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Survey of rice cultivars and landraces of upland ecology for *Phosphorous uptake 1* (*Pup1*) QTL using linked and gene specific molecular markers

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ABSTRACT

The upland rice ecology is usually low in available phosphorus. A major QTL Pup1 *related to P-uptake is very much important for upland rice. In the present study, ninety six genotypes mainly upland cultivars and landraces were genotyped for better P-uptake. Forty six numbers of genotypes possessed the* Pup1 *locus that accounts for 47.92% of the total genotypes considered. Seventy six genotypes (79.17%) showed probable presence of* PSTOL1 *gene. The cluster analysis could group the upland, aerobic and irrigated genotypes into different subgroups. The genotypes N22, Dinoroda, Bowde, Bamawpyan, Tepiboro, Karni, Lalsankari, Surjamukhi, Hazaridhan, and Kalinga III were positive for two closest flanking and two gene specific* Pup1 *markers and formed distinct cluster. The genotypes possessing the* Pup1 *QTL can be taken as donor lines to be used in marker-assisted breeding programmes for incorporation of the QTL into high yielding popular varieties to increase their phosphorus uptake efficiency.*

Key words: *Pup1; Marker validation; upland rice*

Rice is the most important food crop for almost half of the world's population. The global population is increasing and expected to reach 9 billion by the middle of twenty-first century. The targeted food production need to be increased even from the drought-prone areas with a hike of 40% from this crippled ecology by 2025 (Penisi, 2008). Phosphorous (P) is a major component of energy currency of cell and a macronutrient essential for plant growth and development. Approximately 5.7 billion hectares of aerable land lack sufficient P available for plant and almost 50% of rice soils are P-deficient worldwide (Baties, 1997). Research analysis indicates that 42% districts are with low, 38% districts with medium and 20% districts with high phosphorus availability in India (Motsara, 2002). Low phosphorus availability has been a common problem in upland rice ecology and a challenging factor for rice production. 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. Rice genotypes that are efficient in absorption and utilization of phosphorus under low available form are a great help under this situation. Adaptation of such cultivars with higher nutrient use efficiency is relatively easy, since no additional cost is involved and no major changes in cropping systems are necessary (Aziz *et al.*, 2006). Therefore, the development of P-efficient crop varieties that can grow and yield better with low P supply is a key to improve rice production. In rice, a major QTL *Pup1* located on chromosome 12 exhibiting 78.8 % of the total phenotypic variance for phosphorus uptake has been found to be associated with tolerance to P deficiency and efficient P uptake in low phosphorus soil (Wissuwa and Yano, 1998; Wissuwa *et al.*, 2002). Kasalath, a *Pup1* donor variety has a 278 Kb INDEL and near isogenic lines with the QTL exhibited an

increase P uptake (Wissuwa *et al.*, 2002; Heuer *et al.*, 2009) and also 2 to 4- fold increase in grain weight per plant (Heuer *et al.*, 2009).

The *aus* type of rice varieties, reservoir of many tolerance genes, found in north-eastern states of India (Haefele and Hijmans, 2007; 2009) need to be utilized as donors for various breeding programs. *Aus* type rice genotype 'Kasalath', tolerant to P deficiency was found to possess a major quantitative trait locus (QTL) associated with P-deficiency tolerance (Wissuwa *et al.*, 2002). At present, phosphorus uptake 1 (*Pup1*) is the only P-related QTL available for marker-assisted breeding programs, and tolerant *Pup1* breeding lines have proven effective in field trials (Heuer *et al.*, 2009; Chin *et al.*, 2011).

Hence, the present investigation aims to identify the rice genotypes with *Pup1* QTL through molecular and phenotypic analysis that can be used as donors for developing high yielding varieties under drought stress and phosphorous limitation.

MATERIALS AND METHODS

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

Leaves were collected from 20 days old seedlings to extract genomic DNA for molecular screening. Total genomic DNA was extracted after crushing in liquid nitrogen in microfuge tubes using CTAB extraction buffer (100mM Tris-HCl pH 8, 20 mM EDTA pH 8, 1.3M NaCl, 2% CTAB) and chloroform-Isoamyl alcohol extraction followed by RNAase treatment and ethanol precipitation. Agarose gel electrophoresis was used to estimate DNA concentration using Lambda DNA as standard, and each sample was then diluted to approximately 30ng/µL.

DNA amplification was performed in a Gradient Thermal Cycler (Veriti, Applied BioSciences) with a reaction volume of 20ml containing 1.5mM Tris HCL (pH 8.75), 50mM KCL, 2mM $MgCl_2$, 0.1% TrotonX-100, 200ìM each of dATP, dCTP, dTTP, dGTP, 4pMole of each forward and reverse primers, 1 unit of Taq Polymerase and 30ng of genomic DNA. The reaction mixture was first denatured for 4 mins at 94^0C and then subjected to 35 cycles of 1 min denaturation at 94^0C, 1 min annealing at 57-59^0C, and 1 min extension at 72^0C; and then a final extension for 10 mins at 72^0C. Two closest flanking markers RM28073 and RM28102 and two gene specific markers namely *Pup1*-K42 and *Pup1*-K46 were used to screen for *Pup1* QTLs (Table 1; Chin *et al.*, 2010).

PCR amplification products were loaded in 2.5-3% gel containing 0.8mg/ml Ethidium Bromide for electrophoresis in 1X TBE (pH 8.0). One lane was loaded with 50bp DNA ladder. The gel was run at 2.5V/cm for 4 hrs and photographed using a Gel Documentation System (SynGene).

Data scored were analysed on the basis of the presence or absence of amplified products for each genotype-primer combination. An unweighted neighbor joining un-rooted tree was constructed using the calculated dissimilarity index by using NEI coefficient (Nei, 1972) with bootstrap value of 1000 by using FreeTree software (Hampl *et al.*, 2001; Pavalicek *et al.*, 1999) and the dendrograms were visualized by Treeview 32 software (Page, 1996).

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

Marker name	Sequence	Tm(p C)	Gradient range (p C)	Annealing temperature obtained (p C)	Kasalath/IR64 allele (bp)
Pup1-K42	5'-CCCGAGAGTTCATCAGAAGGA-3' (F) 5'-AGTGAGTGGCGTTTGCGAT-3' (R)	59.97(F)57.56(R)	52-57	57	918/nil
Pup1-K46	5'-TGAGATAGCCGTCAAGATGCT-3' (F) 5'-AAGGACCACCATTCCATAGC-3' (R)	58.00(F)57.80(R)	54-59	59	523/nil
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

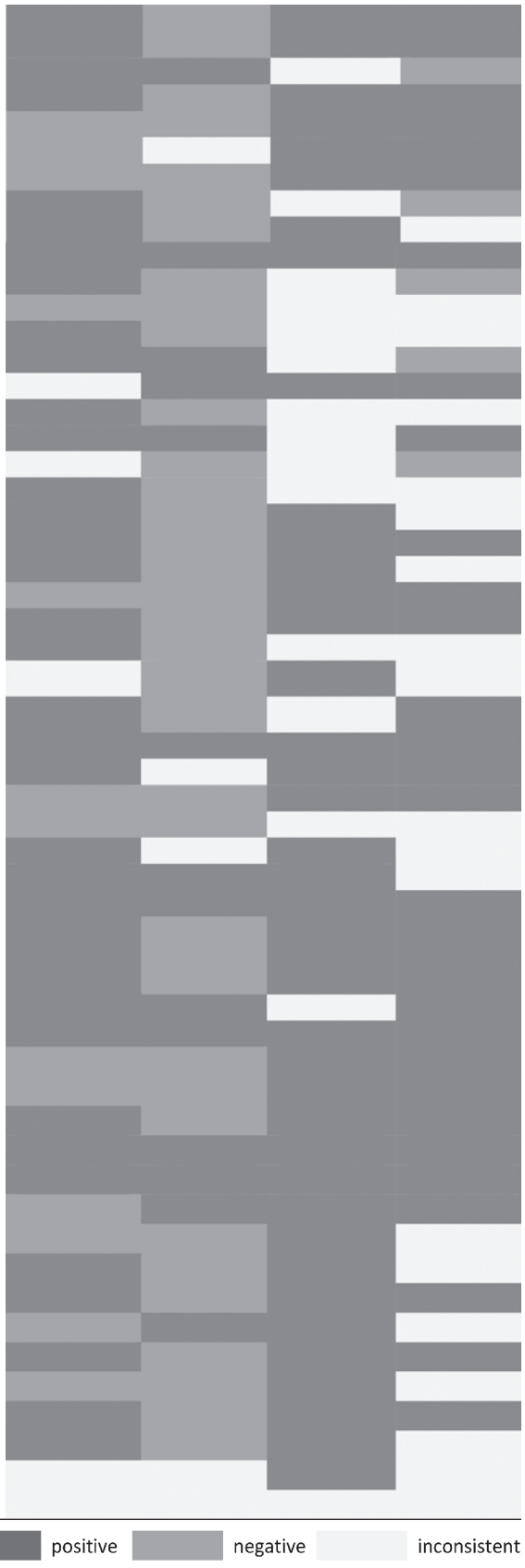
RESULTS AND DISCUSSION

The genotypes were screened to find out better donor lines for efficient Phosphorous uptake. Four markers were used to amplify the *Pup1* locus of the ninety six genotypes including both positive and negative checks (Table 2). The popular variety IR64 and Hazaridhan were taken as the negative check (Heuer *et al.*, 2009; Ni *et al.*, 1998; Tyagi *et al.*, 2012), whereas Kasalath and Sahabhagidhan were used as positive check (Heuer *et al.*, 2009; Chin *et al.*, 2010; Tyagi *et al.*, 2012). Of the four markers considered for the study, the two flanking markers RM28073 and RM28102 were closest markers to *Pup1* QTL (Heuer *et al.*, 2009) whereas the two gene based direct markers *Pup1*-K42 and *Pup1*-K46 were used to detect presence of *Pup1* QTL (Chin *et al.*, 2010). The genotypes showing positive response for all the four markers employed or positive

Table 2. Markers detected for the *Pup1* QTL in the genotypes

Genotypes	Description	RM28073	RM28102	Pup1-K46	Pup1-K42
SAHABHAGIDHAN	Popular upland rice variety of India				
KASALATH	*Aus* landrace of Bangladesh				
IR64	Popular midaearly variety of India				
TEPIBORO	Boro rice cultivar, India				
SURJAMUKHI	Landrace of West Bengal, India				
FULLKATI	Germplasm from Suwon,Korea				
BAILAM	Landrace of Chittagong, Bangladesh				
SERETY	Germplasm from Suwon, Korea				
BOILAN	Germplasm from Suwon, Korea				
DV123	Germplasm from IRRI, Philippines				
BG301	Germplasm from Srilanka				
MANDRIRAVAN	Germplasm from IRRI, Philippines				
CSR90	Rice culture from CSR,Karnal, Haryana				
YN1353-3	Germplasm from IRRI, Philippines				
HABIGONJ BORO6	Boro cultivar of Bangladesh				
BAMAWPYAN	Germplasm from IRRI, Philippines				
NSICRC 106	Germplasm from IRRI, Philippines				
HONGZUI ER	Germplasm from IRRI, Philippines				
ARC12071	Germplasm from Assam, India				
BENAMURI	Landrace from India				
SATHI	Upland rice landrace of India				
DZ78	Germplasm from IRRI, Philippines				
SUDUWEE	Germplasm from IRRI, Philippines				
KHAODAW	Rice cultivar from Thailand				
EZI	Indegineous rice of Yunnan province, China				
MADHABSA	Germplasm from Bangladesh				
ARC 10319	Germplasm from Assam, India				
ARC10818	Germplasm from Assam, India				
AL LANKE	-				
HARBHOONDI	Upland germplasm from Rewa Madhya Pradesh, India				
HUU SHAO TZU WU	Germplasm from IRRI, Philippines				
RAY JAZAYKAYZ	Germplasm from IRRI, Philippines				
AUS 439	Aus landrace, Assam, India				
RR 272-17	Improved upland culture from Jharkhand, India				
RAB-56-50	Rice cultivar from Africa				
HEERA	Upland variety, Odisha, India				
DASARAMATIA	Upland rice landrace from India				
JALIA	Upland rice landrace from India				
N22	Upland landrace from north India				
HASURIDHAN	Upland rice landrace from India				
CR143-2-2	Upland drought tolerant donor from CRRI, Cuttack, India				

SADABAHAR	Improved upland cultivar from Jharkhand, India
RR347-1	Improved upland culture from Jharkhand, India
LALNAKANDA-41	Upland drought tolerant donor from India
RR-2-6	Improved upland culture from Jharkhand, India
BROWNGORA	Upland landrace from Jharkhand, India
DULAR	Upland landrace from Odisha, India
KALAKARI	Upland landrace from Odisha, India
JOGESH	Irrigated rice Variety
KALINGA-2	Irrigated rice variety
DINORADO	Upland rice genotype
RR-348-6	Improved upland culture from Jharkhand, India
RR-665-645	Improved upland culture from Jharkhand, India
ANJALI	Popular upland rice variety
VANAPRABHA	Upland rice variety
RR20	Irrigated rice genotype
RR160-10	Improved upland culture from Jharkhand, India
RR 51-1	Improved upland culture from Jharkhand,India
RR 347-466	Improved upland culture from Jharkhand,India
KHANDAGIRI	Popular upland rice variety of Odisha, India
BLACKGORA	Upland landrace from Jharkhand, India
RR354-1	Improved upland culture from Jharkhand,India
RR 272-1745	Improved upland culture from Jharkhand, India
SATYABHAMA	Recent upland rice variety of Odisha, India
PYARI	Recent aerobic rice variety of Odisha, India
CR DHAN204	Recent aerobic rice variety of Odisha, India
CR DHAN 202	Recent aerobic rice variety of Odisha and Jharkhand, India
CR DHAN 201	Recent aerobic rice variety of Chhattisgarh and Jharkhand, India
KALCHI	Upland landrace of India
SETKA	Upland landrace of India
CHINGERDHAN	Upland landrace of India
BUNDEI	Upland landrace of India
SHELLA	Upland landrace of India
DHOBOSANKARI	Upland landrace of India
KARNI	Upland landrace of India
DENGBOREI	Upland landrace of India
SEKRE	Upland landrace of India
TAWADHAN	Upland landrace of India
KUTIARASI	Upland landrace of India
SEKRADHAN	Upland landrace of India
GURUJIDHAN	Upland landrace of India
KUSUMA	Upland landrace of India
BASARAMATIA	Upland landrace of India
BOWDE	Upland landrace of India
LALSANKRI	Upland landrace of India
BORI	Upland landrace of India
MENKASALA	Upland landrace of India
MURIDANRA	Upland landrace of India
RAISARIA	Upland landrace of India
LAXMIKAJAL	Upland landrace of India
KASARAKUNDA	Upland landrace of India
PUNEI	Upland landrace of India
DUMERPHULLI	Upland landrace of India
LAHDIL	Upland landrace of India
HAZARIDHAN	Irrigated rice variety of Jharkhand, India
KALINGA 3	Irrigated rice variety of Odisha, India



for the two direct markers and one flanking marker were taken as positive for *Pup1* locus. Based on this criteria, forty six out of ninety six genotypes surveyed were positive for *Pup1* QTL (Table 2).

The dominant marker *Pup1*-K42 with expected amplicon size of 918bp was obtained in 66 genotypes (Figure 1), 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 (Figure2; Table 2). The positive checks Sahabhagidhan and Kasalath showed the amplification band and IR64, the negative check did not show any amplification. The genotypes negative for *PSTOL1* gene were Serety, Heera, Hasuridhan, CR143-2-2, Lalnakanda-41, Jogesh, RR348-6, RR-665-645, Anjali, Vanaprabha, RR160-10, RR51-1, RR347-466, CR Dhan 204, CR Dhan 201, Kutiarasi, Bundei and Kalinga III (Figure 2). The Kasalath allele of 656bp was obtained in 58 genotypes by using microsatellite marker RM28073 whereas the IR64 allele in 29 genotypes (Figure 3; Table 2). But the target Kasalath allele of 168bp for RM28102 could be obtained only in 24 genotypes (Figure 4; 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* donor variety, whereas the genotypes Mandriravina, CSR90, YN1353-3, Karni, Gurujidhan, Muridanra, Raisaria, Laxmikajal and Kasarakunda showed strong bands.

Similar kind of germplasm survey had been reported for *Pup1* by Heuer *et al.* (2009) and Tyagi *et al.* (2012). Earlier report of reveals that genotypes Kasalath, Sahabhagidhan, Dagardeshi, RPCL115, N902, Laljagali, Theruvii, Pynthor, Paijong, Sali, Theke, Pokkali were be positive for *Pup1* locus taking *Pup1*-K41, K42, K43, K46, K48, K52, K59 into account (Tyagi *et al.*, 2012). Kasalath and Sahabhagidhan used in the present study showed presence of *Pup1* whereas Hazaridhan was negative which is in agreement with Tyagi *et al.* (2012). The genotype N22 which is a drought tolerant popular upland land race from Northen India was reported to be negative to Kasalath type alleles by (Tyagi *et al.*, 2012). But the present study showed positive for *Pup1* which is quite expected for such a tolerant cultivar (Heuer *et al.*, 2009). There are many accessions and variants of N22 present in IRRI and NRRI Gene bank. Eight accessions of N22 were

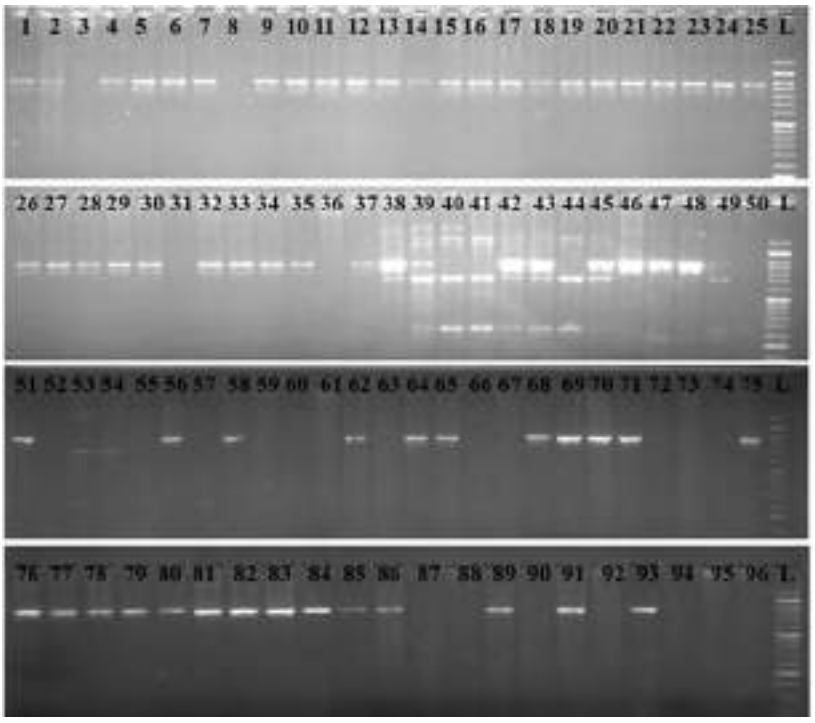


Fig. 1. Amplicons obtained with Pup1-K42. The numbers represent the genotypes listed in Table 2.

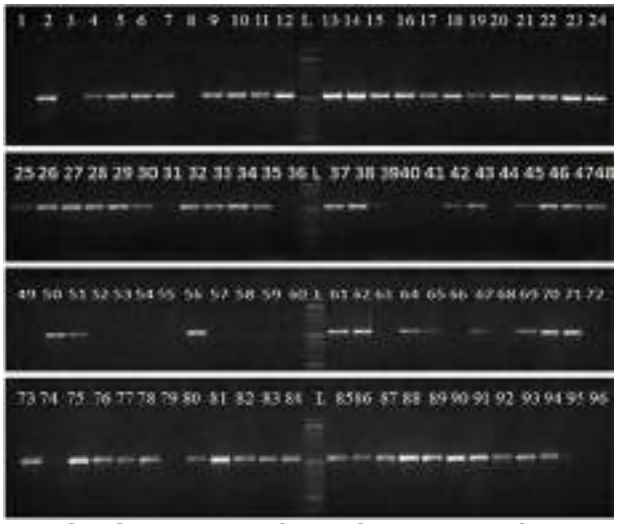


Fig. 2. Amplicons obtained with Pup1-K46. The numbers represent the genotypes listed in Table 2.

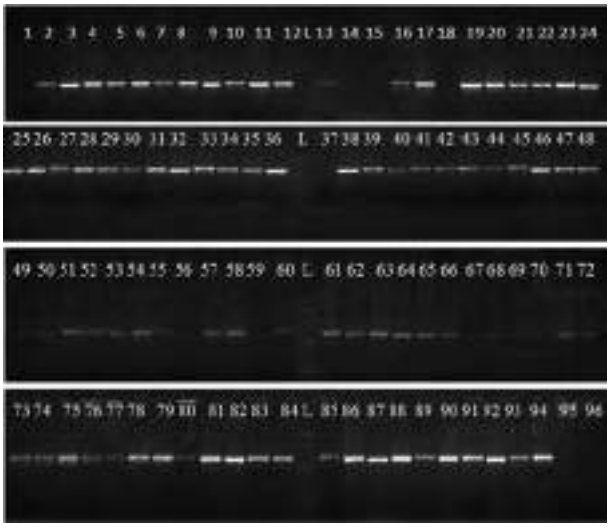


Fig. 3. Amplicons obtained with RM28073. The numbers represent the genotypes listed in Table 2.

registered in the IRRI gene-bank (Heuer *et al.*, 2009). The discrepancy in the results of N22 *Pup1* allele with Tyagi *et al.*, (2012) may be due to different accession of N22 used in the present study. Anjali an upland cultivar found negative for *Pup1* locus which was in agreement with Tyagi *et al.*, (2012) and Heuer *et al.*, (2009). The genotypes Dinorado, Dular, Apo, Vandana, Way Rarem, FR13A and Jalmagna were reported to be positive for *Pup1* (Heuer *et al.*, 2009).

Forty six numbers of genotypes possessed the *Pup1* locus that accounts for 47.92% of the total genotypes considered. Germplasm survey conducted by Heuer *et al.* (2009) also reported that many of the upland varieties i.e. 56.2% of *indica* and 50.7% of *japonica* types possessed the *Pup1* QTL, whereas it was largely absent in lowland and irrigated *indica* and *japonica* type varieties. Out of the markers available for *Pup1*, OsPupK46-2 was reported to be the most obvious candidate gene and named 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). Seventy six genotypes out of ninety six, *i.e.*, 79.17% used in this study showed probable presence of this gene, which was quite obvious as most of the genotypes taken were upland varieties. When

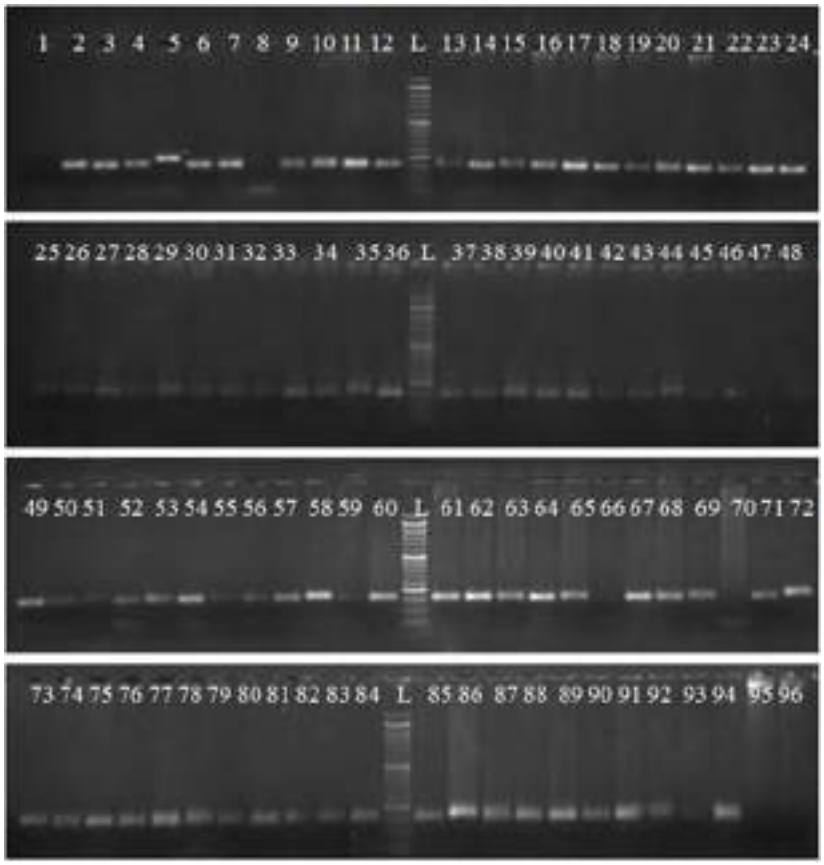


Fig. 4. Amplicons obtained with RM28102. The numbers represent the genotypes listed in Table 2.

the other markers taken along with the *PSTOL1* gene in the study, 46 genotypes were observed to be positive for *Pup1*. These genotypes can be used as donors in breeding programs.

The cluster analysis clearly grouped the genotypes having the *Pup1* QTL and the genotypes lacking the QTL (Figure 5). The genotypes Kasarakanda, Raisaria, Dengborei, RR354-1, RR2-6, Sadabahar, RR272-17, Aus439, Suduwee, YN1353-3, Manidravan, Bailam and Fullkati that were positive for all the markers except RM28102 formed a distinct group. The genotypes positive for all four markers *i.e.* N22, Dinoroda, Bowde, Bamawpyan, Tepiboro, Karni, Lalsankari, Surjamukhi, Hazaridhan, and Kalinga 3 formed another subcluster. Similarly the genotypes negative for all four markers or positive for only one marker were grouped along with IR64, the negative check. The genotypes like Jogesh, RR160-10, CR Dhan 204, Srety, Anjali, CR143-2-2, Lalnakanda-41, RR348-6, Khandagiri, Vanaprava, Kutiarasi and RR51-1 having positive response to one marker and showing inconsistency for other three markers formed a distinct group. Chin *et al.* (2011) classified the upland, upland & lowland and lowland genotypes based on the *Pup1* specific markers. They obtained three distinct groups, one group consisting the genotypes positive for most of the *Pup1* markers, another group having genotypes mostly negative for the markers and another 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. In the present investigation, the genotypes used were mostly of upland ecology. The cluster analysis could group the upland, aerobic and irrigated genotypes into different subgroups.

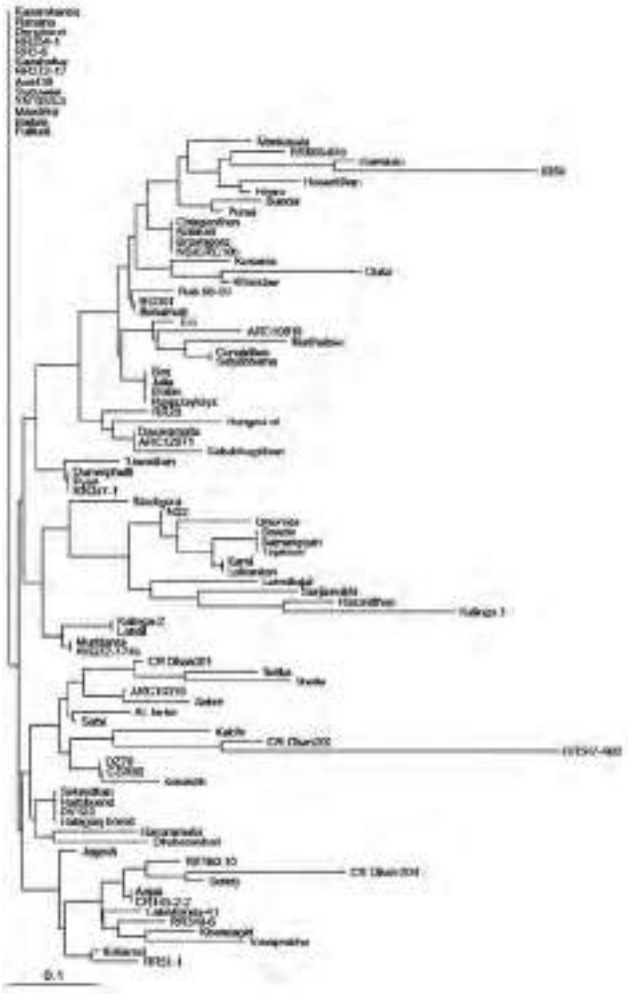


Fig. 5. Phylogram of the ninety six genotypes with respect to the markers used for *Pup1* QTL.

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