | MTU 1010 (susceptible parent) | 96 | 71.8 ± 0.43 | 13.0 ± 0.33 | 13.0 ± 0.33 | 26.2 ± 0.59 | 55.1 ± 0.98 | 20.5 ± 0.58 | 24.2 ± 0.23 | 32.1 ± 0.47 | LS |
| 3 | RP5972-13-1-6-67-129-21 | 92 | 76.4 ± 0.29 | 17.0 ± 0.33 | 17.0 ± 0.33 | 29.5 ± 0.52 | 68.7 ± 1.07 | 23.8 ± 0.38 | 23.7 ± 0.06 | 32.2 ± 0.20 | LS |
| 4 | RP5972-13-1-6-67-129-36 | 87 | 78.9 ± 0.29 | 18.0 ± 0.33 | 18.0 ± 0.33 | 29.1 ± 0.27 | 71.1 ± 0.81 | 22.3 ± 0.20 | 24.5 ± 0.21 | 33.6 ± 0.46 | LS |
| 5 | RP5972-13-1-6-67-129-57 | 89 | 77.7 ± 0.17 | 19.0 ± 0.58 | 19.0 ± 0.58 | 29.2 ± 0.49 | 70.7 ± 0.49 | 23.5 ± 0.55 | 24.4 ± 0.33 | 32.1 ± 0.26 | LS |
| 6 | RP5972-13-1-6-67-129-266* | 90 | 79.4 ± 0.09 | 20.0 ± 0.33 | 20.0 ± 0.33 | 31.7 ± 0.64 | 71.5 ± 0.55 | 24.1 ± 0.54 | 25.5 ± 0.21 | 36.7 ± 0.27 | LS |
| 7 | RP5972-13-1-6-67-129-268 | 94 | 78.3 ± 0.21 | 18.0 ± 0.33 | 18.0 ± 0.33 | 29.4 ± 0.90 | 70.6 ± 0.46 | 23.7 ± 0.24 | 25.2 ± 0.19 | 34.2 ± 0.20 | LS |
| | C. V. value | | 2.50 | 4.08 | 4.08 | 2.74 | 3.28 | 2.79 | 1.14 | 3.64 | |
| | C. D. @ 5% | | 2.74 | 2.40 | 2.40 | 2.95 | 7.60 | 2.51 | 0.99 | 4.22 | |

All the *Pup1* lines in the background of MTU1010 showed equivalent or better performance as compared to their original parent in normal soil P plot (> 18.3 ppm) of IIRR, Hyderabad, India. The line RP5972-4-1-6-129-266 (*Pup1* line in the genetic background of MTU1010) was observed to show better performance than MTU1010. The line have been indicated by an asterisk (*) mark

**Table 2** Agromorphological characters of selected $BC_2F_6$ plants recorded in Low soil P plot of IIRR, Hyderabad

| S. no | Genotype | Days to 50% flowering | Plant height (cm) | Number of tillers per plant | Number of productive tillers | Root length (cm) | Root volume (ml) | Panicle length (cm) | 1000 seed weight (g) | Grain yield per plant (g) | Grain type |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Swarna (donor) | 133 | 55.6 ± 0.72 | 8.0 ± 0.58 | 8.0 ± 0.58 | 29.5 ± 0.92 | 27.7 ± 1.67 | 16.1 ± 1.48 | 17.9 ± 0.49 | 15.2 ± 0.20 | MB |
| 2 | MTU1010 (susceptible parent) | 109 | 65.3 ± 0.59 | 5.0 ± 0.33 | 4.0 ± 0.33 | 18.1 ± 0.15 | 14.5 ± 0.61 | 15.6 ± 0.41 | 23.3 ± 0.53 | 4.2 ± 0.07 | LS |
| 3 | RP5972-13-1-6-67-129-21 | 97 | 76.0 ± 0.48 | 7.0 ± 0.33 | 7.0 ± 0.33 | 31.2 ± 0.23 | 28.3 ± 1.03 | 20.9 ± 0.35 | 23.4 ± 0.17 | 16.3 ± 0.32 | LS |
| 4 | RP5972-13-1-6-67-129-36 | 93 | 78.3 ± 0.61 | 8.0 ± 0.33 | 8.0 ± 0.33 | 29.9 ± 0.15 | 30.0 ± 0.31 | 23.1 ± 0.24 | 24.1 ± 0.36 | 17.1 ± 0.34 | LS |
| 5 | RP5972-13-1-6-67-129-57 | 99 | 73.2 ± 0.41 | 7.0 ± 0.58 | 7.0 ± 0.33 | 30.9 ± 0.17 | 28.3 ± 0.88 | 21.2 ± 0.28 | 23.2 ± 0.21 | 15.9 ± 0.35 | LS |
| 6 | RP5972-13-1-6-67-129-266* | 96 | 79.1 ± 0.29 | 8.0 ± 0.67 | 8.0 ± 0.67 | 32.9 ± 0.23 | 30.6 ± 1.04 | 23.2 ± 0.20 | 25.2 ± 0.23 | 18.0 ± 0.18 | LS |
| 7 | RP5972-13-1-6-67-129-268 | 102 | 78.8 ± 0.23 | 9.0 ± 0.58 | 9.0 ± 0.33 | 32.3 ± 0.32 | 27.7 ± 0.26 | 21.7 ± 0.24 | 24.2 ± 0.20 | 17.2 ± 0.26 | LS |
| | C. V, value | | 3.56 | 6.85 | 9.11 | 8.49 | 7.35 | 3.39 | 2.72 | 5.65 | |
| | C. D. @ 5% | | 8.67 | 1.72 | 2.03 | 8.67 | 6.24 | 2.31 | 1.11 | 2.07 | |

All the *Pup1* lines in the background of MTU1010 showed equivalent or better performance as compared to their original parent in an experimental plot of with low soil P (< 2 ppm) at IIRR, Hyderabad, India. The line RP5972-4-1-6-129-266 (*Pup1* line in the genetic background of MTU1010) was observed to show better performance than MTU1010 in both low P and normal soil P plots. The line have been indicated by an asterisk (*) mark


**Fig. 3** Performance of the *Pup1* plants in the genetic background of MTU1010 in low soil P plot and normal soil P plot. A set of backcross derived lines of MTU1010 possessing *Pup1* were screened in the low soil P plot and normal soil P plot of ICAR-IIRR, Hyderabad, India. **a** Screening of $BC_2F_6$ introgressed lines of MTU1010 in low soil P plot. **b** Comparison of growth of $BC_2F_6$ lines of MTU1010 in normal plot w.r.t to parent MTU1010. The backcross-derived lines were identical to MTU1010 in normal plot. **c** Shoot and root architecture comparison of introgressed lines with that of parental lines in low P plot. The selected backcross–derived lines of MTU1010 possessing *Pup1* were observed to be significantly better than MTU1010. **d** Panicle characteristics of four introgressed lines in comparison with the donor parent-Swarna and recurrent parent—MTU1010. There is a considerable increase in the panicle length and number of grains per panicle in the introgressed lines of MTU1010 and the grains obtained the hull color of the donor parent—Swarna

varieties and breeding lines to assess if the tolerant allele of *Pup1* is widely distributed or confined to a few rice varieties. The analysis established that most of the rice varieties released for/adapted to upland ecosystem had *Pup1* and were showing high degree of tolerance, while most of the varieties released for the lowland and irrigated ecosystem (like MTU 1010) were devoid of *Pup1* and hence are highly sensitive to soils having low P.

Through an earlier study, we identified that one of the very popular, high-yielding, lowland Indian rice variety, Swarna, showed excellent tolerance to low soil P and also possessed the tolerant allele with respect to *Pup1* (Sarkar et al. 2011) when analyzed with a set of markers specific for *Pup1*. Hence, for the present study, we selected Swarna as the donor for *Pup1*, as it is known to be a good general combiner, with very high yield and desirable grain quality along with wider adaptability. The approach of MABB involving foreground, recombinant, and background selection was adopted in the study for quick transfer of *Pup1* into MTU 1010, without any significant changes in the background genome and the number of backcrosses was limited to just two due to a stringent MABB procedure adopted. At each backcross generation, a closely linked co-dominant marker, K20-2, located at a physical distance of 106 kb (~ 1 cM) from *Pup1*, was used for foreground selection in an accurate manner and the plants thus selected were validated with the dominant functional marker, K46-1. Two SSR markers, viz., RM28011 and RM28157, which are located at either ends of *Pup1* genomic region, were used for recombinant selection in order to minimize the linkage drag of the donor parent genome around *Pup1*. Further, we also carried out background selection using a set of 85 parental polymorphic SSR markers for accelerated recovery of MTU 1010 genome in the backcross-derived plants. This was necessary, because, even though Swarna is a good combiner and high yielder, it is a long duration variety with short bold grain type, which is different from the long slender grain type of MTU 1010. As traits like flowering duration (Yano et al. 2001) and grain quality (Sheehy et al. 2001) are determined by multiple genes/QTLs, it was necessary to eliminate unwanted genomic regions of the donor parent and a stringent recombinant selection and

background selection done using markers helped us in recovering desirable attributes like early flowering, long slender grain type, and high yield of MTU 1010 (with ~ 93% recurrent parent genome recovery) within two backcrosses (Supplementary table 1), thus saving time and resources in the present study. In fact, a set of five selected $BC_2F_6$ lines were observed to possess recovery of MTU 1010 genome to an extent of 93–95%, indicating that the strategy of stringent marker–assisted backcross breeding involving only limited backcrossing (i.e., only until $BC_2$ generation) was successful in the present study. In addition to deploying the co-dominant marker K20-2 for foreground selection in $BC_2F_2$, we also deployed a dominant, functional marker, i.e., K46-1, to reconfirm the selected plants in each backcross generation as we apprehended that K20-2 being a linked marker located at a distance of 106 Kb from *Pup1*, there could be some recombinants between the marker and *Pup1*. Fortunately, we did not notice any recombinants among the selected $BC_2F_2$ plants and we propose that K20-2 can be used for routine foreground selection for *Pup1*, as it is a co-dominant marker and facilitates identification of both homozygous and heterozygous individuals with respect to the gene/QTL.

A set of five breeding lines of MTU 1010, possessing *Pup1* (viz., RP5972-13-1-6-67-129-21, RP5972-13-1-6-67-129-36, RP5972-13-1-6-67-129-57, RP5972-13-1-6-67-129-266, and RP5972-13-1-6-67-129-268), which were identical to MTU 1010 in terms of agro-morphological traits and grain quality, and possessing recurrent parent genome recovery ranging from 92.2 to 95.2%, were evaluated in a plot having optimum soil P level and also in the low soil P plot of ICAR-IIRR, Hyderabad, during Kharif 2016 (Tables 1 and 2, Fig. 3), which have most of the traits similar to or better than the original recurrent parent, their advanced backcross–derived lines were not analyzed for the other genes/QTLs, which might have contributed for significant improvement in few of the traits other than low soil P tolerance.

Under the low soil P condition, significant improvement was found among the *Pup1* introgressed lines in respect to plant height, number of productive tillers, root length, root volume, panicle length, and grain yield per plant in comparison to the recipient parent MTU 1010. Under normal soil P plot, there was no significant difference found in respect MTU 1010 and *Pup1* introgressed lines for days to 50% flowering, plant height, and thousand grain weight, but we noticed significant difference in respect to number of productive tillers, root volume, and root length in most of the lines. Improvement in root traits and yield related traits under low P condition can be principally attributed to *Pup1*, as it is responsible for production of more root under low P condition, which in turn helps in improving the P uptake efficiency, leading to significantly better yield under low P condition. Under the normal condition, improvement in root traits is also due to *Pup1*, as Gamuyao et al. (2012) reported that its behavior is partially independent. A single line # RP5972-13-1-6-67-129-266 performed better with respect to number of productive tillers per plant, panicle length, root length, root volume, and grain yield per plant in both normal soil P and low soil P plots. Interestingly, this line possessed a recurrent parent genome recovery of 95.2% with a donor genome introgression of < 5% (Fig. 2). This line was also found to be earlier in flowering than recurrent parent MTU 1010 by 6 days under normal soil P plot and by 13 days under low soil P plot. The earliness in flowering could be useful to farmers by saving input resources and also helps in fitting this new genotype in diverse cropping system. Due to its superior performance, it has been nominated for multi-locational trials. Two other lines, viz., RP5972-13-1-6-67-129-36 and RP5972-13-1-6-67-129-268, which were having a recurrent genome recovery of 94.1% and 93.9%, respectively, also performed well in both low P and normal P soils and these three lines have been nominated for multi-location trials across India. The major objective of this study was to transfer of *Pup1* to MTU 1010. With the development of *Pup1* containing lines of MTU 1010, which exhibited better performance as compared to the original parental lines in the experimental plot at ICAR-IIRR, Hyderabad, having low soil P (< 2 ppm), we have achieved the principal objective of the study. Further, line(s) possessing *Pup1*, showing better performance as compared to MTU 1010 under both normal and low P soils, have also been developed in this study. The improvement of tolerance to low soil P in such lines can be helpful to increase the acreage of elite mega-varieties like MTU 1010 in marginal (i.e., problematic) soils with low P and can assist the resource-poor farmers in increasing their income. Upland farmers growing MTU 1010 possessing *Pup1* will certainly benefit due to the robust root system architecture of *Pup1* plants of MTU 1010, developed through this study. Further, breeding lines of MTU 1010 possessing *Pup1* can also serve as good donors for

targeted transfer of the major QTL to other elite rice varieties and can be helpful to reduce the amount spent on subsidy of phosphatic fertilizers in India.

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