https://doi.org/10.17221/12/2024-CJGPB

Unravelling population structure and marker trait association using SSR markers among the identified drought tolerant rice landraces (*Oryza sativa* L.)

Shanmugam Manju Devi[1], Amalraj John Joel[1], Muthurajan Raveendran[2], Ramamoorthy Pushpam[3], Sengalan Muthuramu[4], Raman Pushpa[5], N. Sritharan[6], Periyasamy Prasanna[1], Ramalingam Suresh[6]**

[1]*Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, India*
[2]*Directorate of Research, Tamil Nadu Agricultural University, Coimbatore, India*
[3]*Department of Forage Crops, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India*
[4]*Agricultural Research Station, Paramakudi, India*
[5]*Tamil Nadu Rice Research Institute, Aaduthurai, India*
[6]*Department of Rice, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India*

**Corresponding authors: jnjoel@gmail.com; suresh.r@tnau.ac.in*

Citation: Manju Devi S., Joel A.J., Raveendran M., Pushpam R., Muthuramu S., Pushpa R., Sritharan N., Prasanna P., Suresh R. (2025): Unravelling population structure and marker trait association using SSR markers among the identified drought tolerant rice landraces (*Oryza sativa* L.). Czech J. Genet. Plant Breed., 61: 1–22.

Abstract: With climate change, plants face numerous stresses, notably drought for rice cultivation. Improving rice drought tolerance is vital for sustainable production in water-scarce regions. Identification of drought tolerant genotypes at the seedling stage of the crop contributes to build a climate resilient genotype during the period of water scarcity and under challenging environmental conditions. Hence, polyethylene glycol-6000 (PEG-6000) induced drought conditions could be used for testing the drought tolerance in rice at an earlier stage of the crop. Optimization of PEG-6000 concentration for screening index at -6 bar was done using three drought-tolerant and two drought-susceptible check varieties based on probit analysis. Subsequently, 100 rice landraces underwent PEG-6000 induced drought screening at –6 bar and a total of 32 genotypes were selected as tolerant. After 14 days of treatment, the nine observations *viz.* germination %, root length (cm), shoot length (cm), number of secondary roots, fresh weight (g), dry weight (g), shoot/root ratio, root/shoot ratio and vigour index were recorded. Variance analysis, revealing significant genetic variation among genotypes for all studied traits, indicating genetic variability. Post hoc analysis confirmed notable variation among treatments. Principal component analysis revealed three components, with the first three accounting for 88.89% of total variability. With respect to the biplot, the ten genotypes viz., IRGC109, IRGC403, IRGC448, IRGC461, IRGC466, IRGC486, IRGC508, IRGC518, IRGC527 and IRGC535 are the seedling stage drought tolerant genotypes based on shoot length, number of secondary roots and vigour index. Population structure classified the accessions into two subpopulations, reflecting diversity. The allele frequency divergence is 0.095 which is a measure of fixation index revealing that the moderate divergence is not extremely pronounced.

Supported by the Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India, Grant No. SAN No.102/IFD/SAN/4188/2019-20).

https://doi.org/10.17221/12/2024-CJGPB

Genetic diversity, assessed through 26 SSR markers selected from drought tolerant QTLs and markers related to vigour index, exhibited 100% polymorphism with 115 alleles and an average PIC value of 0.61 per primer. Shannon index varied between 0.34 (RM212) and 1.96 (RM252), averaging 1.18. Six SSR markers *viz.,* RM246, RM302, RM252, RM219, RM251, and RM486 were associated with the six key traits *viz.,* shoot length, root length, number of secondary roots, dry weight, shoot/root ratio, and root/shoot ratio respectively offering valuable resources for selecting drought-tolerant accessions as it provides the first step in the selection of genotypes based on the key traits.

Keywords: molecular diversity; polyethylene glycol; polymorphic information content; Shannon index; seed vigour

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world, having been cultivated across 165.25 million ha worldwide (FAO 2022) with a global production of 501 million metric tons (Statista 2021; www.statista.com). In India, over 46 million ha of land are used for rice cultivation. With increasing abnormal changes in climate and global warming, plants are experiencing a number of abiotic and biotic stresses (Pandey et al. 2017). Drought affects more than 23 million hectares of rainfed rice in Asia (Kumbhar et al. 2015) and causes 65 to 85% yield loss (Vinod et al. 2019). The major rice-producing states affected due to drought in India are West Bengal, Odisha, Punjab, Uttar Pradesh, Bihar, North eastern states and southern peninsular regions. The yield loss due to drought in these regions is estimated to be around 5 to 10% (USDA 2022; https://ipad.fas.usda.gov). Under this situation, there is a need to improve drought tolerance in rice to have sustainable rice production in water-limiting areas.

The seedling stage of crops is particularly vulnerable to drought stress due to its critical role in seed germination, which is essential for crop establishment and transition phases (Farooq et al. 2019). Limited water availability during germination significantly hampers crop growth and productivity (Rauf et al. 2007). Leaf growth diminishes under drought stress because of reduced water potential (Zhu et al. 2020) which leads to poor cell development and smaller leaf areas (Hussain et al. 2018). Leaf rolling and the onset of early senescence are additional key characteristics observed under drought stress. The impact of drought stress at the seedling stage is experienced by other crops *viz.,* maize, wheat, and pearl millet (Ahmed et al. 2022; Chakraborty et al. 2022; Sheoran et al. 2022). To induce drought stress, different osmotic agents such as sorbitol, mannitol, sucrose, and polyethylene glycol are utilized. Among these, polyethylene glycol (PEG-6000), known for its high molecular weight and safety for humans and other organisms, is commonly employed (Awan et al. 2021). Consequently, seed germination is evaluated under laboratory conditions using PEG-6000 to assess the genotype's tolerance level at seedling stage (Gholami et al. 2009). This method serves as the standard approach for inducing drought stress at an earlier stage of the crop.

Screening a large number of genotypes in natural conditions is not feasible due to limited land area and labour cost, which could be overcome by screening under controlled condition. This method would likely downsize the genotypes based on seedling vigor (Mahpara et al. 2022). Seedling vigour is a complex trait that depends on the seed germination % and seedling length (Wang et al. 2010; Panda et al. 2019; Evamoni et al. 2023). It is the ability of the seed to emerge rapidly from the soil (Huang et al. 2004). The seed with high vigour plays an important role in the seedling establishment (Lou et al. 2007) as well as competes in early germination with respect to biotic and abiotic stresses. Seed vigour also had a positive correlation with seedling dry weight, root length, shoot length and germination rate (Sanghamitra et al. 2021).

India is one of the centres for rice diversity (Singh et al. 2016). Landraces serve as a repository to meet new challenges during stressful condition. The diversity of landraces broadens the genetic base for crop improvement. Genetic diversity can be determined by assessing morphological or molecular data. Evaluation of genetic diversity using DNA marker technology offers non-destructive analysis which is not influenced by environmental factors, requires only a small quantity of samples and eliminates the need for large experimental setups (Kanawapee et al. 2011).

Simple sequence repeats (SSR) markers, known for their high informativeness, codominance, and cost-effectiveness (Garcia et al. 2004), are pivotal in detecting genetic variation among accessions (Ma et al. 2011; Sajib et al. 2012). Widely applied in genetic diversity analysis (Ni et al. 2002), molecular map construction, and gene mapping (Zhang et al. 2007; Ma et al. 2011), SSR markers play a crucial role in assessing germplasm diversity (Zhou et al. 2003;

https://doi.org/10.17221/12/2024-CJGPB

Jin et al. 2010; Ma et al. 2011) and trait association studies. Despite their smaller numbers, SSR markers provide a comprehensive genetic diversity spectrum due to their multi-allelic and highly polymorphic nature (Singh et al. 2016). Understanding the genetic diversity and population structure aids in molecular breeding programs, emphasizing the importance of trait association in breeding. Linkage disequilibrium or association mapping is instrumental in correlating phenotype with genotype, making it vital in analysing germplasm. Therefore, the present study aims to establish the suitability of PEG-6000 for drought screening and the identification of superior drought tolerant genotypes during the seedling stage itself. Following this, the selected genotypes were evaluated for population structure analysis, genetic diversity studies. Also the association of SSR markers with the traits specific to drought QTLs were also detected.

MATERIAL AND METHODS

Plant material. A total of 100 diverse rice landraces, including three tolerant checks, Apo, Wayreram, and Anna (R) 4, as well as two susceptible checks, IR 64 and Jaya, sourced from National Bureau of Plant Genetic Resources (NBPGR), New Delhi, were employed to assess drought tolerance using an optimised concentration of PEG-6000 (Table 1).

Optimization of PEG-6000 concentration. This study utilized a completely randomized design (CRD) featuring five distinct concentrations, with two replications each. The optimization of PEG-6000 concentration was conducted using three tolerant varieties *viz.,* Apo, Wayreram, Anna (R) 4 and two susceptible checks *viz.,* IR 64 and Jaya. Four treatments with the osmotic potentials of –2, –4, –6 and –8 bars were established by adding 12.60, 18.60, 23.20 and 27.10 g of PEG-6000 to 100 mL of distilled water along with control of using only distilled water of 0 bars was used for screening.

Screening of genotypes under optimized concentration. A total of 100 rice genotypes were screened under an optimised concentration of –6 bar (PEG-6000) under laboratory conditions in the Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology (CPMBB), Tamil Nadu Agricultural University (TNAU), Coimbatore. A factorial randomized complete block design with two replications was used to facilitate the combination

Table 1. List of rice landraces used in the present study for screening of drought tolerance

S. No.	Accession No.	S. No.	Accession No.	S. No.	Accession No.	S. No.	Accession No.	S. No.	Accession No.
1	IRGC22	21	IRGC145	41	IRGC264	61	IRGC385	81	IRGC483
2	IRGC47	22	IRGC146	42	IRGC272	62	IRGC403	82	IRGC486
3	IRGC48	23	IRGC155	43	IRGC282	63	IRGC411	83	IRGC487
4	IRGC58	24	IRGC158	44	IRGC291	64	IRGC413	84	IRGC488
5	IRGC88	25	IRGC170	45	IRGC292	65	IRGC414	85	IRGC493
6	IRGC93	26	IRGC173	46	IRGC295	66	IRGC420	86	IRGC495
7	IRGC94	27	IRGC177	47	IRGC297	67	IRGC421	87	IRGC508
8	IRGC95	28	IRGC179	48	IRGC298	68	IRGC424	88	IRGC509
9	IRGC102	29	IRGC216	49	IRGC306	69	IRGC428	89	IRGC516
10	IRGC104	30	IRGC222	50	IRGC310	70	IRGC437	90	IRGC518
11	IRGC105	31	IRGC223	51	IRGC313	71	IRGC439	91	IRGC522
12	IRGC108	32	IRGC224	52	IRGC317	72	IRGC444	92	IRGC526
13	IRGC109	33	IRGC227	53	IRGC318	73	IRGC445	93	IRGC527
14	IRGC111	34	IRGC229	54	IRGC319	74	IRGC446	94	IRGC533
15	IRGC113	35	IRGC230	55	IRGC326	75	IRGC448	95	IRGC535
16	IRGC121	36	IRGC231	56	IRGC336	76	IRGC456	96	IRGC540
17	IRGC125	37	IRGC242	57	IRGC342	77	IRGC460	97	IRGC541
18	IRGC127	38	IRGC251	58	IRGC344	78	IRGC461	98	IRGC542
19	IRGC129	39	IRGC253	59	IRGC361	79	IRGC466	99	IRGC544
20	IRGC136	40	IRGC254	60	IRGC381	80	IRGC467	100	IRGC545

Tolerant check: Apo, Wayreram, Anna (R) 4; susceptible check: Jaya, IR 64

https://doi.org/10.17221/12/2024-CJGPB

of two factors. The first factor was rice genotypes, and the second factor was two levels of PEG-6000, i.e. control and –6 bar concentration of PEG-6000 solution. The desired quantity of PEG-6000 for –6 bar concentration (23.20 g) was measured and mixed in distilled water whereas, for control, seeds were placed in distilled water (Kaufmann et al. 1971). The seeds were surface sterilized with 0.1% sodium hypochlorite solution and washed immediately three to four times with distilled water. A total of ten seeds per genotype were placed in separate sterilized Petri plates covered with blotting paper in two replications. The Petri plates were kept in dark condition until the germination occurred. Nine quantitative observations *viz.* germination %, shoot length (cm), root length (cm), number of secondary roots, root/shoot ratio, shoot/root ratio, fresh weight (g), dry weight (g) and vigour index followed by Gupta (1993) and Addanki et al. (2019) were measured on 14th day of stress as well as in control.

Genomic DNA extraction. The plant genomic extraction was carried out in Plant Molecular Laboratory, CPMBB, TNAU, Coimbatore. The young leaves of 10 to 15 days old seedlings from selected drought tolerant genotypes were clipped and genomic DNA was then extracted using modified CTAB method (Doyle & Doyle 1987). The isolated DNA was quantified using Nanodrop/UV-VIS-Spectrophotometer (ND-1000 Spectrophotometer, M/s. NanoDrop Technologies, USA) by measuring A260/A280 ratio and DNA quality was checked by electrophoresis in 0.8% agarose gel.

SSR markers and PCR amplification. A total of 26 rice SSR markers *viz.,* RM202, RM11, RM276, RM289, RM25, RM413, RM252, RM243, RM106, RM218, RM219, RM251, RM486, RM302, RM404, RM495, RM434, RM164, RM262, RM511, RM11928, RM246, RM5752, RM133, RM152 and RM212 related to drought study were used for molecular diversity analysis (Table 8). The PCR amplification was carried out in 10 µL of reaction mixture containing 100 ng genomic DNA (2 µL), 1× PCR Master Mix- Red (smART Prime) (3 µL), 1 µL 0.4 µM of each forward and reverse primer, and 3 µL sterile water using a thermal cycler (Eppendorf Mastercycler Nexus GSX1 Cycler, Germany). The thermal cycling program involved an initial denaturation at 94 °C for 10 min, denaturation at 94 °C for 30 s, annealing at 2 °C below melting temperature (Tm) of respective primers for 30 s, primer extension at 72 °C for 30 s for 35 cycles, followed by a final extension at 72 °C for 7 min and 4 °C for cooling (McCouch et al. 2002) (Master cycler gradient, Eppendorf). The amplified PCR products, along with a 100 bp ladder (BIO-HELIX, Taiwan), were size fractioned by electrophoresis in 3% agarose gel prepared in 1× TBE buffer and visualized under UV trans-illuminator at 302 nm.

Statistical analyses. The optimized concentration of PEG-6000 based on 50% germination was carried out based on probit analysis using the software MedCalc (Ver. 22.023). Using the R software with the help of the Agricolae package (de Mendiburu 2019) analysis of variance (ANOVA) was tested for its significance at 0.05 and 0.01 level, followed by mean comparison using Tukey's honest significant difference (HSD) test at a significance level of 5%. The principal component analysis of Jolliffee and Cadima (2016) describes the largest contributor to the total variance, which helps to visualize the data better. The principle components with more than 1 eigenvalues were taken for interpretation. The statistical computation was done using R software (Ver. 4.3.1.) with the help of FactoMineR and Factoshiny packages (Vaissie et al. 2021).

SSR data analysis. Using the 100 bp DNA ladder as a reference size, the sizes of the amplified fragments were scored. Population structure analysis was constructed using Bayesian clustering method in Structure (Ver. 2.3.4) (Pritchard et al. 2000). The length of the burn in period and Markov Chain Monte Carlo (MCMC) were set at 1 00 000 iterations (Evanno et al. 2005). To have accurate information, 10 runs for each K value ranging from 1 to 10. The K value was estimated based on the method given by Evanno et al. (2005) using STRUCTURE harvestor programme (Earl &Von Holdt 2012).

Genetic dissimilarity and cluster analysis based on UPGMA. The obtained data was analysed using dissimilarity-based methods, followed by cluster analysis based on the taxonomic distance matrix using the unweighted pair group method with arithmetic mean (UPGMA). A dendrogram was created based on the genetic distance matrix using the DARwin software (Dissimilarity Analysis and Representation, Ver. 6, Apple Inc., 2000).

Polymorphism information content and Shannon diversity index. The polymorphism information content (PIC) value was determined using a formula developed by Powell et al. (1997).

$$PIC = 1 - \Sigma Pi^2$$

where:

Pi –the frequency of the i^{th} locus, summed across all loci and lines.

https://doi.org/10.17221/12/2024-CJGPB

PIC values, which range from 0 (indicating monomorphism) to 1 (indicating high discriminative power with many alleles of equal and low frequency), were estimated for each profile across 37 rice genotypes. The Shannon diversity index (*H*) (Lewontin 1972) was calculated to determine the alleles present at each SSR locus for each individual, as given below:

$$H = -\Sigma_{i=1}^{n} Pi \times \ln(Pi)$$

where:
Pi –the relative abundance of allele *i*;
n – the total number of alleles at the locus.

The analysis was done by using MS EXCEL.

Single marker analysis. The association between the traits and the SSR markers was done based on the general linear statistical model using the software R studio (Ver. 4.3.1) using the Agricolae package (de Mendiburu 2019). The genotypic data and phenotypic data were used for the analysis. The marker-trait association was made significant when $P \leq 0.01$. The phenotypic variation (R^2) explained by the marker was estimated using R software (Ver. 4.3.1) using the Agricolae package (de Mendiburu 2019)

RESULTS AND DISCUSSION

The *in vitro* screening of rice landraces in the seedling stage using PEG-6000 cause drought stress by obstructing the movement of water inside the cell membrane by lowering the water potential (Adkins et al. 1995). Hence, in this study, four concentrations of PEG-6000 *viz.,* –2, –4, –6 and –8 bars were used and a concentration of –6 bars was optimised based on 50% germination (Table 2) using probit analysis (Figure 1). Root length for the three tolerant checks *viz.,* Apo, Wayreram, Anna (R) 4 was higher than two susceptible checks *viz.,* IR 64 and Jaya. This shows that root morphology plays an important role in drought conditions (Pepe et al. 2022). Seed germination is managed by several enzymes and decreasing the osmotic potential disrupts the enzyme activity. This results in a reduction of germination potential. Moreover, seeds require water for imbibitions, but increasing the PEG-6000 concentration lowers the water imbibitions and subsequently reduces the enzyme activity (Mahpara et al. 2022). Earlier studies have observed increased osmotic potential resulted in decreased seed germination in crops as well as in weed crops (Farooq et al. 2019). Subsequently, 100 rice landraces were used for screening of drought tolerance using optimised concentration at room temperature. The Seedling vigour index is the key trait for selecting better performing genotypes in terms of drought tolerance (Gupta 1993). The impact of elevated levels of PEG-6000 on seed germination was assessed to ascertain the water deficit tolerance of different rice genotypes (Islam et al. 2018). The vigour index, which broadly depends on germination % and seedling length, is necessary to select drought tolerant genotypes (Diwan et al. 2013). Germination % shows a negative trend with PEG-6000 concentration.

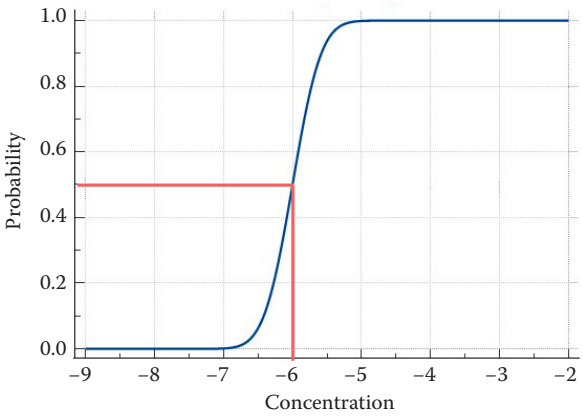

Figure 1. Probit analysis using germination % depicting dose-response curve for optimization of polyethylene glycol-6000 (PEG-6000) concentration

Table 2. Mean performance of tolerant and susceptible checks at –6 bar concentration (polyethylene glycol-6000)

Genotypes	Germination (%)	Shoot length (cm)	Root length (cm)	No. of secondary roots	Fresh weight (mg)	Dry weight (mg)	Vigour index
Apo	60	3.36	5.50	5	180	90	201.6
Wayreram	60	2.44	4.02	5	150	75	146.4
Anna (R) 4	50	2.50	5.54	4	160	84	125
IR 64	20	1.52	1.80	3	123	42	30.4
Jaya	20	2.00	2.50	2	110	35	40

https://doi.org/10.17221/12/2024-CJGPB

Table 3. List of 32 rice genotypes selected for further analyses based on germination % and vigour index

S. No.	Accession No.	S. No	Accession No.	S. No	Accession No.	S. No	Accession No.
1	IRGC93	11	IRGC381	21	IRGC487	31	IRGC540
2	IRGC95	12	IRGC403	22	IRGC493	32	IRGC542
3	IRGC108	13	IRGC411	23	IRGC495		
4	IRGC109	14	IRGC437	24	IRGC508		
5	IRGC121	15	IRGC445	25	IRGC509		
6	IRGC129	16	IRGC448	26	IRGC516		
7	IRGC146	17	IRGC461	27	IRGC518		
8	IRGC158	18	IRGC466	28	IRGC527		
9	IRGC177	19	IRGC467	29	IRGC533		
10	IRGC291	20	IRGC486	30	IRGC535		

Based on 50% germination and vigour index, a total of 32 genotypes were selected as seedling stage drought tolerant genotypes (Table 3). These 32 genotypes had satisfactory performance, achieving a 50% germination rate, allowing for the observation of other traits. The remaining drought susceptible genotypes failed to reach the 50% germination and did not survive.

The analysis of variance revealed significant genetic variation among the genotypes for all the traits studied, explaining the genetic heterogeneity among them, and Tukey's post hoc analysis indicated significant variation among the treatments (Table 4). The germination % expressed significant differences among the genotypes under drought stress conditions. Maximum germination % of 90% was observed by eleven genotypes *viz.,* IRGC93, IRGC146, IRGC291, IRGC467, IRGC486, IRGC487, IRGC509, IRGC516, IRGC527, IRGC535 and IRGC542. In contrast, shoot and root length were notably reduced under stress compared to control conditions. The longest shoot length of 8.85 and 5.45 cm was observed in IRGC109 under both control and stress conditions, while IRGC177 had the shortest length. The minimal difference in shoot length between control and drought treatment was observed by IRGC516, followed by IRGC488. Similarly, IRGC486 showed the longest root length (8.1 cm) under stress, whereas IRGC533 had the shortest (1.2 cm). The primary reason for the inhibition of root emergence is the reduction in the water potential gradient between the seed's external environment and the seed itself. This reduction subsequently hampers seedling shoot and root length (Sokoto & Muhammad 2014).

Secondary root development was affected upon PEG-6000 treatment, with IRGC486 observed the highest and IRGC542 the lowest number of roots. IRGC486 was also observed to have a minimal difference in a number of secondary root developments between non-stress and stress conditions. Fresh and dry weights were significantly influenced by PEG-6000 treatment, with IRGC509 showing the highest fresh weight and IRGC95 the lowest (Table 5). IRGC445, IRGC467 and IRGC403 were observed to have the highest dry weight, and IRGC95 had the lowest. The genotype IRGC535 exhibited the highest dry weight under non-stress conditions;

Table 4. Analysis of variance for different traits among the selected accessions with Tukey's post hoc test

Sources of variation	Df	Germination (%)	Shoot length	Root length	No. of secondary roots	Fresh weight	Dry weight	Shoot/root ratio	Root/shoot ratio	Vigour index
Control *vs.* stress	1	22 703.5*	903.53*	682.67*	1 110.4*	3.14*	0.07*	2.02*	4.43*	1 133.24*
Genotypes	36	384.7*	4.91*	8.63*	9.99*	0.01*	0.01*	0.4*	0.75*	419.77*
Control *vs.* stress × genotypes	36	384.7*	1.94*	7.35*	6.92*	0.01*	0.01*	0.3*	0.60*	218.42*
Error	74	0.1	1.57	3.57	5.63	0.01	0.01	0.3	0.43	117.95

Df – degree of freedom; *significant at 0.05 level

https://doi.org/10.17221/12/2024-CJGPB

Table 5. Effect of drought stress on germination %, shoot length, root length, no. of secondary roots, and fresh weight on different genotypes of rice

S. No.	Accession	Germination (%)		Shoot length (cm)		Root length (cm)		No. of secondary roots		Fresh weight (g)	
		control	drought	control	drought	control	drought	control	drought	control	drought
1	IRGC93	90.00 a	90.00 a	8.45 abcdefgh	2.23 opqrstu	6.25 abcdefg	3.03 defg	11.00 abcde	2.10 cdef	0.45 abcdefghijklmno	0.19 klmnopqr
2	IRGC95	90.00 a	63.40 b	8.70 abcdefg	2.62 klmnopqrstu	8.00 abcdefg	4.03 defg	12.50 ab	3.80 abcdef	0.58 abcd	0.11 qr
3	IRGC108	90.00 a	63.40 b	7.35 abcdefghijklmno	3.14 hijklmnopqrstu	4.95 bcdefg	2.48 defg	12.50 ab	3.20 abcdef	0.44 abcdefghijklmno	0.13 pqr
4	IRGC109	90.00 a	63.40 b	8.85 abcde	5.45 abcdefghijklmnopqrstu	5.60 bcdefg	5.36 bcdefg	9.00 abcdef	4.10 abcdef	0.44 abcdefghijklmno	0.24 fghijklmnopqr
5	IRGC121	90.00 a	50.80 c	7.15 abcdefghijklmnopq	1.34 stu	7.95 abcdefg	3.99 defg	12.50 ab	2.40 bcdef	0.46 abcdefghijklm	0.15 nopqr
6	IRGC129	90.00 a	63.40 b	6.70 abcdefghijklmnopqrs	1.73 qrstu	8.75 abcdef	3.60 defg	7.50 abcdef	1.60 def	0.49 abcdefghijk	0.13 qr
7	IRGC146	90.00 a	90.00 a	8.40 abcdefghi	2.26 opqrstu	14.30 a	3.06 defg	11.50 abcd	4.10 abcdef	0.57 abcde	0.20 hijklmnopqr
8	IRGC158	90.00 a	63.40 b	8.65 abcdefg	2.79 jklmnopqrstu	5.50 bcdefg	3.76 defg	8.00 abcdef	4.50 abcdef	0.47 abcdefghijklm	0.17 mnopqr
9	IRGC177	90.00 a	63.40 b	6.95 abcdefghijklmnopqr	1.16 tu	12.50 abc	1.99 defg	10.50 abcde	1.70 cdef	0.49 abcdefghijk	0.15 nopqr
10	IRGC291	90.00 a	90.00 a	7.25 abcdefghijklmnop	1.85 pqrstu	8.75 abcdef	5.79 bcdefg	7.00 abcdef	2.10 cdef	0.49 abcdefghijk	0.19 jklmnopqr
11	IRGC381	90.00 a	63.40 b	9.85 a	2.53 lmnopqrstu	8.25 abcdefg	3.19 defg	8.00 abcdef	3.50 abcdef	0.51 abcdefghi	0.25 fghijklmnopqr
12	IRGC403	90.00 a	26.60 e	8.40 abcdefghi	3.46 efghijklmnopqrstu	6.25 abcdefg	3.50 defg	8.50 abcdef	3.10 abcdef	0.50 abcdefghij	0.21 hijklmnopqr
13	IRGC411	90.00 a	63.40 b	6.15 abcdefghijklmnopqrst	2.30 nopqrstu	8.25 abcdefg	4.42 bcdefg	10.00 abcdef	4.20 abcdef	0.59 abc	0.28 defghijklmnopqr
14	IRGC437	90.00 a	50.80 c	8.55 abcdefgh	3.50 efghijklmnopqrstu	12.65 ab	5.47 bcdefg	12.00 abc	3.30 abcdef	0.52 abcdefg	0.20 jklmnopqr
15	IRGC445	90.00 a	50.80 c	6.55 abcdefghijklmnopqrst	2.94 ijklmnopqrstu	8.20 abcdefg	5.15 bcdefg	9.50 abcdef	4.30 abcdef	0.39 abcdefghijklmnopq	0.24 fghijklmnopqr
16	IRGC448	90.00 a	63.40 b	6.75 abcdefghijklmnopqrs	3.96 bcdefghijklmnopqrstu	4.95 bcdefg	5.74 bcdefg	8.50 abcdef	6.70 abcdef	0.48 abcdefghijkl	0.30 cdefghijklmnopq
17	IRGC461	90.00 a	50.80 c	9.35 ab	3.50 efghijklmnopqrstu	5.35 bcdefg	3.93 defg	10.50 abcde	5.00 abcdef	0.49 abcdefghijk	0.20 jklmnopqr
18	IRGC466	90.00 a	63.40 b	6.40 abcdefghijklmnopqrst	3.36 fghijklmnopqrstu	7.95 abcdefg	2.97 defg	8.00 abcdef	4.40 abcdef	0.51 abcdefghi	0.19 jklmnopqr
19	IRGC467	90.00 a	90.00 a	7.55 abcdefghijklmno	2.19 opqrstu	9.60 abcde	2.10 defg	11.50 abcd	2.60 bcdef	0.50 abcdefghij	0.13 qr
20	IRGC486	90.00 a	90.00 a	9.00 abcd	5.76 abcdefghijklmnopqrst	12.55 ab	8.10 abcdefg	8.00 abcdef	8.80 abcdef	0.54 abcdef	0.23 ghijklmnopqr
21	IRGC487	90.00 a	90.00 a	8.00 abcdefghijkl	2.62 klmnopqrstu	9.00 abcdef	4.26 cdefg	11.50 abcd	3.40 abcdef	0.36 bcdefghijklmnopq	0.16 mnopqr

Czech Journal of Genetics and Plant Breeding, 61, 2025 (1): 1–22

https://doi.org/10.17221/12/2024-CJGPB

Table 5 to be continued

S. No.	Accession	Germination (%)		Shoot length (cm)		Root length (cm)		No. of secondary roots		Fresh weight (g)	
		control	drought	control	drought	control	drought	control	drought	control	drought
22	IRGC493	90.00 a	63.40 b	8.05 abcdefghijk	1.83 pqrstu	7.60 abcdefg	2.17 defg	10.50 abcde	2.60 bcdef	0.51 abcdefgh	0.14 opqr
23	IRGC495	90.00 a	63.40 b	7.90 abcdefghijklm	1.58 rstu	7.70 abcdefg	2.22 defg	9.50 abcdef	1.80 cdef	0.41 abcdefghijklmnopq	0.17 mnopqr
24	IRGC508	90.00 a	50.80 c	9.40 ab	5.40 abcdefghijklmnopqrstu	5.55 bcdefg	5.04 bcdefg	13.00 a	5.70 abcdef	0.51 abcdefghi	0.27 efghijklmnopqr
25	IRGC509	90.00 a	90.00 a	8.70 abcdefg	5.43 abcdefghijklmnopqrstu	6.55 abcdefg	6.82 abcdefg	7.50 abcdef	7.30 abcdef	0.53 abcdefg	0.32 bcdefghijklmnopq
26	IRGC516	90.00 a	90.00 a	5.65 abcdefghijklmnopqrst	3.55 defghijklmnopqrstu	7.80 abcdefg	3.94 efg	9.50 abcdef	5.30 abcdef	0.46 abcdefghijklm	0.26 efghijklmnopqr
27	IRGC518	90.00 a	63.40 b	8.80 abcdef	3.23 ghijklmnopqrstu	6.50 abcdefg	3.56 defg	7.50 abcdef	4.60 abcdef	0.49 abcdefghijk	0.20 ijklmnopqr
28	IRGC527	90.00 a	90.00 a	8.25 abcdefghij	4.58 abcdefghijklmnopqrstu	5.70 bcdefg	4.01 defg	12.00 abc	5.90 abcdef	0.38 bcdefghijklmnopq	0.16 mnopqr
29	IRGC533	90.00 a	63.40 b	6.65 abcdefghijklmnopqrs	2.47 mnopqrstu	10.20 abcd	1.20 fg	6.50 abcdef	3.20 abcdef	0.28 defghijklmnopqr	0.15 nopqr
30	IRGC535	90.00 a	90.00 a	8.10 abcdefghij	4.41 abcdefghijklmnopqrstu	7.65 abcdefg	2.71 defg	7.50 abcdef	5.80 abcdef	0.25 fghijklmnopqr	0.18 lmnopqr
31	IRGC540	90.00 a	50.80 c	9.80 a	1.47 stu	8.45 abcdef	2.06 defg	7.00 abcdef	2.60 bcdef	0.45 abcdefghijklmn	0.17 mnopqr
32	IRGC542	90.00 a	90.00 a	8.65 abcdefg	1.67 rstu	8.15 abcdefg	1.57 efg	7.50 abcdef	1.10 ef	0.33 bcdefghijklmnopq	0.19 jklmnopqr
33	Apo	90.00 a	50.80 c	9.05 abc	3.55 defghijklmnopqrstu	7.15 abcdefg	2.85 defg	6.50 abcdef	2.00 cdef	0.45 abcdefghijklmno	0.15 nopqr
34	Wayreram	90.00 a	63.40 b	7.75 abcdefghijklmn	3.75 cdefghijklmnopqrstu	7.30 abcdefg	4.45 bcdefg	7.00 abcdef	3.00 abcdef	0.62 ab	0.26 fghijklmnopqr
35	Anna (R) 4	90.00 a	45.00 d	8.00 abcdefghijkl	4.20 bcdefghijklmnopqrstu	7.40 abcdefg	3.80 defg	7.50 abcdef	5.00 abcdef	0.69 a	0.14 opqr
36	IR64	90.00 a	18.40 f	6.20 abcdefghijklmnopqrst	0.00 u	4.95 bcdefg	0.00 g	6.00 abcdef	0.00 f	0.44 abcdefghijklmnop	0.00 r
37	Jaya	90.00 a	26.60 e	4.70 abcdefghijklmnopqrstu	0.00 u	7.05 abcdefg	0.00 g	4.50 abcdef	0.00 f	0.53 abcdefg	0.00 r

Numbers followed by the same letter in the same column are not significantly different in the Tukey's test at 0.05

https://doi.org/10.17221/12/2024-CJGPB

Table 6. Effect of drought stress on dry weight, shoot /root ratio, root/shoot ratio, vigour index on different genotypes of rice

S. No.	Accession	Dry weight (g)		Shoot/root ratio		Root/shoot ratio		Vigour index	
		control	drought	control	drought	control	drought	control	drought
1	IRGC93	0.07 abcdefgh	0.02 ijklmnop	1.36 a	1.11 a	0.74 abc	2.74 abc	845.00 abc	223.00 ijklmnopqr
2	IRGC95	0.06 abcdefghijklmno	0.01 op	1.09 a	0.65 a	0.92 abc	1.67 abc	870.00 abc	209.60 jklmnopqr
3	IRGC108	0.07 abcdefghij	0.02 ghijklmnop	1.85 a	1.22 a	0.65 bc	1.13 abc	735.00 abcdefg	251.20 hijklmnopqr
4	IRGC109	0.05 abcdefghijklmno	0.04 abcdefghijklmnop	1.60 a	1.01 a	0.60 bc	0.99 abc	885.00 abc	436.00 cdefghijklmnopqr
5	IRGC121	0.06 abcdefghijklmno	0.03 defghijklmnop	0.90 a	0.34 a	1.11 abc	2.96 ab	715.00 abcdefgh	80.40 qr
6	IRGC129	0.07 abcdefghij	0.02 ijklmnop	0.77 a	0.55 a	1.32 abc	2.40 abc	670.00 abcdefghijk	138.40 opqr
7	IRGC146	0.08 abc	0.03 fghijklmnop	0.59 a	0.74 a	1.71 abc	1.35 abc	840.00 abc	226.00 ijklmnopqr
8	IRGC158	0.06 abcdefghijklmno	0.04 abcdefghijklmnop	2.30 a	0.74 a	0.63 bc	1.42 abc	865.00 abc	223.20 ijklmnopqr
9	IRGC177	0.08 abcdef	0.03 abcdefghijklmnop	0.56 a	0.59 a	1.82 abc	1.80 abc	695.00 abcdefghi	92.80 pqr
10	IRGC291	0.08 abcde	0.01 nop	0.87 a	0.29 a	1.25 abc	3.62 a	725.00 abcdefgh	185.00 lmnopqr
11	IRGC381	0.08 abcd	0.01 mnop	1.26 a	0.93 a	0.84 abc	1.90 abc	985.00 a	202.40 jklmnopqr
12	IRGC403	0.08 ab	0.04 abcdefghijklmnop	1.37 a	1.14 a	0.74 abc	0.91 abc	840.00 abc	69.20 qr
13	IRGC411	0.08 abcdef	0.03 fghijklmnop	0.77 a	0.59 a	1.39 abc	2.00 abc	615.00 abcdefghijklmn	184.00 lmnopqr
14	IRGC437	0.08 abc	0.02 klmnop	0.68 a	0.64 a	1.47 abc	1.56 abc	855.00 abc	210.00 jklmnopqr
15	IRGC445	0.06 abcdefghijklmn	0.04 abcdefghijklmnop	0.80 a	0.51 a	1.26 abc	2.11 abc	655.00 abcdefghijkl	176.40 mnopqr
16	IRGC448	0.07 abcdefghij	0.02 jklmnop	1.73 a	0.93 a	0.76 abc	1.19 abc	675.00 abcdefghij	316.80 efghijklmnopqr
17	IRGC461	0.07 abcdefghij	0.04 abcdefghijklmnop	1.77 a	0.97 a	0.58 bc	1.14 abc	935.00 ab	210.00 jklmnopqr
18	IRGC466	0.08 abc	0.04 abcdefghijklmnop	0.80 a	1.14 a	1.26 abc	0.88 abc	640.00 abcdefghijklm	268.80 ghijklmnopqr
19	IRGC467	0.06 abcdefghijklm	0.04 abcdefghijklmnop	0.79 a	1.01 a	1.28 abc	1.00 abc	755.00 abcdef	219.00 jklmnopqr
20	IRGC486	0.07 abcdefghi	0.03 efghijklmnop	0.72 a	0.77 a	1.40 abc	1.41 abc	900.00 abc	576.00 abcdefghijklmno
21	IRGC487	0.07 abcdefghijk	0.03 defghijklmnop	0.92 a	0.53 a	1.15 abc	2.23 abc	800.00 abcd	262.00 ghijklmnopqr

https://doi.org/10.17221/12/2024-CJGPB

Table 6 to be continued

S. No.	Accession	Dry weight (g)		Shoot/root ratio		Root/shoot ratio		Vigour index	
		control	drought	control	drought	control	drought	control	drought
22	IRGC493	0.06 abcdefghijkl	0.03 cdefghijklmnop	1.09 a	2.27 a	0.93 abc	1.79 abc	805.00 abcd	146.40 nopqr
23	IRGC495	0.07 abcdefghi	0.02 hijklmnop	1.18 a	0.82 a	1.02 abc	1.41 abc	790.00 abcde	126.40 opqr
24	IRGC508	0.08 abcdefg	0.02 ijklmnop	1.74 a	1.07 a	0.59 bc	0.93 abc	940.00 ab	324.00 efghijklmnopqr
25	IRGC509	0.08 abc	0.02 jklmnop	1.35 a	0.80 a	0.78 abc	1.26 abc	870.00 abc	543.00 abcdefghijklmnopq
26	IRGC516	0.06 abcdefghijklmn	0.03 defghijklmnop	0.72 a	0.90 a	1.47 abc	1.15 abc	565.00 abcdefghijklmnop	355.00 defghijklmnopqr
27	IRGC518	0.07 abcdefghi	0.03 cdefghijklmnop	1.35 a	0.95 a	0.74 abc	1.06 abc	880.00 abc	258.40 hijklmnopqr
28	IRGC527	0.07 abcdefghij	0.03 defghijklmnop	1.47 a	1.20 a	0.71 bc	0.85 abc	825.00 abcd	458.00 cdefghijklmnopqr
29	IRGC533	0.08 abc	0.02 jklmnop	0.67 a	2.07 a	1.52 abc	0.48 bc	665.00 abcdefghijk	197.60 klmnopqr
30	IRGC535	0.09 a	0.02 jklmnop	1.14 a	1.78 a	0.99 abc	0.58 bc	810.00 abcd	441.00 cdefghijklmnopqr
31	IRGC540	0.05 abcdefghijklmno	0.01 nop	1.21 a	0.72 a	0.86 abc	1.44 abc	980.00 a	88.20 qr
32	IRGC542	0.07 abcdefghi	0.02 ijklmnop	1.10 a	1.11 a	0.96 abc	0.91 abc	865.00 abc	167.00 mnopqr
33	Apo	0.03 defghijklmnop	0.03 bcdefghijklmnop	1.31 a	1.24 a	0.78 abc	0.86 abc	775.00 abcde	300.00 fghijklmnopqr
34	Wayreram	0.08 abc	0.02 jklmnop	1.10 a	0.85 a	0.94 abc	1.19 abc	905.00 abc	213.00 jklmnopqr
35	Anna (R) 4	0.05 abcdefghijklmno	0.01 lmnop	1.08 a	1.11 a	0.93 abc	0.91 abc	620.00 abcdefghijklmn	0.00 r
36	IR64	0.06 abcdefghijk	0.00 p	1.25 a	0.00 a	0.80 abc	0.00 c	470.00 bcdefghijklmnopqr	0.00 r
37	Jaya	0.04 abcdefghijklmnop	0.00 p	0.68 a	0.00 a	1.51 abc	0.00 c	800.00 abcd	0.00 r

Numbers followed by the same letter in the same column are not significantly different in the Tukey's test at 0.05

https://doi.org/10.17221/12/2024-CJGPB

however, under stress conditions, it showed a lower dry weight. This reduction in dry weight under low soil moisture could be attributed to decreased leaf area and photosynthesis rate (Zubarer et al. 2007). In contrast, the genotype IRGC445 was observed to retain leaf moisture content under drought stress, suggesting it might have dehydration tolerance, which allows the plants to maintain metabolic processes despite low leaf water potential.

Notable differences were also seen in shoot/root and root/shoot ratios between control and stress conditions, with IRGC493 showing the highest shoot/root ratio and IRGC291 the highest root/shoot ratio under stress (Table 6). Similarly, Sobahan et al. (2022) observed that BRRI dhan71 exhibited a lower percentage of weight reduction in terms of both fresh weight and dry weight under the PEG-6000 treatment compared to the control. Conversely, BRRI dhan49 showed a higher percentage of weight reduction compared to the control. The vigour index, calculated based on germination percentage and shoot length, varied significantly among genotypes, with IRGC486 recording the highest and IRGC403 the lowest values. A similar reduction in germination %, shoot length, root length, fresh weight, dry weight and vigour index when the concentration of PEG-6000 increases was observed by several researchers in rice genotypes (Priya et al. 2022; Evamoni et al. 2023; Fatimah et al. 2023).

The principal component analysis on several traits of different genotypes yielded three principal components (PC) with more than one eigenvalues (Table 7). Several studies reported more than one eigenvalue in different crops *viz.* rice (Nachimuthu et al. 2015), Blackgram (Ghafoor & Arshad 2008), wheat (Adilova et al. 2020), barley (Enyew et al. 2019) and maize (Hazif et al. 2015).

The first three principal components (PC1, PC2 and PC3) explained 88.89% of total variability, and the remaining principal components explained 11.11% of the variability. The characters shoot length, root length, number of secondary roots, fresh weight, dry weight, shoot/root ratio and vigour index observed positive loading in PC 1. The second PC was positively affected by root length, fresh weight, dry weight and root/shoot ratio, whereas shoot length, root length, number of secondary roots, root/shoot ratio and vigour index observed positive loading factor in PC3 (Table 7). Traits clustering within diverse principal components might receive increased emphasis in breeding endeavors owing to their tendency to co-occur (Chakravorty et al. 2013). Hence, the characters associated with the first three PCs are more important for differentiating among the genotypes (Ponsiva et al. 2019).

The screeplot explains the variation % between eigen values and the principal components (Christina et al. 2021). In this study, PC1 expressed 51.09% of the variance with eigenvalue of 4.08 whereas, PC2 and PC3 resulted in 24.92% and 12.88% of variance with eigenvalue of 1.99 and 1.03 respectively (Figure 2).

The biplot describes the interaction between the traits and the performance of genotypes linked to the traits. The vector length shows the contribution of the trait to total divergence (Kasanaboina et al. 2022), i.e., the longer the vector length, the more the contribution of the trait towards divergence and vice versa. Shoot length followed by a number of secondary roots and vigour index expressed maximum vector length depicting its total divergence (Figure 3). Under drought stress, root growth is limited and enhances secondary growth, whereas shoot growth is ceased (Lipiec et al. 2013). An angle < 90° between each vector indicates its positive relationship, whereas the right-angled vector indicates that the traits are not correlated to each other, and a wide-angle depicts a negative relationship (Christina et al. 2021). Here, except for shoot/root ratio and root/shoot ratio all the traits vector had a positive relationship. The tolerant checks were placed in the positive quadrant whereas, the susceptible checks were observed under fourth negative quadrant (Figure 3). The genotypes falling

Table 7. Eigenvalues, variability and factor loadings of the first two principal components (PC) of principal components analysis depicted on various traits of rice landraces

	PC1	PC2	PC3
Parameter			
Eigenvalue	4.08	1.99	1.03
% of variance	51.09	24.92	12.88
Cumulative (%)	51.09	76.02	88.90
Factor loadings			
Shoot length	0.46	–0.17	0.15
Root length	0.39	0.32	0.31
No. of secondary roots	0.44	–0.08	0.14
Fresh weight	0.39	0.17	–0.37
Dry weight	0.18	0.28	–0.78
Shoot/root ratio	0.01	–0.60	–0.26
Root/shoot ratio	–0.12	0.61	0.12
Vigour index	0.46	–0.11	0.10

https://doi.org/10.17221/12/2024-CJGPB

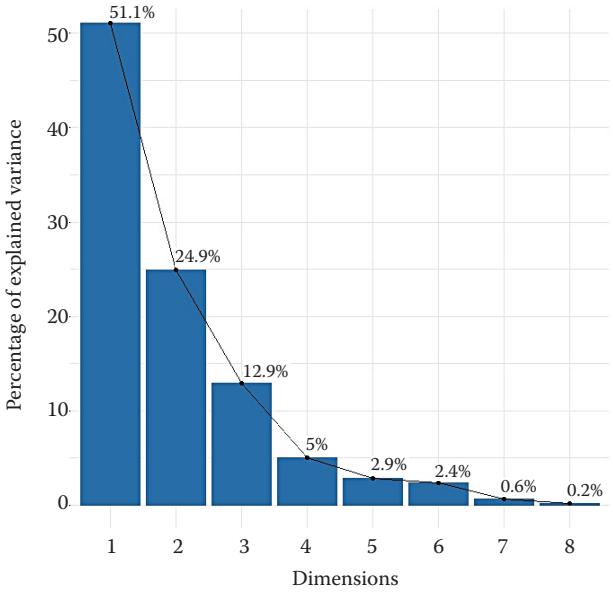

Figure 2. Scree plot diagram based on principal components of 37 rice landraces

under the first quadrant *viz.* IRGC109, IRGC403, IRGC448, IRGC461, IRGC466, IRGC486, IRGC508, IRGC518, IRGC527 and IRGC535 are the drought tolerant genotypes based on seedling shoot length, number of secondary roots and vigour index. Based on seedling root length, fresh weight and dry weight the five genotypes *viz.* IRGC381, IRGC411, IRGC437, IRGC445 and IRGC509 were found to perform better under drought conditions. These identified genotypes could be further evaluated under target production environment for testing the stability for drought tolerance and to evolve best pre-breeding drought tolerant donors. A similar result of drought tolerance in bread wheat cultivars based on the principal component analysis was described by Bilgili et al. (2019).

To understand the knowledge of diversity among the selected landraces. Population structure and molecular diversity analyses were performed using 26 SSR markers exclusively on those germinated 32 genotypes. However, for comparative purposes, both drought-tolerant and susceptible checks were included. Model-based approach by STRUCTURE for studying population structure was implemented frequently by various researchers (Garris et al. 2005; Jin et al. 2010; Courtois et al. 2012). The SSR markers were selected based on the drought QTLs. As per the log-likelihood LnP (D) and Evanno's ad hoc measure, ΔK expressed a high peak at an optimal K value of 2 (ΔK=2). A higher ΔK value was chosen with respect to a number of clusters (Evanno et al. 2005) indicating that the genotypes could be grouped into two subpopulations. As per the Evano table output, the K = 2 was observed to be the best based on a high ΔK value of 5.8. Genotypes having > 0.80 were admitted as pure line populations, while those that were < 0.80 as admixtures (Anandan et al. 2016a, b). Population structure grouped the thirty-seven genotypes into two subpopulations. Fifteen rice genotypes representing

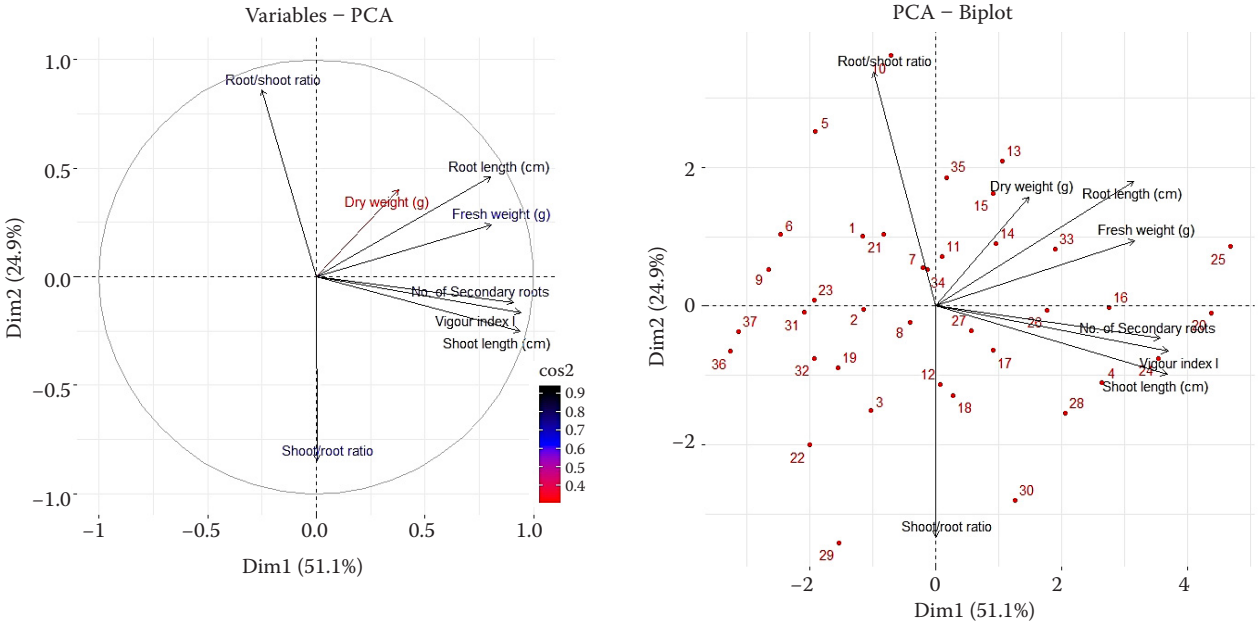

Figure 3. Biplot depicting two principal components

https://doi.org/10.17221/12/2024-CJGPB

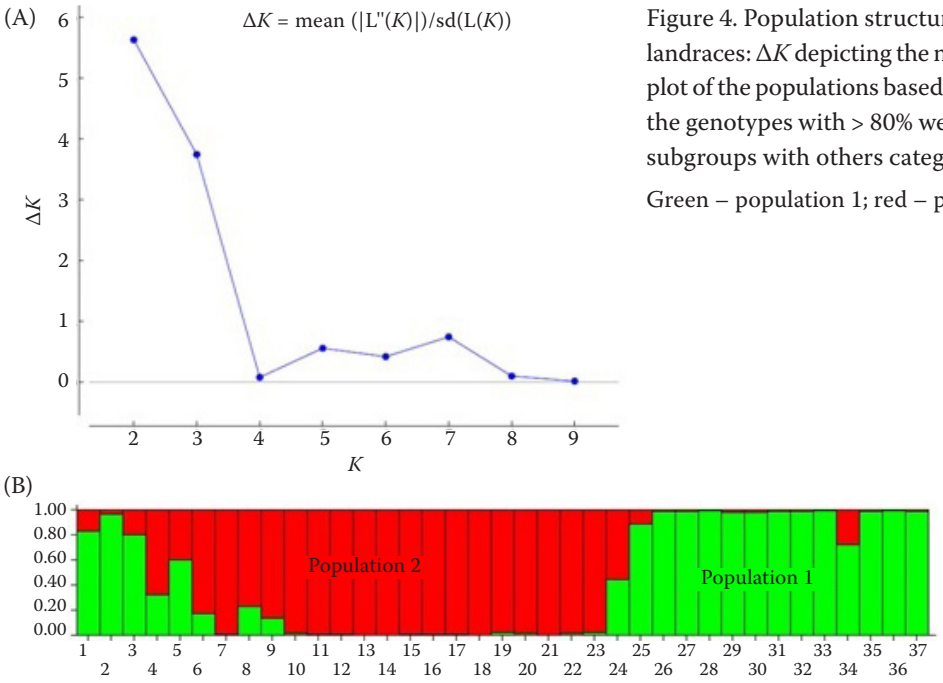

Figure 4. Population structure analysis of thirty seven rice landraces: Δ*K* depicting the number of populations (A), bar plot of the populations based on the membership fractions, the genotypes with > 80% were assigned to corresponding subgroups with others categorized as admixtures (B)

Green – population 1; red – population 2

40% of the population were assigned as subpopulation 1 represented in green colour and the remaining 22 genotypes were categorized as fifteen pure genotypes and seven admixtured ones representing 60% were assigned as subpopulation 2 (red colour) (Figure 4). The genotypes identified based on higher shoot length, number of secondary roots and vigour index was grouped under subpopulation 2 with few admixture lines. The reason for the admixture may be due to the diverse ancestral backgrounds with different domestication process through which the landraces have evolved. Jin et al. (2010) observed seven subpopulations among 416 rice accessions. Roy et al. (2016) identified two subpopulations among 126 rice genotypes by population structure analysis. The fixation index (Fst) of 0.13 for subpopulation 1 and 0.18 for subpopulation 2 indicates moderate divergence between the two groups. The allele frequency divergence is measured at 0.095 which is a measure of fixation index revealing that the moderate divergence is not extremely pronounced. The alpha value observed as 0.08 revealing 8% of the population have admixture which indicates low level of admixture. Pradhan et al. (2016) observed lower alpha value among 240 rice germplasm which was grouped into three subpopulations. In subpopulation 1 and 2, the expected heterozygosity is 0.56 and 0.53 respectively, suggesting that approximately 50% of genotypes within the population have the chance of being heterozygotes.

All the markers produced polymorphism and resulted in a total of 115 alleles with an average of 4.42 alleles per marker. For each marker, the number of alleles ranged from 2 to 9 alleles (Figure 5, 6). The highest alleles were generated by the marker RM252. The markers *viz.* RM302 (2), RM11928 (2), RM133 (2) and RM212 (2) produced the fewest number of alleles. Earlier studies observed number of alleles ranged from 2 (RM19) to 7 (RM44) among *Oryza rufipogon* population (Song et al. 2003). The amplified fragments varied in size from 90 to 295 bp.

Polymorphic information content (PIC) describes the frequency of each allele and represents the allelic diversity (Ashraf et al. 2016). The PIC values in this study ranged from 0.192 to 0.837 (Table 8), with an average PIC value of 0.613 per primer. The PIC value of > 0.5 is regarded as highly polymorphic, and the SSR primers employed in this work showed an average PIC value of 0.613, indicating that they were highly informative (Serrote et al. 2020). The SSR primer, RM252, revealed the highest (0.837) PIC value, whereas the primer RM212 revealed the lowest (0.19) (Figure 7). It was demonstrated that primers that have fewer alleles revealed less gene variability than those that observed more alleles, which revealed more gene diversity (Islam et al. 2023). The markers expressing more than 0.5 PIC value could be further used for phylogenetic studies (Sakina et al. 2022). Measures on the Shannon diversity index described the heterozygous nature of the genotypes studied. The

https://doi.org/10.17221/12/2024-CJGPB

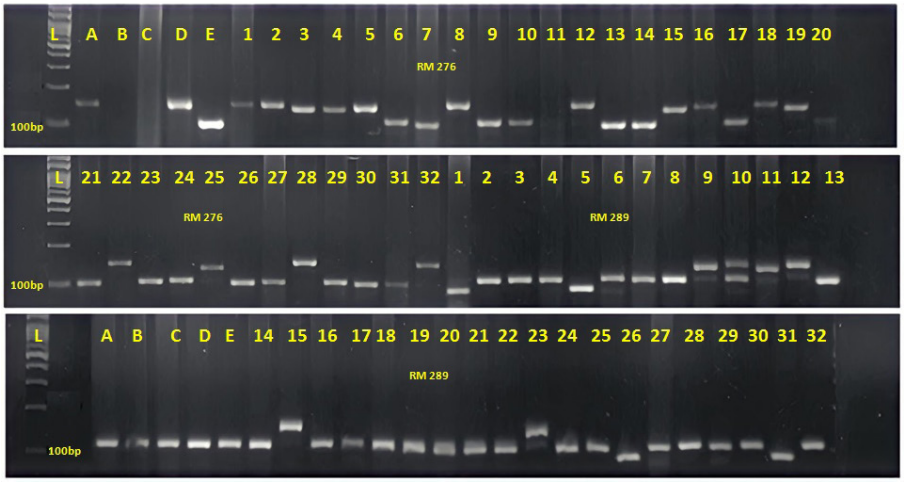

Figure 5. Screening of rice landrace using SSR markers using drought linked QTL SSR markers

L – 100bp ladder; A – Apo; B – Wayreram; C – Anna (R) 4; D – IR64; E – Jaya; 1 – IRGC93; 2 – IRGC95; 3 – IRGC108; 4 – IRGC109; 5 – IRGC121; 6 – IRGC129; 7 – IRGC146; 8 – IRGC158; 9 – IRGC177; 10 – IRGC291; 11 – IRGC381; 12 – IRGC403; 13 – IRGC411; 14 – IRGC437; 15 – IRGC445;16 – IRGC448; 17 – IRGC461; 18 – IRGC466; 19 – IRGC467; 20 – IRGC486; 21 – IRGC487; 22 – IRGC493; 23 – IRGC495; 24 – IRGC508; 25 – IRGC509; 26 – IRGC516; 27 – IRGC518; 28 – IRGC527; 29 – IRGC533; 30 – IRGC535; 31 – IRGC540; 32 – IRGC542

index ranged between 0.34 for RM212 and 1.96 for RM252 with a mean of 1.18. In this study, Shannon's information index served as an additional indicator highlighting the considerable genetic diversity present within the rice germplasm. Yang et al. (2021) studied the Shannon diversity index of 0.13 to 0.48, with a mean of 0.28 observed from a total of 48 SSR markers in rice while screening genotypes for several

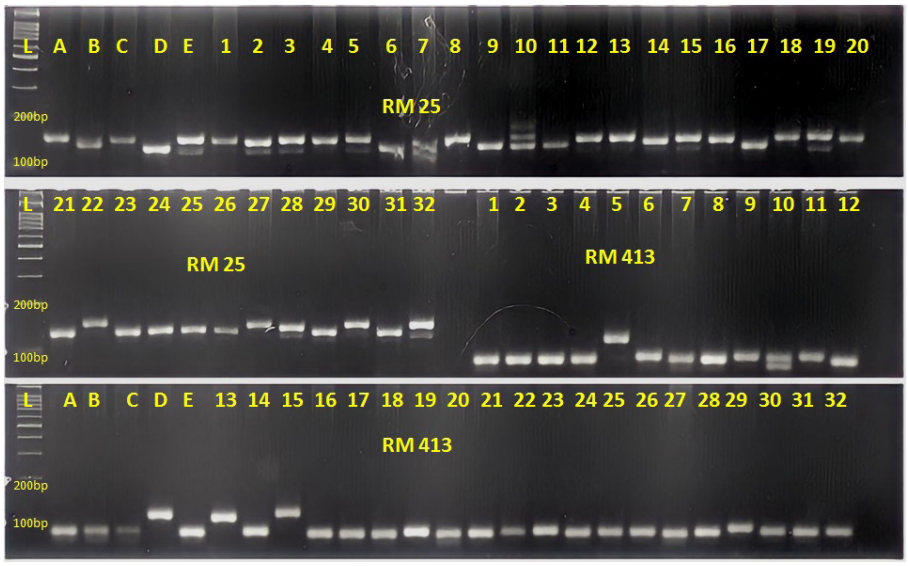

Figure 6. Screening of identified rice landrace using drought linked QTL SSR markers

L – 100bp ladder; A – Apo; B – Wayreram; C – Anna (R) 4; D – IR64; E – Jaya; 1 – IRGC93; 2 – IRGC95; 3 – IRGC108; 4 – IRGC109; 5 – IRGC121; 6 – IRGC129; 7 – IRGC146; 8 – IRGC158; 9 – IRGC177; 10 – IRGC291; 11 – IRGC381; 12 – IRGC403; 13 – IRGC411; 14 – IRGC437; 15 – IRGC445; 16 – IRGC448; 17 – IRGC461; 18 – IRGC466; 19 – IRGC467; 20 – IRGC486; 21 – IRGC487; 22 – IRGC493; 23 – IRGC495; 24 – IRGC508; 25 – IRGC509;26 – IRGC516; 27 – IRGC518; 28 – IRGC527; 29 – IRGC533; 30 – IRGC535; 31 – IRGC540; 32 – IRGC542

https://doi.org/10.17221/12/2024-CJGPB

Table 8. List of SSR markers used for molecular diversity analysis

S. No.	Marker name	Forward and reverse sequence	Tm (°C)	Chromosome No.	Expected product size (bp)	PIC value	Shannon diversity index	Reference
1	RM202	CTCGTTTATTACCTACAGTACC CTACCTCCTTTCTAGACCGATA	55	11	189	0.52	0.79	Rejeth et al. (2020)
2	RM11	TCTCCTCTTCCCCCGATC ATAGCGGGCGAGGCTTAG	55	7	140	0.72	1.33	Mahender et al. (2015)
3	RM276	CTCAACGTTGACACCTCGTG TCCTCCATCGAGCAGTATCA	56	6	149	0.80	1.80	Panda et al. (2019)
4	RM289	TTCCATGGCACACAAGCC CTGTGCACGAACTTCCAAAG	56	5	108	0.64	1.35	Gaballah et al. (2021)
5	RM25	GGAAAGAATGATCTTTTCATGG CTACCATCAAAACCAATGTTC	49	8	146	0.60	1.09	Singh et al. (2017)
6	RM413	CCAATCTTGTCTTCCGGATCTTGC AGATAGCCATGGGCGATTCTTGG	52	5	80	0.37	0.73	Salem and Sallam (2016)
7	RM252	TTCGCTGACGTGATAGGTTG ATGACTTGATCCCGAGAACG	55	4	216	0.84	1.96	Singh et al. (2012)
8	RM243	GATCTGCAGACTGCAGTTGC AGCTGCAACGATGTTGTCC	55	1	116	0.73	1.59	Noryan et al. (2021)
9	RM106	CGTCTTCATCATCGTCGCCCCG GGCCCATCCCGTCGTGGATCTC	55	2	297	0.69	1.35	Anandan et al. (2016a, b)
10	RM218	TGGTCAAACCAAGGTCCTTC GACATACATTCTACCCCCGG	55	3	148	0.83	1.75	El-Gamal et al. (2015)
11	RM219	CGTCGGATGATGTAAAGCCT CATATCGGCATTCGCCTG	55	9	202	0.63	1.25	Mas-ud et al. (2022)
12	RM251	GAATGGCAATGGCGCTAG ATGCGGTTCAAGATTCGATC	55	3	147	0.75	1.69	Kotla et al. (2013)
13	RM486	CCCCCCTCTCTCTCTCTCTC TAGCCACATCAACAGCTTGC	55	1	104	0.70	1.33	Sanghamitra et al. (2021)
14	RM302	TCATGTCATCTACCATCACAC ATGGAGAAGATGGAATACTTGC	55	1	156	0.27	0.51	Bhattarai et al. (2019)
15	RM404	CCAATCATTAACCCCTGAGC GCCTTCATGCTTCAGAAGAC	55	8	236	0.73	1.56	Srividhya et al. (2011)
16	RM495	AATCCAAGGTGCAGAGATGG CAACGATGACGAACACAACC	55	1	159	0.68	1.23	Noryan et al. (2021)
17	RM434	GCCTCATCCCTCTAACCCTC CAAGAAAGATCAGTGCGTGG	55	9	152	0.66	1.07	Srividhya et al. (2011)
18	RM164	TCTTGCCCGTCACTGCAGATATC GCAGCCCTAATGCTACAATTCT	55	5	246	0.74	1.37	Gaballah et al. (2021)
19	RM262	CATTCCGTCTCGGCTCAACT CAGAGCAAGGTGGCTTGC	55	2	154	0.65	1.07	Venuprasad et al. (2009)
20	RM511	CTTCGATCCGGTGACGAC AACGAAAGCGAAGCTGTCTC	55	12	130	0.53	1.08	Tabkhkar et al. (2018)
21	RM11928	TAAACCAGATCATGCCCTCATCC AGCAGTAACGGTTGGGTACTTGG	55	1	280	0.60	0.88	Renuprasath et al. (2023)

https://doi.org/10.17221/12/2024-CJGPB

Table 8 to be continued

S. No.	Marker name	Forward and reverse sequence	Tm (°C)	Chromosome No.	Expected product size (bp)	PIC value	Shannon diversity index	Reference
22	RM246	GAGCTCCATCAGCCATTCAG CTGAGTGCTGCTGCGACT	55	1	116	0.51	0.85	Subashri et al. (2009)
23	RM5752	TTGCAATTAATTCGATCTCC GCAGATCGATTCGTTAGTTC	55	7	138	0.56	0.95	Mohanty et al. (2021)
24	RM133	TTGGATTGTTTTGCTGGCTCGC GGAACACGGGGTCGGAAGCGAC	60	6	230	0.31	0.56	Noryan et al. (2021)
25	RM152	GAAACCACCACACCTCACCG CCGTAGACCTTCTTGAAGTAG	61	8	151	0.59	0.98	Bhattarai et al. (2019)
26	RM212	CCACTTTCAGCTACTACCAG CACCCATTTGTCTCTCATTATG	56	1	117	0.19	0.34	Salam et al. (2017)

Tm – melting temperature; PIC – polymorphism information content

agronomic traits. Hence, the allelic diversity measures help in further dissecting the overall variations present in the population.

The dendrogram based on dissimilarity matrix by UPGMA method grouped the genotypes into seven clusters on the basis of SSR marker alleles (Figure 8). High dissimilarity was found between the genotypes *viz.* IRGC93 & IRGC437; IRGC509 & Jaya; IRGC509 & IRGC93; IRGC509 &IRGC129 (0.88). The lowest dissimilarity matrix was found between IR64 & Anna (R) 4 (0.27). Cluster analysis showed a broad genetic background among selected landraces (Pascual et al. 2020). Among the seven clusters, the highest number of genotypes was present in cluster V with 13 genotypes (IRGC466, IRGC448, IRGC445, IRGC461, IRGC411, IRGC146, IRGC437, IRGC381, IRGC177, IRGC125, IRGC291, IRGC403 and IRGC158) followed by the cluster VI with eight genotypes (Apo, Wayreram, Anna (R) 4, IRGC121, IRGC108, IRGC109, IRGC93 and IRGC95). Only one

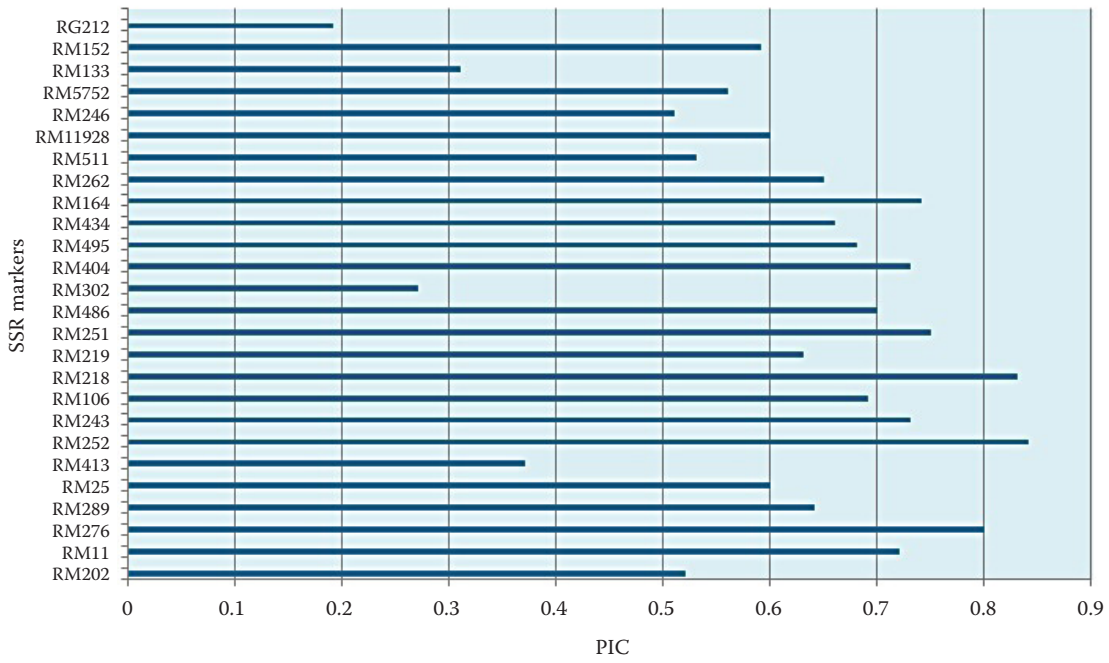

Figure 7. Polymorphic information content (PIC) of 26 SSR markers utilized in this study

https://doi.org/10.17221/12/2024-CJGPB

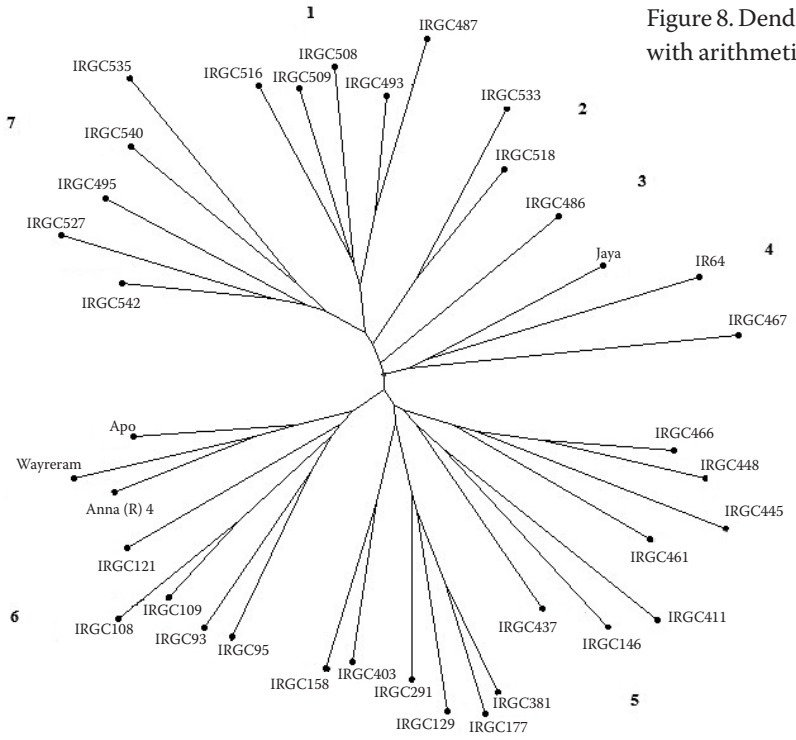

Figure 8. Dendrogram using unweighted pair group method with arithmetic mean (UPGMA) method for 37 genotypes

genotype, IRGC486, having a positive relationship with shoot length and vigour index was observed in cluster III.

Moreover, a marker trait association study conducted among the germplasm collection could be useful for the identification of molecular markers linked to the particular trait of interest (Pradhan et al. 2016). The marker trait association depends on the genetic distance between the genotypes and the strength of linkage disequilibrium between the markers (Ashfaq et al. 2014). A total of seven SSR markers were significantly associated with all the studied traits in general linear model at $P \leq 0.05$ (Table 9). Marker trait association was tested between the studied SSR markers and the traits studied. The markers *viz.* RM246, RM302, RM252, RM219, RM251, and RM486 were significantly associated with shoot length, root length, number of secondary roots, dry weight, shoot/root ratio and root/shoot ratio, respectively, with phenotypic variance ranging from 11.57% to 32.71%. The marker RM302 expresses a pleiotropic effect governing root length and root/shoot ratio. Ashfaq et al. (2014) observed RM302 to be associated with root traits due to strong linkage disequilibrium in rice. Likewise, several markers were reported to be associated with different agronomic traits with phenotypic variance ranging from 11% to 32 %, respectively. For example, RM215 is associated with decreased plant height under drought stress in rice (Wang et al. 2016). Similarly, RM152 was associated with drought score in rice (Ramchander et al. 2016).

CONCLUSION

Drought stress significantly affects the sensitivity of rice genotypes during seed germination. The observation revealed that increasing PEG-6000 concentrations had an impact on seed germination and seedling growth in rice genotypes, representing drought stress. Consequently, –6 bars emerged as the optimized dose among all concentrations, enabling the screening of genotypes based on probit analysis. The identified accessions *viz.* IRGC109, IRGC403, IRGC448, IRGC461, IRGC466, IRGC486, IRGC508, IRGC518, IRGC527 and IRGC535 expressing positive relationship with shoot length, number of secondary roots and vigor index. The observation of molecular diversity among the identified accessions revealed greater divergence, and the marker RM252 with the highest PIC value ought to be used further in drought tolerant studies in rice. Significant associations were observed between the markers RM246, RM302, RM252, RM219, RM251, and RM486 in the respective traits, *viz.* shoot length, root length, number

https://doi.org/10.17221/12/2024-CJGPB

Table 9. Association of SSR markers with respective traits

Trait S. No.	Trait	Marker	Chromosome No.	*P* value	R^2 (%)
1	shoot length	RM413	5	0.07	11.24
		RM218	3	0.38	15.05
		RM246	1	0.03	12.63
2	root length	RM252	4	0.06	21.23
		RM251	3	0.13	12.87
		RM486	1	0.19	16.89
		RM302	1	0.04	11.57
		RM404	8	0.14	12.40
3	number of secondary roots	RM252	4	0.04	24.64
		RM251	3	0.23	25.49
		RM164	5	0.17	13.79
4	fresh weight	RM252	4	0.23	32.03
		RM106	2	0.06	13.93
		RM434	9	0.10	16.62
5	dry weight	RM276	6	0.07	18.01
		RM289	5	0.28	20.75
		RM252	4	0.14	14.67
		RM219	9	0.04	17.68
		RM495	1	0.07	10.94
		RM434	9	0.13	15.43
6	shoot/root ratio	RM252	4	0.14	14.55
		RM243	1	0.46	16.17
		RM251	3	0.01	32.71
		RM486	1	0.12	19.55
		RM302	1	0.02	16.45
		RM262	2	0.09	12.92
7	root/shoot ratio	RM252	4	0.30	29.37
		RM243	1	0.21	22.95
		RM486	1	0.02	19.79
		RM302	1	0.02	15.23
		RM262	2	0.11	12.03
8	vigor index	RM246	1	0.07	14.42

of secondary roots, dry weight, shoot/root ratio, and root/shoot ratio. The phenotypic variance for these associations varied from 11.57% to 32.71%. Notably, marker RM302 displayed a pleiotropic effect, impacting both root length and root/shoot ratio. These markers underwent additional validation in both drought-tolerant and drought-susceptible lines, revealing polymorphic distinctions between them. Based on marker trait association, the identified markers ought to be used in marker assisted drought tolerant breeding programmes.

REFERENCES

Addanki K.R., Balakrishnan D., Yadavalli V.R. (2019): Swarna × *Oryza nivara* introgression lines: A resource for seedling vigour traits in rice. Plant Genetic Resources: Characterization and Utilization, 17: 12–23.

Adilova S.S., Qulmamatova D.E., Baboev S.K., Bozorov T.A., Morgunov A.I. (2020): Multivariate cluster and principle component analyses of selected yield traits in Uzbek bread wheat cultivars. American Journal of Plant Sciences, 11: 903.

https://doi.org/10.17221/12/2024-CJGPB

Adkins S.W., Kunanuvatchaidach R., Godwin I.D. (1995): Somaclonal variation in rice- drought tolerance and other agronomic characters. Australian Journal of Botany, 43: 201–209.

Ahmed H.G., Zeng Y., Shah A.N., Yar M.M., Ullah A., Ali M. (2022): Conferring of drought tolerance in wheat (*Triticum aestivum* L.) genotypes using seedling indices. Frontiers in Plant Science, 13: 961049.

Anandan A., Anumalla M., Pradhan S.K., Ali J. (2016a): Population structure, diversity and trait association analysis in rice (*Oryza sativa* L.) germplasm for early seedling vigor (ESV) using trait linked SSR markers. PLoS ONE, 11: e0152406.

Anandan A., Mahender A., Kumari R., Pradhan S.K., Subudhi H.N., Thirugnanakumar S., Singh O.N. (2016b): Appraisal of genetic diversity and population structure in assorted rice genotypes for early seedling vigour trait linked markers. ORYZA – An International Journal on Rice, 53: 113–125.

Ashfaq M., Saleem Haider M., Ali A., Ali M., Hanif S., Mubashar U. (2014): Screening of diverse germplasms for genetic studies of drought tolerance in rice (*Oryza sativa* L.). Caryologia, 67: 296–304.

Ashraf H., Husaini A.M., Ashraf Bhat M., Parray G.A., Khan S., Ganai N.A. (2016): SSR based genetic diversity of pigmented and aromatic rice (*Oryza sativa* L.) genotypes of the western Himalayan region of India. Physiology and Molecular Biology of Plants, 22: 547–555.

Awan S.A., Khan I., Rizwan M., Zhang X., Brestic M., Khan A., El-Sheikh M.A., Alyemeni M.N., Ali S., Huang L. (2021): Exogenous abscisic acid and jasmonic acid restrain polyethylene glycol-induced drought by improving the growth and antioxidative enzyme activities in pearl millet. Physiologia Plantarum, 172 (Special Issue): 809–819.

Bhattarai U., Subudhi P.K. (2019): Genetic diversity, population structure, and marker-trait association for drought tolerance in US rice germplasm. Plants, 8: 530.

Bilgili D., Mehmet A., Kazım M. (2019): Effects of peg-induced drought stress on germination and seedling performance of bread wheat genotypes. YYU Journal of Agricultural Science, 29: 765–771.

Chakraborty A., Viswanath A., Malipatil R., Semalaiyappan J., Shah P., Ronanki S., Rathore A., Singh S.P., Govindaraj M., Tonapi V.A., Thirunavukkarasu N. (2022): Identification of candidate genes regulating drought tolerance in Pearl Millet. International Journal of Molecular Sciences, 23: 6907.

Chakravorty A., Ghosh P.D., Sahu P.K. (2013): Multivariate analysis of landraces of rice of West Bengal. American Journal of Experimental Agriculture, 3: 110–123.

Christina G.R., Thirumurugan T., Jeyaprakash P., Rajanbabu V. (2021): Principal component analysis of yield and yield-related traits in rice (*Oryza sativa* L.) landraces. Electronic Journal of Plant Breeding, 12: 907–911.

Courtois B., Julien F., Raffaella G., Gianluca B., Gaeetan D., Chantal H., Manuel R., et al. (2012): Genetic diversity and population structure in a European collection of rice. Crop Science, 52: 1663–1675.

de Mendiburu F. (2019): Package Agricolae. R Package, Version 1(3). Available on https://cran.r-project.org/web/packages/agricolae/index.html

Diwan J., Channbyregowda M., Shenoy V., Salimath P., Bhat R. (2013): Molecular mapping of early vigour related QTLs in rice. Research Journal of Biology, 1: 24–30.

Doyle J.J., Doyle J.L. (1987): A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, 19: 11–15.

Earl D.A., VonHoldt B.M. (2012): STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetic Resources, 4: 359–361.

El-Gamal W.H., Draz A.E., Abo El-Maksoud M.M., El-Moghazy A.M. (2015): Genetical and molecular analysis of rice yield and some anatomical root traits under water deficit conditions. Egyptian Journal of Agricultural Research, 93.

Enyew M., Dejene T., Lakew B., Worede F. (2019): Clustering and principal component analysis of barley (*Hordeum volugare* L.) landraces for major morphological traits from North Western Ethiopia. International Journal of Agricultural Science and Food Technology, 5: 58–63.

Evamoni F.Z., Nulit R., Yap C.K., Ibrahim M.H., Sidek N.B. (2023): Assessment of germination performance and early seedling growth of Malaysian indica rice genotypes under drought conditions for strategic cropping during water scarcity. Chilean Journal of Agricultural Research, 83: 281–292.

Evanno G., Sebastien R., Jerome G. (2005): Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Molecular Ecology, 14: 2611–2620.

FAO (2022): Faostat Food and Agriculture Organization Statistical Databases. Available on https://www.fao.org/faostat/en/data/QCL

Farooq S., Onen H., Ozaslan C., Baskin C.C., Gunal H. (2019): Seed germination niche for common ragweed (*Ambrosia artemisiifolia* L.) populations naturalized in Turkey. South African Journal of Botany, 123: 361–371.

Fatimah S., Amzeri A., Syafii M., Purwaningsih Y. (2023): Screening of red rice (*Oryza sativa* L.) landraces for drought tolerance at early stages using PEG 6000. AGRIVITA, Journal of Agricultural Science, 45: 199–208.

https://doi.org/10.17221/12/2024-CJGPB

Gaballah M.M., Fiaz S., Wang X., Younas A., Khan S.A., Wattoo F.M., Shafiq M.R. (2021): Identification of genetic diversity among some promising lines of rice under drought stress using SSR markers. Journal of Taibah University for Science, 15: 468–478.

Garcia A.A., Benchimol L.L., Barbosa A.M., Geraldi I.O., Souza Jr C.L., Souza A.P. (2004): Comparison of RAPD, RFLP, AFLP and SSR markers for diversity studies in tropical maize inbred lines. Genetics and Molecular Biology, 27: 579–588.

Garris A.J., Tai T.H., Coburn J., Kresovich S., McCouch S. (2005): Genetic structure and diversity in *Oryza sativa* L. Genetics, 169: 1631–1638.

Ghafoor A., Arshad M. (2008): Multivariate analyses for quantitative traits to determine genetic diversity of blackgram *Vigna mungo* (L.) Hepper germplasm. Pakistan Journal of Botany, 40: 2307–2313.

Gholami A., Sharafi S., Sharafi A., Ghasemi S. (2009): Germination of different seed size of pinto bean cultivars as affected by salinity and drought stress. Journal of Food, Agriculture & Environment, 7: 555–558.

Gupta P.C. (1993): Seed vigour testing. In: Agrawal P.K. (ed.): Handbook of Seed Testing. New Delhi, Ministry of Agriculture: 242–249.

Hafiz S.B., Jehanzeb F., Bibi T., Tariq M. (2015): Cluster and principle component analyses of maize accessions under normal and water stress conditions. Journal of Agricultural Sciences (Belgrade), 60: 33–48.

Huang Z., Yu T., Su L., Yu S.B., Zhang Z.H., Zhu Y.G. (2004): Identification of chromosome regions associated with seedling vigor in rice. Yi Chuan Xue Bao, 31: 596–603.

Hussain H. A., Hussain S., Khaliq A., Ashraf U., Anjum S.A., Men S., Wang L. (2018): Chilling and drought stresses in crop plants: implications, cross talk, and potential management opportunities. Frontiers in Plant Science, 9: 393.

Islam M.M., Kayesh E., Zaman E., Urmi T.A., Haque M.M. (2018): Evaluation of rice (*Oryza sativa* L.) genotypes for drought tolerance at germination and early seedling stage. The Agriculturists, 16: 44–54.

Islam M.A., Alam M.S., Maniruzzaman M., Haque M.S. (2023): Microsatellite marker-based genetic diversity assessment among exotic and native maize inbred lines of Bangladesh. Saudi Journal of Biological Sciences, 30: 103715.

Jin L., Lu Y., Xiao P., Sun M., Corke H., Bao J. (2010): Genetic diversity and population structure of a diverse set of rice germplasm for association mapping. Theoretical and Applied Genetics, 121: 475–487.

Jolliffe I.T., Cadima J. (2016): Principal component analysis: A review and recent developments. Philosophical Transactions of the Royal Society A, 374: 20150202.

Kanawapee N., Sanitchon J., Srihaban P., Theerakulpisut P. (2011): Genetic diversity analysis of rice cultivars (*Oryza sativa* L.) differing in salinity tolerance based on RAPD and SSR markers. Electronic Journal of Biotechnology, 14: 2.

Kasanaboina K., Chandra M.Y., Krishna L., Parimala G., Jagadeeshwar R. (2022): Multivariate analysis based prediction of phenotypic diversity associated with yield and yield component traits in germplasm lines of rice (*Oryza sativa* L.). Electronic Journal of Plant Breeding, 13: 764–771.

Kaufmann M.R., Eckard A.N. (1971): Evaluation of water stress control with polyethylene glycols by analysis of guttation. Plant Physiology, 47: 453–456.

Kotla A., Agarwal S., Yadavalli V.R., Vinukonda V.P., Chakravarthi Dhavala V.N., Neelamraju S. (2013): Quantitative trait loci and candidate genes for yield and related traits in Madhukar × Swarna RIL population of rice. Journal of Crop Science and Biotechnology, 16: 35–44.

Kumbhar S.D., Kulwal P.L., Patil J.V., Sarawate C.D., Gaikwad A.P., Jadhav A.S. (2015): Genetic diversity and population structure in landraces and improved rice varieties from India. Rice Science, 22: 99–107.

Lewontin R.C. (1972): The apportionment of human diversity. In: Dobzhansky T., Hecht M.K., Steere W.C. (eds.): Evolutionary Biology. Springer, New York.

Lipiec J., Doussan C., Nosalewicz A., Kondracka K. (2013): Effect of drought and heat stresses on plant growth and yield: A review. International Agrophysics, 27: 463–477.

Lou Q., Chen L., Sun Z., Xing Y., Li J., Xu X., Mei H., Luo L. (2007): A major QTL associated with cold tolerance at seedling stage in rice (*Oryza sativa* L.). Euphytica, 158: 87–94.

Ma H., Yin Y., Guo Z., Chen L., Zhang L., Zhong M., Shao G. (2011): Establishment of DNA fingerprinting of Liaojing series of Japonica rice. Middle East Journal of Scientific Research, 8: 384–392.

Mahender A., Anandan A., Pradhan S.K. (2015): Early seedling vigour, an imperative trait for direct-seeded rice: An overview on physio-morphological parameters and molecular markers. Planta, 241: 1027–1050.

Mahpara S., Zainab A., Ullah R., Kausar S., Bilal M., Latif M.I., Arif M., Akhtar I., Al-Hashimi A., Elshikh M.S., Zivcak M. (2022): The impact of PEG-induced drought stress on seed germination and seedling growth of different bread wheat (*Triticum aestivum* L.) genotypes. PLoS ONE, 17: e0262937.

Mas-ud M.A., Matin M.N., Fatamatuzzohora M., Ahamed M.S., Chowdhury M.R., Paul S.K., Hossain M.S. (2022): Screening for drought tolerance and diversity analysis of Bangladeshi rice germplasms using morphophysiology and molecular markers. Biologia, 77: 21–37.

https://doi.org/10.17221/12/2024-CJGPB

McCouch S.R., Teytelman L., Xu Y., Lobos K.B., Clare K., Walton M., Fu B., Maghirang R., Li Z., Xing Y., Zhang Q., Kono I., Yano M., Fjellstrom R., DeClerck G., Schneider D., Cartinhour S., Ware D., Stein L. (2002): Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Research, 9: 199–207.

Mohanty S., Donde R., Das S., Panda D., Mishra B., Pradhan S.K., Behera L. (2021): Utilization of genetic diversity and population structure to reveal prospective drought-tolerant donors in rice. Gene Reports, 23: 101151.

Nachimuthu V.V., Muthurajan R., Duraialaguraja S., Sivakami R., Pandian B.A., Ponniah G., Gunasekaran K., Swaminathan M., Suji K.K., Sabariappan R. (2015): Analysis of population structure and genetic diversity in rice germplasm using SSR markers: An initiative towards association mapping of agronomic traits in *Oryza sativa*. Rice, 8: 1–25.

Ni Z.F., Zhang Y.R., Liang R.Q., Liu G.T., Sun Q.X. (2002): Genetic diversity of D-genome revealed by SSR markers in synthesized hexaploid wheat Introduced from CIMMYT. Yi Chuan Xue Bao, 29: 542–548.

Noryan M., Hervan I.M., Sabouri H., Kojouri F.D., Mastinu A. (2021): Drought resistance loci in recombinant lines of Iranian *Oryza sativa* L. in germination stage. BioTech, 10: 26.

Panda D., Mishra S.S., Mohanty S.K., Behera P.K., Lenka S.K. (2019): Data on genetic potentiality of folk rice (*Oryza sativa* L.) genotypes from Koraput, India in reference to drought tolerance traits. Data in Brief, 25: 104363.

Pandey P., Irulappan V., Bagavathiannan M.V., Senthil Kumar M. (2017): Impact of combined abiotic and biotic stresses on plant growth and avenues for crop improvement by exploiting physio-morphological traits. Frontiers in Plant Science, 8: 537.

Pascual L., Ruiz M., Lopez-Fernandez M., Perez-Pena H., Benavente E., Vazquez J.F., Sansaloni C., Giraldo P. (2020): Genomic analysis of Spanish wheat landraces reveals their variability and potential for breeding. BMC Genomics, 21: 1–7.

Pepe M., Crescente M.F., Varone L. (2022): Effect of water stress on physiological and morphological leaf traits: A comparison among the three widely-spread invasive alien species *Ailanthus altissima*, *Phytolacca americana*, and *Robinia pseudoacacia*. Plants, 11: 899.

Ponsiva S.T., Sabarinathan S., Senthilkumar N., Thangavel P., Manivannan K., Thirugnanakumar S. (2019): Principal components analysis in rice genotypes grown over seasons. Plant Archives, 19: 3118–3120.

Powell W., Thomas W.T., Baird E., Lawrence P., Booth A., Harrower B., McNicol J.W., Waugh R. (1997): Analysis of quantitative traits in barley by the use of amplified fragment length polymorphisms. Heredity, 79: 48–59.

Pradhan S.K., Barik S.R., Sahoo A., Mohapatra S., Nayak D.K., Mahender A., Meher J., Anandan A., Pandit E. (2016): Population structure, genetic diversity and molecular marker-trait association analysis for high-temperature stress tolerance in rice. PLoS ONE, 11: e0160027.

Pritchard J.K., Stephens M., Rosenberg N.A., Donnelly P. (2000): Association mapping in structured populations. The American Journal of Human Genetics, 67: 170–181.

Priya S.J., Vincent S., Joel A.J., Sritharan N. (2022): Screening root traits of rice landraces seedlings (*Oryza sativa* L.) under induced drought stress using hydroponic system. International Journal of Plant and Soil Science, 34: 582–591.

Ramchander S., Raveendran M., Robin S. (2016): Mapping QTLs for physiological traits associated with drought tolerance in rice (*Oryza sativa* L.). Journal of Investigative Genomics, 3: 00052.

Rauf M., Munir M., Ul Hassan M., Ahmad M., Afzal M. (2007): Performance of wheat genotypes under osmotic stress at germination and early seedling growth stage. African Journal of Biotechnology, 6: 971–975.

Rejeth R., Manikanta C.L., Beena R., Stephen R., Manju R.V., Viji M.M. (2020): Water stress mediated root trait dynamics and identification of microsatellite markers associated with root traits in rice (*Oryza sativa* L.). Physiology and Molecular Biology of Plants, 26: 1225–1236.

Renuprasath P., Boominathan P., Suresh R. (2023): Marker assisted pyramiding of qDTY QTLs governing yield under drought stress into rice variety ADT (R) 45. Electronic Journal of Plant Breeding, 14: 60–68.

Roy P.S., Samal R., Rao G.J., Patnaik S.S., Jambhulkar N.N., Patnaik A., Mohapatra T. (2016): Differentiation and description of aromatic short grain rice landraces of the eastern Indian state of Odisha based on qualitative phenotypic descriptors. BMC Ecology, 16: 1–4.

Sajib A.M., Hossain M.M., Mosnaz A.T., Hossain H., Islam M.M., Ali M.S., Prodhan S.H. (2012): SSR marker-based molecular characterization and genetic diversity analysis of aromatic landraces of rice (*Oryza sativa* L.). Journal of BioScience & Biotechnology, 1: 107–116.

Sakina A., Sofi N.R., Shikari A.B., Mir R.R., Bhat M.A., Waza S.A., Jan S., Rafiqee S., Khan G.H., Wani S.H. (2022): DNA marker-based diversity across rice genotypes and advanced breeding lines bred for temperate regions of North-West India. Molecular Biology Reports, 49: 7145–7155.

Salam S.A., Sindhumole P., Waghmare S.G., Sajini S. (2017): Molecular characterization of rice (*Oryza sativa* L.) genotypes for drought tolerance using two SSR markers. Electronic Journal of Plant Breeding, 8: 474–479.

Salem K.F., Sallam A. (2016): Analysis of population structure and genetic diversity of Egyptian and exotic rice (*Oryza sativa* L.) genotypes. Comptes Rendus Biologies, 339: 1–9.

Sanghamitra P., Nanda N., Barik S.R., Sahoo S., Pandit E., Bastia R., Pradhan S.K. (2021): Genetic structure and mo-

https://doi.org/10.17221/12/2024-CJGPB

lecular markers-trait association for physiological traits related to seed vigour in rice. Plant Gene, 28: 100338.

Serrote C.M., Reiniger L.R., Silva K.B., Rabaiolli S.M., Stefanel C.M. (2020): Determining the polymorphism information content of a molecular marker. Gene, 726: 144175.

Sheoran S., Kaur Y., Kumar S., Shukla S., Rakshit S., Kumar R. (2022): Recent advances for drought stress tolerance in maize (*Zea mays* L.): Present status and future prospects. Frontiers in Plant Science, 13: 872566.

Singh M., Singh S.K., Vennela P.R., Singh D.K., Kumar D. (2017): Genetic divergence studies for drought tolerance in rice (*Oryza sativa* L.) using morphological traits and molecular markers. ORYZA – An International Journal on Rice, 54: 385–391.

Singh N., Choudhury D.R., Tiwari G., Singh A.K., Kumar S., Srinivasan K., Tyagi R.K., Sharma A.D., Singh N.K., Singh R. (2016): Genetic diversity trend in Indian rice varieties: An analysis using SSR markers. BMC Genetics, 17: 1–3.

Singh S., Pradhan S.K., Singh A.K., Singh O.N. (2012): Marker validation in recombinant inbred lines and random varieties of rice for drought tolerance. Australian Journal of Crop Science, 6: 606.

Sobahan M.A., Akter N., Rana M.M. (2022): Polyethylene glycol mediated drought stress impacts on germination, growth and accumulation of proline in rice (*Oryza sativa* L.). SAARC Journal of Agriculture, 20: 107–119.

Sokoto M.B., Muhammad A. (2014): Response of rice varieties to water stress in Sokoto, Sudan Savannah, Nigeria. Journal of Bioscience Medicine, 2: 68–74.

Song Z.P., Xu X., Wang B., Chen J.K., Lu B.R. (2003): Genetic diversity in the northernmost *Oryza rufipogon* populations estimated by SSR markers. Theoretical and Applied Genetics, 107: 1492–1999.

Srividhya A., Vemireddy L.R., Sridhar S., Jayaprada M., Ramanarao P.V., Hariprasad A.S., Siddiq E. (2011): Molecular mapping of QTLs for yield and its components under two water supply conditions in rice (*Oryza sativa* L.). Journal of Crop Science and Biotechnology, 14: 45–56.

Subashri M., Robin S., Vinod K.K., Rajeswari S., Mohanasundaram K., Raveendran T.S. (2009): Trait identification and QTL validation for reproductive stage drought resistance in rice using selective genotyping of near flowering RILs. Euphytica, 166: 291–305.

Tabkhkar N., Rabiei B., Samizadeh Lahiji H., Hosseini Chaleshtori M. (2018): Genetic variation and association analysis of the SSR markers linked to the major drought-yield QTLs of rice. Biochemical Genetics, 56: 356–374.

Vaissie P., Monge A., Husson F. (2021): Factoshiny: Perform factorial analysis from FactoMineR with a shiny application. R package version, 2(4). Available on http://factominer.free.fr/graphs/factoshiny.html

Venuprasad R., Dalid C.O., Del Valle M., Zhao D., Espiritu M., Sta Cruz M.T., Atlin G.N. (2009): Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. Theoretical and Applied Genetics, 120: 177–190.

Vinod K.K., Krishnan S.G., Thribhuvan R., Singh A.K. (2019): Genetics of drought tolerance, mapping QTLs, candidate genes and their utilization in rice improvement. In: Rajpal V.R., Sehgal D., Kumar A., Raina S.N. (eds.): Genomics Assisted Breeding of Crops for Abiotic Stress Tolerance. Vol. II, Springer: 145–186.

Wang J.X., Sun J., Li C.X., Liu H.L., Wang J.G., Zhao H.W., Zou D.T. (2016): Genetic dissection of the developmental behavior of plant height in rice under different water supply conditions. Journal of Integrative Agriculture, 15: 2688–2702.

Wang Z.F., Wang J.F., Bao Y.M., Wang F.H., Zhang H.S. (2010): Quantitative trait loci analysis for rice seed vigor during the germination stage. Journal of Zhejiang University Science B, 11: 958–964.

Yang G., Yang Y., Guan Y., Xu Z., Wang J., Yun Y., Yan X., Tang Q. (2021): Genetic diversity of shanlan upland rice (*Oryza sativa* L.) and association analysis of SSR markers linked to agronomic traits. BioMed Research International, 2021: 7588652.

Zhang S.B., Zhu Z., Zhao L., Zhang Y.D., Chen T., Lin J., Wang C.L. (2007): Identification of SSR markers closely linked to *eui* gene in rice. Yi Chuan, 29: 365–370.

Zhou H.F., Xie Z.W., Ge S. (2003): Microsatellite analysis of genetic diversity and population genetic structure of a wild rice (*Oryza rufipogon* Griff.) in China. Theoretical and Applied Genetics, 107: 332–339.

Zhu R., Wu F., Zhou S., Hu T., Huang J., Gao Y. (2020): Cumulative effects of drought – flood abrupt alternation on the photosynthetic characteristics of rice. Environmental and Experimental Botany, 169: 103901.

Zubarer M.A., Chowdhury A.K.M.M.B., Islam M.Z., Ahmed T., Hasan M.A. (2007): Effects of water stress on growth and yield attributes of Aman rice genotypes. International Journal of Sustainable Crop Production, 2: 25–30.

Received: February 8, 2024
Accepted: July 15, 2024
Published online: October 4, 2024