Oryza Vol. 57 Issue No. 2, 2020 (94-99)
DOI https://doi.org/10.35709/ory.2020.57.2.2

Assessment of molecular divergence using microsatellite markers linked to drought-yield QTLs of rice (*Oryza sativa* L.)

ND Rathan*, SK Singh, RK Singh and PR Vennela

Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Corresponding author e-mail: rathanndnagl27@gmail.com

Received : 12 March 2020 Accepted: 18 June 2020 Published : 30 June 2020

ABSTRACT

The drought stress is the major limitation for the production and yield stability in the rice (Oryza sativa L.) growing areas particularly under rainfed conditions. The assessment of genetic diversity among the rice genotypes using molecular markers linked to drought-yield QTLs will help in the selection of divergent parents to produce superior recombinants and transgressive segregants to develop high yielding cultivars for drought conditions. So the current study was conducted to estimate the genetic diversity among the set of twenty four advanced drought tolerent rice genotypes and commercial checks using SSR markers linked to major drought-yield QTLs. A total of 31 alleles were detected by 13 polymorphic markers across 24 rice genotypes with a mean of 1.94 alleles per polymorphic marker. Among the polymorphic markers, 11 markers formed 2 alleles each, 2 markers have produced 3 alleles each. The PIC value observed in the contemporary study ranged from 0.239 to 0.499 with an average PIC value of 0.346. The dendrogram displayed a total five clusters on the basis of dissimilarity coefficient values, the cluster II had maximum ten genotypes followed by cluster IB-a with five genotypes and clusters IA-a, IA-b, IB-b with three genotypes each. IR 82475-110-2-2-1-2 and IR92937-178-2-2 (R-155) were found to be the most divergent genotypes.

Key words: *Genetic diversity, SSR markers, rice, PIC, clusters, drought*

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple food crops for nearly half of the global population. Approximately three billion people around the world consume rice as a basic food which provides about 50 to 80% of their daily calories (Jasim Al jumaili et al., 2018). Globally rice is grown in an area of 163 million hectares with the production of 758.9 million tonnes (FAO Rice Market Monitor, 2017). But over half of the global rice producing area is under rainfed condition where drought is the major limiting factor for increased production. So considering the fact that the huge area is under rainfed condition, it is of paramount importance to enhance the rice production in water deficit conditions to meet the increasing global population demand. The selection of appropriate parents which are genetically diverse is important for any hybridization programme to be successful as it helps in developing superior recombinants.Classification of the genetic variation and their relationship are critically required for enhancing the management and utilization of the germplasm resource (Rao and Hodgkin, 2002) and understanding the genetic relationship within germplasm resource provides an insight for breeders to increase efficiency of parental selection for future rice breeding programs (Singh et al., 2016). Traditionally morphological or physiological traits as well as protein or isozyme markers were used to assess genetic diversity in plants. But they are greatly influenced by environmental conditions, need long time for assessment and show low polymorphism between the genotypes (Chakravarthi and Naravaneni, 2006). In contrast, modern biotechnology provides us molecular markers which are independent of environmental factors, show high polymorphism between the genotypes, allow easy and

quick analysis of loci distributed among the plant genome (Chakravarthi and Naravaneni, 2006). Thus, DNA based markers such as AFLP (Amplified Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), SSR (Simple Sequence repeat) and SNP (Single Nucleotide Polymorphism) have been routinely used to assess the genetic divergence among the genotypes. Multi-allelic nature and high polymorphism of SSR markers help to establish the relationship among the individuals even with less number of markers (McCouch et al., 1997). SSR markers are preferred as they are abundant in the genome, well-distributed throughout the genome, hyper-variable, multi-allelic and co-dominant nature, ease of assaying, highly reproducible and highly informative(Gupta &Varshney, 2000; Vieira et al., 2016).

So by considering the above points in mind, the current study was aimed at assessingthegenetic diversity of drought toletent advanced IRRI rice (*Oryza sativa* L.) genotypes using microsatellite markers linked to major drought-yield QTLs to identify the most diverse genotypes for future hybridization programmes to develop high yielding drought tolerant cultivars.

MATERIALS AND METHODS

The present study was conductedduring the kharifseason 2016-2017 using twenty four rice genotypes, which include drought tolerant advanced generation lines along with commercial checks received from IRRI Networking Project, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. The list of selected genotypes along with the brief description about their sources is given in Table 1. The experiment was laid out in randomized block design (RBD) with three replications. Twenty five days old seedlings were transplanted in main research plot. Each plot consisted of five rows of 1.5 m length with spacing 15 × 20 cm. The crop was maintained as per the standard agronomic practices.

Genomic DNA extraction

The leaves are collected from 21 days old seedlings of each genotypes from the field and carried it to the lab using Ice-box and stored the samples at -80°C until further use. Genomic DNA was then extracted from 24 rice genotypes using CTAB method proposed by Doyle and Doyle (1987) and diluted this DNA for 30mg/μl. The quality of DNA was checked by using 0.8% Agarose gel electrophoresis.

Table 1. List of genotypes along with their sources.

S.no.	Genotypes	Source of genotypes
1	IR 92522-47-2-1-4	IRRI, Philippines
2	IR 92522-45-3-1-4	IRRI, Philippines
3	IR 91648-B-85-B-1-1	IRRI, Philippines
4	IR 91648-B-215-B-2-1	IRRI, Philippines
5	IR 91648-B-58-B-7-3	IRRI, Philippines
6	IR 96321-315-402-B-1	IRRI, Philippines
7	IR 96321-327-300-B-1-1	IRRI, Philippines
8	Swarna-Sub1	IRRI, Philippines
9	Swarna	APRRI, Maruteru, AP
10	Lalat	OUAT, Orissa
11	MTU1010	APRRI, Maruteru, AP
12	IR 94391-587-1-2-B	IRRI, Philippines
13	IR 85604-19-2-3-2-2	IRRI, Philippines
14	IR 82475-110-2-2-1-2	IRRI, Philippines
15	IR 96248-16-3-3-1-B	IRRI, Philippines
16	IR 96248-16-3-3-2-B	IRRI, Philippines
17	IR 97477-110-3-1-B	IRRI, Philippines
18	IR92978-192-1-2 (R-306)	IRRI, Philippines
19	IR91953-141-2-1-2 (R-119)	IRRI, Philippines
20	IR92937-178-2-2 (R-155)	IRRI, Philippines
21	R-RHZ-7	IGKV, Raipur
22	CGZR-1	IGKV, Raipur
23	IR64	IRRI, Philippines
24	SambaMahsuri	APRRI, Maruteru, AP

PCR amplification using SSR markers

The list of SSR markers along with the information about their repeat motif, drought-yield traits for which they are linked, annealing temperature is presented in the Table 2. The sequence of the primers of each marker is retrieved from the online database GRAMENE (https://archive.gramene.org/). PCR amplification using sixteen SSR primers was carried out using sterilized thin-walled PCR tubes according to the protocol of Channamallikarjuna et al. (2010). The PCR amplification was carried out in 15 μl of reaction mixture containing 30 ng genomic DNA, 1.5 mM PCR buffer (MBI Fermentas, USA), 400 μM dNTPs (MBI Fermentas), 1 U Taq DNA polymerase (MBI Fermentas) and 0.4 μM primer using a thermal cycler (Master cycler gradient, Eppendorf). Thermal cycling program involved an initial denaturation at 94°C for 45 sec, annealing at annealing temperature of respective

Table 2. The details of the SSR markers and their association with various drought-yield traits/QTLs.

Sl.no.	SSR marker	Repeat motiff	Traits linked	Reference	Annealing temp (°C)	Average alleles	PIC
1	RM 1	(GA)26	GYP, GYPa, SPP, FGP, SL, SDW, RDW, RSR	Brondani et al., 2002, Srividya et al. 2011	50	3	0.499
2	RM 28166	(CT)12	BIO, HI , PH, PPP, DF	Bernier et al., 2007	55	3	0.424
3	RM 495	(CTG)7	SL, SDW, RDW, RSR	Srividya et al., 2011	52	2	0.368
4	RM 283	(GA)18	TGW, PL, PPP	Rabiei et al., 2015	55	2	0.328
5	RM 259	(CT)17	FGP, RFW, RN	Brondani et al., 2002, Mu et al., 2003	51	2	0.373
6	RM 5	(GA)14	RDW, RFW	Mu et al., 2003	51	2	0.275
7	OSR 13	(GA)n	GY, KB	Li et al., 2011	55	2	0.375
8	RM 338	(CTT)6	GL, TGW	Aluko et al., 2004, Tang et al., 2013	55	2	0.239
9	RM 514	(AC)12	GY, TGW	Zou et al., 2005	51	2	0.365
10	RM 22	(GA)22	DF, BIO, GY, HI	Vikram et al., 2011	53	2	0.373
11	RM 431	(AG)16	BIO, DTF, HI, PH	Bernier et al., 2007, Vikram et al., 2011	57.5	2	0.328
12	RM334	(CTT)20	SDWT, RGER,VI,	Diwan et al., 2013	55	2	0.315
13	RM 11943	(GA)11	DF, PH, GY, HI, BIO	Vikram et al., 2011	53	2	0.239
14	RM 237	(CT)18	PH, SSP, LDS, DIDRV	Brondani et al., 2002, Yue et al., 2006	53.5	1	----
15	RM 489	(ATA)8	LDS, RVD, HI	Yue et al., 2006, Vikram et al., 2011	53	1	----
16	RM 507	(AAGA)7	RBM	Yue et al., 2006	52.5	1	----

Grain Yield per Panicle (GYPa), Grain Yield per Plant (GYP), Spikelets per Panicle (SPP), Shoot length (SL), Shoot dry weight (SDW), Root dry weight (RDW), Root shoot ratio (RSR), Biomass (BIO), Harvest index (HI), Panicle/Plant(PPP), Days to flowering (DF), 1000 grain weight (TGW), Panicle length (PL), Filled Grains per Panicle (FGP), Root fresh weight (RFW), Grain yield (GY), Kernels/ branch(KB), Grain length (GL), Seedling dry weight (SDWT), Rate of germination (RGER), Vigour Index (VI), Leaf-drying score(LDS), Deep root rate in volume induced by drought conditions (%) (DIDRV), Root volume under drought conditions (ml) (RVD), Relative biomass (%) (RBM).

primers for 30 sec, primer extension at 72°C for 30 sec, followed by a final extension at 72°C for 7 min. The amplified PCR products were size fractioned by electrophoresis in 2.5% agarose gel prepared in TAE buffer and visualized by staining with ethidium bromide (0.5 µg/ml) in a gel documentation system (BIO-RAD, USA).

Scoring of PCR amplified fragments

Band position in comparative SSR profile for each genotype and primer combination was scored from the respective gel images. SSR profile from only that genotype x primer combination, which gave consistent amplification for all the genotypes and without any blank lane/unclear bands, was included in this study. The amplified fragments were scored as "1" for the presence and "0" for the absence of a band generating the 0 and 1 matrix. These binary data matrix was then utilized to generate genetic similarity data among the 24 rice genotypes.

Statistical analysis of molecular data

The effects of different scales of measurement for different quantitative traits were minimized by standardizing the data for each trait separately prior to cluster analysis. Standardization was done by dividing the deviation of mean for a line from the mean for twenty four lines with the standard deviation for the given trait; the STAND module of NTSYS (Rohlf, 1997) software was used to furnish the same. The binary data matrix generated by polymorphic SSR markers, which were subjected to further analysis using NTSYS-pc version 2.11W (Rohlf, 1997). The SIMQUAL programme was used to calculate the Jackard' dissimilarity coefficient. The dissimilarity matrix was used as an input for analysis of clusters. UPGMA-based clustering was done using SAHN module of NTSYSpc for dendogram construction. In unweighted pair-group average (UPGMA) clusters are joined based on the average distance between all members in the two groups. PIC for SSR markers was calculated as per the formula: PIC= 1-Pij^2, where PICi is the polymorphic information contents of a marker i and the summation extends over n patterns.

RESULTS AND DISCUSSION

The level of polymorphism among advanced generation drought tolerant rice genotypes was assessed by computing allelic number and PIC values for each of the sixteen SSR markers linked to drought-yield related traits (Table 2).

A total of 31 alleles were noticed with the help of 13 polymorphic markers across 24 rice genotypes with a mean of 1.94 alleles per polymorphic marker. This result is in accordance with the work of Umadevi et al., 2014, who found a total of 98 alleles with an average of 2.78 alleles per locus, Ramadan et al., 2015 found a mean of 2.76 alleles per locus and average number of effective alleles ranged from 1.78 to 2.49 in the study of Kibria et al., 2009. Among the polymorphic markers, 11 markers formed 2 alleles each, 2 markers have produced 3 alleles each. PIC value is an indication of allelic diversity and frequency among genotypes. The PIC value observed in the contemporary study ranged from 0.239 to 0.499 with an average PIC value of 0.346, comparable to earlier assessments of SSR analysis in rice *viz*., 0.14 to 0.71 with an average of 0.48 in the study by Sajib et al., 2012, 0.28-0.50 with an average of 0.45 reported by Umadevi et al., 2014, 0.21-0.79 with a mean of 0.46 in the study by Ramadan et al., 2015 and Singh et al., 2015 reported PIC value from 0.26 to 0.65 with a mean of 0.47. The greater the PIC value of a locus, the higher the number of alleles spotted. RM 1 and RM 28166 were found to be the most suitable markers to categorize among the rice genotypes owing to the highest PIC value of 0.499 and 0.424 respectively.

The dendrogram displayed a total five clusters on the basis of Jackards's dissimilarity coefficient values (Fig. 1).In the dendogram, cluster II had maximum ten genotypes followed by cluster IB-a with five genotypes and clusters IA-a, IA-b, IB-b with three genotypes each (Table 3).

On the basis of dendrogram, the highest diversity were found between the genotypes IR 82475-110-2-2-1-2 and IR92937-178-2-2 (R-155) (0.846) followed by IR 91648-B-215-B-2-1 and IR 96248-16-3-3-2-B (0.807), IR 94391-587-1-2-B and IR 82475-110-2-2-1-2 (0.807). Hence these genotypes can be utilized as parental combinations in hybridization programme to obtain potential transgressivesegregants for drought tolerance. The dissimilarity coefficient was calculated by Jaccard IJ distance analysis, and result showed that the value ranged from 0.00 to 0.846. According to this dissimilarity coefficient and dendrogram, we can understand the diversityand relatedness among the genotypes. So, genotypes with maximum divergence can be used as parents in breeding programme. The dissimilarity coefficient varied from the maximum value 0.846 between the cultivar IR 82475-110-2-2-1-2 and IR92937-178-2-2 (R-155) and minimum value 0.00 between the cultivars IR 96321-315-402-B-1 and Swarna-Sub1 which showed highest similarity between them,and the greater similarity between the genotypes may be because of the common parentage or the total number of genome wide SSR markers used were rather less to differentiate between these genotypes. The SSR markers used in the current study were linked with the Drought-Yield QTLs and hence able to evaluate the diversity among the genotypes particularly for drought and yield related traits and hence very helpful in selecting genetically diverse genotypes to use in breeding programs to develop high yielding drought tolerant cultivars.

Table 3. Grouping of twenty four rice genotypes into different clusters based on Jaccard's IJ coefficient.

Cluster name	No. of genotypes	Name of the genotypes
IA-a	3	IR 92522-47-2-1-4 IR 92522-45-3-1-4 IR 82475-110-2-2-1-2
II	10	IR 91648-B-85-B-1-1 IR 91648-B-215-B-2-1 IR 96321-315-402-B-1 Swarna-Sub1 Swarna Lalat, IR 94391-587-1-2-B IR 85604-19-2-3-2-2 IR92937-178-2-2 (R-155) R-RHZ-7
IB-a	5	IR 91648-B-58-B-7-3 IR 96321-327-300-B-1-1 IR91953-141-2-1-2 (R-119) CGZR-1 SambaMahsuri
IA-b	3	MTU1010 IR 96248-16-3-3-1-B IR 96248-16-3-3-2-B
IB-b	3	IR 97477-110-3-1-B IR92978-192-1-2 (R-306) IR64

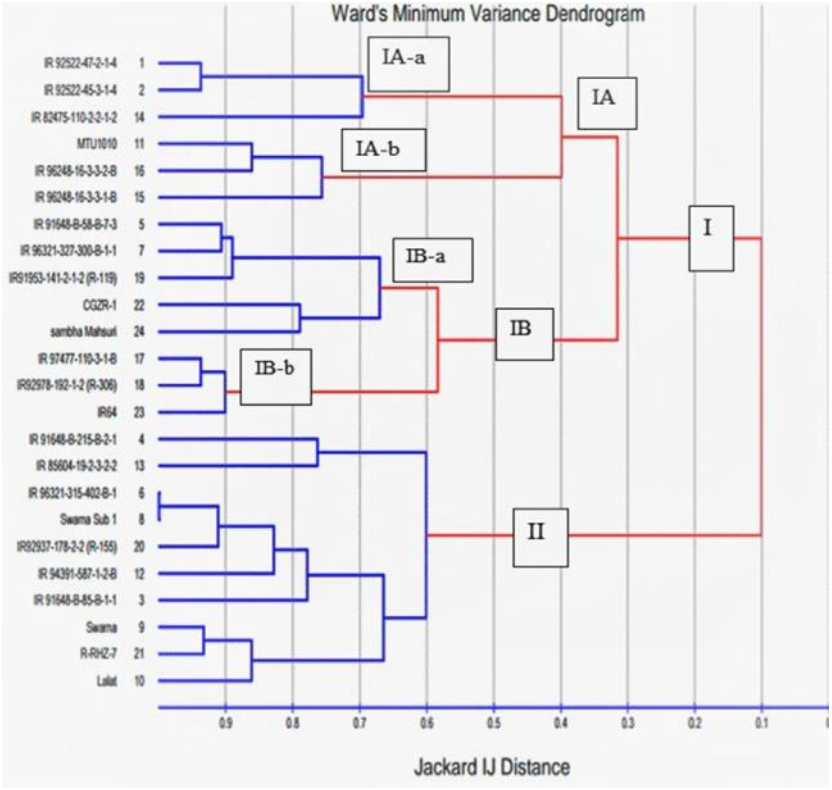

Fig. 1. The dendrogram for Grouping the twenty four rice genotypes into different clusters based on Jaccard's IJ coefficient.

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