Indian J. Genet. Plant Breed., (2024); 84(3): 329-335
https://doi.org/10.31742/ISGPB.84.3.3
ISSN: 0975-6906
www.isgpb.org

RESEARCH ARTICLE

Marker-trait association of the major pyruvate pathway genes regulating yield related traits in rice (*Oryza sativa* L.)

Gyanisha Nayak[$], C. Parameswaran, Nairita Vaidya, Madhuchhanda Parida, Ram Lakhan Verma, Prabharani Chaudhari[1], Pallavi Sinha[2], Vikas Kumar Singh[2], Sanghamitra Samantaray and Jawahar Lal Katara[*]

Abstract

Pyruvate is the primary respiratory substrate used to provide energy for growth and development in plants. The role of genetic variants of pyruvate pathway genes with yield and its attributing traits in rice is not properly understood. In this study, association of sixteen yield traits with SNPs and In/Dels of nine pyruvate metabolism pathway genes (*Os01g0649100*, *Os01g0743500*, *Os04g0671700*, *Os05g0186300*, *Os06g0246500*, *Os07g0630800*, *Os10g0159800*, *Os11g0210300* and *Os11g0216000*) were analyzed using the mixed linear models with principal component analysis (MLM+PCA) based population correction approach. The majority of the traits were found to follow a normal distribution pattern. Besides, MLM+PCA analysis showed a significant association of five markers with six different yield-related traits and the proportion of phenotypic variation explained ranged from 3.56 to 4.56%. Furthermore, the pyruvate dehydrogenase E1 alpha subunit (*Os06g0246500*) gene (Chr06:7602782) showed the highest mean percent difference (19.36%) for plant height. Moreover, most of the IRRI elite lines possessed favorable alleles for plant height (74.55%) and flag leaf area (79.28%) and in contrast, popular varieties like Swarna have inferior alleles for the yield trait-associated SNPs. Thus, genetic variation in the pyruvate pathway genes associated with yield-related traits could be exploited in rice improvement programs.

Keywords: Allelic effect, marker-trait association, pyruvate pathway, rice, favorable alleles.

Introduction

Pyruvate is an intermediary that links respiration and photosynthesis and also acts as a center for the mutual conversion of sugars, lipids, and amino acids. It serves as the main respiratory substrate for the synthesis of energy necessary for plant growth and development (Le and Millar 2023). It also acts as the base for many additional pathways. For instance, it can conduct a decarboxylation reaction to make acetyl-CoA in an aerobic environment and produce ethanol and lactic acid. In addition to the tricarboxylic acid cycle, acetyl-CoA also contributes to the synthesis of fatty acids and other metabolites (Li et al. 2021). The enzyme Pyruvate Kinase (PK) promotes the final phase of glycolysis, producing adenosine triphosphate (ATP) and pyruvate by irreversibly converting the phosphate group of phosphoenolpyruvate (PEP) to adenosine diphosphate (ADP) (Yang et al. 2022a).

For one-third of the world's population, rice (*Oryza sativa* L.) is the most adaptable staple food, and Asia produces about 90% of the world's rice (Hasan-Ud-Daula and Sarker 2020). In the Asian diet, rice continues to be the main source of protein, fiber, and nutrients. Because of the rising demand for rice and to cope with climate change, it is essential to create exceptional genotypes that can flourish in a wide range of environments without reducing yield. The contribution of genes to the growing environment along

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Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack 753 006, India

[1,$] Depatment of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya, Raipur 492 012, India

[2] International Rice Research Institute (IRRI)-South-Asia Hub, International Crops Research Institute for the Semi-Arid Tropics, Hyderabad 502 324, India

***Corresponding Author:** Jawahar Lal Katara, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack 753006, India, E-Mail: jawaharbt@gmail.com

How to cite this article: Nayak G., Parameswaran C., Vaidya N., Parida M., Verma R.L., Chaudhari P., Sinha P., Singh V.K., Samantaray S. and Katara J.L. 2024. Marker-trait association of the major pyruvate pathway genes regulating yield related traits in rice (*Oryza sativa* L.). Indian J. Genet. Plant Breed., **84**(3): 329-335.

Source of support: SERB and DST, New Delhi 110012, (Grant No: EEQ/2021/000506)

Conflict of interest: None.

Received: Oct. 2023 **Revised:** July 2024 **Accepted:** August 2024

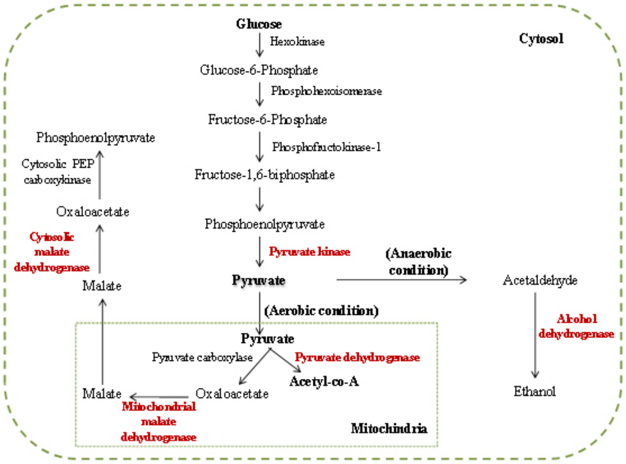


Fig. 1. Metabolic pathway of pyruvate-related genes

with the degree and types of genotypic variability, show an impact on the complicated trait of grain yield (Faysal et al. 2022). Moreover, it is also associated with other agronomic characteristics like plant height, panicle length, tiller per plant, and grains per panicle directly or indirectly (Beena et al. 2021). To create a preference index, plant breeders should focus on selecting combinations of desirable traits to provide each with an economic benefit based on grain yield (Singh et al. 2023).

According to recent research on *Arabidopsis*, the pyruvate pathway enzymes play a crucial role in controlling aerobic growth (Ventura et al. 2020). Particularly, it was discovered that the pyruvate decarboxylase and alcohol dehydrogenase growth penalties were larger in mutant *Arabidopsis* plants grown in aerobic situations. This demonstrates the pyruvate pathway gene's broader function in controlling plant growth and development. In sugarcane, pyruvate kinase (Sspon*02G0043670-1B*) shows a positive association with growth and development as well as a significant contribution to the regulation of the crop's ability to withstand drought (Yang et al. 2022b). In rice, the pyruvate kinase *OsPK1* plays a crucial role in controlling sugar transport and monosaccharide metabolism. Reduced pyruvate content as a consequence of lower cytosolic PK (PKc) expression in the mutant *ospk1* caused dwarf plants and panicle enclosure (Zhang et al. 2012). Similarly, *OsPK2* controls rice grain filling, complex granule formation, and endosperm starch synthesis (Cai et al. 2018). Besides, rice grain filling and the buildup of storage chemicals were negatively impacted by the drastically reduced expression of *OsPK3* and *OsPK4* in leaves and endosperm (Hu et al. 2020). Additionally, disruption of *OsPK3*, *OsPK5*, *OsPK7*, *OsPK8*, and *OsPK10* and *OsPK4*, *OsPK5*, *OsPK6* and *OsPK10* significantly reduced the seed setting rate and test weight (Dong et al. 2022). Further, over-expression of the pyruvate carboxylase gene enhanced the photosynthesis rate and abiotic stress tolerance in mulberry (Sarkar et al. 2024), and it was proposed that regulating the carbon gain and net loss involving genes in the pyruvate pathway would enhance the crop yield (Karthick et al. 2024). However, the genetic diversity and trait association of pyruvate pathway genes for yield related traits is not understood properly. Thus, the present study used the 3k rice panels and attempted to associate the genetic variants of the major pyruvate pathway genes, which were up-regulated during drought stress with yield-related traits in rice.

Materials and methods

Plant materials

The genotypes used in the present investigation comprised of 339 lines of 3k-MCP (Mini Core Panel) of rice received from IRRI-SA Hub, ICRISAT Campus, Hyderabad, India. The experiment was conducted in *Kharif* 2022 using an augmented randomized block design in three blocks with five checks (Vandana, IR64, MTU1010, Swarna and BPT5204) on the experimental field of the Crop Improvement Division, ICAR-National Rice Research Institute (NRRI), Cuttack. The checks were repeated three times. The crop was grown using the suggested agronomic practices and harvested in December 2022. Three plants at random from each genotype were chosen to take observations on the following traits: plant height (cm), panicle length (cm), number of panicles per plant (nos.), flag leaf length (cm), flag leaf width (cm), flag leaf area (cm^2), number of total tillers per plant (nos.), number of filled grains per panicle (nos.), number of chaffy grains per panicle (nos.), number of total grains per panicle (nos.), spikelet fertility (%), biological yield per plant (g), seed yield per plant (g), harvest index (%) and 100 seed weight (g). Additionally, the number of days to 50% flowering was also recorded. Leaf area was calculated using the formula, Leaf area = Leaf length × Breadth × 0.71 (Yoshida et al. 1976). Similarly, the harvest index was calculated according to the formula Harvest index (%) = (Grain yield/ Biological yield) × 100 (Donald, 1962) and spikelet fertility was calculated using the formula Spikelet fertility (%) = (Number of filled grains/ Number of total grains) × 100 (Virmani et al. 1997).

Statistics analysis

Data compilation, calculation of mean, and analysis of descriptive statistics were performed using Microsoft Excel 2013. The mean value for all the characters in three replications of each genotype was subjected to a two-way analysis of variance (ANOVA) following the method suggested by Singh and Chaudhury (1985). The distribution of the trait values was analyzed in R-studio (version R 4.2.2) using the ggplot2 package.

Association analysis

Using the rice SNP-Seek database (https://snp-seek.irri.org/), 1566 SNP markers for nine genes of the pyruvate metabolism pathway (Table 1) were retrieved for 334

Table 1. List of genes involved in the pyruvate metabolism pathway

Gene	Common name
Os01g0649100	Mitochondrial malate dehydrogenase
Os01g0743500	Cytosolic NADP malic enzyme 3
Os04g0671700	DJ-1/PfpI domain containing protein C, DJ-1 protein C
Os05g0186300	Cytosolic NADP malic enzyme 2
Os06g0246500	Pyruvate dehydrogenase E1 alpha subunit
Os07g0630800	Malate dehydrogenase 7.1
Os10g0159800	Alcohol dehydrogenase superfamily, zinc-containing protein
Os11g0210300	Alcohol dehydrogenase 1
Os11g0216000	Pyruvate kinase 5

genotypes (excluding checks). The heterozygous alleles were considered missing alleles, and genotype data were filtered for the rare alleles (frequency = 0.05) (Breseghello and Sorrels 2006), which made a working data set of 471 SNP sites. Principal Component Analysis (PCA) and kinship matrix (Centered_IBS method) were obtained from TASSEL (version 5) software (Bradbury et al. 2007). TASSEL was also used to identify SNP and phenotypic trait associations by generating a mixed linear model (MLM) and the significant SNPs were identified using the Manhattan plot (Wang et al. 2016). Significant marker-trait associations were determined based on a threshold of LOD score of more than 2.5 and $p \leq 0.05$. Z-test at 5% and 1% levels of significance were used to check the significance of mean differences for different traits between the alternate alleles (Sprinthall 2011). The proportion of favorable alleles in the IRRI elite lines and popular rice varieties or genotypes has been studied using the SNP seek database (https://snp-seek.irri.org/).

Results and discussion

ANOVA and descriptive statistics

The two-way ANOVA indicated the existence of a highly significant variation among traits, and genotypes and also in trait × genotype interaction ($p = 0$) (Table 2) and also high genetic variability for all the traits studied. This indicates that there is ample scope for the selection of promising genotypes from the present diverse genotypes for yield-related traits as previously reported by Saharia et al. (2024). Besides, skewness, a third-degree statistic, was negligible, indicating the symmetric distribution of phenotypes and kurtosis, a fourth-degree statistic was platykurtic for all the traits (Table 3). Therefore, traits are governed by quantitative inheritance involving minor genes with additive effects (Anilkumar et al. 2023).

Frequency distribution of various yield-related traits

The frequency distribution pattern of different yield-related traits is depicted in Fig. 2. Out of 339 genotypes, most of the genotypes showed 50% flowering in 90 to 110 days and only nine genotypes flowered early, that is, ≤ 70 days. Further, the majority of genotypes have plant heights between 120 and 140 cm except 37 showed lesser height. Further, six genotypes possessed panicles longer than 28 cm and the mean length was 22.36 cm. Similar to this, two genotypes had flag leaves that are extremely short (15.74 and 16.44 cm long), and two genotypes had leaves that are extremely long (43 and 45 cm long). Flag leaf area varied from 20 to 38 cm^2 in 76.18% of genotypes in the association panel, showing a fairly symmetrical distribution. While the majority of the genotypes (247 nos.) showed a tillering capacity of 7 to 14, eight of them showed a strong tillering capacity of more than 17. Similarly, only four genotypes showed a stronger grain-filling ability of more than 180 filled grains per panicle. Besides, more than 200 total grains per panicle were seen in three genotypes and in 56.47% of genotypes, spikelet fertility was greater than 80%. In 80 genotypes, less than 50 g of biological yield per plant was observed and a total of 47 genotypes showed more seed yield per plant than 25 g, with a maximum yield of 60.47 g. The harvest indexes ranged from 15 to 28%, but remarkably, five genotypes showed harvest indices above 50%. Hundred seed weight was well distributed in the panel ranging from 2.00 to 3.00 g in 232 genotypes. The present finding is supported by the recent report of a high range of frequency distribution for yield-related traits in rice (Ashfaq et al. 2023). Besides, a wide phenotypic variation in the association panel suggests there is the potential for novel donors to manipulate traits through breeding (Kakar et al. 2021).

Marker-trait association analysis

Using association analysis through the MLM approach and 471 SNP genetic variations for the targeted genes, six significant marker trait associations (MTAs) were identified (Table 4).

Table 2. ANOVA of yield related traits for selected genotypes of 3k rice panels (including checks)

Source of Variation	Sum of square	Degree of freedom	Mean sum of square	F-value	p-value	F critical
Traits	15140156	10	1514016	13814.09	0	1.831965
Genotypes	590840.9	339	1742.894	15.9024	0	1.133043
Trait x Genotype Interaction	2175360	3390	641.6993	5.854952	0	1.049081
Within groups	819803.5	7480	109.5994			
Total	18726161	11219				

Table 3. Descriptive statistics of yield related traits for selected genotypes of 3k rice panels (including checks)

Traits	DFF	PH	PL	PP	FLL	FLW	FLA	TTP	FGP	CGP	TGP	SF	BYP	SYP	HI	HSW
Mean	97.90	117.66	22.36	9.06	29.43	1.35	28.49	10.32	81.02	19.74	100.76	80.07	75.88	17.22	24.07	2.56
Range	68.00	150.40	15.57	21.00	30.03	1.60	45.39	22.33	188.67	56.00	213.00	48.12	179.26	58.58	55.43	2.8
Minimum	57.00	67.83	15.40	3.00	15.74	0.57	9.28	3.33	28.34	1.67	32.33	49.37	17.16	1.88	7.20	0.88
Maximum	125.00	218.23	30.97	24.00	45.77	2.17	54.67	25.67	217.00	57.67	245.33	97.49	196.42	60.47	62.63	3.68
Skewness	-0.06	0.12	0.04	0.93	0.21	-0.05	0.36	0.70	1.00	0.91	0.90	-0.67	0.64	1.18	0.91	-0.05
Kurtosis	0.08	1.09	-0.12	2.11	-0.25	0.15	0.11	1.08	1.99	0.84	1.62	0.11	0.57	2.80	1.19	0.10

DFF = Days to 50% flowering; PH = Plant height (cm); PL = Panicle length (cm); PP = Number of panicles per plant; FLL = Flag leaf length (cm); FLW = Flag leaf width (cm); FLA = Flag leaf area (cm^2); TTP = Number of total tillers per plant; FGP = Number of filled grains per panicle; CGP = Number of chaffy grains per panicle; TGP = Number of total grains per panicle; SF = Spikelet fertility (%); BYP = Biological yield per plant (g); SYP = Seed yield per plant (g); HI =: Harvest index (%) and HSW = 100 seed weight (g)

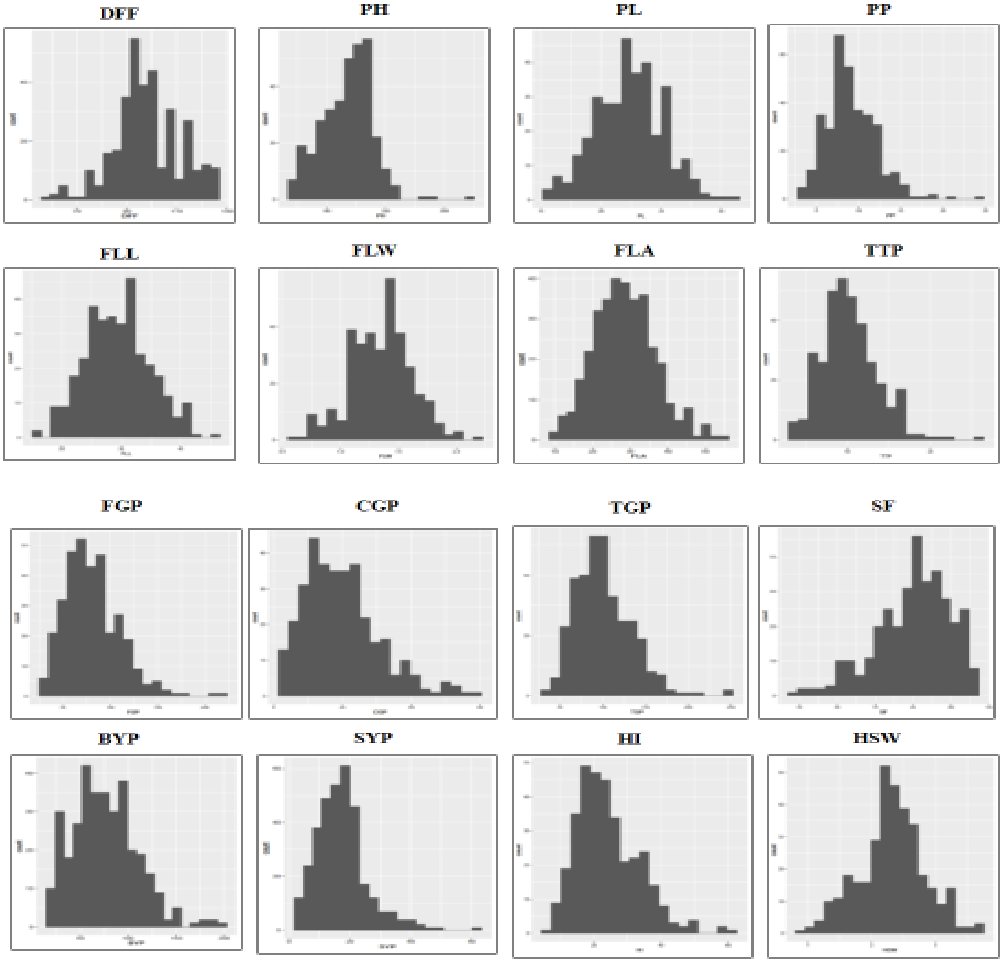


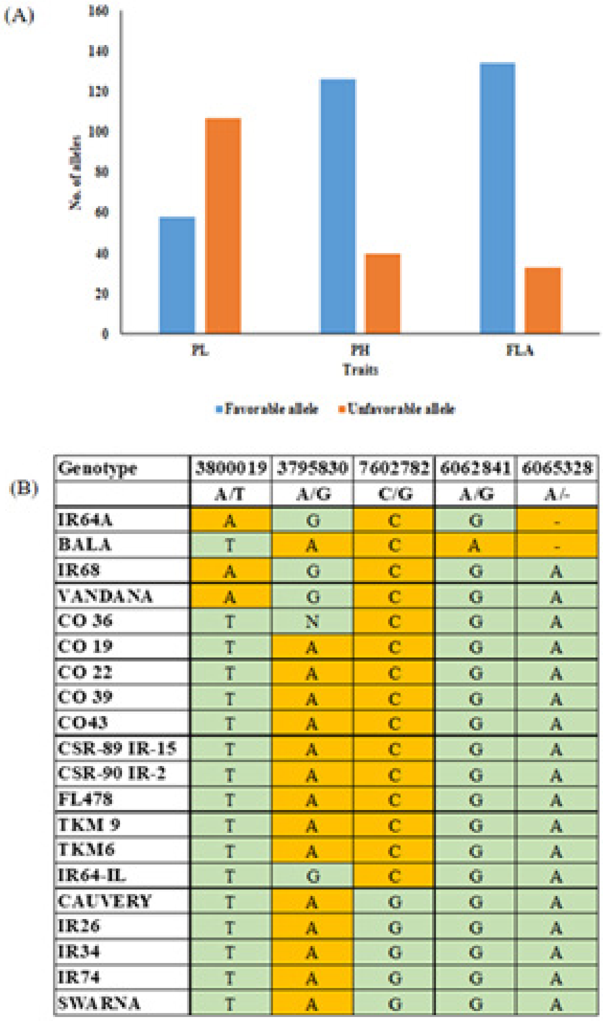
Fig. 2. Distribution of different yield related traits in selected genotypes of 3k rice panels

A SNPs (SNP: 3800019) of a gene *Os10g0159800* (Alcohol dehydrogenase) was found to be associated with the spikelet fertility ($p = 0.00064$), chaffy grains per panicle (p=0.00231), and panicle length (p=0.00195) traits in this analysis. Among these three traits, mean allelic difference for chaffy grains per panicle was found to be 9.76%. This indicates this gene might be involved in the regulation of grain filling in rice. In one of the recent reports, it was reported that alcohol dehydrogenase activity was required for aerobic growth in *Arabidopsis* (Ventura et al. 2020). Further, expression of ADH was higher in the leaves of *Arabidopsis* and rice implicating its role in leaf development (Singh et al. 2024). The *Adh1* mutants in rice demonstrated impaired embryo development and increased sensitivity to glucose and sucrose during germination, supporting this observation (Takahashi et al. 2014). Furthermore, it was discovered that

Table 4. Significant marker-trait associations based on MLM model for grain yield

Trait	SNP ID (Gene)	Chr No.	Position	LOD	Genetic R^2	p-value	Major Allele (A1)	Minor Allele (A2)	MAF (%)	Mean of A1	Mean of A2	Difference between A1 & A2 (%)	Phenotypic P-value
SF	3800019 (*Os10g0159800*)	10	Intron	3.192	0.0456	0.00064	273 (T)	58 (A)	21.25	79.63	81.85	2.71	0.0450
CGP	3800019 (*Os10g0159800*)	10	Intron	2.637	0.0356	0.00231	273 (T)	58 (A)	21.25	20.12	18.33	9.76	0.0939
PL	3795830 (*Os10g0159800*)	10	Intron	2.710	0.0377	0.00195	191 (G)	129 (A)	67.54	22.12	22.71	2.60	0.0322
PH	7602782 (*Os06g0246500*)	6	Exon	2.724	0.0376	0.00189	290 (C)	38 (G)	13.10	120.78	101.19	19.36	4.89E-09
FLA	6062841 (*Os11g0216000*)	11	Intron	3.027	0.0427	0.00094	240 (G)	79 (A)	32.92	28.28	29.50	4.14	0.1229
FLL	6065328 (*Os11g0216000*)	11	3'UTR	2.859	0.0399	0.00139	269 (A)	60 (-)	22.31	28.90	29.56	2.23	0.1802

SF = Spikelet fertility (%); FLA = Flag leaf area in (cm^2); FLL = Flag leaf length (cm); PH = Plant height (cm); PL = Panicle length (cm); CGP = Number of chaffy grains per panicle, and MAF =: Minor allele frequency



Genotype	3800019	3795830	7602782	6062841	6065328
	A/T	A/G	C/G	A/G	A/-
IR64A	A	G	C	G	-
BALA	T	A	C	A	-
IR68	A	G	C	G	A
VANDANA	A	G	C	G	A
CO 36	T	N	C	G	A
CO 19	T	A	C	G	A
CO 22	T	A	C	G	A
CO 39	T	A	C	G	A
CO43	T	A	C	G	A
CSR-89 IR-15	T	A	C	G	A
CSR-90 IR-2	T	A	C	G	A
FL478	T	A	C	G	A
TKM 9	T	A	C	G	A
TKM6	T	A	C	G	A
IR64-IL	T	G	C	G	A
CAUVERY	T	A	G	G	A
IR26	T	A	G	G	A
IR34	T	A	G	G	A
IR74	T	A	G	G	A
SWARNA	T	A	G	G	A

Fig. 3. (A) Distribution of favorable and unfavorable alleles in IRRI Elite lines for PL, PH and FLA, (B) Allelic variation of 20 popular rice varieties/ genotypes at five different SNP positions; orange color depicts the distribution of the favorable allele, while the green color is employed to represent the unfavorable allele; N is missing allele and - represents deletion

the inferior rice spikelets had a down-regulated ADH protein level, which increased spikelet fertility (Zhang et al. 2014). Therefore, the function of alcohol dehydrogenase on seed filling needs to be studied further.

A SNP in the exon region of the gene *Os06g0246500* (Pyruvate dehydrogenase E1 alpha subunit; SNP: 7602782) was found to be associated with the plant height. Further, the major allele and minor allele's phenotypic means were 120.78 and 101.19 cm, respectively which differ significantly (p = 4.89E-09), having a percent difference of 19.36%. In *Arabidopsis*, it was reported that the mutations in pyruvate dehydrogenase complex genes affected plant growth and development (Song and Liu 2015). The development of amyloplasts in grains is also reportedly impacted by the plastid pyruvate dehydrogenase E1 alpha subunit in rice (Lei et al. 2022). Besides, PDH forms the basic substrate for histone acetylation and regulation of epigenetic changes in plants (Huang and He 2024). Therefore, the functional role of pyruvate dehydrogenase in regulating plant height can be better understood by the development of loss-of-function mutants of these genes in rice.

Two SNPs in Pyruvate kinase 5 (*OsPK5*: *Os11g0216000*) (6065328 and 6062841) were found to be associated with flag leaf area and length. The phenotypic mean value of allele G (Chr11: 6062841) was 28.28 and allele A was 29.50 cm^2 and the percent difference between them was 4.14%. In rice, the *OsPK5* gene regulates the equilibrium of GA and ABA, glycolytic metabolism, and seed germination. Previous studies revealed that *OsPK5* gene dysfunction affected the GA/ABA balance, prevented glycolytic metabolism, delayed seed germination and seedling growth, elevated glucose levels, and decreased energy levels (Yang et al. 2022a). However, the mean difference for the alternate alleles of the gene didn't show significant differences under the experimental conditions. Recently, it was reported that the soybean PK21 gene regulates salt responsiveness (Liu et al. 2024). Thus, the effect of the favorable allele of PK5 needs to be further validated.

Interestingly, most of the IRRI Elite Lines (75%) had a favorable allele for the markers associated with the plant height (SNP:7602782) and flag leaf area (SNP:6062841). However, 107 elite lines had unfavorable alleles for the panicle length (Fig. 3A). In contrast to IRRI elite lines, the allelic difference for the identified MTAs and 20 popular varieties/genotypes (Fig. 3B) showed seven varieties (CO36, IR64-IL, CAUVERY, IR26, IR34, IR74 and Swarna) possessed inferior/unfavorable alleles at four out of five associated SNPs. IRRI elite lines are those having the higher breeding values (Juma et al. 2021) and this analysis indicates the scope for the improvement of popular Indian rice varieties like Swarna for the SNPs involved in the pyruvate pathway genes. In summary, this work identified six marker-trait associations in the nine genes involved in the pyruvate pathway. Further, as compared to the IRRI elite lines, popular varieties possessed inferior alleles for the MTA. This provides a scope for targeting the pyruvate pathway genes in marker-assisted breeding programs.

Author's contributions

Conceptualization of research (PC, JLK, SS); Designing of the experiments (PC, JLK, PRC); Contribution of experimental materials (PS, VKS); Execution of field/lab experiments and data collection (GN, NV, MP); Analysis of data and interpretation (GN, RLV); Preparation of the manuscript (PC, JLK, PRC, SS).

Acknowledgments

The authors are thankful to Dr. A.K. Nayak, Competent Authority, ICAR-National Rice Research Institute, Cuttack, Odisha, India, for providing all the necessary facilities and SERB (Science and Engineering Research Board), DST, New Delhi, India, for providing the necessary fund (Grant No: EEQ/2021/000506) to carry out this research work.

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