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Molecular marker and phenotypic analyses for low phosphorus stress tolerance in cultivars and landraces of upland rice under irrigated and drought situations

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Abstract

Low available phosphorous (P) is a major problem for upland rice cultivation. *Pup1*, a major QTL responsible for efficient P uptake is very important for upland rice. The present investigation aimed to identify rice genotypes having *Pup1* QTL, thereby showing better P use efficiency. Ninety six upland rice cultivars and landraces were screened of which 46.88% showed presence of the *Pup1* QTL. Seventy six genotypes accounting for 79.17% of total population showed probable presence of *PSTOL1* gene. The cluster analysis distinguished the irrigated, upland and aerobic genotypes forming different sub-branches. The genotypes Dinoroda, N22, Bowdel, Tepiboro, Bamawpyan, Karni, Lalsankari, Hazaridhan, Surjamukhi and Kalinga 3 being positive for all the molecular markers considered in the study formed a distinct branch in the tree. The phenotyping study also confirmed the usefulness of the *Pup1* positive genotypes. The *Pup1* positive genotypes showed better P-uptake than the *Pup1* negative genotypes. More P-uptake was observed in the genotypes Bowdel, Lalsankari, Karni, N22, Tepiboro, Dular and Surjamukhi as compared to Kasalath taken as the positive check. But the genotypes that showed promisingly/significant better uptake than Kasalath were Surjamukhi (irrigated P normal); Karni (irrigated P deficient); Lalsankari (drought P normal); Tepiboro, N22, Karni and Lalsankari (drought P deficient). A general correspondence of irrigated situation showed decreasing trend of P-uptake in P-deficient condition as compared to P-sufficient condition. The genotypes Surjamukhi, Karni, Lalsankari, Tepiboro, N22 and Bowdel can be considered as donors to be used in marker-assisted breeding programs for incorporation of *Pup1* QTL into high yielding popular varieties to increase their phosphorus uptake efficiency.

Key words: *Pup1*, *PSTOL1*, phosphorus deficiency, donor identification, upland rice

Introduction

Rice, being the major carbohydrate source of daily dietary intake for more than half of the human population all over the world, is considered to be the most important food crop. The human population is continuously growing with a rapid rate and expected to cross the margin of 9 billion by the middle of twenty-first century. Phosphorous (P) is major macronutrient required for plant growth and development as it forms the major component in ATP, the energy currency of the cell. P deficiency is observed in soils those are highly weathered, acidic and inherently low in P (Hammond et al. 2004). Ten to twenty percent of the phosphatic fertilizers applied are available to the rice plant whereas the rest of the phosphorous gets fixed to the soil (Wissuwa et al. 1998). To meet the future food grain target, rice production need to be increased even from the drought-prone areas with a hike of 40% (Penisi 2008). Worldwide, around 5.7 billion hectares of cultivable land are reported to be P deficient (Baties 1997). Around 80% of the districts in India are with low or medium in phosphorus availability while only 20% districts are with high phosphorus availability (Motsara 2002). The yield limitation of upland rice is mainly due to drought stress, however a common problem of low phosphorus availability in the ecology become a challenging factor for rice breeders. The cost of phosphatic fertilizers is high in India and farmers are not able to provide required quantity of the fertilizers for higher rice yield. Under such condition, the rice genotypes having better efficiency to absorb and utilize

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phosphorous in P deficient soils can help to serve the purpose because adaptation of such varieties/cultivars require neither additional cost nor major changes in cropping system (Aziz et al. 2006). Hence, the high yielding popular varieties can be improved for their P uptake efficiency that can grow and yield better with low P supply. This can be a key to improve rice production.

P uptake in rice has been reported to be governed by a major QTL, *Phosphorous uptake 1 (Pup1)* accounting for 78.8% phenotypic variance. This QTL, located on chromosome 12, contributes towards tolerance to P deficiency and efficient P uptake in low phosphorus soil (Wissuwa and Yano 1998; Wissuwa et al. 2002). *Pup1* QTL consists of 68 genes of which *PSTOL1* gene is identified to be most probable candidate gene for efficient P uptake (Gamuyao et al. 2012). The other genes present within the QTL may have additional supportive function for better P uptake. The near isogenic lines possessing *Pup1* QTL showed enhanced P uptake as well as 2-to 4- fold increase in grain weight per plant (Wissuwa et al. 2002; Heuer et al. 2009). The P-efficient rice varieties with inbuilt *Pup1* developed through marker-assisted breeding programs are very effective in the field trials (Heuer et al. 2009; Chin et al. 2011). Therefore, in the present study a large number of rice genotypes comprising released varieties, landraces, germplasm lines from irrigated, aerobic and upland ecology were screened to identify the presence of *Pup1* QTL through molecular analysis as well as phenotypic confirmation for P uptake.

Materials and methods

Plant materials

The seeds of ninety six genotypes of which majority are upland cultivars and landraces were collected from gene bank of ICAR-National Rice Research Institute, Cuttack and were germinated in tray under controlled condition of RGA-cum-Phytotron facility.

Genomic DNA extraction

Genomic DNA was extracted from the leaves of 20 days old seedlings. The leaf samples were crushed in liquid nitrogen with the help of tissue lyser II (Qiagen). The total genomic DNA was extracted using CTAB buffer (2% CTAB, 100mM Tris-HCl, 20mM EDTA, 1.3M NaCl with pH adjusted to 8.0) along with phenol-chloroform-isoamyl alcohol followed by RNAase treatment and isopropanol precipitation. The DNA was washed with 70% ethanol and dissolved in TE buffer (10mM Tris-HCl, 1mM EDTA). The DNA was quantified by agarose gel electrophoresis using commercial Lambda DNA as standard.

Amplification of Pup1 markers by polymerase chain reaction

The amplification of target markers were performed in a reaction volume of 2μl containing 30ng of genomic DNA, 1 unit of Taq Polymerase, 200μM each of dATP, dCTP, dTTP, dGTP, 4pMole of each forward and reverse primers,1.5mM Tris HCL (pH 8.5), 50mM KCL, 2mM $MgCl_2$, and 0.1% TritonX-100 in a Gradient Thermal Cycler (Veriti, Applied BioSciences). The temperature profile applied to the reaction mixture is as follows: an initial denaturation at 94^{o}C for 4 M followed by 35 cycles of 1 min. denaturation at 94^{o}C, 1 M annealing at 57-59^{o}C, and 1 M extension at 72^{o}C, then final extension at 72^{o}C for 10 M. Two gene specific markers Pup1-K42 and Pup1-K46 along with two closest flanking markers RM28073 and RM28102 were used (Table 1).

Gel electrophoresis and documentation of amplified products

Agarose gel (2.5-3%) containing 0.8mg/ml Ethidium Bromide was used for electrophoresis of the PCR products. 10ml of sample was loaded onto the gel and electrophoresed in 1X TBE (pH 8.0). 50bp DNA ladder was loaded at least to one lane to know the size of the amplicons. Electrophoresis was carried out at 3V/cm for 3.5 h and photographed using a Gel Documentation System (SynGene).

Scoring and analysis of data

The data were scored in a binary matrix on the basis of the presence (1) or absence (0) of amplified products for each genotype-primer combination. The un-rooted tree was constructed following unweighted neighbor joining method with calculated dissimilarity index as described in earlier publications (Pandit et al. 2017; Mohanty et al. 2016; Pandit et al. 2016; Pradhan et al. 2016). The bootstrap value of 1000 was considered to check the robustness of the clustering pattern. The tree was constructed by using FreeTree software (Hampl et al. 2001; Pavalicek et al. 1999) and the tree was visualized by Treeview 32 software (Page 1996).

Phenotyping for phosphorus uptake

A pot culture experiment was conducted in the rainout shelter of ICAR-National Rice Research Institute, Cuttack, India for phosphorus uptake of rice genotypes under four conditions, *i.e.* with normal and deficient phosphorous soil under irrigated and drought situation. The pot culture experiment was laid out in a randomized blocks design with two phosphorus levels. The average P content of random soil sample from the site of upland soil collection was 6.8 mg/kg (P deficient soil). The collected soil samples were supplemented with P to bring it to sufficient level (13.3 mg kg^{-1}). Seven genotypes observed to be positive for both *Dro* and *Pup* QTLs along with the negative and positive checks were used in the pot study using three replications. Nitrogen was applied as three equal splits *viz.,* basal, active tillering and panicle initiation and full dose of phosphorus and potassium applied as basal application. Seeds were direct seeded in the pots and after germination thinning was done to maintain two seedlings per pot. Vegetative drought stress was applied at 35 days after sowing and plant samples were collected at –60kPa pascal soil moisture tension and normal situation. The collected samples were dried in a hot air oven at 80°C and the dry weight was recorded. The oven dried plant materials were chopped and grounded in a Willey mill and stored in wide-mouthed stoppard bottles. After suitable sub sampling, the samples were analyzed for total phosphorus by Vanadomolybdate yellow colour method (Piper 1966).

Results and discussion

Screening of germplasm for presence of Pup1 QTL

Molecular screening was performed to identify better donor lines for efficient phosphorous uptake. Both gene specific as well as flanking markers were used to detect the presence of *Pup1* locus in 96 genotypes (Table 1). Sahabhagidhan and Kasalath were used as positive checks (Heuer et al. 2009; Chin et al. 2010; Tyagi et al. 2012), whereas IR64 and Hazaridhan were taken as the negative checks (Heuer et al. 2009; Ni et al. 1998; Tyagi et al. 2012). RM28073 and RM28102, the two closest markers to *Pup1* QTL (Heuer et al. 2009) and four gene based direct markers Pup1-K41, Pup1-K42, Pup1-K46 and Pup1-K59 (Chin et al. 2010) were used to detect presence of *Pup1* QTL. The genotypes showing positive response for all the six markers employed or positive for the four direct markers and one flanking marker were taken as positive for *Pup1* locus. Based on this criteria, 45 genotypes were observed to be positive for *Pup1* QTL (Table 2). Fifteen genotypes namely Kasalath, Tepiboro, DV123, CSR90, Habigonj Boro6, Bamawpyan, DZ78, Harbhoondi, N22, Dinoroda, Kalchi, Karni, Sekradhan, Bowdel, Lalsankri were positive for all the six markers considered.

Table 1. List of markers used to screen presence of *Pup1* QTL

Markers*	Sequence	Tm (°C)	Gradient range (p C)	Annealing temperature obtained (p C)	Kasalath/ IR64 allele (bp)
Pup1-K41	5'-TGATGAATCCATAGGACAGCGT-3' (F) 5'-TCAGGTGGTGCTTCGTTGGTA-3' (R)	58.21(F) 59.97(R)	55-60	57	382/null
Pup1-K42	5'-CCCGAGAGTTCATCAGAAGGA-3' (F) 5'-AGTGAGTGGCGTTTGCGAT-3' (R)	59.97(F) 57.56(R)	52-57	57	918/null
Pup1-K46	5'-TGAGATAGCCGTCAAGATGCT-3' (F) 5'-AAGGACCACCATTCCATAGC-3' (R)	58.00(F) 57.80(R)	54-59	59	523/null
Pup1-K59	5'-GGACACGGATTCAAGGAGGA-3' (F) 5'-TGCTTTCCATTTGCGGCTC-3' (R)	59.85(F) 57.56(R)	55-60	58	550/null
RM28073	5'-GTGTTGGTGGTGATGAAGCAAGG-3' (F) 5'-GGACGAAGGATGTATGTGTCTGTACC-3' (R)	61.95(F) 63.57(R)	52-57	57	656/600
RM28102	5'-CACTAATTCTTCGGCTCCACTTTAGG-3' (F) 5'GTGGAAGCTCCGAGAAAGTGC-3' (R)	61.99(F) 61.92(R)	52-57	57	168/180

*Chin et al. (2010)

The dominant marker Pup1-K42 with expected amplicon size of 918bp was obtained in 66 genotypes, whereas *Pup1*-K46 being a dominant marker associated directly with the *PSTOL1* gene showed the expected amplicon of 523bp in 78 upland drought tolerant genotypes used in this study (Table 2).

Similarly, 54 and 42 genotypes were positive for Pup1-K41 and Pup1-K59, respectively. Representative electrophoregram of the amplification pattern of the genotypes has been presented in Fig. 1. The positive checks Sahabhagidhan and Kasalath showed the amplification band and IR64, the negative check did not show any amplification in all the gene specific markers used. The genotypes negative for *PSTOL1* gene were Serety, RR347-466, Heera, CR143-2-2, Hasuridhan, Lalnakanda-41, RR348-6, Jogesh, RR-665-645, Vanaprabha, RR160-10, Anjali, RR51-1, CR Dhan 201, CR Dhan 204, Kutiarasi, Kalinga III, Bundei, (Figure 1A, Table 2), while rest all were positive for *PSTOL1* gene. The target Kasalath allele of 656bp was obtained in 58 genotypes by using flanking co-dominant marker RM28073 and the IR64 allele in 29 genotypes (Fig. 1B), but only 24 genotypes showed Kasalath allele with RM28102 (Table 2). Some genotypes, namely, Sahabhagidhan, ARC 12071, Ezi, N22, Sadabahar, RR-2-6, Pyari and CR Dhan 202 exhibited weak bands with *PSTOL1* specific marker based on sequence of *Pup1* donors whereas the genotypes Mandriravina, CSR90, YN1353-3, Karni, Gurujidhan, Muridanra, Raisaria, Laxmikajal and Kasarakunda showed strong bands. Some genotypes showed inconsistency in the amplification pattern when repeated thrice (Table 2).

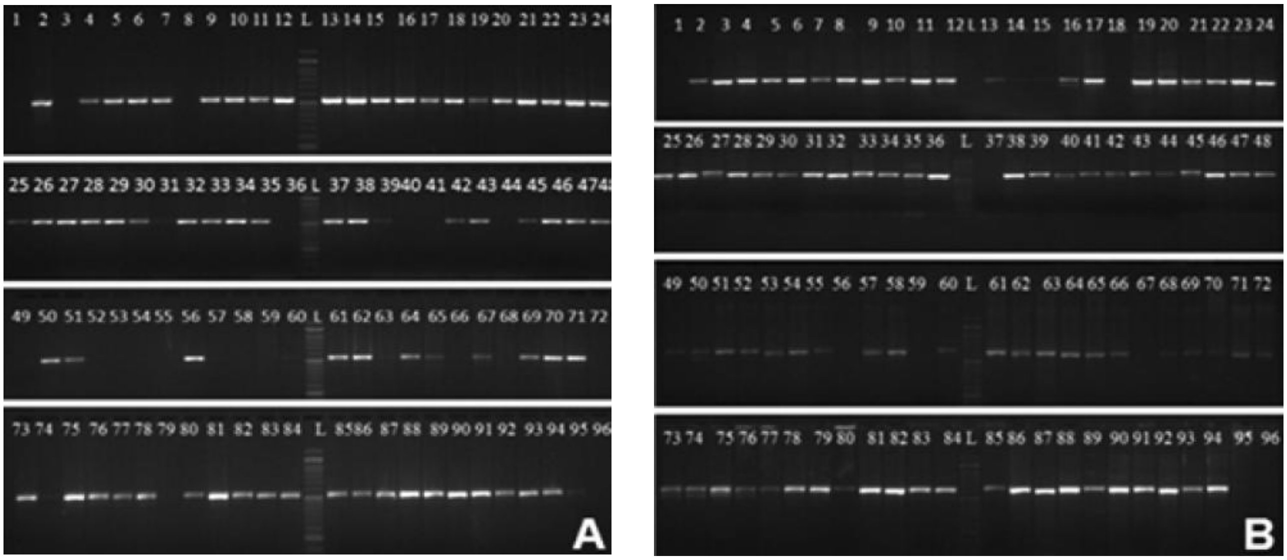

Fig. 1. Representative electrophoregram showing amplification pattern obtained with (A) Pup1-K46 and (B) RM28073. The numbers represent the genotypes listed in Table 1

Similar kind of germplasm survey was carried out for *Pup1* by Heuer et al. (2009) and Tyagi et al. (2012). These reports reveal that genotypes Kasalath, Sahabhagidhan, Dagardeshi, RPCL115, N902, Laljagali, Theruvii, Pynthor, Paijong, Sali, Theke, Pokkali were positive for *Pup1* locus taking Pup1-K41, K42, K43, K46, K48, K52, K59 into account whereas Hazaridhan was negative which is in agreement with the present investigation. Various contradictory results have been presented for presence of *Pup1* locus in N22, a drought tolerant popular upland landrace from Northern India. In the present investigation, we found N22 to be positive for *Pup1* locus which is quite expected for such a tolerant cultivar (Heuer et al. 2009). Many accessions and variants of N22 are available in IRRI and NRRI Gene banks. The disagreement in results of N22 *Pup1* allele may be due to the use of different accessions of N22. The genotypes Dular, Dinoroda, Vandana, FR13A, Way Rarem, Apo and Jalmagna were observed to be positive, whereas Anjali an upland cultivar found negative for *Pup1* locus which was in agreement with earlier reports of Tyagi et al. (2012) and Heuer et al. (2009).

In order to identify donor lines having *Pup1* locus, most of the upland genotypes were considered in the panel population leading to identification of 46.88% of the genotypes to be positive for the locus. Similar germplasm survey showed that large number of upland genotypes possess this QTL whereas it is largely absent in lowland and irrigated varieties. Heuer et al. (2009) reported that 56.2% of *indica* and 50.7% of *japonica* upland varieties possessed the *Pup1* QTL, but very few lowland and irrigated *indica* and *japonica*

Table 2. Markers detected the *Pup1* QTL in the genotypes originated from different geographical regions

S.No.	Genotypes	Description	RM 28073	Pup1-K41	Pup1-K42	Pup1-K46	Pup1-K59	RM 28102
1	SAHABHAGIDHAN	Popular upland rice var., India (I)	⬜	⬜	🟩	🟩	⬜	⬜
2	KASALATH	*Aus* landrace of Bangladesh	🟩	🟩	🟩	🟩	🟩	🟩
3	IR64	Popular midearly var. (I)	⬛	⬛	⬛	⬛	⬛	⬛
4	TEPIBORO	Boro rice cultivar (I)	🟩	🟩	🟩	🟩	🟩	🟩
5	SURJAMUKHI	Landrace of West Bengal (I)	⬛	🟩	🟩	🟩	🟩	⬛
6	FULLKATI	Germplasm from Suwon, Korea	🟩	🟩	🟩	🟩	⬛	⬛
7	BAILAM	Landrace of Chittagong, Bangladesh	🟩	🟩	🟩	🟩	⬛	⬛
8	SERETY	Germplasm from Suwon, Korea	🟩	⬜	⬜	⬜	⬜	⬛
9	BOILAN	Germplasm from Suwon, Korea	⬛	⬛	🟩	🟩	🟩	🟩
10	DV123	Germplasm from IRRI, Philippines	🟩	🟩	🟩	🟩	🟩	🟩
11	BG301	Germplasm from Srilanka	⬛	⬛	🟩	🟩	🟩	⬛
12	MANDRIRAVAN	Germplasm from IRRI, Philippines	🟩	🟩	🟩	🟩	⬛	⬛
13	CSR90	Rice culture from CSR, Karnal (I)	🟩	🟩	🟩	🟩	🟩	🟩
14	YN1353-3	Germplasm from IRRI, Philippines	🟩	🟩	🟩	🟩	⬛	⬛
15	HABIGONJ BORO6	Boro cultivar of Bangladesh	🟩	🟩	🟩	🟩	🟩	🟩
16	BAMAWPYAN	Germplasm from IRRI, Philippines	🟩	🟩	🟩	🟩	🟩	🟩
17	NSICRC 106	Germplasm from IRRI, Philippines	⬛	⬛	🟩	🟩	⬛	⬛
18	HONGZUI ER	Germplasm from IRRI, Philippines	⬜	⬜	🟩	🟩	⬛	⬛
19	ARC12071	Germplasm from Assam (I)	🟩	🟩	🟩	🟩	🟩	⬛
20	BENAMURI	Landrace (I)	⬛	⬜	🟩	🟩	⬜	⬛
21	SATHI	Upland rice landrace (I)	🟩	🟩	🟩	🟩	⬜	⬛
22	DZ78	Germplasm from IRRI, Philippines	🟩	🟩	🟩	🟩	🟩	🟩
23	SUDUWEE	Germplasm from IRRI, Philippines	🟩	🟩	🟩	🟩	⬜	⬛
24	KHAODAW	Rice cultivar from Thailand	⬛	🟩	🟩	🟩	⬜	⬛
25	EZI	Indegineous rice Yunnan, China	⬛	⬜	🟩	🟩	⬜	⬛
26	MADHABSA	Germplasm from Bangladesh	⬛	🟩	🟩	🟩	🟩	🟩
27	ARC 10319	Germplasm from Assam (I)	🟩	🟩	🟩	🟩	🟩	⬛
28	ARC10818	Germplasm from Assam (I)	⬛	⬜	🟩	🟩	🟩	🟩
29	AL LANKE	-	🟩	🟩	🟩	🟩	🟩	⬛
30	HARBHOONDI	Upland germplasm, Rewa, MP (I)	🟩	🟩	🟩	🟩	🟩	🟩
31	HUU SHAO TZU WU	Germplasm from IRRI, Philippines	⬛	🟩	⬜	⬜	🟩	⬛
32	RAY JAZAYKAYZ	Germplasm from IRRI, Philippines	⬛	🟩	🟩	🟩	🟩	🟩
33	AUS 439	Aus landrace, Assam, India	🟩	🟩	🟩	🟩	🟩	⬛
34	RR 272-17	Improved upland culture, Jhar. (I)	🟩	🟩	🟩	🟩	⬜	⬛
35	RAB-56-50	Rice cultivar from Africa	⬛	⬜	🟩	🟩	⬛	⬛
36	HEERA	Upland variety, Odisha (I)	⬛	⬛	⬜	⬜	⬛	⬛
37	DASARAMATIA	Upland rice landrace (I)	⬜	⬜	🟩	🟩	⬛	⬛
38	JALIA	Upland rice landrace (I)	⬛	⬜	🟩	🟩	🟩	🟩
39	N22	Upland landrace from north India	🟩	🟩	🟩	🟩	🟩	🟩
40	HASURIDHAN	Upland rice landrace (I)	⬛	⬛	⬛	⬜	⬜	⬛
41	CR143-2-2	Upland drought tolerant var. CRRI (I)	🟩	⬛	⬛	⬜	⬛	⬛
42	SADABAHAR	Improved upland cultivar, Jhark. (I)	🟩	🟩	🟩	🟩	🟩	⬛
43	RR347-1	Improved upland culture, Jhark. (I)	🟩	🟩	🟩	🟩	⬜	⬛
44	LALNAKANDA-41	Upland drought tolerant donor (I)	🟩	⬛	⬛	⬜	⬜	🟩
45	RR-2-6	Improved upland culture, Jhar. (I)	🟩	🟩	🟩	🟩	⬜	⬛
46	BROWNGORA	Upland landrace, Jhark. (I)	⬛	🟩	🟩	🟩	🟩	⬛

47	DULAR	Upland landrace from Odisha (I)	⬛	🟩	🟩	🟩	🟩	⬜
48	KALAKARI	Upland landrace from Odisha (I)	⬛	⬛	🟩	🟩	🟩	⬛
49	JOGESH	Irrigated rice variety	🟩	⬛	⬛	⬜	⬛	⬛
50	KALINGA-2	Irrigated rice variety	🟩	⬜	⬜	🟩	⬛	⬛
51	DINORODA	Upland rice genotype	🟩	🟩	🟩	🟩	🟩	🟩
52	RR-348-6	Improved upland culture, Jhark. (I)	🟩	⬜	⬛	⬜	⬛	⬛
53	RR-665-645	Improved upland culture, Jhark. (I)	⬛	⬛	⬜	⬜	⬛	⬛
54	ANJALI	Popular upland rice variety	🟩	⬜	⬜	⬜	⬜	⬛
55	VANAPRABHA	Upland rice variety	🟩	⬛	⬛	⬜	⬜	🟩
56	RR20	Irrigated rice genotype	⬜	⬜	🟩	🟩	🟩	🟩
57	RR160-10	Improved upland culture, Jhark. (I)	🟩	⬜	⬜	⬜	⬛	⬛
58	RR 51-1	Improved upland culture, Jhark. (I)	🟩	🟩	🟩	⬜	⬜	🟩
59	RR 347-466	Improved upland culture, Jhark. (I)	🟩	⬛	⬛	⬜	⬛	⬛
60	KHANDAGIRI	Popular upland rice variety, Odisha (I)	🟩	⬛	⬜	⬜	⬜	⬛
61	BLACKGORA	Upland landrace, Jharkhand (I)	🟩	🟩	⬜	🟩	🟩	⬛
62	RR354-1	Improved upland culture, Jhark. (I)	🟩	🟩	🟩	🟩	🟩	⬛
63	RR 272-1745	Improved upland culture, Jhark. (I)	🟩	⬜	⬜	🟩	⬛	⬛
64	SATYABHAMA	Recent upland rice var. Odisha (I)	⬛	⬜	🟩	🟩	⬜	⬛
65	PYARI	Recent aerobic rice var. Odisha (I)	🟩	🟩	🟩	🟩	🟩	⬛
66	CR DHAN204	Recent aerobic rice var. Odisha (I)	🟩	🟩	⬜	⬜	⬜	⬛
67	CR DHAN 202	Recent aerobic, var. Odisha & Jhark. (I)	⬜	🟩	⬜	🟩	⬛	⬛
68	CR DHAN 201	Recent aerobic, var. Chh. & Jhark. (I)	🟩	🟩	🟩	⬜	⬜	⬛
69	KALCHI	Upland landrace (I)	🟩	🟩	🟩	🟩	🟩	🟩
70	SETKA	Upland landrace (I)	🟩	🟩	🟩	🟩	🟩	⬜
71	CHINGERDHAN	Upland landrace (I)	⬛	⬜	🟩	🟩	⬜	⬛
72	BUNDEI	Upland landrace (I)	⬛	⬛	⬜	⬜	⬜	⬛
73	SHELLA	Upland landrace (I)	🟩	⬜	⬜	🟩	⬜	⬜
74	DHOBOSANKARI	Upland landrace (I)	🟩	🟩	⬜	🟩	🟩	🟩
75	KARNI	Upland landrace (I)	🟩	🟩	🟩	🟩	🟩	🟩
76	DENGBOREI	Upland landrace (I)	🟩	🟩	🟩	🟩	🟩	⬛
77	SEKRE	Upland landrace (I)	🟩	🟩	🟩	🟩	🟩	⬛
78	TAWADHAN	Upland landrace (I)	🟩	🟩	🟩	🟩	🟩	⬛
79	KUTIARASI	Upland landrace (I)	🟩	🟩	🟩	⬜	⬜	🟩
80	SEKRADHAN	Upland landrace (I)	🟩	🟩	🟩	🟩	🟩	🟩
81	GURUJIDHAN	Upland landrace (I)	⬛	🟩	🟩	🟩	⬜	⬛
82	KUSUMA	Upland landrace (I)	⬛	⬜	🟩	🟩	⬜	⬛
83	BASARAMATIA	Upland landrace (I)	🟩	🟩	🟩	🟩	⬜	⬛
84	BOWDEL	Upland landrace (I)	🟩	🟩	🟩	🟩	🟩	🟩
85	LALSANKRI	Upland landrace (I)	🟩	🟩	🟩	🟩	🟩	🟩
86	BORI	Upland landrace (I)	⬛	⬜	🟩	🟩	🟩	🟩
87	MENKASALA	Upland landrace (I)	⬛	⬜	⬜	🟩	⬜	⬛
88	MURIDANRA	Upland landrace (I)	🟩	⬜	⬜	🟩	⬜	⬛
89	RAISARIA	Upland landrace (I)	🟩	🟩	🟩	🟩	⬜	⬛
90	LAXMIKAJAL	Upland landrace (I)	⬛	⬛	⬜	🟩	⬜	🟩
91	KASARAKUNDA	Upland landrace (I)	🟩	🟩	🟩	🟩	⬜	⬛
92	PUNEI	Upland landrace (I)	⬛	⬛	⬜	🟩	⬛	⬛
93	DUMERPHULLI	Upland landrace (I)	🟩	🟩	🟩	🟩	⬜	⬛
94	LAHDIL	Upland landrace (I)	🟩	🟩	⬜	🟩	⬛	⬛
95	HAZARIDHAN	Irrigated rice var.Jhar. (I)	⬜	⬜	⬜	🟩	🟩	⬜
96	KALINGA 3	Irrigated rice var.Odisha (I)	⬜	⬜	⬜	⬜	⬜	⬜

Chh. = Chhattisgarh; Jhar. = Jharkhand; 🟩 Positive; ⬛ Negative; ⬜ Inconsistent type varieties showed presence of this QTL. OsPupK46-2 marker was reported to be the candidate gene for *Pup1* and subsequently termed as Phosphorous-starvation tolerance 1 (*PSTOL1*). Over expression of this gene enhances tolerance to P deficiency as well as increase in total root length and surface area (Gamuyao et al. 2012). In the panel population, 79.17% genotypes showed presence of this *PSTOL1* gene, but only 46.88% genotypes showed presence of entire *Pup1* QTL considering presence of all the markers used in the study. These genotypes can be used as donors in future breeding programs to increase the P use efficiency of popular varieties.

Cluster analysis

The un-rooted tree constructed with Nei's method clearly grouped the genotypes having the *Pup1* QTL and the genotypes lacking the QTL (Fig. 2). The genotypes Raisaria, Kasarakanda, Bailam Dengborei, RR2-6, Sadabahar, RR354-1, RR272-17, Suduwee, Aus439, Manidravan, YN1353-3 and Fullkati that were positive for all the markers except RM28102 formed a distinct branch. The genotypes positive for all the markers *i.e.* Surjamukhi, N22, Bowdel, Dinoroda, Karni, Bamawpyan, Kalinga 3, Tepiboro, Hazaridhan and Lalsankari formed another sub-branch. Similarly the genotypes negative for all the markers or positive for only one marker were grouped along with IR64, the negative check. The genotypes like Khandagiri, Jogesh, Vanaprava, RR160-10, Serety, CR Dhan 204, Anjali, Lalnakanda-41, CR143-2-2, RR348-6, Kutiarasi and RR51-1 positive for one marker and showing inconsistency for other three markers formed a distinct group. The upland, upland & lowland and lowland genotypes were classified on the basis of *Pup1* specific markers by Chin et al. (2011). A clear distinction between the groups could be observed in the present study as in case of previous report of Chin et al. (2011). They obtained three distinct groups, one group

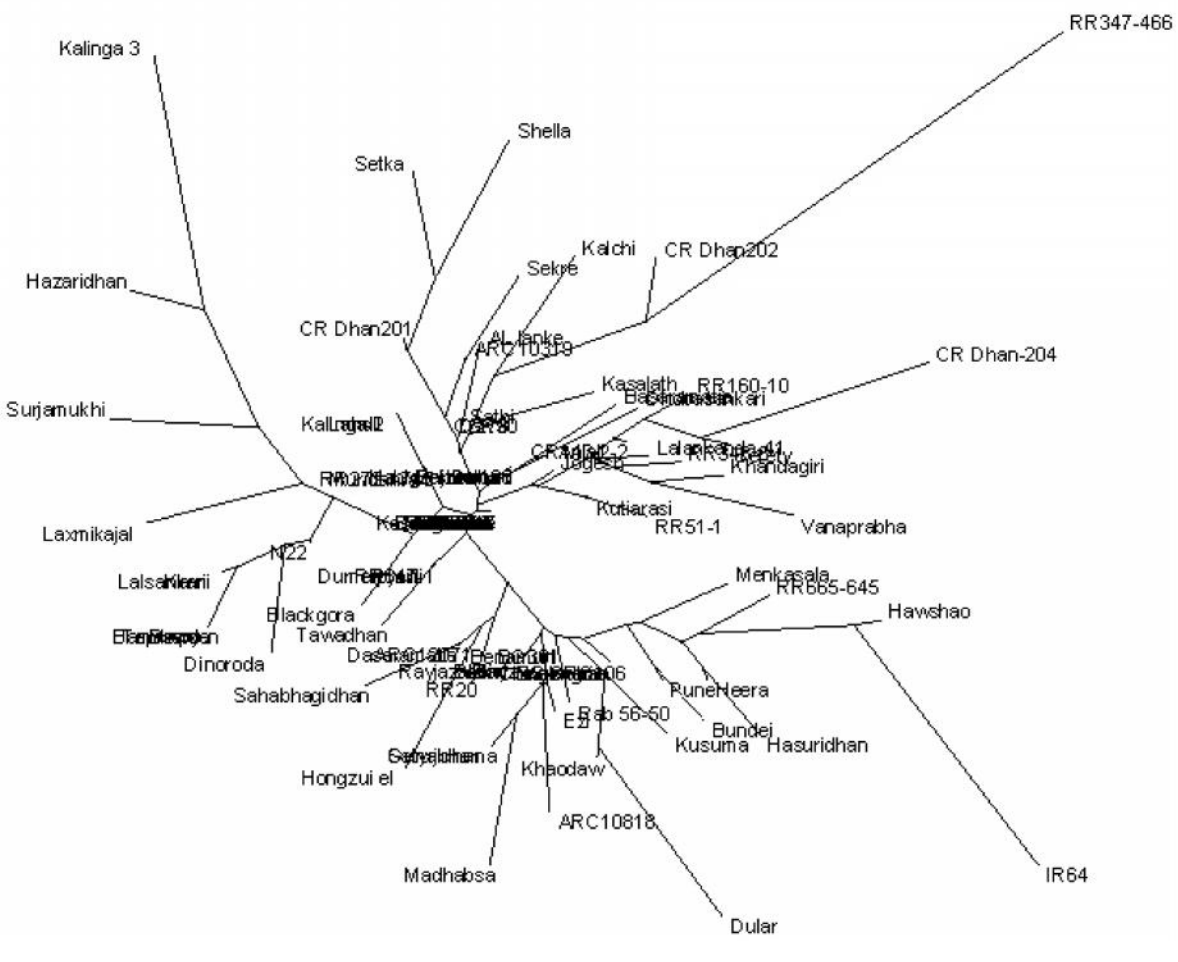

Fig. 2. Unrooted tree illustrating the genetic relationship among the ninety six genotypes with respect to the markers used for *Pup1* QTL

consisted the genotypes positive for most of the *Pup1* markers, another group having genotypes mostly negative for the markers and the third one is an intermediate group. The group with the genotypes having positive response to the markers were mostly upland genotypes and group with negative response to the markers were mostly lowland genotypes. The present cluster analysis could group the upland, aerobic and irrigated genotypes into different subgroups as most of the genotypes were of upland ecology.

Phenotyping for phosphorus uptake in rice

The P uptake ability of the genotypes was studied in pots by using randomized block design in four conditions, *i.e*., irrigated situation with normal/deficient phosphorous and drought situation with normal/deficient phosphorous. Eight *Pup1* positive and four *Pup1* negative genotypes were used for phenotyping study of which six genotypes (Kasalath, Bowde, Lalsankari, Karni, N22, Tepiboro) were positive for all four markers used in the study, whereas two (IR64, Hasuridhan) were negative for all four markers. The genotypes Jogesh and RR-348-6 were negative for three markers and positive for only one flanking marker, whereas Surjamukhi was positive for two gene specific markers and other one (Dular) was positive for the two gene specific markers, inconsistent positive for one flanking marker and negative for another flanking marker. Significant difference in P-uptake was observed among the genotypes under irrigated and drought situation. The P-uptake ranged from 0.096 to 0.385 (Table 3).

Under irrigated situation, there was a decreasing trend of P-uptake in P-deficient condition as compared to P-sufficient condition with an exception of Bowdel, Karni and N22. These genotypes showed increased P-uptake by 2.8, 27.3 and 6%, respectively under P-deficient irrigated situation. Similar trend in P-uptake in P-deficient condition as compared to P-sufficient condition was observed under drought stress where the genotypes Bowdel, Karni, N22 and Tepiboro showed enhanced P-uptake by 29, 2, 8.3 and 3.3%. Bowdel showed better P-uptake efficiency under P-deficient situation both under irrigated and drought situation. This result shows that the lack of available P has impact on the P-uptake of the genotypes both under irrigated and drought condition. Only those which are equipped with the ability to uptake P from the soil can only show better P-uptake as the genotypes, Bowdel, Karni, N22 and Tepiboro possessed the *Pup1* QTL being positive to all the four markers used in the

Table 3. Phosphorous uptake (irrigated and drought stress situation) by the genotypes possessing *Pup1* QTL

Genotypes	Mean P uptake under Irrigated (mg/g dry weight of tissue)		Mean P uptake under drought situation (mg/g dry weight of tissue)		Response to Pup1 QTL
	Phosphorus normal situation	Phosphorus deficient situation	Phosphorus normal situation	Phosphorus deficient situation	
Bowde	0.239	0.246	0.152	0.214	Positive
Lal Sankari	0.270	0.221	0.248	0.193	Positive
Karni	0.280	0.385	0.194	0.198	Positive
N22	0.204	0.217	0.188	0.205	Positive
Tepi Boro	0.283	0.250	0.205	0.212	Positive
Dular	0.234	0.162	0.188	0.182	Positive
Surjamukhi	0.290	0.254	0.169	0.150	Positive
RR-348-6	0.185	0.161	0.121	0.110	Negative
Jogesh	0.186	0.157	0.122	0.112	Negative
Hasuridhan	0.181	0.153	0.116	0.107	Negative
IR64 (Negative check)	0.174	0.146	0.112	0.096	Negative
Kasalath (Positive check)	0.228	0.206	0.172	0.148	Positive
CV%	10.3	12.4	13.2	11.4	
$CD_{5\%}$	0.052	0.056	0.052	0.043	

study. When compared the effect of drought stress under P-sufficient and P-deficient condition, it is evident that drought has negative impact on P-uptake in either conditions. The effect of drought was observed to be severe in *Pup1* negative genotypes as compared to the *Pup1* positive genotypes. Dular performed better when drought stress applied under P-deficient condition as compared to irrigated P-deficient condition. The genotypes positive for only gene specific markers, but negative for flanking ones showed moderate result indicating the additive effect of the entire *Pup1* locus. The positive check Kasalath showed more P-uptake as compared to IR64, the negative check in all situations. More P-uptake was observed in the genotypes Bowdel, Lalsankari, Karni, N22, Tepiboro, Dular and Surjamukhi as compared to Kasalath taken as the positive check. But the genotypes that showed promisingly/significant better uptake than Kasalath were Surjamukhi (irrigated P normal); Karni (irrigated P deficient); Lalsankari (drought P normal); Tepiboro, N22, Karni and Lalsankari (drought P deficient).

The genotypes N22, Dinoroda, Bowdel, Bamawpyan, Tepiboro, Karni, Lalsankari, Surjamukhi, Hazaridhan, and Kalinga 3 that formed distinct cluster and positive for all the markers are promising genotypes. The phenotyping study through representative ones also confirmed the usefulness of the *Pup1* positive genotypes. Hence, the genotypes possessing the *Pup1* QTL can be taken as donor lines to be used in marker-assisted breeding programs for incorporation of the QTL into high yielding popular varieties to increase their phosphorus uptake efficiency.

Authors' contribution

Conceptualization of research (SKP, EP, DRP); Designing of the experiments (SKP, RKP, SS, DRP); Contribution of experimental materials (SKP, DRP); Execution of field/lab experiments and data collection (EP, RKP, RC); Analysis of data and interpretation (EP, RKP); Preparation of manuscript (SKP, EP, SS).

Declaration

The authors declare no conflict of interest.

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