Indian J. Genet. Plant Breed., (2024); 84(3): 425-436
https://doi.org/10.31742/ISGPB.84.3.13
ISSN: 0975-6906
www.isgpb.org

RESEARCH ARTICLE

Discerning of Ahu rice (*Oryza sativa* L.) landraces of Assam for phosphorus deficiency tolerance using molecular and morphological approaches

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Abstract

A set of 32 Ahu rice landraces was analyzed for the identification of P-efficient genotypes at three levels of P under field conditions. Based on molecular data of 7 *Pup1* markers, the genotypes were grouped into two major clusters. Out of the 32 genotypes, 13 having consistent amplification of the entire 7 *Pup1* markers and grouped in the same subcluster with Kasalath were identified as *Pup1* positive group. Pool mean analysis showed highly significant genotypic variations ($p \leq 0.01$ or 0.05) with G X E interaction for various yield traits, P-uptake and use efficiency. Heat map quantitative clustering groups the genotypes into 4 major clusters, indicating wide variation in response to differential P- levels of the environment. Analysis of genetic variability revealed moderate to high phenotypic and genotypic coefficient of variability, heritability and genetic advance for various critical yield contributing traits. The PCA analysis extracted 4 principal components, of which the first two components accounted for 71.50% of the total variance. Pearson's correlation analysis revealed a significant positive association of grain yield with various yield parameters, P-uptake and use efficiency, while traits like sterility percent, plant height and days to flowering showed a negative correlation with grain yield. Path co-efficient analysis using yield per plant as the resultant dependent variable revealed direct positive effects by 11 traits with negligible residual effect of (0.0077). The present inquest revealed that genotypes with *Pup1* gene have a lesser percent yield reduction than genotypes lacking *Pup1*. However, landraces like Gopinath with, devoid of *Pup1* gene, gave at par yield potential with *Pup1* positive genotypes. This indicates the probable presence of unidentified P-deficiency tolerance locus among the landraces. Following landraces Kolong, Ikhojoi, Koimurali and Sadakara with *Pup1* positive were identified as promising in P-use efficiency.

Keywords: *Pup1*, phosphorus use efficiency, ahu rice, genetic variation

Introduction

Rice is one of the most important staple food crops shaping the diet and culture of millions around the world. Various factors are hampering its production and productivity. The deficiency of available Phosphorus (P) under acidic soil conditions has been considered as one of the major constraints worldwide (Zhang et al. 2014). Due to the binding effects of free ions with native and applied P, it often remains unavailable to crops (Shimizu et al. 2004; Kochian 2012; Zeng et al. 2016). In Northeast India, soil acidity has been a major constraint due to the prevailing high rainfall climatic conditions, which leads to the leaching down of bases in topsoil. As a result, many rice landraces in the region harbor desirable alleles for various adaptive traits.

P, an essential nutrient, is vital for the growth and development of plants (Abel et al. 2002; Hawkesford et al. 2012). As a result, its deficiency exposed the plants to various physiological disorders (Chaudhary et al. 2008). Thus, low P-stress remains one of the limiting factors of augmenting yield in the rice production system (Wissuwa et al. 1998). Unfortunately no known substitute of P fertilizers has been

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How to cite this article: Touthang L., Sarma D., Sarma R.N., Baruah A., Kumar A., Singh B.K., Philanim W.S., Jaiswal S. and Kaur S. 2024. Discerning of Ahu rice (*Oryza sativa* L.) landraces of Assam for phosphorus deficiency tolerance using molecular and morphological approaches. Indian J. Genet. Plant Breed., **84**(3): 425-436.

Source of support: Nil

Conflict of interest: None.

Received: Jan. 2024 **Revised:** July 2024 **Accepted:** July 2024

reported yet that can sustain crop plants (Van Kauwenbergh 2010; Cordell and White 2011). Hence, the best cost-effective solution would be the exploration and development of P-efficient cultivars that can be sustained even in soil with P-deficiency conditions (Veneklaas et al. 2012; Swamy et al. 2019; Revadi et al. 2023).

The identification of the novel locus of Phosphorus uptake 1 (*Pup1*) by (Wissuwa et al. 1998; Chin et al. 2011) and its candidate gene named as phosphorus starvation tolerance 1 (PSTOL1) on chromosome 12 specific to high P-uptake by (Gamuyao et al. 2012), has opened a new opportunity for genetic improvement of rice for low P stress tolerance. The efficiency of breeding lines with *Pup1* locus in low P- conditions has been testimonial through earlier reports (Chin et al. 2011; Anila et al. 2018; Chithrameenal et al. 2018). Generally, P efficiency is categorized into improved P acquisition efficiency (PAE) or uptake efficiency from soils and enhanced internal P utilization efficiency (Wang *et al.* 2010). The internal P-use efficiency mostly relate to the ability of crop plants to produce more yield or biomass per unit P-uptake from soil (Rose and Wissuwa 2012). Thus, the development of high P uptake and use efficiency cultivars is crucial to overcoming over-exploitation of exhaustible phosphatic fertilizers and sustaining rice production (Heuer et al. 2017; Anandan et al. 2022). The existence of wide variability of rice landraces and breeding lines at genotypic and phenotypic levels in P deficiency environments has been reported by various authors (Fageria and Baligar 1997; Wissuwa and Ae 2001a**;** Sarkar et al. 2011; Tyagi et al. 2012; Vejchasarn et al. 2016; Neelam et al. 2017; Aluwahire et al. 2018, Chankaew et al. 2019; Swamy et al. 2019). Such huge genetic variability for low P indicates the scope and possibility of genetic improvement of rice tolerance to low P in a cost-effective way (Fageria et al. 1988 a,b; Nirubana et al. 2020). In a situation of Phosphorus deficiency, crop plants have evolved various morphological, physiological, biochemical and molecular adaptive mechanisms such as root architecture modifications to explore more of available P (Aziz et al. 2011; Kumar et al. 2021; Verbeeck et al. 2023). Such an adaptive mechanism makes the plant tolerance to low soil P (Nord and Lynch 2008; Kumar et al. 2021). Since the north-eastern region of India has extremely diverse rice-growing climatic conditions, the landraces often harbor various desirable allelic variations and genetic diversity (Roy et al. 2014a). Keeping all this in view, the present study has been undertaken to assess the genotypic variation in P-deficient tolerance in a set of a selected set of Ahu rice landraces for the identification of promising donors in a breeding program.

Materials and methods

Molecular survey of Pup1 locus

The study material comprises of 32 diverse ahu rice landraces originating from Assam except for Krishna and Tamado (Table 1). Genotyping was done using seven *Pup1*-specific dominant SSR markers using Kasalath as a positive check *viz*., *Pup1*-K41, *Pup1*-K42, *Pup1*-K43, *Pup1*-K46-1, *Pup1*-K46-2, *Pup1*-K52-1 and *Pup1*-K59 (Chin et al. 2011). Genomic DNA Isolation was done from leaf tissue of 20-25 days old seedlings following the method described by Dellaporta et al. (1983). The isolated DNA was quantified with the help of NanoDrop (Thermo Scientific) at 260 nm and 280 nm absorbance. The working sample of genomic DNA was diluted with sterile distilled water with a concentration of about 30 to 40 ng μL^{-1}. The Polymerase Chain Reaction (PCR) amplification was performed in a reaction volume of 10 µL with a mixture composition of 2 µL of Template DNA, 4.74 µL of sterile distilled water, 1-µL PCR buffer, 0.1 µL 2.5 dNTPs, 1-µL (for both forward and reverse primers) and 0.16 µL of 3U/µL taq DNA polymerase. The PCR thermal profile was performed in initial denaturation at 94°C for 4 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 1-minute, and one cycle of final extension at 72°C for 10 minute. PCR products were then resolved on 3.5% metaphor gel at 130 V for 3.0 hours. Gels were visualized under UV and photographed using a gel documentation system (Syngene G-Box, U.K.). The presence or absence of marker locus was determined based on the amplicon size of Kasalath (Chin et al. 2011) using 100 base pair DNA ladder.

Phenotyping under graded P-levels

Field experiment was conducted in three graded doses of phosphorus *viz*. 0 kg P_2O_5 ha^{-1}, 20 kg P_2O_5 ha^{-1} and 40 kg P_2O_5 ha^{-1} during 2018-2019. Field experiment was conducted at the Instructional-cum-Research (ICR) Farm of College of Agriculture, Assam Agricultural University, Jorhat, situated (26°46′ N latitude; 94°16′ E longitude). The experiment was designed in RBD (Randomized Block Design) with 3 replications. Each genotype was sown in 3 rows of 1 m length with a spacing of 25x15 cm following standard agronomic practices. Pool RBD analysis was performed from the data generated across the three graded levels of P. The experiment was conducted in a place where limited Phosphorus application was maintained.

Determination P-content and P-use efficiency parameters

The Shoot P-content (SPC) and grain P-content (GPC) were estimated following the phosphovanadate molybdate yellow color method as described by (Hanson, 1950). Digestion of the sample was done tri acid mixture of HNO_3: H_2SO_4: $HClO_4$ (800: 80: 360 ml). The sample color intensity was read in with spectrophotometer at 470 wavelengths. The total amount of P-uptake from the soil by the plant tissue, shoot P-uptake (SPU) = shoot dry weight (SDW) x shoot P-content (SPC) (mg/g). While grain P-uptake (GPU) = Grain

Table 1. List of 32 rice genotypes used in the present investigation

S. No.	Genotype	Pedigree	S. No.	Genotype	Pedigree	S. No.	Genotype	Pedigree
1	Pyajihari	Landrace	12	Haru Begunigootia	Landrace	23	Rangoli	Landrace
2	Koijapuri	Landrace	13	Dimrou	Landrace	24	Kolong	Chilarai/Kalinga III
3	Saiamura	Landrace	14	Suryamukhi	Landrace	25	Bau Murali	Landrace
4	TBK-7-1	Tamdao/ Banglami/Kasalath	15	Kmj-13AB-1-12-3	Mahsuri/Luit	26	Koimurali	Pureline selection in landrace
5	Lal Aus	Landrace	16	TBK-7-2	Tamdao/Banglami/ Kasalath	27	Mantetoi	Landrace
6	Ikhojoi	Landrace	17	Dikhow	Heera/Annada	28	Kapilee	Heera/Annada
7	Sadakara	Landrace	18	Kasalath	Pureline selection in landrace	29	Krishna Early	Selection in Krishna
8	Lewly	Landrace	19	Tamdao#	Introduction	30	Basmoti Red	Landrace
9	Bormekohi Dhan	Landrace	20	Gopinath	Pusa 2-21/IR 36	31	Lachit	CRM 13-3241/ Kalinga II
10	Balighungoor	Landrace	21	Aus Joria	Landrace	32	Banglami	Pureline selection in landrace
11	Krishna*	GEB 24/TN 1	22	Luit	Heera/Annada			

*From Odisha; # From Vietnam

yield/hill x grain P-content (GPC) (mg/g). Shoot P utilization efficiency (SPUE) = SDW/SPU= 1/SPC (mg/g) and grain P-use efficiency (GPUE)= 1/GPC (mg/g). Physiological efficiency (PE), apparent recovery efficiency (ARE) and phosphorus use efficiency (PUE=ARE x PE) were estimated according to (Baligar et al. 2001 and Fageria et al. 2007). In contrast, the percent yield reduction (PYR) was estimated according to Golestani Araghi and Assad 1998.

Statistical analysis

Pool RBD analysis was performed in an excel sheet. Descriptive statistics, Principal Component Analysis (PCA) and multidimensional scale analysis were performed in SPSS version 20.0 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows Version 20.0. Armonk, NY: IBM Corp.). The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) hierarchical clustering based on the dissimilarity matrix of 7 *Pup1* markers data was computed in the DARwin software version 6.0.12 (Perrier and Jacquemoud 2006) DARwin software http://darwin.cirad.fr/. Heat map and quantitative clustering was performed by Pheatmap package, PCA genotype and trait biplot by analysis by FactoMineR and factoextra packages and Pearson's correlation analysis by metan packages in R-studio version 4.2.2. (R Core Team 2022) https://www.R-project.org. The phenotypic and genotypic variance was estimated according to Burton and Devane (1953), heritability as per Hanson et al. (1956), genetic advance by Johnson et al. (1955) and Path co-efficient analysis by Wright (1923).

Results and discussion

Genotyping based on Pup1 markers

The molecular screening revealed that the frequency of *Pup1* gene varied widely across the 32 genotypes. The highest Kasalath (K) allele frequency was detected by K-46-2 (75%) followed by K-46-1 (68.79%), K-52 (65.62%), K-41 (62.5%), K-43 (59.37%), K-5 (56.25%) and K-42 (50%) respectively. The UPGMA clustering based on 7 core *Pup1* markers grouped the genotypes into two major clusters (Fig. 1). Cluster-I with 11 genotypes, were mostly *Pup1* negative. It comprises of two sub-clusters. The first sub-cluster-I with 6 genotypes viz. Kapilee, Mantetoi, Gopinath, Krishna, Saiamura and Koijapuri were completely devoid of *Pup1* gene. The second sub-cluster-I comprised of 5 genotypes viz. Kmj-13AB-1-12-3, Haru, Begunigootia, Lal Aus and Luit. These genotypes inconsistently amplified one or two markers randomly. While the major cluster II included 21 genotypes and was sub-grouped into three sub-clusters. The first sub-cluster-II consisted of 3 genotypes, namely Lachit, Koimurali and Bormekohi Dhan. This group showed the amplification of 5 *Pup1* markers, except K-52 and K-59. The second sub-cluster-II comprised of 4 genotypes viz. Basmoti Red, Rangoli, Dikhou and Lewly. This sub-group exhibits amplification of 6 *Pup1* markers except K-42. While the third sub-cluster-II distinctly grouped 14 genotypes including Kasalath. This Kasalath grouped with consistent amplification of all the 7-dominant *Pup1* specific markers (K-41, K-42, K-43, K-46-1, K-46-2, K-52, K-59) were identified

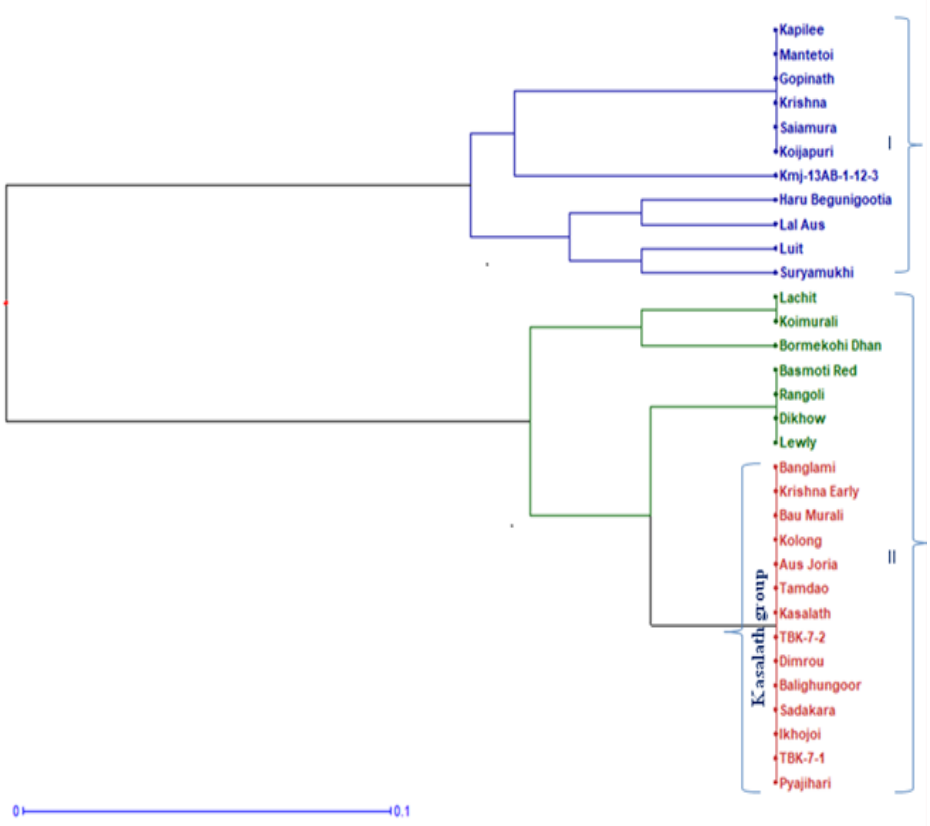

Fig. 1. The UPGMA clustering of 32 Ahu rice genotypes based on 7 core Pup1 markers

as *Pup1* positive group/genotypes. The genotypes were Banglami, Krishna Early, Bau Murali, Kolong, Aus Joria, Tamdao, Kasalath, TBK-7-2, Dimrou, Balighungoor, Sadakara, Ikhojoi, TBK-7-1 and Pyajihari.

The frequency of Kasalath (K) allele detected in the present study by K-46-2 (75%) and K-46-1 (68.79%) was found to be higher than the frequency detected among the 31 rice genotypes by Roy et al. (2021) and Revadi et al. (2023) on validating 143 rice lines by detecting 67.13 and 38.46% amplification by K46-2 and K46-1 respectively. This *Pup1* locus is associated with low P tolerance identified from Kasalath (Wissuwa *et al.* 2002). The candidate gene PSTOL1 acts as an enhancer of early root growth (Gamuyao et al. 2012). The distribution pattern of landraces in our study indicates the presence of considerable molecular diversity, which has been evolved year after year by the selection of the farmers to suit the local climatic conditions. Overall 6 genotypes exhibit complete devoid of *Pup1* gene, 12 genotypes with partial amplification and 13 genotypes with consistent amplification of all the 7 core *Pup1* markers. Anandan et al. (2022) also elucidated the *Pup1* specific markers based genetic diversity of 120 diverse rice genotypes into three major clusters. A similar trend of either the complete or partially presence of *Pup1* locus in northeast rice germplasm lines was also reported earlier (Swamy et al. 2019) and in popular rice varieties by Roy et al. (2021). Being most of the rice growing ecology in North-East India is rainfed with a highly acidic environment, the rice landraces evolved in the region generally possess *Pup1* either completely or partially (Mahajan and Gupta 2009; Ngachan et al. 2011). Such nature might have led to the positive selection of *Pup1,* which thereby played a crucial role in conserving this locus across the rice genotypes adapted in the region (Swamy et al 2019). This major locus (*Pup1*) was estimated to contribute about 70% of total P-uptake variation in rice (Wissuwa et al. 2002). It is reported to be prevalent in varieties and landraces adapted to rainfed, drought-prone and acidic soil conditions (Chin et al. 2011; Swamy et al. 2019). Thus core *Pup1* specific markers would be helpful in a varietal development programme for the selection of suitable parents (Roy et al. 2021).

Phenotypic and P use efficiency variations

Analysis of variance revealed significant difference ($p \leq 0.01$) among the 32 genotypes as well as significant G X E interaction ($p \leq 0.01$ or 0.05) in yield and its attribute parameters viz., plant height (PH), productive tiller number (PT), panicle length (PL), average panicle weight (APW), filled grain number per panicle (FG/P), chaffy grain per panicle (C/P), spikelet sterility% (SS), test grain weight (TGW),

maximum root length (MRL), root volume (RV), root dry weight (RDW), shoot P content (SPC), P harvest index (PHI), shoot dry weight (SDW), grain yield per panicle (GY/Pl), shoot P uptake (SPU), grain P uptake (GPU), shoot P use efficiency (SPUE), grain P use efficiency (GPUE) and grain yield per hectare GY/HA across the three graded level of P (0, 20 and 40 KgP_2O_5/ha). This indicates that the variation of these traits highly depended on the differential response of the genotypes, whereas four traits viz., DFF, GPC, GHI, and PUE exhibit significant variation (($p \leq 0.01$) among the genotypes with non-significant G X E interaction. This suggests that the variation of these traits depended more on the impacts of P-availability rather than the genotype (Table 2).

PH varied from 79.44 to 141.89 cm, with a mean of 110.5 cm. PT ranged from 7.22 to 13.78 with a mean of 9.8. The recorded pool mean of DFF was 73.87, with a range of 68.78 to 79.78 days. Although non-significant, the days to flowering and maturity of genotypes were delayed under a P-stress environment than normal conditions. PL varied from 22.27 cm to 26.58 cm, with an average of 24.21 cm. APW ranged from 1.64 to 3.17 g, with a mean of 2.34g per panicle. FG/P revealed a pool mean of 132.35, which varied from 92 to 195.33. C/P (%) varied from 22 to 45.56, with a mean of 32.61%. In contrast, SS (%) recorded a mean of 20.85%, with a range of 11.47 to 32.14%. TGW of 1000 seeds revealed a pool mean of 22.96 g, which varied from 16.4 to 29.13 g. The mean of MRL was 28.51 cm, having a variation from 20.57 cm to 38.18 cm. RV varied from 9.89 to 21.11 cc. In contrast, the RDW varied from 1.74 to 4.25g, with an average of 2.77 g per hill.

From the P-content analysis, the SPC mean of 2.11 mg/g with a range of 1.6 to 2.5 mg/g and GPC mean of 2.16 mg/g with a range of 1.43 to 2.65 mg/g were recorded among the 32 genotypes. The SPU varied from 31.47 to 66.59 mg/plant with a mean of 46.85 mg/plant, whereas the GPU varied from 9.36 to 39.67 mg/plant with a mean of 23.27 mg/plant. The PHI and GHI ranged from 0.21 to 0.36 and 0.24 to 0.35, with pool means of 0.31 and 0.30, respectively. The SPUE and GPUE ranged from 0.42 to 0.69 mg/g and 0.4 to 0.8 mg/g with the mean of 0.50 and 0.51 mg/g respectively. In contrast, the analysis of overall P-use efficiency (PUE) based on the product of Physiological efficiency (PE) and apparent recovery efficiency (ARE) revealed a mean of 8.79 g/g with a range from 3.4 to 15.6 g/g.

The grain yield (GY/HA) varied significantly in response to the availability of P among the genotypes from 14.1 to 37.93 q/ha with a pool mean yield of 27.03 q/ha across the three environments of P levels. However, landraces like Gopinath without *Pup1* gene exhibit very promising yields equivalent to those genotypes with *Pup1* gene. The following genotypes were identified as promising in response to different P-treatments viz. Kolong (37.93 q/ha), Ikhojoi (36.42 q/ha), Gopinath (35.72 q/ha), Koimurali (35.43 q/ha), Kasalath (35.22 q/ha) and Sadakara (33.19 q/ha), respectively (Fig. 2).

The Principal component analysis (PCA) extracted four principal components (eigen value >1) cumulative variance of 82.29% phenotypic variance. The first principal components PC1, PC2 and PC3 accounted for 61.93, 9.57 and 5.87% of the total variance, respectively. The score plot distribution depicted that genotypes with *Pup1* gene mostly confined in the right half of the biplot. The Pearson's correlations showed highly significant positive association between grain yield per hectare (GY/HA) with GY/Pl (0.93^{***}), GPU (0.94^{***}), PT (0.47^{**}), TGW (0.55^{***}), FG/P (0.58^{***}), GHI (0.86^{***}), PHI (0.58^{***}), RV (0.71^{***}), MRL (0.77^{***}), RDW (0.83^{***}), SDW (0.92^{***}), APW (0.58^{***}) and PUE (0.64^{***}), while negative association was shown by C/P (-0.53^{**}), SS (-0.61^{***}), PH (-0.27) and DFF (-0.09). The path coefficient analysis for direct and indirect effects of traits on dependent variable grain yield per plant revealed that the following traits have direct positive effects viz. SPC (0.55), GPU (0.50), SDW (0.43), GHI (0.34), SF (0.27), SPUE (0.17), PHI (0.15), PT (0.072), PL (0.038), RV (0.026), and RDW (0.001) respectively (data not shown). This suggests that these traits could be an important selection criterion of P-efficient genotypes.

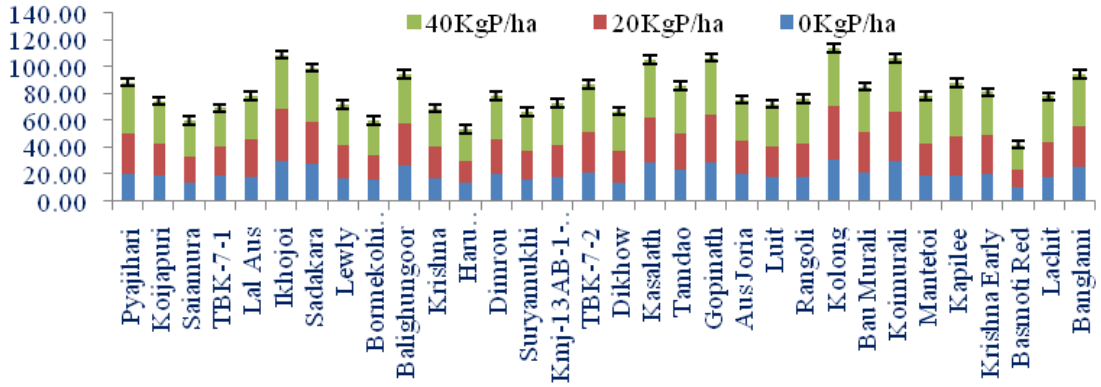

Fig. 2. The variation in grain yield (q/ha) in response to graded P-levels among the 32 ahu rice genotypes

Table 2. Pool traits mean and genetic variability acroos the graded P-levels

Traits	Mean	Min.	Max.	CV	SEM(±)	Genotype	GxE	PCV	GCV	h^2_{bs}	GA	GAM
PT	9.8	7.22	13.78	15.37	0.7	**	**	22.84	16.89	54.72	2.52	25.74
DFF	73.87	68.78	79.78	2.58	0.64	**	NS	4.59	3.8	68.44	4.78	6.48
PL	24.21	22.27	26.58	23.06	0.6	**	**	7.27	4.93	45.99	1.67	6.89
APW	2.34	1.64	3.17	14.65	0.17	**	**	23.84	18.8	62.23	0.71	30.56
FG/P	132.35	92	195.33	12.1	8.16	**	**	22.99	19.55	72.32	45.33	34.25
C/P	32.61	22	45.56	13.74	3.36	**	**	24.88	20.74	69.49	11.61	35.61
SS	20.85	11.47	32.14	13.08	2.12	**	**	30.4	27.45	81.48	10.64	51.04
TGW	22.96	16.4	29.13	3.02	0.34	**	**	13.88	13.54	95.25	6.25	27.23
MRL	28.51	20.57	38.18	9.57	1.29	**	**	18.84	16.22	74.18	8.21	28.79
RV	13.31	9.89	21.11	14.16	1.15	**	**	22.65	17.67	60.9	3.78	28.41
RDW	2.77	1.74	4.25	8.6	0.29	**	**	26.88	25.46	89.75	1.38	49.7
SPC	2.11	1.6	2.5	5.9	0.06	**	**	12.42	10.93	77.46	0.42	19.82
GPC	2.16	1.43	2.65	10.3	0.07	**	NS	18.58	15.46	69.28	0.57	26.51
PHI	0.31	0.21	0.36	12.4	0.02	**	*	17.37	12.16	49.03	0.05	17.54
GHI	0.31	0.24	0.35	9.52	0.01	**	NS	13.18	9.11	47.8	0.04	12.97
SDW	21.69	19.36	26.05	7.16	0.83	**	**	10.03	7.03	49.06	2.2	10.14
GY/H	9.92	6.11	14.21	11.82	0.52	**	**	22.7	19.38	72.9	3.38	34.09
SPU	46.85	31.47	66.59	10.09	2.84	**	**	20.6	17.97	76.02	15.12	32.27
GPU	23.27	9.36	39.67	17.82	2.55	**	**	36.63	32.01	76.33	13.4	57.6
SPUE	0.5	0.42	0.69	6.29	0.03	**	**	14.84	13.44	82.04	0.13	25.08
GPUE	0.51	0.4	0.8	10.71	0.05	**	**	22.57	19.87	77.48	0.18	36.02
PUE	8.79	3.4	15.6	35.16	1.26	**	NS	42.69	24.2	32.13	2.48	28.26
GY/HA	27.03	14.1	37.93	3.5	4.41	**	*	33.27	21.63	50.94	6.57	31

*&**=siginificance at P≤0.05 or 0.01 respectively

The variation observed in phenotypic, P-uptake and use efficiency across the genotypes indicates that phosphorus significantly influenced crop growth and yield. In congruence with the present findings, significant G x P interaction of traits under low P-condition was also reported by (Fageria and Baligar 1997; Manoj et al. 2023). This suggests the presence of substantial genetic diversity among the Ahu rice landraces. The present study also observed prolonged days to flowering/maturity under a P-deficient environment. In consistent with the present finding, phenological delays in flowering and maturity under P-deficient conditions have also been reported by earlier researchers (Rodriguez et al. 1998; Swamy et al. 2019). Likewise, a reduction in plant height, productive tiller and other traits under low P encounter in our study was in agreement with earlier reports (Fageria and Baligar 1997; Dobermann and Fairhurst 2000; Swamy et al. 2019; Yan et al. 2023). Phosphorus plays an important role in rice tillering. Thus, the tillering potential of rice is inhibited under low P in soil (Takehisa and Sato 2019). The reduction in phenotypic mean values and biological yield under P deficiency stress observed in our study is also in congruence with (Yan et al. 2023). Such reduction could be due to reduced in net photosynthesis under deficient conditions (Wissuwa *et al.* 2005). As compared to normal P, low P stress significantly reduced leaf photosynthetic rate in plants (Deng et al. (2020). The genotypes in our study also exhibit higher unfilled grain in response to P-deficiency stress. In consistent with our study, a reduction in grain yield per plant due to low P-stress has also been reported by Yan et al. (2023). An adequate P-level is necessary for more filled grain and test grain weight and biomass (Qadir and Ansari 2006). Similarly, an increase in grain yield in rice with the increase in optimum P-level was reported by (Sudhakar et al. 2004; Qadir and Ansari 2006). The study also reported the significant effect of phosphorus on the grain harvest index of rice (Fageria and Santosa 2002)**.** The reduction in biomass and plant height under P-stress indicates that the expansion of the tissue growth zone is directly associated

with P-availability (Assuero et al. 2004; Kavanova et al. 2006). The P acquisition strategies by the crops in low-P conditions undergo root architectural modification. Such architectural traits are linked with improved topsoil foraging that enhances the P-acquisition efficiency (Lynch 2011). As far as the plant P-uptake and PUE are concerned, root architectural and physiological traits are the two critical determinants (Van de Wiel *et al.* 2016). The dry shoot weight and root characters are vital parameters for indicating low soil P tolerance (Fageria et al. 1988a; Wissuwa and Ae 2001a; Wissuwa 2005; Li et al. 2009; Tian et al. 2017). Such root architectural modification for extensive foraging of P in the soil has been attributed to the *Pup1* locus located on chromosome 12. In general, the P use efficiency decreased with increasing P-uptake (Fageria et al. 1988a,b; Osborne and Rengel 2002 a, b; Saleque et al. 2001). The highest efficiency is usually obtained with the first increment of nutrients as compared to the additional increments (Fageria and Baligar 1997). In the present investigation, a decreasing trend of P-utilization efficiency with increasing P-rates was observed if per unit P-uptake use efficiency was taken into account, *i.e.,* Shoot P utilization Efficiency (SPUE) = SDW/SPU = 1/SPC (mg/g) [where SPU = SDW x SPC]. Thus low P-uptake genotypes showed higher PUE value, which was mostly poor yield potential. Taking this into account, our study estimated PUE from the product of apparent recovery efficiency (ARE) and physiological efficiency (PE), which was proportionately related to the yield potential of genotypes (Baligar et al. 2001; Fageria et al. 2007).

From the molecular clustering (Fig. 1**)**, the genotypes with identified *Pup1* positive group/Kasalath group have lesser percent yield reduction, higher mean performance for grain yield and its contributing traits across the graded P-levels as compared to the group without *Pup1* gene. The pool mean number of productive tillers in the Kasalath group was 11.25, while in non Kasalath group was 8.67. Likewise mean value of filled per panicle (149.79, 118.79), shoot P-uptake (53.52, 41.65) mg/g, grain P- uptake (28.5, 19.20) mg/g, grain yield per hill (11.09, 9.07) g and grain yield (30.23, 24.54) q/ha in *Pup1* positive group and *Pup1* negative group respectively (Fig. 3). Thus the grain yield potential of genotypes with *Pup1* gene group exhibit about 23.19% higher yields than *Pup1* negative group. This indicates that the *Pup1* locus plays an important role in crop tolerance to P-deficiency stress. The efficiency of breeding lines with *Pup1* locus in low P-conditions has been well documented (Chin et al. 2011; Anila et al. 2018; Chithrameenal et al. 2018). However, landraces like Gopinath without *Pup1* gene exhibit very promising yields equivalent to those genotypes with *Pup1* gene. Similar findings of P-deficient tolerant rice germplasm lines devoid of *Pup1* gene have been reported earlier (Kale et al. 2021). A study on phosphorus starvation tolerance attributes in *aus* rice germplasm also reported that genotypes with *PSTOL1*-positive exhibit more tolerance and higher yield under low-P stress. However there were a few *PSTOL1*-negative genotypes showing higher levels of tolerance (Sar et al. 2024). This further substantiated the present findings and suggests that there could be a novel QTL for P-deficiency tolerance. Exploration of gene expression in root and shoot tissues of contrasting rice genotypes for P-starvation stress reported that up-regulation expression of gene played a major role in stress tolerance (Kumar et al. 2021). So far, the Phosphorus uptake 1 (*Pup1*) is the only low P-tolerant QTL that has been cloned and functionally identified (Wissuwa et al. 2002). The candidate gene PSTOL1 encodes a protein kinase that promotes early root growth and enhances the plant P-uptake from the soil (Wissuwa and Ae 2001b; Chin et al. 2010; Gamuyao et al. 2012). The over-expression of PSTOL1 significantly enhances the tolerance to P deficiency and increases P-uptake and grain yield in P-deficient soil (Gamuyao et al. 2012). Such genotypic differences in phosphorus uptake of under low phosphorus were explained by root biomass or architectural modification in genotypes with P-uptake locus (Madhusudan et al. 2022; Verbeeck et al. 2023). To cope with the P-deficiency stress, plants have evolved several morpho-physiological, biochemical and molecular mechanisms. However, the underlying molecular mechanism for low P tolerance remains largely unexplored (Manoj et al. 2023). Further, not all P deficiency-tolerant genotypes contain the PSTOL1 gene. There are genotypes without P-uptake locus having

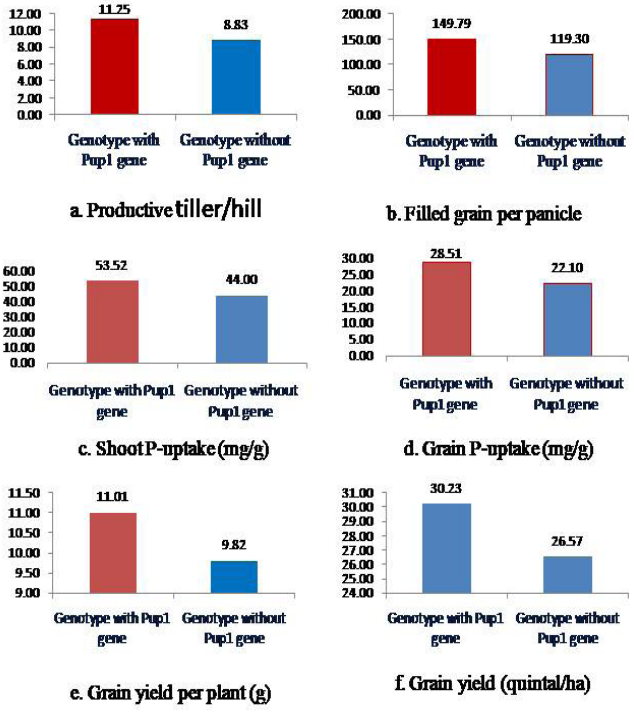

Fig. 3. The variation between Pup1 positive and negative group in yield, tiller, filled grain and P-uptake potential

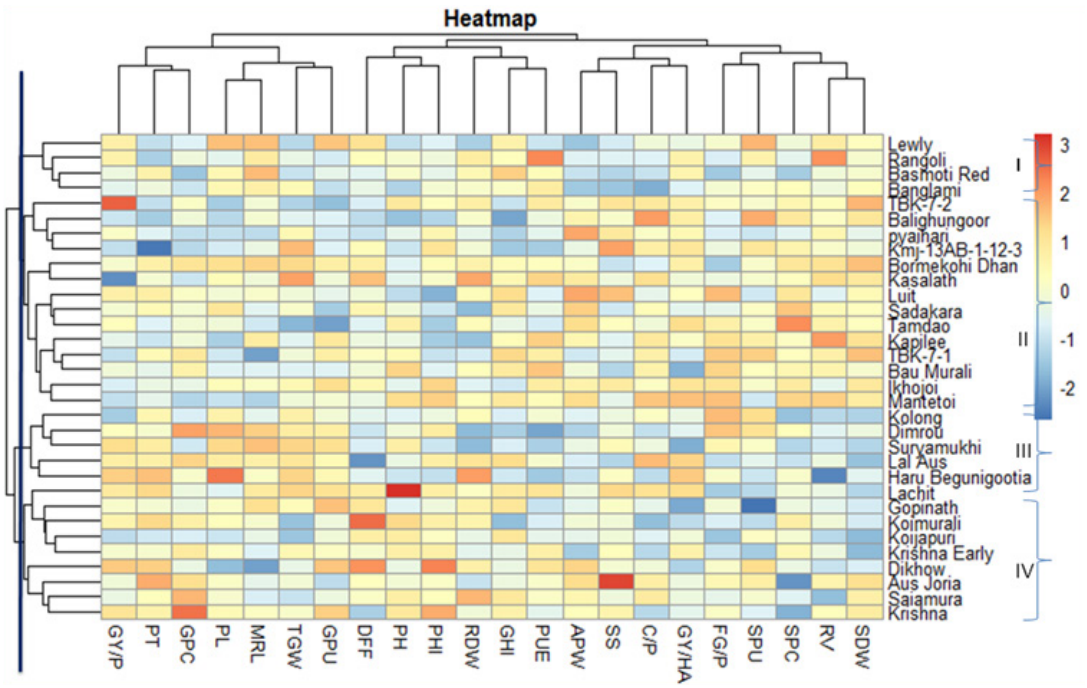

Fig. 4. Heat map clustering of 32 rice genotypes based on quantitative traits across the graded P-levels

shown tolerance to low P (Gamuyao et al. 2012; Kale et al. 2021; Sar et al. 2024).

Being one of the key macronutrients essential for the survival and biological activity of plants, soil phosphorus (P) deficiency constitutes one of the major constraints on rice productivity (Tyagi et al. 2021). So the identification of donors with better uptake and yield under low soil P is crucial for augmenting the yielding potential of rice in the breeding progamme (Revadi et al. 2023). Hence the identified landraces with promising low P efficiency or tolerance in the present study would be helpful in the varietal development programme.

Study on genetic variability

The higher value of PCV than the corresponding GCV in our study indicates the impacts of the environment (P-availability) on the expression of the trait (Table 3). The PCV, GCV, heritability and GAM were moderate to high for most of the critical yield traits under study. Out of the 24 traits, the following 5 traits C/P, SS/ RDW/ GPU and PUE, exhibit high PCV (>20), GCV (>20), heritability (>60) and GAM (>20). Whereas moderate PCV, GCV, and moderate to high heritability with high GAM (>20) were revealed by the traits PH, PT, APW, FG/P, TGW, MRL, RV, GPC, GY/Pl, SPUE, GPUE and GY/HA. This indicates that the differential expression of these traits was highly under additive gene action and would be rewarding as a selection criterion. The lowest value of PCV, GCV, and GAM on days to fifty percent flowering (DFF) revealed that the trait is highly under non-additive gene action and flowering as a selection criterion for P-efficient selection would not be rewarding. Tolerance to P-deficiency stress is a complicated trait with low heritability influenced by both environmental and genetic mechanisms (Veneklaas et al. 2012). The magnitude of its heritability highly determines the transmission of specific trait in advanced generations. High heritability in concurrence with high genetic advance is a prerequisite in predicting genetic gains (Johnson *et al.* 1955). Thus the selection based on high heritability coupled with high genetic advance or trait with low heritability with high genetic advance would be more effective due to additive gene action. A similar trend of high heritability with high GAM for yield and its components has also been reported earlier by (Singh *et al.* 2007; Kumar *et al.* 2013).

Heatmap clustering based on phenotypic and P-use efficient parameters

Based on the quantitative trait variation across the graded level of P using the Euclidean distance coefficient the 32 genotypes into four major clusters (Fig. 4). The horizontal dendrogram represents the genotypes and the traits by vertical dendrogram. The heat map color gradient showed that P- availability has dynamic effects on differential response and expression of traits across the genotypes. The cluster mean of various traits is illustrated in Table 4. The distribution pattern shown that Cluster-I comprises of 4 genotypes (Lewly, Rangoli, Basmoti Red, and Banglami). The genotypes in this group have higher cluster mean values of PH (117.25 cm), PL (24.34 cm), C/P (36.75%) and SS (23.97%). These genotypes also recorded the lowest cluster mean

Table 4. Cluster mean of quantitative traits among 32 rice genotypes in graded P-levels

Cluster	PH	PT	DFF	PL	APW	FG/P	C/P	SS	MRL	RV	RDW
C-I	117.25	8.89	73.84	24.34	2.28	129	36.75	23.97	25.66	12.61	2.56
C-II	111.79	10.56	73.87	24.42	2.44	138.01	32.06	19.86	30.16	13.83	2.91
C-III	111.83	8.78	76.38	24.26	2.4	131.4	33.67	21.42	26.94	13.56	2.89
C-IV	104.76	9.59	72.51	23.85	2.16	125.57	31.05	20.69	28.08	12.65	2.57
Mean	104.76	8.78	72.51	23.85	2.16	125.57	31.05	19.86	25.66	12.61	2.56
Min	104.76	8.78	72.51	23.85	2.16	125.57	31.05	19.86	25.66	12.61	2.56
Max	117.25	10.56	76.38	24.42	2.44	138.01	36.75	23.97	30.16	13.83	2.91
SeM(±)	2.56	0.41	0.81	0.13	0.06	2.63	1.25	0.89	0.95	0.31	0.1
Std.Dev.	5.12	0.82	1.62	0.25	0.13	5.25	2.49	1.78	1.91	0.62	0.19
CV%	4.89	9.34	2.23	1.05	6.02	4.18	8.02	8.96	7.44	4.92	7.42

Cluster	SPC	GPC	PHI	GHI	SDW	GY/Pl	TGW	SPU	GPU	PUE	GY/HA
C-I	2.02	1.99	0.29	0.3	20.68	9.1	21.97	42.91	19.97	8.48	23.78
C-II	2.19	2.31	0.32	0.31	22.11	10.42	22.77	49.51	25.58	8.9	28.53
C-III	2.05	2.01	0.28	0.29	21.48	9.21	23.86	45.53	20.82	8.59	26.04
C-IV	2.05	2.09	0.31	0.31	21.6	9.91	23.19	45.19	22.51	8.87	26.68
Mean	2.02	1.99	0.28	0.29	20.68	9.1	21.97	42.91	19.97	8.48	23.78
Min	2.02	1.99	0.28	0.29	20.68	9.1	21.97	42.91	19.97	8.48	23.78
Max	2.19	2.31	0.32	0.31	22.11	10.42	23.86	49.51	25.58	8.9	28.53
SeM(±)	0.04	0.07	0.01	0	0.3	0.31	0.4	1.37	1.24	0.1	0.98
Std.Dev.	0.08	0.15	0.02	0.01	0.59	0.62	0.79	2.74	2.48	0.21	1.96
CV%	3.96	7.54	7.14	3.45	2.85	6.81	3.6	6.39	12.42	2.48	8.24

value of MRL (25.66 cm), TV (12.61cc), RDW (2.56 g), GY/Pl (9.1 g/pl) and GY/HA (23.78 q/ha). Cluster-II with 14 genotypes was mostly *Pup1* psoitive genotypes (TBK-7-2, Balighungoor, Pyajihari, Kmj-13AB-1-12-3, Bormekhohi Dhan, Kasalath, Luit, Sadakara, Tamdao, Kapilee, TBK-7-1, Bau Murali, Ikhojoi and Mantetoi). These group have high cluster mean value of PT(10.56), PL (24.42cm), APW (2.44g), FG/P (138.01), MRL (30.16cm), RV (13.83cc), RDW (2.91g), GY/Pl (10.42 g) and GY/HA (28.53 q/ha). Cluster-III consisted of 5 genotypes (Kolong, Dimrou, Suryamukhi, Lal Aus, and Haru Begonigootia). Genotypes in this cluster have longer days to flowering (76.38 days) and low cluster mean of PT (8.78). Cluster-IV with 9 genotypes (Lachit, Gopinath, Koimurali, Koijapuri, Krishna Early, Dikhow, Aus Joria, Saiamura and Krishna). Compared to other clusters, the genotypes in this group have low cluster mean values of traits like PH (104.70cm), DFF (72.51), PL (23.85cm), FG/P (125.57) and moderate mean values of GY/HA (26.68 q/ha). Thus, genotypes in these clusters could be useful for breeding short stature and early maturing.

A similar trend has also been apparent in other studies based on agronomic traits (Roy et al. 2014b; Chakravorty *et al.* 2013). Nirubana *et al.* (2020) reported the morphological variation of 30 rice genotypes based on phosphorus starvation tolerance and clustered them into six clusters. Chankaew et al. (2019) grouped Thai indigenous upland rice into three groups based on the P-deficiency tolerance index. Swamy et al. (2019) reported a similar grouping of rice landraces of northeast India based on this P-tolerance index. The systemic analysis of traits both at morphological and molecular levels is immensely helpful in the selection and identification of potential lines in the breeding programme (Dhillon et al. 2004; Nadeem *et al.* 2018). Thus, the information generated in our study through molecular and morphological traits suggests the existence of desirable features among the landraces under study. The information from clustering could be useful for trait-specific lines breeding objectives. Consistent with our study, the morpho-physiological variations and higher tolerance potential of *Pup1* introgressed lines in graded P-level environments have been reported by Madhusudan et al. (2022). Such phenotypic variability of traits related to differential response to graded P levels implies a considerable possibility of improving rice cultivars for P-deficiency tolerance (Fageria *et al.* 1988 a, b; Revadi et al. 2023).

In conclusion, the study revealed the presence of wide genetic variability among the Ahu rice landraces in response to low P environments. The genotype with *Pup1* positive group and high P-use efficiency, such as Kolong, Ikhojoi, Koimurali, Sadakara, Balighungor, Banglami, and Pyajihar, could be a good donor in future breeding programmes. Although *Pup1* gene conferred low P-tolerance, non-*Pup1* linked genotypes also expressed tolerant characters like in Gopinath. Thus there could be probable presence of novel sources of P-tolerant locus yet to be identified. Hence, the information on the present investigation would be useful for future breeding programmes for low P tolerance.

Authors' contribution

Conceptualization of research (LT, DS, RNS); Desigssning of the experiments (LT, DS, RNS and AB); Contribution of experimental materials (DS, RNS, AB); Execution of field/ lab experiments and data collection (LT); Analysis of data and interpretation (LT, DS, RNS, AK); Preparation of the manuscript (LT, DS, AK, BKS, WSP, SJ, SK).

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