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RESEARCH ARTICLE

Identification of *Pup1* QTL linked DNA marker haplotypes for molecular breeding of phosphorous deficiency tolerant rice varieties

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Abstract: The development of phosphorous deficiency (PD) tolerant cultivars is important for rice farming. The DNA markers linked to *Pup1*, the major quantitative trait locus (QTL) for PD tolerance, have been developed using reference rice cultivars *Kasalath* and *Nipponbare*, however these markers cannot be directly employed in regional breeding programmes as specific cultivars that are important to a region/country may contain diverse QTL haplotypes complicating marker assisted breeding (MAB). Thus identification of the *Pup1* linked DNA marker haplotypes present in local cultivars is the prerequisite for MAB of PD tolerance in rice. Therefore, the objective of this study was to identify the *Pup1* haplotypes from a panel of 30 rice cultivars important for breeding programmes in Sri Lanka. A total of 17 *Pup1* linked DNA markers were used to genotype the rice cultivars previously characterised for PD tolerance in comparison to *Kasalath* and *Nipponbare*. Overall 28 *Pup1* haplotypes were identified in the marker allele based clustergram and the *Pup1* hapoltype in *Kasalath* was observed in four PD tolerant cultivars *Sudubalawee*, Bw 364, *Kaluheenati* and *Marss*. However, the PD tolerant cultivars Bg 94-1, Bg 403, H-7, H-4 and *Murungakayan* contain haplotypes that are clustered separately from *Kasalath* indicating the possibility of conferring PD tolerance in the absence of *Kasalath* haplotype. The marker-trait association analysis for the PD tolerance revealed that selection of the favourable alleles such as 433 bp allele of *K46-K2* marker could increase PD tolerance by 0.73. The detected positively associated alleles with PD tolerance can be readily employed in MAB of rice in Sri Lanka.

Keywords: Marker assisted breeding of rice, phosphorous uptake, rice breeding in Sri Lanka, rice landraces.

INTRODUCTION

Phosphorous (P) is the most problematic macronutrient in fertiliser management of rice farming because it forms complexes with Fe^{3+} and Al^{3+} ions and becomes unavailable to the plants (Bieleski, 1973; Holford & Mattingly, 1976; Shen *et al*., 2011). Application of artificial P fertiliser is very expensive and can significantly contribute to environmental pollution (Reddy *et al*., 1999; Cordell *et al*., 2009; Bennett *et al*., 2001). Therefore, the current situation of P fertiliser management in rice farming is at a critical state, thus sustainable, fast acting solutions such as marker assisted breeding (MAB) of P deficiency (PD) tolerant rice varieties are required. MAB (i.e. molecular breeding) is one of the most powerful and the least controversial biotechnological tools available for genetic improvement of plants and animals (Beuzen *et al*., 2000; Collard & Mackill, 2008; Moose & Mumm, 2008). QTL mapping, validation and establishment

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of linked marker haplotypes or alleles, systematically designed breeding programmes and deployment of DNA testing to check the defined haplotypes are the key steps in molecular breeding (Ribaut & Hoisington, 1998; Collard *et al*., 2005).

In order to layout an efficient MAB programme towards PD tolerance, the underlying genetics must be unravelled as the first step. Genetics of PD tolerance in rice has been a central research focus in rice science since 1998 (Ni *et al*., 1998; Wissuwa *et al*., 1998). A major quantitative trait locus (QTL), *Pup1*, has been identified (Wissuwa *et al*., 1998; Wissuwa & Ae, 2001), validated (Chin *et al*., 2011), fine mapped (Wissuwa *et al*., 2002; Heuer *et al*., 2009) and characterised for the genes present within the region using the reference rice cultivars *Kasalath* and *Nipponbare* (Heuer *et al*., 2009; Chin *et al*., 2010; 2011). *Kasalath* contains the entire *Pup1* locus and exhibits PD tolerance whereas *Nipponbare* has a 130 kb deletion within *Pup1* locus and exhibits PD sensitivity. *Pup1* has attracted much attention from the rice breeders because of its higher effect (80 %) on the overall variability of PD tolerance (Wissuwa *et al*., 2002). A set of DNA markers linked to *Pup1* locus have been developed based on the sequence polymorphisms of underlying genes. These markers could be used to introgress the favourable haplotypes of *Pup1* QTL into important rice varieties that are sensitive to PD (Chin *et al*., 2011).

Rice breeding programmes are specific to countries and diverse geographical locations because of the requirement of diverse breeding objectives and the utilisation of varied genetic material as the breeding parents. The QTLs detected in one location cannot be directly utilised in MAB of another region because the QTLs are environment dependent. Therefore, the flanking DNA markers of a QTL in published studies cannot be directly employed in country or location specific breeding programmes without validation. Therefore, the *Pup1* linked DNA markers published in many studies using *Kasalath* and *Nipponbare* cannot be directly recruited to a specific MAB programme in a country. PCR amplification of the DNA markers and detection of their allelic states, understanding informative polymorphisms and establishment of marker-allele haplotypes for the entire *Pup1* QTL are essential groundwork before implementing them in MAB (Aluwihare *et al*., 2016a). The validation of published QTLs in this manner is essential before adapting them as tools in breeding programmes (Mengistu *et al*., 2012; Yan *et al*., 2014; Zhang *et al*., 2014).

Application of MAB for rice in Sri Lanka is still at its infancy. Recently a set of rice landraces and improved varieties that represent the breeding pool and panel of mega production varieties in Sri Lanka were characterised for PD tolerance and they were grouped into three PD tolerant levels, namely tolerant, moderately tolerant and sensitive (Aluwihare *et al*., 2016b). The objective of the present study was to identify the *Pup1* linked marker-allele haplotypes of the rice landraces and improved varieties characterised for PD tolerance by Aluwihare *et al*. (2016b) to facilitate the MAB programmes to produce PD tolerant rice varieties for Sri Lanka.

METHODOLOGY

Rice cultivars and PD tolerance

A set of rice genotypes containing 12 landraces, three old improved (released before 1970) and 15 newly improved (released after 1970) varieties (herein after collectively referred to as cultivars) that are predominantly used in Sri Lankan rice breeding and production were assessed in the present study (Table 1). Seeds from authenticated stocks available at the Rice Research and Development Institute (RRDI), Bathalagoda, Sri Lanka were taken as the planting material. PD tolerance of these 30 rice genotypes were assessed in *Yala* and *Maha* seasons in 2012 and published in Aluwihare *et al*. (2016b). The same dataset was used in the present study to find the association of the PD traits with DNA marker-alleles linked to *Pup1* QTL.

Detection of DNA marker polymorphism

Immature leaf samples were collected from each cultivar and subjected to DNA isolation using Dnaeasy® plant mini kit (Qiagen, Solna, Sweden). Genomic DNA samples of *Kasalath* and *Nipponbare* were also included as the standards for PCR. *matK*, a universal plant DNA barcoding primer pair (Hollingsworth *et al*., 2011), was used to substantiate the quality and aptness of DNA for PCR amplification. DNA samples were amplified using markers developed for the *Pup1* QTL region (Table 2) (Heuer *et al*., 2009; Chin *et al*., 2010; 2011; Pariasca-Tanaka *et al*., 2014). The PCR conditions were provided using a Thermal Cycler (Takara, Otsu Shiga, Japan) as follows; initial denaturation at 94 ºC for 5 min, then 35 cycles of 94 ºC for 30 s, primer annealing temperature (Ta) (Table 2) for 90 s, and 72 ºC for 2 min, final extension at 72 ºC for 10 min. The amplified PCR products were size separated using 1.5 % ethidium bromide stained agarose gel electrophoresis.

Table 1: Rice cultivars characterised for *Pup1* linked DNA marker haplotypes

PD tolerance score[a]	Name of the cultivar[b]	Released year	Parental cultivars	Maturity class (months)	Mean yield (mt/ha)[c]
1	At 306	2004	OB 2273 / At 05	3.0	3.80
	At 354	1992	Bg 94-1 / *Pokkali*	3.5	5.00
	Bg 357[d]	1997	Bg 797 / Bg 300 // 85-1580 / *Senerang* M-17	3.5	6.70
	Bg 300[d]	1987	Bg 367-7 // IR 841/Bg 276-5	3.0	6.00
2	*Suwandel*	-	-	4.0	2.75
	Suduheenati	-	-	4.0	2.75
	Hondarawala	-	-	4.0	2.75
	Pokkali	-	-	4.0	2.75
	Rathuheenati	-	-	3.5	2.75
	Sudurusamba	-	-	4.0	2.75
	At 353	1992	Bg 94-1(R) / Bg 400-1 // Bg 94-1	3.5	4.00
	Bg 250	2005	Farmer field selection	2.5	4.00
	Bg 358[d]	1999	Bg 12-1 / Bg 1492	3.5	6.00
	Bg 450[d]	1985	Bg 12-1*[2] / IR 42	4.0- 4.5	5.00
	Bg 352[d]	1992	Bg 380 / Bg 367-4	3.5	6.80
	Bg 379-2	1980	Bg 96-3*[2] / Ptb 33	4.0- 4.5	6.00
	Bw 364	2006	IR 36 / Bw 267-3-11M	3.5	4.00
	Ld 356	1994	Bw 451 / Bw 351	3.5	4.30
3	*Murungakayan*	-	-	4.0	4.00
	Sudubalawee	-	-	4.0	2.50
	Marss[e]	1950[f]	-	4.5	2.75
	Kokuwellai	-	-	4.0	2.75
	Kaluheenati	-	-	3.5	2.75
	Rathel	-	-	5.0	2.75
	H-10	1968	PP / *Marss* // H-5	3.0	3.00
	H-4	1958	*Murungakayan* / *Marss*	4.0- 4.5	4.50
	H-7	1964	PP / *Marss* // H-5	3.5	3.50
	At 362[d]	2002	At 85-2 / Bg 380	3.5	7.00
	Bg 403	1993	83-1026 / Bg 379-2	4.0- 4.5	4.00
	Bg 94-1[d]	1975	IR 262 / Ld 66	3.5	6.00

[a] PD tolerance scores are indicated as described in Aluwihare *et al*. (2016b). 1: Sensitive; 2: Moderately tolerant; 3: Tolerant

[b] Standard rice variety names as given in the Database of Rice Varieties, Department of Agriculture, Sri Lanka (2006).

[c] The yield data available at RRDI, Sri Lanka.

[d] Mega rice varieties grown in Sri Lanka.

[e] *Marss* is also known as *Mas*.

[f] *Marss* was introduced to Sri Lanka in 1950.

The symbol '-' indicates the unavailability of information for landraces.

Table 2: The details of the *Pup1* linked DNA markers used to detect haplotypes

Marker[x]	Forward and reverse primer sequences (5'→3')	Band sizes[y] (bp)	Ta (°C)	Reference
RM28073	GTGTTGGTGGTGATGAAGCAAGG GGACGAAGGATGTATGTGTCTGTACC	656	55	Chin *et al*., 2010
K20	TCAGGTGATGGGAATCATTG TGTTCCAACCAAACAACCTG	240, 243	55	
K29	CCATAGTAGCACAAGAAACCGACA GCTTCAATGAGCCCAGATTACGAA	491	55	
K41	TGATGAATCCATAGGACAGCGT TCAGGTGGTGCTTCGTTGGTA	382	57	
K42	CCCGAGAGTTCATCAGAAGGA AGTGAGTGGCGTTTGCGAT	918	57	
K43	AGGAGGATGAGCCTGAAGAGA TCGCACTAACAGCAGCAGATT	912	57	
K46	TGAGATAGCCGTCAAGATGCT AAGGACCACCATTCCATAGC	523	57	
K46-K1	TGAGATAGCCGTCAAGATGCT TGAGCCAGTAGAATGTTTTGAGG	342	57	
K46-K2	CTGAAGTGAAAAGAATGACTAA TGAGCCAGTAGAATGTTTTGAGG	433	57	Pariasca-Tanaka *et al*., 2014
K46-3	TCCAAAGATCTCTGATTTTGGC GCTTTCCAACATCTCAAGGACT	400	57	
K46-CG1	CTAGAGTATCTCCACAGTCGTT AAGGACCACCATTCCATAGC	258	57	
K46-CG2	CCGAAGTAAGAAGAATGACGGA TGATCCAGGAGAATGTTTTGTGG	433	57	
K48	CAGCATTCAGCAAGACAACAG ATCCGTGTGGAGCAACTCATC	847	57	
K52	ACCGTTCCCAACAGATTCCAT CCCGTAATAGCAACAACCCAA	505	57	Chin *et al*., 2010
K59	GGACACGGATTCAAGGAGGA TGCTTTCCATTTGCGGCTC	550	57	
Ba76H14_7154	GAAACGGGGTCAAATAAGC GGGTTCGTCCAACAGGAGTA	292, 259	55	Heuer *et al*., 2009
RM28102	CACTAATTCTTCGGCTCCACTTTAGG GTGGAAGCTCCGAGAAAGTGC	168	55	Chin *et al*., 2010

[x] Markers are listed according to their order in the *Kasalath* genome.

[y] Band sizes are reported in the references indicated.

Data analysis

DNA marker polymorphism and cluster analysis

The presence or absence of each marker allele was detected by the presence/absence of the PCR fragments on agarose gel and was recorded for each cultivar. Marker polymorphism of each marker was calculated based on gene diversity (GD) using *GDdom* Software (Abuzayed *et al*., 2016). *GDdom* employs the following formula to calculate GD.

$$GD_i = 2f_i(1 - f_i)$$ Roldan - Ruiz *et al*., (2000)

GD_i = gene diversity of marker 'i', f_i = frequency of the amplified allele (band presence), and 1-f_i = frequency of the null allele (band absence)

Based on the binary data of DNA marker polymorphism, the dissimilarity matrix was calculated using unweighted pair group method with arithmetic means (UPGMA) (Nei & Kumar, 2000) in Phylip Package version 3.697 (Felsenstein, 2005) and the tree

was visualised in FigTree v1.4.3. (Rambaut, 2018). The tree was edited by adding labels for the rice cultivars, their PD tolerance levels and the cluster labels. The cluster position and the PD tolerance score of each cultivar were subjected to a chi-square test using the statistical package Minitab 17 (Minitab Inc., 2010) to detect the association between clustering and PD tolerance scores.

Marker-trait association analysis

The marker-allele haplotypes were identified and drawn as a clustergram, graphically presented and the polymorphic allelic data were subjected to association analysis with the traits for PD tolerance, plant height (PIH), number of tillers (NT), shoot dry weight (SDW), shoot P concentration (SPC), shoot P uptake (SPU), P utilisation efficiency (PUE) and overall PD tolerance score using general lineal model (GLM) procedure in TASSEL 5.0 software (Bradbury *et al*., 2007).

RESULTS AND DISCUSSION

Marker polymorphism

The expected monomorphic band was detected for DNA of all the cultivars in *matK* based PCR revealing the good quality of the extracted DNA samples. All the *Pup1* DNA markers provided clear polymorphic bands in PCR for the studied set of cultivars. A total of 23 alleles were detected for all 17 markers. Out of these, 14 markers accounted for 18 of the polymorphic alleles. The markers *K20*, *Ba76H14_7154* and *RM28102* were monomorphic for the 32 cultivars. However, they could be used as candidate loci to detect any possible variations within the sequence tagged sites (STS) present among different cultivars (Aluwihare *et al*., 2017). The highest GD (0.50) was detected for *K46*, *K46-K1*, *K48* and *K59* (Table 3). A total of five novel bands were detected for Sri Lankan (SL) cultivars (not present in either *Kasalath* or *Nipponbare*) in the markers *K29*, *K46-K2*, *K46-CG2* and *K52* indicating potentially specific or unique *Pup1* QTL haplotypes and hence unique PD tolerance mechanisms in these cultivars (Table 3).

Cluster analysis

The UPGMA clustergram yielded 11 main clusters (C1 – C11). The C1 contained 12 rice cultivars including the standard PD tolerant cultivar, *Kasalath*. These 12 cultivars contained eight PD tolerant, three moderately PD tolerant and one PD sensitive genotypes (Table 1). Within C1, five cultivars were identified as 100 % similar to *Kasalath Pup1* and they were labelled as "Sub C *Kasalath*". Except Bw 364 (a moderately PD tolerant cultivar), all the others in Sub C *Kasalath* were PD

Table 3: The polymorphism of the *Pup1* linked DNA markers in studied rice cultivars

Marker	No. of alleles detected	No. of polymorphic alleles	No. of novel bands (present only in SL cultivars)	Average GD
RM28073	1	1	0	0.30
K20	1	0	0	0.00
K29	2	2	1	0.19
K41	1	1	0	0.43
K42	1	1	0	0.06
K43	1	1	0	0.47
K46	1	1	0	0.50
K46-K1	1	1	0	0.50
K46-K2	2	2	1	0.46
K46-3	2	1	0	0.12
K46-CG1	2	1	0	0.47
K46-CG2	2	2	2	0.34
K48	1	1	0	0.50
K52	2	2	1	0.42
K59	1	1	0	0.50
Ba76H14_7154	1	0	0	0.00
RM28102	1	0	0	0.00

tolerant. Seven cultivars including *Nipponbare* were clustered individually. The cultivar *Nipponbare* was the most distant genotype. The clusters C6 and C8 contained the most frequently grown mega rice varieties (which are PD sensitive) in Sri Lanka. C7 contained seven moderately tolerant rice cultivars and one tolerant cultivar. The C11 is unique because it contains only PD tolerant Sri Lankan cultivars / landraces that are genetically distant from Sub C *Kasalath* (Figure 1). The existence of separate C11 to Sub C *Kasalath* implies the occurrence of unique PD tolerant mechanisms in Sri Lankan rice germplasm. Aluwihare *et al*. (2017) also confirms the existence of this group by studying the sequence polymorphism within *K20* locus of *Pup1*. The association between cluster positions (Figure 1) and PD tolerance scores (Table 1) was found to be significant at $p < 0.01$. This indicates significant association of the groups (clusters) with the response to P starvation.

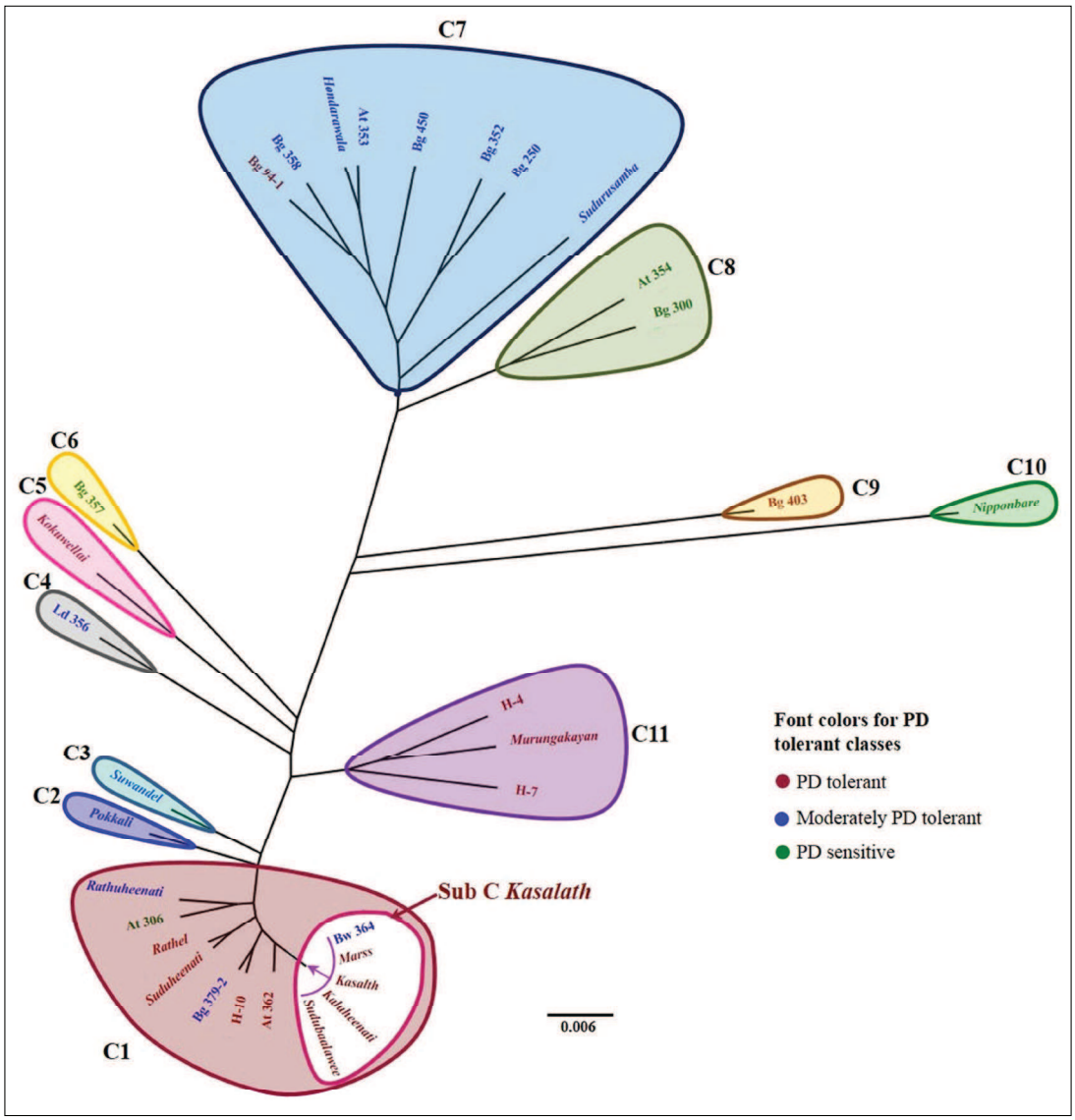

Figure 1: The cluster diagram constructed for 32 rice cultivars based on the *Pup1* linked DNA marker polymorphism. The dissimilarity matrix was obtained using unweighted pair group method with arithmetic means (UPGMA) in Phylip Package version 3.697 (Felsenstein, 2005) and the tree was visualised in FigTree v1.4.3. (Rambaut, 2018). The cluster (C) and other labels were separately added to the FigTree output.

Table 4: Association between polymorphic marker alleles and PD tolerance traits of the rice cultivars

Marker	Allele (bp)	Trait	Growing condition	Mean trait value		Gain of selection	P value
				When allele present	When allele absent		
RM28073	656	PlH	*Maha*-P_{30}-GH	79.25	64.78	14.47	0.0115
K29	850	NT	*Maha*-P_0-F	3.00	2.21	0.79	0.0457
	850	NT	*Yala*-P_0-F	3.20	2.28	0.92	0.0040
	850	NT	*Yala*-P_{30}-GH	6.44	3.99	2.45	0.0206
K41	491	NT	*Yala*-P_0-F	2.25	2.88	-0.63	0.0130
	382	PlH	*Maha*-P_0-GH	77.6	65.21	12.39	0.0461
	382	PUE	*Maha*-P_{30}-GH	1.25	1.55	-0.30	0.0400
K43	382	SPU	*Maha*-P_0-GH	11.40	7.73	3.67	0.0033
K46	912	SPU	*Yala*-P_{30}-GH	18.01	13.87	4.14	0.0122
	523	PUE	*Maha*-P_{30}-GH	1.16	1.48	-0.32	0.0122
	523	NT	*Yala*-P_0-GH	3.38	4.73	-1.35	0.0094
K46-K2	433	PlH	*Maha*-P_0-GH	81.25	62.82	18.43	0.0007
	433	PlH	*Maha*-P_{30}-GH	81.63	68.43	13.2	0.0031
	433	SDW	*Maha*-P_0-GH	18.36	13.26	5.10	0.0086
	433	SPU	*Maha*-P_0-GH	11.26	8.85	2.41	0.0390
	433	PDT Score	*Maha*-P_0-GH	2.57	1.84	0.73	0.0028
	433	PlH	*Yala*-P_0-GH	81.03	61.24	19.79	0.0416
	433	PlH	*Yala*-P_{30}-GH	72.81	54.25	18.56	0.0128
	433	SPU	*Yala*-P_{30}-GH	19.1	14.22	4.88	0.0405
K46-CG1	110	PlH	*Maha*-P_0-GH	63.51	79.1	-15.59	0.0084
	110	PlH	*Maha*-P_{30}-GH	67.85	80.6	-12.75	0.0070
	110	SPU	*Yala*-P_{30}-GH	12.95	19.13	-6.18	0.0064
	258	PUE	*Yala*-P_{30}-GH	1.57	1.16	0.41	0.0356
	258	SDW	*Yala*-P_0-GH	23.71	16.29	7.42	0.0330
	258	SPC	*Yala*-P_{30}-GH	0.67	0.88	-0.21	0.0389
K46-CG2	433	NT	*Yala*-P_0-F	3.20	2.29	0.91	0.0040
	433	NT	*Yala*-P_{30}-GH	6.44	3.99	2.45	0.0206
	130	PlH	*Maha*-P_0-GH	64.68	80.02	-15.34	0.0063
	130	SDW	*Maha*-P_0-GH	13.55	18.18	-4.63	0.0182
	130	SPU	*Maha*-P_0-GH	8.82	11.28	-2.46	0.0349
	130	PDT Score	*Maha*-P_0-GH	1.91	2.5	-0.59	0.0225
	130	PlH	*Yala*-P_{30}-GH	51.75	71.58	-19.83	0.0090
	130	SPU	*Yala*-P_{30}-GH	13.15	19.01	-5.86	0.0118
K48	847	PUE	*Maha*-P_{30}-GH	1.16	1.55	-0.39	0.0017
K52	700	NT	*Maha*-P_0-GH	1.78	1.36	0.42	0.0333
	700	PUE	*Yala*-P_{30}-GH	1.54	1.10	0.44	0.0245
	700	SPC	*Yala*-P_{30}-GH	0.68	0.91	-0.23	0.0196
K59	505	SPU	*Maha*-P_0-GH	10.99	7.50	3.49	0.0179
	550	SPU	*Maha*-P_0-GH	11.55	9.04	2.51	0.0242
	550	SPC	*Yala*-P_0-F	19.93	25.47	-5.54	0.0066

Gain of selection is calculated by subtracting the mean trait value when allele is absent from the mean trait value when allele is present. p value indicates the probability level for significant mean differences calculated using Tassel 5.0 software. PlH: plant height (cm); NT: number of tillers; SDW: shoot dry weight (grams per pot); SPC: shoot P concentration [amount of P (mg) in 1 gram of dry shoot]; SPU: shoot P uptake (mg P per pot); PUE: P utilisation efficiency (biomass produced per unit P accumulated in shoot tissue); PDT: P deficiency tolerance score assigned in Aluwihare *et al*. (2016b).

Haplotype analysis

When 23 alleles of the 17 markers were considered together for 32 cultivars, a total of 28 *Pup1* marker allele haplotypes were detected implying a huge genomic diversity within the *Pup1* QTL region (Figure 1). The cultivars *Kasalath*, *Sudubalawee*, Bw 364, *Kaluheenati* and *Marss* shared the same haplotype (Figure 1) and all these five cultivars were PD tolerant (the score of 3) or moderately tolerant (the score of 2). Therefore, this particular haplotype can be readily employed in MAB for PD tolerance. The marker locus *K46-CG2*, which was developed for African rice (Pariasca-Tanaka *et al.*, 2014), was missing in these five cultivars (C_*Kasalath*). A total of 16 Sri Lankan cultivars contained an allele for the marker *K46-CG2* implying their genetic relatedness to African rice than *Kasalath*, *Nipponbare* and the rest of the Sri Lankan cultivars. *Nipponbare* exhibited a unique *Pup1* genomic haplotype with entire INDEL region missing as observed by Chin *et al.* (2010). However, the variable loci of this INDEL region were observed in many of the Sri Lankan cultivars that would be either PD tolerant or moderately tolerant or sensitive indicating that the degree of PD tolerance cannot be directly linked to the presence and absence of the INDEL region. For example, the PD tolerant cultivars like *Murungakayan*, H-4, H-7, Bg 403 and Bg 94-1 contained some loci of the INDEL region complicating the MAB for PD tolerance using *Pup1* markers in Sri Lankan rice cultivars. If MAB is done solely based on selecting the *Kasalath* haplotype, the PD tolerant mechanism/s conferred by the cultivars such as H-4, H-7 and *Murungakayan* will eventually be lost during MAB and from developed lines.

It is logical to hypothesise that some of the Sri Lankan cultivars may exhibit PD tolerance in the absence of *Kasalath Pup1* haplotype. Within *Pup1* locus, the Sri Lankan cultivars exhibited a very high level of genomic diversity, which is very useful in rice breeding but often complicates the process of MAB lowering its applicability as explained in Collard and Mackill (2008) and Hospital (2009). This sort of genetic diversity within *Pup1* QTL was found to be caused by higher transposon and retro-transposon activities operated within this genomic region (Heuer *et al.*, 2009). However, it shows the need of diversely adapted mechanisms for PD tolerance in rice cultivars of different geographic locations. As most of the rice growing soils lack available P in the soil and diverse nature of the soil chemistry and biology worldwide, this adaptive evolution or unintentional selection by our ancestral farmers would have contributed to this much of higher diversity in *Pup1* genomic region.

Association between the marker alleles and PD tolerance traits

Out of the 18 polymorphic marker alleles detected, 15 alleles showed significant effects on the traits of PD tolerance ($p < 0.05$). Table 4 exhibits the mean trait value difference when the allele is present and absent in the studied cultivars. Overall the presence of 433 bp band of *K46-K2* marker conferred higher PD tolerance. Conversely, when 130 bp allele in *K46-CG2* is absent, higher PD tolerance was observed. The gain of selection must be higher when considering the means of allelic states for MAB and Table 4 clearly displays the specific alleles to look for when conducting MAB for PD tolerance. The haplotype or allelic state based breeding is the core practice in molecular breeding and recently coined as 'DNA informed breeding' (Iezzoni *et al*., 2010; Yunbi, 2010). The allelic states and the haplotypes established in the present study could be used to conduct MAB of rice for PD tolerance.

CONCLUSION

The haplotype analysis of 30 Sri Lankan rice cultivars using 17 DNA markers for *Pup1* QTL revealed 27 unique haplotypes excluding *Kasalath* type. The PD tolerant rice cultivars *Sudubalawee*, Bw 364, *Kaluheenati* and *Marss* share the same haplotype which is also present in the standard PD tolerant cultivar, *Kasalath*. Other PD tolerant SL cultivars contain haplotypes that are more similar to *Pup1* haplotype of *Nipponbare*, the standard PD sensitive cultivar, than to that of *Kasalath* indicating the presence of novel PD tolerant mechanism/s and QTLs within the SL rice germplasm. The association analysis between marker alleles and the traits of PD tolerance shows that a higher gain of selection is possible through selecting the favourable alleles of the associated markers such as 433 bp allele of *K46-K2* marker that favours 0.73 of increased PD tolerance.

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Conflict of interest

Authors declare no conflict of interest on the results reported.

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