



Molecular Diversity Analysis and Screening of Drought Tolerance Rice (*Oryza sativa* L.) based on Morpho-physiological and SSR Marker

Mukh Ram¹, Shiv Prakash Shrivastav¹, Deshraj Gurjar¹, Durga Prasad¹

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ABSTRACT

Background: Drought prone areas have been increasing day by day because of raising temperature or heat. The assessment of genetic divergence existing in the germplasm collections is very important for success of hybridization programme leading to development of high yielding varieties under drought stress condition.

Methods: The present investigation comprising of 123 rice genotypes was carried out at LPU Phagwara punjab in *Kharif* 2022 and plants were grown in augmented design under controlled environment. 28 SSR markers were selected for the genetic diversity analysis. The software NTSYS-pc version 2.1 (Exeter Software, Setauket, NY) was used for the statistical analyses of the morphological and SSR marker data.

Result: The analysis of variance for genotypes was reported to be highly significant for all the characters studied. This indicated sufficient variability available among the genotypes. The leaf rolling was recorded by visual observation in the field condition in the scale 0 to 9 to screen for drought tolerance. The molecular diversity analysis and clustering of diverse rice germplasm into different groups confirm the presence of wide genomic variation among selected genotypes. The range of polymorphic information content (PIC) varied from 0.352 (RM204) to 0.698 (RM480). The markers RM480, RM170, RM316 and RM20 were found most appropriate for discriminating rice genotypes owing to their highest PIC values (0.690 to 0.698). The genotypes were grouped to construct a dendrogram by using the unweighted pair group method based on Arithmetic. In cluster II most of the moderately tolerant genotypes were included, while in cluster III highly tolerant 11 genotypes were grouped. The UPGMA clustering also correlated with drought tolerance characters such as days to maturity, leaf rolling and chlorophyll content, proline content and relative water contents. The genotypes APO, RASI and Nagina-22 were observed to be highly tolerant of drought stress conditions. Among the genotypes, Vandana, Rajendra Bhagwati and RAU-3 were moderately tolerant whereas BPT-5204, MTU-7029 and Sarjoo-52 were susceptible to drought stress. Four genotypes viz. APO, RASI, Nagina-22 and RAU-4 can be used as a source of drought-tolerant genotypes for further breeding programs.

Key words: Diversity, Dendrogram, Drought tolerant, SSR markers, Rice.

INTRODUCTION

Rice (*Oryza sativa* L.), a member of the family Poaceae, is the largest widely cultivated major crop in the world. Approximately 90% of the world's rice is grown in the Asian continent and constitutes a staple food for 2.7 billion people worldwide. About 30% of the 700 million people in absolute poverty (with an income of less than US\$ 1 per day) live in rain-fed rice-growing areas in South Asia (IRRI, Philippines, 2012). Rice is grown widely under rainfed conditions in Asia. South Asia alone holds 37% of the world's rice area, 50% of which is rainfed (Dawe *et al.*, 2010). About 45% of the total area is estimated to have no irrigation input (Crosson, 1995). It threatens sustainable agriculture with its rapid and unpredictable effects, making it particularly difficult for agricultural scientists and farmers to respond to challenges of water stress. Fluctuation in normal period of monsoon either delayed or early withdrawal affects the growing season of crops and causes detrimental effects on yield. Water stress may cause up to 50% yield reduction in rice-growing areas. To overcome this problem, it is important to select those rice genotypes, which have greater genetic diversity and genetic potential for the improvement

¹Department of Genetics and Plant Breeding, School of Agriculture, Lovely Professional University, Phagwara-144 411, Jalandhar, Punjab, India.

Corresponding Author: Shiv Prakash Shrivastav, Department of Genetics and Plant Breeding, School of Agriculture, Lovely Professional University, Phagwara-144 411, Jalandhar, Punjab, India. Email: shiva.26060@lpu.com.in

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of desired traits against drought stresses. The genetic diversity is also very important in rice breeding from the standpoint of selection, conservation and proper utilization. Screening tests for drought tolerance are time-consuming, labour intensive and not so effective in identifying the material, which is actually drought tolerant (O'Toole and Chang, 1979). Molecular marker-based genetic diversity

analysis has potential for assessing changes in genetic diversity over time and space. A molecular marker is a DNA sequence that is readily detected and whose inheritance can be easily monitored. Recently, DNA profiles based on various molecular markers have been widely applied across different fields. Simple sequence repeat (SSR) markers are class of repetitive DNA sequences usually 2-6 bp distributed throughout the whole genome and flanked by highly conserved region. Microsatellite or simple sequence repeat (SSR) markers are appropriate for assessment of genetic diversity and variety identification because of their ability to detect large numbers of discrete alleles repeatedly, accurately and efficiently (Smith and Helentjaris, 1996; Varshney *et al.*, 2005). Therefore, the present study was carried out to screen out the available rice genotypes based on phenotyping (various morphological, physiological and seed parameters traits) and genotyping (using various SSR markers at a molecular level) to assess the genetic diversity among the genotypes for the selection of diverse parents that would be used in a cross-breeding programme.

MATERIALS AND METHODS

The present work was carried out during *Kharif* 2022 in Agricultural Research Farm LPU, Phagwara, Punjab. The experimental material comprised of 123 rice genotypes received from the Institute of Agricultural Sciences, B.H.U., Varanasi, U.P., India. (Table 1), were grown in augmented design under controlled environment for Screening purposes. The crop was maintained as per the standard agronomic practices. Observations were recorded on days to 50 per cent flowering (DF), days to maturity (DM). Plant height (cm) (PH), number of effective tillers per plant (ET), panicle length (cm) (PL), panicle weight (g) (PW), number of fertile spikelet per panicle (FSP), total number of spikelet per panicle (TSP), spikelet fertility (SF%), test weight (g) (TW), grain yield per plant (g) (GY), biological yield per plant (g) (BY), harvest Index (HI), leaf rolling (LR), leaf angle (LA), stay green (SG), chlorophyll content (CC), stomatal conductance (SC), proline content (PC) and relative water content (%) (RWC). Fully expanded leaves were excised at 10.00 AM and relative water content was determined by method of Matin *et al.* (1989). Chlorophyll content was measured by chlorophyll meter (model CCM-200). Stomatal conductance (mmol/m²/s) was measured by Porometer just before heading. The free proline content (mg g⁻¹) based on fresh weight of leaves was estimated at the anthesis stage according to the method by Bates *et al.* (1973). The leaf rolling was recorded by visual observation in the field condition in the scale 0 to 9 to screen for drought tolerance, where scale 0 corresponds to no symptom of stress and scale 9 corresponds to fully rolled leaf (Abd Allah, 2009). Analysis of variance for Augmented Design by Federer, 1956. The DNA was extracted from plant samples using the CTAB method (Doyle and Doyle, 1987). Major contaminations in crude DNA preparation are RNA, protein and polysaccharides. Inclusion of CTAB in extraction buffer

Table 1: List of 123 rice genotypes.

Code no.	Name of genotype
R-1	RAU 1417-2-1-5-7-7
R-2	RAU 1421-12-1-7-4-3
R-3	RAU 1428-43-2-7-26
R-4	RAU 1428-52-2-4-6
R-5	RAU 1421-15-3-2-5-3-7
R-6	RAU 1421-12-1-7-4-4
R-7	RAU 1417-9-7-22-5-7-3
R-8	RAU 1451-66-1-1-5-2
R-9	RAU 1421-15-3-2-5-7-3
R-10	RAU 1415-35-7-6-9-5-1
R-11	RAU 1426-43-2-5-4
R-12	RAU 1428-31-5-4-3-2-2-2
R-13	RAU-4
R-14	APO
R-15	NAGINA-22
R-16	NDR-359
R-17	RAU 1415-35-76-9-5-3-4
R-18	RAU 1401-18-1-4
R-19	RAU 1401-18-1-5
R-20	RAU 1428-54-35-5-5
R-21	RAU 1451-66-1-1-5-1
R-22	RAU 1417-11-1-74-3-2
R-23	RAU 1463-16
R-24	RAU 1471-10
R-25	RAU 1415-8-6-4-3-3
R-26	RAU 1415-9
R-27	RAU 1428-7-3-6
R-28	RAU 1421-12-1-7-3
R-29	RAU 1417-11-4-74-3-2
R-30	Rajendra Bhagwati
R-31	Vandna
BH-1	HUBR-2-1
BH-2	HUR-3022
BH-3	IDR-763
BH-4	Shambha
BH-5	Sahbhagi (check)
BH-6	BPT-5204
B-42	MAS-946
B-31	NDR-1159
BH-7	IR-64
B-98	B-3688-TB-25-MR-2
B-67	CB-05-755-2
B-122	GK-5022
B-37	R-1700-2240-4
B-103	CR-3423-1
BH-8	SARJOO-52
B-145	BVS-1
B-116	IR-78755-190-B-1-3
B-146	BVD-111
B-133	IR-32252-B-43-745-1
B-32	CR-3630-1-3
B-141	IR-83867-B—250-CRA-1-1
B-104	OR-1946-2-1
B-44	RP-BIO-4918-70-11
B-109	IR-82635-B-B-23-1

Table 1: Continue...

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B-89	IR-71146-97-1-2-1-3
B-123	ANJALI
B-3	REWA-1207-15
B-19	OR-1968-2
B-50	CB-09-512
B-129	BD-104
B-6	CR-2994-5-3-2-1-1-1
B-137	UPLRI-7
B-130	IR-77298-14-1-2-13
B-115	IR-83182-6-4
B-26	RP-5214-18-6-2-1-1-1
B-35	RP-BIO-4918-166-9
B-87	IR-82635-B-B-93-2
B-63	IR-87710-2-1-3-3
B-43	RP-BIO-4918-14-3-11
B-10	CR-3625-1-1
B-64	IR-74371-70-1-1
B-53	CRR-554-3-1-1
B-5	CR-3635-1-1
B-83	IR-82912-B-B-1
B-69	TB-409B-TB-14-3
B-71	IR-70175-51-2-1-1-2
B-72	IR-84881-B-132-CRA-1-1
B-73	IR-79971-B-309-REWA-49
B-75	BAU-446-06
B-143	IR-60080-46-A
B-117	IR-82635-B-B-25-4
B-38	UPRI-2012-15
B-39	RP-512517-6-3-2-1
B-41	CRR-614-4-1
B-21	CB-09-516
B-88	BVD-109
B-34	RASI
B-54	CRR-680-B-B-25-4
B-131	CR-422-63-51-B-2-1
B-59	IR-73000-160-3-3-2
B-17	CRR-599-5-1
B-60	B-10580E-KN-28-1-1
B-118	RP-5345-9-6-3
B-27	CRR-599-4-1
B-12	CR-2721-81-3
B-23	CR-3634-1-3
B-101	BP-1351D-1-1-PK-3-1
B-40	RP-5333-5-2-13
B-1	REWA-1207-15
B-120	IR-55423-01
B-127	BAU-411-05
B-85	CT-15673-8-2-3-3-1
B-96	IR-87756-20-2-2-3
B-61	RR-646-B-93-6-B-3
B-56	CRR-452-23-1-1-1-1
B-33	CR-3633-1-3
B-107	IR-82635-B-B-47-1
B-28	RP-1525-12-5-3-B
B-134	NDR-H-40
B-65	IR-78908-193-B-3-B

Table 1: Continue...

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B-29	CR-36171-1-3-1-1
B-15	CRR-599-6-1
B-66	CRR-265-9
B-20	R-1240-913-2-1013-1
B-110	CRR-660-2
B-99	BP-19768-2-3-7-78-1-1
M-4	HUR-105
M-7	IR-36
M-1	MTU-7029
M-2	PR-113
BH-9	NDR-97
BH-10	SHUSHK SAMRAT (check)

helps the elimination of polysaccharides. RNA was removed by RNase treatment and proteins were removed by phenol-chloroform extraction. In the present study, quantification was done by agarose gel electrophoresis of the isolated DNA samples along with known quantity of uncut lambda DNA marker. 28 SSR markers were selected for the genetic diversity analysis on the basis of published rice microsatellite framework map. These markers cover almost all the 12 chromosomes of rice (Table 2). The original source of markers sequence and the chromosomal positions of these markers can be found from rice genome database (<http://www.gramene.org>). Statistical analyses for the morphological and SSR marker data were conducted using the software NTSYS-pc version 2.1 (Exeter software, Setauket, NY). The morpho-physiological characters were standardized prior to cluster analysis and visual observation on the basis of leaf rolling under drought stress conditions. The matrix of average taxonomic distance for individuals and morphological traits was then computed using SIMINIT function and EUCLIDIAN distance coefficient. This dissimilarity coefficient is based on interval measure data collected for the morpho-physiological traits. Cluster analysis was then conducted on the taxonomic distance matrix with the Unweighted Pair Group Method based on Arithmetic (UPGMA). The relevant markers were used based on polymorphism extent. The polymorphic information content (PIC) value of each SSR marker was calculated according to the following formula (Weir, 1996).

$$PIC = 1 - (\sum p_i)^2$$

Where,

i = Total number of alleles detected for SSR marker.

pi = Frequency of the ith plus allele in a set of 120 genotypes of rice.

The frequencies of the null alleles were not included in the calculation of PIC values. The genetic similarity between 120 genotypes of rice was determined according to method developed by (Nei, 1973). All the calculations were performed using the software package power marker (Liu and Muse, 2005).

Table 2: The details of 28 polymorphic SSR markers used for genetic diversity analysis.

SSR marker	Linkage group	Forward sequence	Reverse sequence
RM237	1	CAAATCCCGACTGCTGTCC	TGGGAAGAGAGCACTACAGC
RM302	1	TCATGTCATCTACCATCACAC	ATGGAGAAGATGGAATACTTGC
RM555	2	TTGGATCAGCCAAAGGAGAC	CAGCATTGTGGCATGGATAC
RM279	2	GCGGGAGAGGGATCTCCT	GGCTAGGAGTTAACCTCGCG
RM5474	3	GAAGTCCTTGCGTGGCAC	AAGGCGACCTTCAGCCTC
RM53	2	ACGTCTCGACGCATCAATGG	CACAAGAACTTCTCGGTAC
RM7389	3	AGCGACGGATGCATGATC	TTGAGCCGGAGGTAGTCTTG
RM335	4	GTACACACCCACATCGAGAAG	GCTCTATGCGAGTATCCATGG
RM551	4	AGCCCAGACTAGCATGATTG	GAAGGCGAGAAGGATCACAG
RM13	5	TCCAACATGGCAAGAGAGAG	GGTGGCATTTCGATTCCAG
RM480	5	GCTCAAGCATTCTGCAGTTG	GCGCTTCTGCTTATTGGAAG
RM8225	6	ATGCGTGTTCCAGAAATTAGG	TTGTTGTATACCTCATCGACAG
RM439	6	TCATAACAGTCCACTCCCCC	TGGTACTCCATCATCCCATG
RM3826	7	TTAGCTTTCTCCAGTCTCC	ACGGGTATCTGAAACACAAC
RM125	7	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC
RM316	9	CTAGTTGGGCATACGATGGC	ACGCTTATATGTTACGTCAAC
RM38	8	ACGAGCTCTCGATCAGCCTA	TCGGTCTCCATGTCCAC
RM264	8	GTTGCGTCTACTGCTACTTC	GATCCGTGTCGATGATTAGC
RM160	9	AGCTAGCAGCTATAGCTTAGCTGGAGATCG	TCTCATCGCCATGCGAGGCCCTC
RM304	10	TCAAACCGGCACATATAAGAC	GATAGGGAGCTGAAGGAGATG
RM316	9	CTAGTTGGGCATACGATGGC	ACGCTTATATGTTACGTCAAC
RM1125	10	GGGGCCAGAGTTTTCTTCAG	GTACGCGCAGAAAATGAGAG
RM4601	11	CATACATGTGAACCTGACTG	CTAGCTTAGCATCTCCTCAA
RM170	12	TCGCGCTTCTCCTCGTCGACG	CCCGCTTGCGAGGAAGCAGCC
RM227	3	ACCTTTCGTCATAAAGACGAG	GATTGGAGAGAAAAGAAGCC
RM194	5	GCCCTGCTTCTTGCCCACCACC	TCCAGGGAGGGCAAGGCTGAGC
RM20	12	ATCTTGCCCTGCAGGTCAT	GAAACAGAGGCACATTTTCATTG
RM17	12	TGCCCTGTATTTCTTCTCTC	GGTGATCCTTTCCATTTC

RESULTS AND DISCUSSION

The data pertaining to the analysis of variance are presented in Table 3. All the genotypes showed highly significant variations for all the traits studied, which indicated presence of genetic diversity among the genotypes.

On the basis of visual observation of leaf rolling under drought stress condition in a controlled atmosphere, all the germplasm were categorized in the scale 0 to 9, where scale 0 corresponds to no symptom of stress and scale 9 corresponds to fully rolled leaf (Table 4). The genotypes RASI, APO, ANJALI, NAGINA-22 were highly resistant, while the genotypes BPT-5204, MTU-7029, SARJOO-52 were highly susceptible to drought stress. The days to maturity, chlorophyll content, proline content, relative water content, grain yield and leaf rolling are the most important attributes for screening of rice germplasm under drought stress conditions. On the basis of these traits, nine genotypes performing better with respect to yield and yield contributing traits under water stress condition were selected (Table 5). This finding conforms with the findings of Singh *et al.* (2014).

Total of 28 SSR primers was used to assess molecular diversity among 123 genotypes. The number of polymorphic bands per primer was observed with an average value of 2.82. The PIC of the 28 SSR primers ranged from 0.352 to

Table 3: Analysis of Variance for 19 traits in rice under drought stress condition.

Traits	MSS		
	Replication	Treatment	Error
DF	47.64	328.90**	5.13
DM	499.46	572.96**	7.77
PH	112.35	586.18**	15.17
ET	8.86	10.00**	0.31
PL	13.53	21.30*	0.70
PW	3.70	1.13**	0.08
FSP	4006.40	665.89**	31.12
TSP	5178.77	1448.25**	84.88
SF%	39.73	152.81**	6.27
TW	42.29	14.97*	1.40
GY	0.07	69.98**	0.86
BY	23.21	1497.15**	8.63
HI	1.33	23.25*	1.58
LA	25.24	348.70**	2.80
SG	2.11	465.30**	8.89
CC	23.73	48.51*	8.60
SC	120660.70	43010.69**	1633.33
PC	0.04	0.08**	0.01
RWC	11.22	185.87**	4.62

*Significant at 5%; **Significant at 1%.

0.698 with an average PIC of 0.53 (Table 6). Thus, the selected primers were highly polymorphic with higher diversity among the genotypes. The largest PIC value was observed for locus RM 480 (0.698), RM 170 (0.696) and RM 316 (0.696) followed by RM 20 (0.690) and lowest for RM 201 (0.352) followed by RM 237 (0.358). The number of

Table 4: Grouping of rice germplasm based on leaf rolling under drought stress conditions.

Score	Reaction	Symptom	Germplasm
0	Highly resistant	No symptoms of stress	RASI,APO,ANJALI,NAGINA-22
1	Resistant	No rolling	Shambha, Sahbhagi (Check), RAU 1417-2-1-5-7-7, RAU 1428-43-2-7-26, RAU 1415-9, HUBR-2-1,RAU 1421-12-1-7-3,BVD-111,B-3688-TB-25-MR-2,CB-05-755-2,GK-5022, OR-1946-2-1,R-1700-2240-4,CR-3423-1, RAU 1401-18-1-4,RAU 1428-54-35-5-5,RAU 1401-18-1-5, RAU 1451-66-1-1-5-1,RAU 1417-11-1-74-3-2.
3	Moderately resistant	Partially rolled, unrolled in the evening	BAU-411-05,CT-15673-8-2-3-3-1,IR-55423-01,REWA-1207-15,NDR-97,SHUSHK SAMRAT (Check),PR-113, R-1240-913-2-1013-1,IR-36,BP-19768-2-3-7-78-1-1,HUR-105,CRR-660-2, REWA-1207-15 , RAU 1421-12-1-7-4-3, IR-83182-6-4, RAU 1463-16,RAU 1415-8-6-4-3-3, BVS-1,IR-78755-190-B-1-3,IR-32252-B-43-745-1,IR-77298-14-1-2-13,CR-2994-5-3-2-1-1-1,UPLRI-7,CR-3630-1-3,CR-3635-1-1,CRR-554-3-1-1,RAU 1428-52-2-4-6,Rajendra Bhagwati, NDR-359, RAU 1421-15-3-2-5-3-7, RAU 1428-31-5-4-3-2-2-2, IR-64,RAU-4,RP-BIO-4918-166-9, RAU 1415-35-76-9-5-3-4,RAU 1451-66-1-1-5-2,RAU 1421-15-3-2-5-7-3,RAU 1417-9-7-22-5-7-3,RAU 1415-35-7-6-9-5-1,RAU 1426-43-2-5-4,RAU 1421-12-1-7-4-4, RAU 1417-11-4-74-3-2,Vandna, RP-5214-18-6-2-1-1-1,IR-82912-B-B-1,RP-BIO-4918-14-3-11,IR-74371-70-1-1,IR-82635-B-B-93-2,IR-87710-2-1-3-3,CR-3625-1-1,TB-409B-TB-14-3.
5	Intermediate	Partially; unrolled in late evening and early morning	IR-84881-B-132-CRA-1-1,IR-79971-B-309-REWA-49,IR-70175-51-2-1-1-2,IR-82635-B-B-25-4,RP-512517-6-3-2-1,UPRI-2012-15,RP-5345-9-6-3,CB-09-516,BVD-109,CR-422-63-51-B-2-1,BAU-446-06,IR-60080-46-A,CRR-680-B-B-25-4,CRR-614-4-1,IR-73000-160-3-3-2,CRR-599-4-1,CR-2721-81-3,CRR-599-5-1,B-10580E-KN-28-1-1, IR-83867-B-250-CRA-1-1,CB-09-512,OR-1968-2,BD-104,CR-3634-1-3,RP-5333-5-2-13,BP-1351D-1-1-PK-3-1,CRR-599-6-1,CRR-265-9, IR-87756-20-2-2-3,CR-36171-1-3-1-1,IR-78908-193-B-3-B,RR-646-B-93-6-B-3,CR-3633-1-3,IR-82635-B-B-47-1,NDR-H-40,RP-1525-12-5-3-B,CRR-452-23-1-1-1-1.
7	Susceptible	Complete rolling, unrolling in morning	RP-BIO-4918-70-11, IR-82635-B-B-23-1, IR-71146-97-1-2-1-3, IDR-763.
9	Highly susceptible	Like tube; no unrolling in morning	BPT-5204,MTU-7029, SARJOO-52.

Table 5: Mean Performance of selected nine rice genotypes for drought tolerant traits and grain yield under drought stress condition.

Name of genotype	Mean					Status
	Days to maturity	Chlorophyll content	Proline content	Relative water content	Grain yield	
Apo	89.67	42.63	0.85	83.08	29.93	Highly tolerant
Nagina-22	87.67	42.77	0.88	83.41	30.62	Highly tolerant
Rasi	90	41.66	0.86	81.16	28.91	Highly tolerant
RAU-4	91	42	0.80	80.83	28.15	Moderately tolerant
Vandana	93.33	36.93	0.79	81.15	27.56	Moderately tolerant
Rajendra bhagawati	93.67	39.63	0.82	80.32	27.93	Moderately tolerant
MTU-7029	143.67	25.02	0.19	51.10	9.07	Susceptible
BPT-5204	140.67	24.86	0.18	51.47	8.91	Susceptible
Sarjoo-52	140.33	25.28	0.19	52.64	9.14	Susceptible

alleles ranged from 2 to 4 with an average of 2.82. Seventy-nine microsatellite alleles were amplified, demonstrating considerable variability among the cultivar. Maximum alleles (4) were showed by RM 480, RM 20, RM 316 and RM 170. The amplification of lower number of alleles per locus may be due to poor resolution of agarose gel as compared to polyacrylamide gel. Therefore, most of the primers were selected based on the positions of QTL for drought tolerance on corresponding chromosomes with high and low tolerance bands in most of the genotypes.

A dendrogram was obtained using Jaccard's similarity coefficient to cluster diverse rice genotypes. The Jaccard's similarity coefficients ranged from 0.09 to 0.92. The highest dissimilarity coefficient (0.92) was observed between genotypes RP-5214-18 and NDR-1159, IR-83867-B-250-CRA-1-1 and RAU 1421-1, IR-83867-B and RAU 1428-31-5-4-3-2-2-2 whereas, lowest (0.09) was observed between genotypes BVD-109 and CB-09-516 followed by CB-09-516 and CR-422-63-51-B-2-1(0.11). In cluster analysis, all the genotypes were broadly grouped into seven clusters, proving the presence of wide genetic diversity among selected rice genotypes (Table 7). Cluster II consisted of a total of 38 genotypes, followed by cluster VII consisting of 20 genotypes whereas cluster VI has only 6 genotypes (Table 4). Most of the moderately tolerant genotypes were included in cluster II, while highly tolerant 11 genotypes were grouped in cluster III (Verma *et al.* 2019 and Shrivastav *et al.*, 2025).

In molecular diversity study, out of 28 SSR markers RM20, RM316, RM170 and RM480 showed higher PIC value. Therefore, these markers could be used further for improvement of drought tolerance/resistance in rice. On the basis of molecular diversity analysis and performance in morphological characteristics, it was concluded that the four genotypes viz. APO, RASI, Nagina-22 and RAU-4 can be used as a source of drought-tolerant genotypes for further breeding programs. This study indicated ample scope for selecting promising genotypes from present set of cultivars for grain yield along with drought tolerance improvement programme. Ravi *et al.* (2003) in his experiment have also reported that the genetic variation

Table 6: PIC value of 28 SSR markers.

Locus	Allels	HET	PIC
RM201	2.000	0.462	0.352
RM237	2.000	0.467	0.358
RM7389	2.000	0.477	0.363
RM125	2.000	0.486	0.368
RM279	2.000	0.488	0.369
RM439	2.000	0.493	0.371
RM8225	2.000	0.493	0.371
RM53	2.000	0.498	0.374
RM551	2.000	0.499	0.375
RM555	3.000	0.572	0.509
RM227	3.000	0.600	0.523
RM17	3.000	0.625	0.546
RM335	3.000	0.647	0.574
RM13	3.000	0.651	0.577
RM160	3.000	0.654	0.581
RM302	3.000	0.655	0.581
RM1125	3.000	0.662	0.588
RM5474	3.000	0.663	0.589
RM264	3.000	0.663	0.589
RM304	3.000	0.663	0.589
RM38	3.000	0.663	0.589
RM4601	3.000	0.664	0.590
RM194	3.000	0.666	0.592
RM3826	3.000	0.666	0.592
RM20	4.000	0.739	0.690
RM316	4.000	0.744	0.696
RM170	4.000	0.744	0.696
RM480	4.000	0.745	0.698

identified by morphological characters and molecular markers may prove useful in breeding programme, for the developing hybrids resistant to abiotic stresses. Chakravarthi and Naravaneni (2006) also reported similar findings; Herrera *et al.* (2008) and Singh *et al.* (2014). Liu *et al.* (2009) showed that the response of different rice lines to drought tolerance was moderately tolerant.

Table 7: Grouping of rice germplasm on the basis of drought related 28 SSR markers.

Cluster	Name of accession	No. of accession
I	RAU 1417-2-1-5-7-7, RAU 1428-43-2-7-26, RAU 1415-9, HUBR-2-1, RAU 1421-12-1-7-3, BVD-111, B-3688-TB-25-MR-2, CB-05-755-2, GK-5022, OR-1946-2-1, R-1700-2240-4, CR-3423-1, RAU 1401-18-1-4, RAU 1428-54-35-5-5, RAU 1401-18-1-5, RAU 1451-66-1-1-5-1, RAU 1417-11-1-74-3-2.	17
II	RAU 1421-12-1-7-4-3, IR-83182-6-4, RAU 1463-16, RAU 1415-8-6-4-3-3, BVS-1, IR-78755-190-B-1-3, IR-32252-B-43-745-1, IR-77298-14-1-2-13, CR-2994-5-3-2-1-1-1, UPLRI-7, CR-3630-1-3, CR-3635-1-1, CRR-554-3-1-1, RAU 1428-52-2-4-6, Rajendra Bhagwati, NDR-359, RAU 1421-15-3-2-5-3-7, RAU 1428-31-5-4-3-2-2-2, IR-64, RAU-4, RP-BIO-4918-166-9, RAU 1415-35-76-9-5-3-4, RAU 1451-66-1-1-5-2, RAU 1421-15-3-2-5-7-3, RAU 1417-9-7-22-5-7-3, RAU 1415-35-7-6-9-5-1, RAU 1426-43-2-5-4, RAU 1421-12-1-7-4-4, RAU 1417-11-4-74-3-2, Vandna, RP-5214-18-6-2-1-1-1, IR-82912-B-B-1, RP-BIO-4918-14-3-11, IR-74371-70-1-1, IR-82635-B-B-93-2, IR-87710-2-1-3-3, CR-3625-1-1, TB-409B-TB-14-3.	38
III	RAU 1471-10, RAU 1428-7-3-6, RASI, HUR-3022, APO, Sahbhagi (Check), MAS-946, ANJALI, NDR-1159, Shambha, NAGINA-22.	11
IV	IR-83867-B-250-CRA-1-1, CB-09-512, OR-1968-2, BD-104, CR-3634-1-3, RP-5333-5-2-13, BP-1351D-1-1-PK-3-1, CRR-599-6-1, CRR-265-9, IR-87756-20-2-2-3, CR-36171-1-3-1-1, IR-78908-193-B-3-B, RR-646-B-93-6-B-3, CR-3633-1-3, IR-82635-B-B-47-1, NDR-H-40, RP-1525-12-5-3-B, CRR-452-23-1-1-1-1.	18
V	BAU-411-05, CT-15673-8-2-3-3-1, IR-55423-01, REWA-1207-15, NDR-97, SHUSHK SAMRAT (Check), PR-113R-1240-913-2-1013-1, IR-36, BP-19768-2-3-7-78-1-1, HUR-105, CRR-660-2, REWA-1207-15.	13
VI	IR-82635-B-B-23-1, IR-71146-97-1-2-1-3, BPT-5204, RP-BIO-4918-70-11, MTU-7029, SARJOO-52.	6
VII	IR-84881-B-132-CRA-1-1, IR-79971-B-309-REWA-49, IR-70175-51-2-1-1-2, IR-82635-B-B-25-4, RP-512517-6-3-2-1, UPRI-2012-15, RP-5345-9-6-3, CB-09-516, BVD-109, CR-422-63-51-B-2-1, BAU-446-06, IR-60080-46-A, IDR-763, CRR-680-B-B-25-4, CRR-614-4-1, IR-73000-160-3-3-2, CRR-599-4-1, CR-2721-81-3, CRR-599-5-1, B-10580E-KN-28-1-1.	20

CONCLUSION

The present study successfully identified significant genetic diversity among 123 rice genotypes under drought stress conditions using morpho-physiological traits and SSR markers. The genotypes APO, RASI, Nagina-22 and RAU-4 were found to exhibit high drought tolerance and can be valuable resources for breeding programs aimed at enhancing drought resistance. High PIC values observed for markers RM480, RM170, RM316 and RM20 indicate their potential applicability in future genetic improvement efforts. The study demonstrates the efficacy of combining phenotypic and molecular diversity analyses for precise selection of drought-tolerant genotypes, which can ultimately contribute to the development of high-yielding rice varieties under water-stressed conditions.

Conflict of Interest

The authors declare that there is no conflict of interest regarding this manuscript.

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