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Genetic divergence studies for drought tolerance in rice (*Oryza sativa* L.) using morphological traits and molecular markers

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ABSTRACT

Assessing diversity is a prerequisite for any crop improvement programme. In the present investigation, genetic divergence was estimated by employing Mahalanobis'D2-statistics and molecular markers (SSR) to examine diverse rice landraces. Analysis of variance revealed the highly significant differences among the landraces for all the characters under study. Based on the inter-se genetic distance, 20 rice landraces were grouped into five clusters. Clustering pattern indicated that 13 out of 20 belong to cluster I. On the other hand, four genotypes belongs to cluster II and cluster III, IV, V consists of one genotype each. To access the molecular diversity among 20 rice landraces, 16 random SSR markers were used out of which, 10 were found polymorphic. A total of 33 alleles were detected by 10 polymorphic markers across 20 rice landraces with an average of 3.3 alleles per polymorphic marker. PIC value ranged from 0.345 to 0.775 and marker RM152 was found to be the most appropriate marker to discriminate among the rice genotypes owing to the highest PIC value of 0.775. On the basis of dendrogram, the highest similarity observed between cultivar Ekha Keha and B-6149FMR-7 and most diverse cultivar was Swarna and Xinuozao.

Key words: *Drought, molecular diversity, polymorphic and SSR marker*

INTRODUCTION

Rice is a cereal crop, belongs to genus *Oryza* of Poaceae family. It is cultivated in 114 countries across the globe, but 90 percent of world's rice is grown in Asia (FAO, 2013). Rice production, consumption and trade are mostly concentrated in Asia. One third of Asia's rice production is consumed in China and one fifth in India. Among the rice growing countries in the world, India has the largest area under rice crop (about 44 million ha.) and ranks second in production next to China. About 25% of the world's rice area is under rainfed lowlands. Water is the critical and most important factor in rice production. Accordingly, 70 percent of the world's food-growing areas turn increasingly parched. Drought is one of the abiotic stresses which, affect the yield of the rice crop. Drought reduces yield by 15-50 per cent , depending on the stress intensity and crop growth period at which the stress occurs in rice (Srividhya et al., 2011). The main challenge for rice breeders is to raise yield of water intensive crop under drought environment. Characterization and quantification of high yield potential has long been a major goal in evolutionary biology. Information on the genetic diversity within and among closely related crop varieties is essential for a rational use of genetic resources. The usage of diverse genotypes may lead to the production of elite cultivars with improved traits. Thus, the study was undertaken with an objective of evaluating the extent of diversity at field and molecular level among rice landraces for drought tolerance which in future can be used in breeding programme for development of drought tolerant varieties.

MATERIALS AND METHODS

The field experiment was conducted in *kharif*, 2016 at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, and laboratory experiment was conducted at Molecular Breeding Laboratory (DBT), Institute of Agricultural

Sciences, Banaras Hindu University, Varanasi. The present research work confined with 20 rice landraces (drought donors including checks) which were received from the project of Stress Tolerant Rice for Africa and South Asia (STRASA), IRRI, Philippines (Table 1).

The experiment was laid out in randomized block design (RBD) with three replications. The nursery was raised on uniform raised beds applied with recommended fertilizer dose. Twenty one days old seedlings were transplanted in main research plot with one seedling per hill. The recommended agronomic practices were followed to raise a good and healthy crop. A bund was made all around the field and water was removed from the field to create drought environment.

Data was recorded for five competitive plants from each treatment in each replication randomly to record the following observations for 27 quantitative traits *viz.,* days to 50 per cent flowering, days to maturity, plant height (cm), number of tillers per plant, number of effective tillers per plant, panicle length (cm), number of spikelets per panicle, number of grains per panicle, number of sterile spikelets per panicle, grain weight per panicle (g), 1000- grain weight (g), grain yield per plant (g), grain yield per plot (g), biomass (kg/ha), harvest Index, grain quality characters, hulling recovery, milling recovery, kernel length (mm), kernel breadth (mm), kernel L/B ratio, amylose content, canopy temperature depression (CTD), stomatal conductance ($mmol/m^2/s$), chlorophyll content (SPAD value) and proline content (µmol/g fresh weight).

Table 1. List of 20 landraces and their sources.

S. No.	Name of Genotype	Source
1	B 6149 F-MR-7	I.R.R.I., Philippines (S.A. Hub)
2	DZ 78	I.R.R.I. , Philippines (S.A. Hub)
3	E khakeha	I.R.R.I. , Philippines (S.A. Hub)
4	E ZI 124	I.R.R.I. , Philippines (S.A. Hub)
5	Gopal	I.R.R.I. , Philippines (S.A. Hub)
6	Kaukhmwe	I.R.R.I. , Philippines (S.A. Hub)
7	NP 125	I.R.R.I. , Philippines (S.A. Hub)
8	NS 252	I.R.R.I. , Philippines (S.A. Hub)
9	RTS 4	I.R.R.I. , Philippines (S.A. Hub)
10	SOLOI	I.R.R.I. , Philippines (S.A. Hub)
11	Tchampa	I.R.R.I. , Philippines (S.A. Hub)
12	Vellaiseenetti	I.R.R.I. , Philippines (S.A. Hub)
13	Wannidahanala	I.R.R.I. , Philippines (S.A. Hub)
14	WAR 72-2-1-1	I.R.R.I. , Philippines (S.A. Hub)
15	XI Nuozao	I.R.R.I. , Philippines (S.A. Hub)
16	IR -74371-54-1-1	I.R.R.I. , Philippines (S.A. Hub)
17	IR -119	I.R.R.I. , Philippines (S.A. Hub)
18	IR-64	I.R.R.I. , Philippines (S.A. Hub)
19	Swarna	ANGRAU, Hayderabad
20	Local check (NDR 359)	NDUAT, Faizabad

IRRI - International Rice Research Institute, Philippines, S.A. Hub - South Ashia Hub, ANGRAU - Acharya N. G. Ranga Agricultural University, NDUAT - Narendra Deva University of Agriculture and Technology

For DNA extraction young leaves of 15-20 days old were clipped and stored in ice-box to carry it to the lab, which is then stored in -80 ^{0}C till DNA extraction. Genomic DNA was then extracted using CTAB method of Doyle and Doyle (1987). Ten random SSR primers were used for molecular diversity analysis. The details of SSR primers with their forward and backward sequences and chromosome number in which that marker is located are presented in Table 2.

The data was subjected to Mahalanobis D^2 statistics to measure the genetic divergence as suggested by Rao (1952). Tocher´s method as described by Rao (1952) was followed for cluster formation. The dissimilarity matrix was used for clustering of genotypes based on Unweighted Pair Group Method on Arithmetic Average (UPGMA) and a dendrogram was generated based on the genetic distance matrix. The data was analyzed by windostat version 9.2 with indostat services.

RESULTS AND DISCUSSION

In the present study, evaluation of genetic divergence in rice was carried out by using data at field and molecular level. χ^2 test applied to 'V' statistic indicated significant difference between the means in respect of pooled effect of 27 traits under study and between different populations. Hence, further analysis was carried out for estimating D^2 values to study genetic divergence. The composition of different clusters obtained from the D^2 analysis has been presented in the Table 3. Twenty rice landraces were grouped into five different clusters based on the inter-cluster genetic distances. This indicated presence of considerably diverse rice genotype in the set of material under study. The genotypes were grouped into five clusters. Clustering pattern indicated that 13 out of 20 genotypes belong to the same cluster i.e. cluster I. On the other hand, four genotypes belongs to cluster II and III, IV, V consists of one genotype each. D^2 and D values among five clusters have been presented in the Table

Table 2. Details of the SSR primers used in present study.

S.No.	SSR Primer	Sequence	Chr. No.	Tm(ºC)
1.	RM259 F	TGGAGTTTGAGAGGAGGG	1	55
	RM259 R	CTTGTTGCATGGTGCCATGT		
2.	RM154 F	ACCCTCTCCGCCTCGCCTCCTC	2	61
	RM154 R	CTCCTCCTCCTGCGACCGCTCC		
3.	RM283 F	GTCTACATGTACCCTTGTTGGG	1	61
	RM283 R	CGGCATGAGAGTCTGTGATG		
4.	RM 431F	TCCTGCGAACTGAAGAGTTG	1	55
	RM 431 R	AGAGCAAAACCCTGGTTCAC		
5.	RM 316 F	CTAGTTGGGCATACGATGGC	9	55
	RM 316 R	CTAGTTGGGCATACGATGGC		
6.	RM105 F	GTCGTCGACCCATCGGAGCCAC	9	63
	RM105 R	TGGTCGAGGTGGGGATCGGGTC		
7.	RM215 F	CAAAATGGAGCAGCAAGAGC	9	55
	RM215 R	TGAGCACCTCCTTCTCTGTAG		
8.	RM152 F	GAAACCACCACACCTCACCG	8	53
	RM152 R	CCGTAGACCTTCTTGAAGTAG		
9.	RM25 F	GGAAAGAATGATCTTTTCATGG	8	53
	RM25 R	CTACCATCAAAACCAATGTTC		
10.	RM 408 F	CAACGAGCTAACTTCCGTCC	8	55
	RM 408 R	ACTGCTACTTGGGTAGCTGACC		

4. The diagrammatic representation of clusters with intra and inter cluster D values has been presented in fig. 1. The highest intra-cluster distance was observed in the cluster I (41.42) which comprised of 13 genotypes. The highest inter-cluster distance (105.62) was found between cluster II and V followed by cluster I and V (79.20) and cluster III and V (70.08). The smallest inter-cluster distance (46.61) was observed between I and III followed by cluster III and IV (49.27) and between cluster II and IV (54.99). These findings were similar to the findings of Sinha et al., 1991; Banumathy et al., 2010; Ovung et al., 2012. The greater

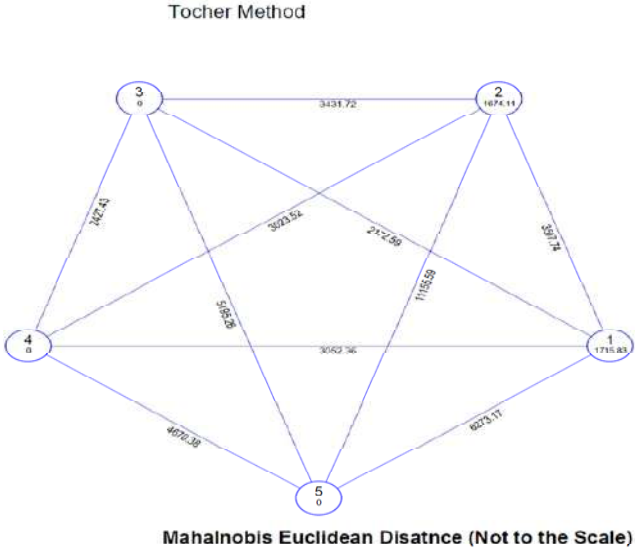

Fig. 1. Cluster by Tocher method

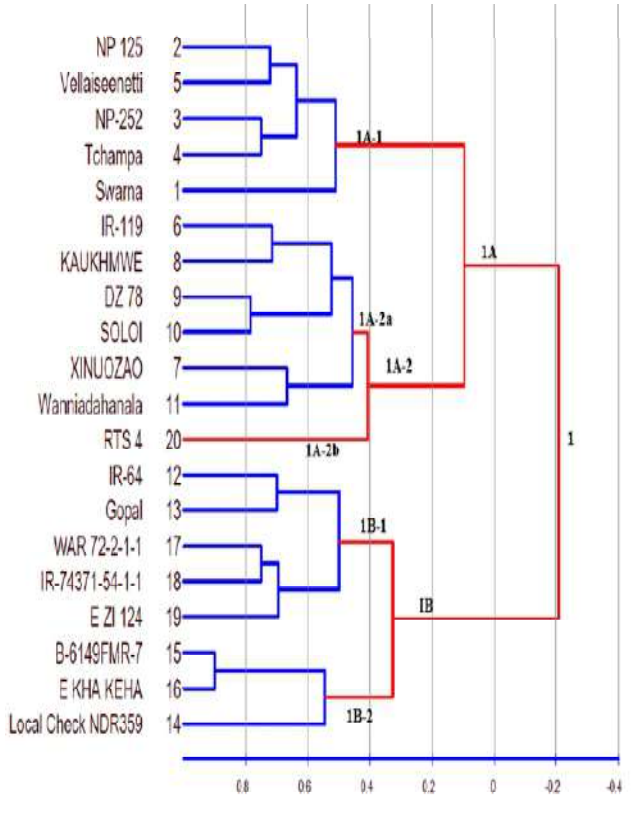

Fig. 3. Dendrogram depicting the relationship of 20 genotypes of rice.

Table 3. Grouping of 20 rice landraces into five clusters (by Tocher method).

Clusters	Germplasm	Number
I	B 6149 F-MR-7, Local check (NDR 359), IR -119, DZ 78, Gopal, RTS 4, IR -74371-54-1-1, XI Nuozao, WAR 72-2-1-1, Wannidahanala, Kaukhmwe, NP 125 and NS 252.	13
II	E ZI 124, SOLOI, Tchampa, Vellaiseenetti.	4
III	E khakeha	1
IV	IR-64	1
V	Swarna	1

the distance between two clusters, wider is the expected genetic advance between them. Therefore, indication of the genetically diverse genotypes would help in selecting parents for hybridizing programme. However, while selecting parents for hybridization programmes their yield potential should not be overlooked (Singh et al., 1991).

The relative contribution of twenty seven characters towards diversity is presented in fig. 2. The characters appeared in first rank more it contributed towards diversity. Among all the characters, biomass (kg/ha)contributed the maximum (31.05%) to the diversity by taking first rank in 59 times, followed by grain yield/ plant (g) (16.84% with 32 times ranked first), harvest index (11.58% with 22 times ranked first), grain yield/ plot(kg) (8.95 with 17) and kernel breadth (mm) (7.89% with 15 times ranked first).

Molecular Diversity

Table 4. Inter and intra cluster distance of D^2 and D values of 20 rice landraces.

Cluster	I	II	III	IV	V
I	1715.825 (41.42)	3507.736 (59.23)	2172.594 (46.61)	3052.358 (55.25)	6273.167 (79.20)
II		1674.107 (40.92)	3431.723 (58.58)	3023.518 (54.99)	11155.591 (105.62)
III			0.000 (0.000)	2427.430 (49.27)	5195.264 (70.08)
IV				0.000 (0.000)	4670.376 (68.34)
V					0.000 (0.000)

Ten polymorphic SSR primers were utilised for molecular assesment of twenty rice genotypes. These polymorphic markers are spread on chromosome 1, 2, 9 and 8. Cluster analysis was done to construct dendogram using Jaccard's dissimilarity coefficient.

Dendrogram (Fig. 3) based on Jaccard's dissimilarity coefficient using UPGMA analysis grouped

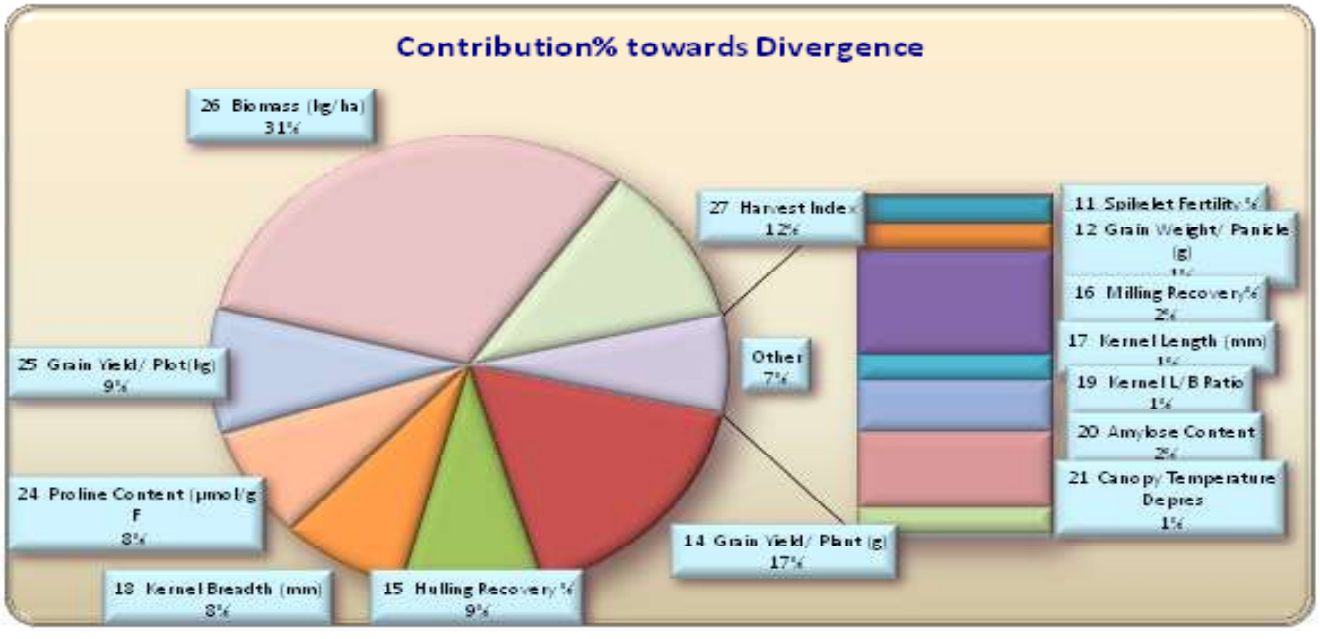

Fig. 2. Character contribution of 27 traits of 20 landraces of rice.

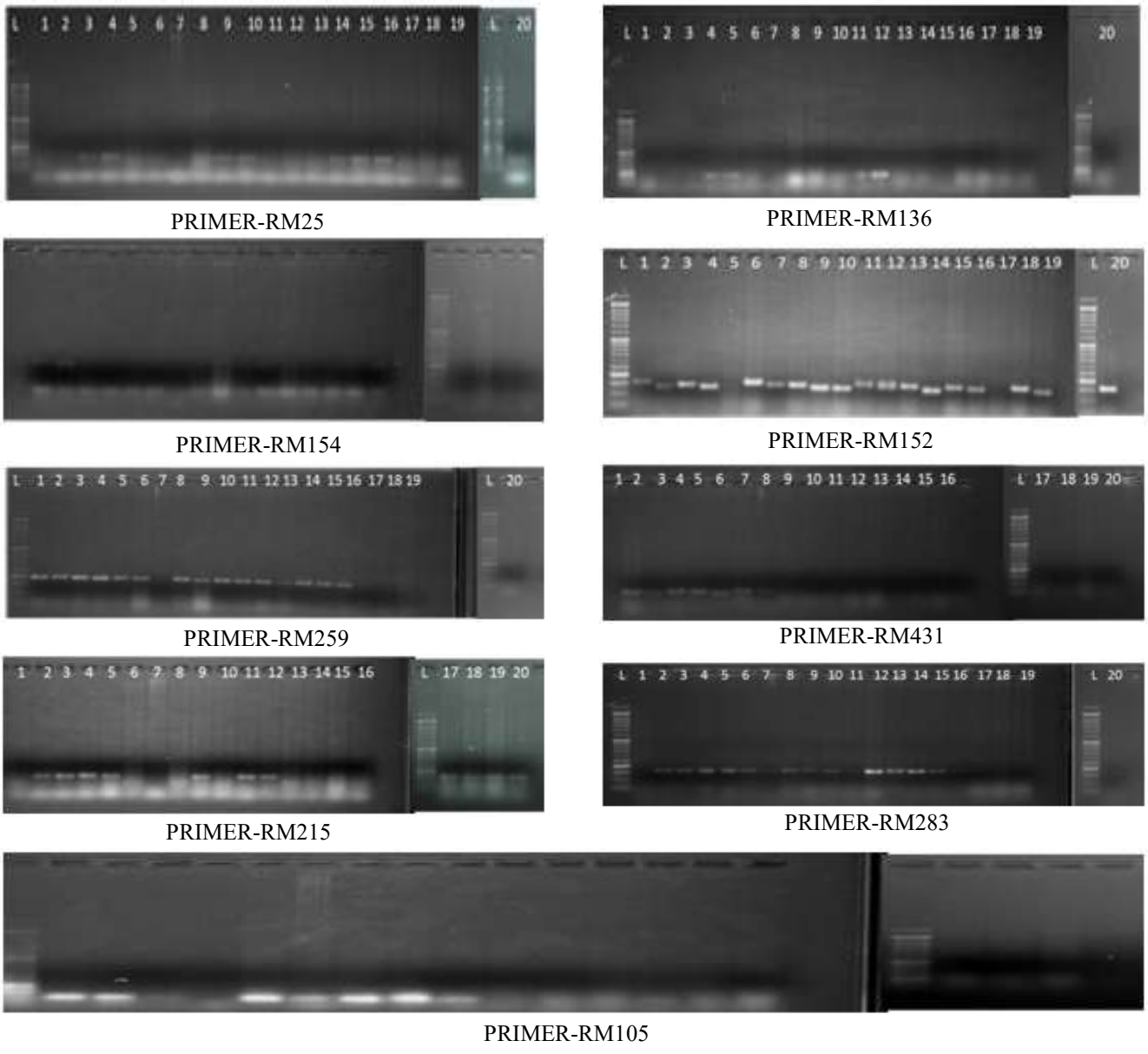

Fig. 4. Banding pattern obtained using four SSR primers between 20 genotypes. L-MARKER (100bp) 1-SWARNA, 2- NP 125, 3- NP-252, 4- TCHAMPA, 5- VELLAISEENETTI, 6- IR-119, 7-XINUOZAO, 8- KAUKHMWE, 9- DZ 78, 10- SOLOI, 11- WANNIADAHANALA, 12- IR-64, 13- GOPAL, 14- LOCAL CHECK NDR359, 15- B-6149FMR-7, 16- E KHA KEHA, 17- WAR 72-2-1-1, 18- IR-74371-54-1-1, 19- E ZI 124, 20-RTS-4.

the 20 landraces into different clusters. Twenty rice landraces fall in a major cluster *i.e.,* cluster I. Cluster I was further sub-divided into two sub groups IA and IB. Sub group IA was further divided in sub cluster IA-1 and 1A-2, Cluster IB was subdivided into clusters IB-1 and 1B-2. The sub cluster IA-2 was further partitioned into IA-2a and IA-2b. Cluster indicated that six genotypes out of twenty belong to the cluster IA-2a followed by cluster IA-1 which has five genotypes and cluster IB-1 with five genotypes. Clusters IB-2 comprises of three genotypes and cluster IA-2b have only one genotype.

The dissimilarity coefficient varies from one to zero, closer to one show high dissimilarity, while closer to zero shows high similarity. The high dissimilarity was found between the most of the genotypes like SWARNA and XINUOZAO, SWARNA

Table 5. PIC value of 10 polymorphic markers.

Sl.No.	Locus	Alleles	PIC
1	RM152	6.000	0.775
2	RM 408	4.000	0.667
3	RM25	4.000	0.547
4	RM259	3.000	0.567
5	RM 431	3.000	0.535
6	RM283	3.000	0.449
7	RM154	3.000	0.375
8	RM 316	3.000	0.346
9	RM105	2.000	0.375
10	RM215	2.000	0.346

and DZ 78, Vellaiseenetti and XI Nuozao, XI Nuozao and SOLOI, Local Check NDR359 and Wannidahanala having value 1.00. The lowest value 0.20 was observed for E khakeha and B-6149FMR-7 followed by DZ 78 and SOLOI (0.429), NP 252 and Tchampa (0.50), Tchampa and Vellaiseenetti (0.50), Kaukhmwe and Tchampa (0.50), IR-64 and IR-119 (0.875), shows high similarity. Similar results were found by Sajib et al. (2012), while working on aromatic landraces of rice and Souroush et al. (2004) on genetic divergence in indigenous upland rice varieties. The 10 polymorphic primers yielded a total of 33 fragments (amplified products). Maximum fragments were produced by primers RM152 (six fragments), RM408 and RM25 which yielded four fragments each and an average of 3.3 fragments was produced per primer which showed polymorphic amplification.

The polymorphic information content (PIC) was employed for each locus to assess the information of each marker and its discriminatory ability. The PIC value is an evidence of diversity and frequency among the varieties (Pervais et al., 2009). The PIC value of SSR markers ranged from 0.346 to 0.775 with a mean PIC of 0.498. Markers RM152, RM408, RM259 and RM25 were the most informative primers on the basis of highest PIC of 0.775, 0.667, 0.643 and 0.602 respectively. SSR marker RM215, RM316, RM105 and RM154 showed least PIC value of 0.346, 0.346, 0.375 and 0.375 respectively (Table 4). The PIC values observed in this study were comparable to those reported in some studies of Sajib et al. (2012) and Ashfaq and Khan (2013) and Sonkar et al. (2016).

CONCLUSION

The foremost idea for conducting this experiment was to identify the most diverse drought tolerant rice landraces with that of local checks. Therefore, diverse landraces can be utilized in the breeding programme for the development of drought tolerant varieties with enhanced yield and quality. In the present investigation along with the drought traits, quality traits were also studied for improving both the traits simultaneously.

The present study has revealed valuable information on different yield traits in rice improvement. The most diverse pair of cultivars were Swarna and XI Nuozao, Swarna and DZ 78, NDR359 and Wannidahanala. These genotypes can be utilized in future breeding programme to obtain potential transgressive segregants with drought tolerance.

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