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Assessment of genetic diversity in a set of rice (*Oryza sativa* L.)genotypes using molecular markers

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Abstract

The assessment of genetic diversity in the germplasm is an essential pre requisite for a breeding programme. In the present study genetic diversity was analysed in a set of 94 genotypes evaluated for various yield attributes under high temperature stress using 48 SSR primers. A total of 129 alleles were detected by 48 primers among 94 rice genotypes with an average of 2.7 alleles per primer. The number of alleles generated by each marker varied from 1 to 4. The Polymorphism Information Content values ranged from 0.19 to 0.60 with an average of 0.41. Twenty six SSR primers revealed PIC values higher than the average. The genetic dissimilarity index ranged from 0.04 to 0.92. The dendrogram generated using DAR with indicated that the 94 genotypes were clustered into three distinct clusters with two sub clusters in each cluster. The selected genotypes will be useful in the on-going breeding programme and will help in broadening the genetic base of the breeding material

Keywords: Rice; genetic diversity; SSR markers; polymorphism information content

1. Introduction

Rice (*Oryza sativa* L.) is one of the most widely cultivated cereal crop occupying about 23% of the total area under cereal production in the world. It is one of the most diverse crops which is cultivated over a wide range of climatic conditions. India is the second largest producer of rice after China. It is the predominant *Kharif* crop of Punjab. During 2019, it occupied an area of 29.2 lakh hectares in Punjab with a total production of 186 lakh tonnes and an average paddy yield of 63.84 quintals per hectare. Genetic improvement of any plant system depends upon the existence of genetic variability available for manipulation. Genetic diversity in rice has been investigated using morphological, biochemical and DNA markers. However, both of these being highly influenced by environment may not provide accurate genetic classification of the crop.

In rice, high temperature stress at the flowering and grain-filling stages has a detrimental effect on spikelet fertility and grain quality which ultimately is associated with reduction in grain yield.Considerable genetic variability exists for tolerance to high temperature stress in rice germplasm which can be exploited to develop high temperature tolerant cultivars (Waugh and Powell 1992). The genetic resources can be effectively characterised using molecular markers for efficient utilisation in breeding programmes (Nachimuthu *et al* 2015). Use of simple sequence repeats (SSRs), also known as the microsatellite DNA markers, is one of the most effective tools for identifying genetic differences among germplasm resources with advantages of convenience, simplicity, high polymorphism, codominance and stability (Liao *et al* 2011). Thus, it is widely applied in characterizing genetic diversity of germplasm resources, especially in identifying species having close genetic relationships (Mao *et al* . 2010).

Considering the current and predicted rates of increase in day/night temperature under Punjab conditions, the negative impact of diurnal temperature on rice production is likely to be felt on a much wider scale, with significant

yield losses. It is estimated that maximum and minimum temperature will go up by 2.4°C and 4°C respectively, by 2050. Rice yields have been estimated to be reduced by 41% due to heat stress by the end of the 21st century (Shah *et al* 2011). The identification of heat tolerant genotypes in relation to spikelet sterility and grain yield will be helpful in developing varieties better adapted to future climates. Therefore, the present study was undertaken with an objective to estimate genetic diversity among a set of genotypes which were evaluated for various yield attributes under high temperature stressusing SSR markers.

2. Material and methods

The material for this study comprised of a set of 94 genetically diverse genotypes of rice (*Oryza sativa* L.) which were received from International Rice Research Institute, Los Banos, Laguna, Philippines as part of Heat Tolerance Nursery. The total genomic DNA was extracted from 30 days old seedlings of germplasm lines using Cetyl Trimethyl Ammonium Bromide (CTAB) method. Murray and Thomson, 1980). The DNA was further subjected to RNase treatment adding 5µL of RNase (10mg/ml) (Genetix) at 37^0C for 1 hour. The DNA pellet was stored at -80^0C for long term stability.A total of forty eight markers spanning all the 12 rice chromosomes were employed to assess the molecular diversity and genetic relationship among 94 genotypes. In vitro amplification using Polymerase Chain Reaction (PCR) was performed in an Eppendorf Master cycler to study the polymorphism. PCR analysis was carried out in the reaction volume of 20µL containing the 2µL template genomic DNA, 1.2µL of 25 mM MgCl2, forward and reverse primers, 0.2µl of 25mM dNTPs, 4µl of 5X PCR buffer and 0.2µl of Taq polymerase (Promega) (3U/ µl). The amplified products were resolved by electrophoresis on 4% agarose gel. After electrophoresis, the gel was visualized under UV trans-illuminator and photographed using Alpha Innotech Multi Imager gel documentation system software programme from Alpha Innotech, California, USA.

The total number of alleles for each micro satellite marker was recoded for all the genotypes by assigning numbers to the amplified alleles i.e. 0 for absence and 1 for presence of allele. Polymorphic information content (PIC) was estimated using the equation of Botstein *et al* (1980).The PIC value provides an estimate of the discriminatory power of a locus or loci, by taking into account the number of alleles that are expressed as well as the relative frequencies of those alleles. The genetic diversity among the germplasm lines was computed by the computer software programme – DAR win 5.0 (Perrier and Jacquemond-Collet 2006). Dissimilarity matrix for SSR primers was constructed using Dice coefficient of associations to find out genetic relationships. The data was subjected to unweighted pair groups method with arithmetic mean (UPGMA) analysis to generate dendrogram. Data from 48 primers were used to estimate the dissimilarity based on the number of shared amplified bands.

3. Results and discussion

Unlike morpho-physiological traits used earlier as discussed by Choudhary *et al* (2013) to estimate genetic variability/relatedness, molecular markers have become quite handy in precisely understanding the extent of genetic divergence among varieties being chosen as parental sources in breeding programs. Genetic diversity among germplasm with a varied degree of stress tolerance has been well documented by several researchers (Reynolds *et al.* 2007, Sun *et al.* 2013, Sharma *et al.* 2014). Moreover, according to Chakravarthi and Naravaneni (2006) use of molecular markers is a reliable tool for assessing genetic variation among rice varieties.

In the present study, a set of 48 SSR primers was employed to evaluate the genetic diversity among 94 genotypes (Fig. 1). These genotypes were selected on the basis of their tolerance or susceptibility to high temperature stresses studied by Kaur (2016). An overall of 129 alleles were detected by 48 primers among 94 rice genotypes with an average of 2.7 alleles per primer. The number of alleles indicates the richness of the germplasm. All the markers showed good polymorphism with primer RM 16493 which showed least polymorphism. The number of alleles generated by each marker varied from 1 (RM 16493) to 4 (RM 547). The SSRs being short tandem repeats, allele numbers of 2 to 7 per locus are considered fairly good. The lower level of polymorphism may be attributed to the narrow genetic diversity. Similar results have been reported by Mahajan *et al.* (2012). The current study showed slightly lower average number of alleles than reported by Zhang *et al.* (2011) where in rice core collection with 150 rice varieties from South Asia and

Brazilthe average number of alleles was 3.88 alleles/locus. However, similar results have been shown by Nachimuthu *et al*.(2015). Several scientists have reported variable allelic diversity in SSR markers (Akagi *et al.* 1999, McCouch *et al.* 2001, Ravi *et al.* 2003).

Polymorphic Information Content (PIC) is the reflection of allelic diversity and frequency among the genotypes and it varied greatly for all SSR loci tested. The PIC values ranged from 0.19 (RM 16493) to 0.60 (RM 151) with an average of 0.41 (Table 1). Twenty six SSR primers revealed above average PIC values and among these RM 221, RM 18614, RM 6193, RM 212, RM 7434, RM 70, RM 2136, RM 547, RM 410, RM 507, RM 216 and RM 151 showed PIC values higher than 0.50. These markers are highly informative as they indicate high polymorphism. The PIC value can be looked as the measurement of usefulness of each marker in distinguishing one individual from another (Bousba *et al.* 2012). De Woody *et al.* (1995) emphasized that markers with PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of marker at specific locus. Vaniarajan *et al.* (2012) reported that PIC value ranged from 0.09 to 0.60 in the rice landraces collected from Tamil Nadu.Pardhan *et al.* (2016) showed average PIC value of 0.2998 and attributed this low to moderate value of alleles, gene diversity and PIC to the use of limited numbers of linked markers for a single phenotypic trait.

Genetic dissimilarity was calculated from the matrix of binary data using software DAR win 5.0 where '0' and '1' were standardized as the least and maximum of dissimilarity respectively. Bootstrapping of the data (1000 permutations) was performed in order to ascertain the statistical strength of genetic relationships identified through this analysis. Based on unweighted neighbour joining method, the dissimilarity coefficients were used for cluster analysis and a dendrogram was generated with the aim of analyzing the relationships between the 94 genotypes tested. The genetic dissimilarity index ranged from 0.04 to 0.92. The lowest value 0.04 was obtained between MTU 9 and IR 8 while highest dissimilarity value (0.92) calculated was between the Teqing and Pao Tou Hung genotypes.

Figure 2A shows dendrogram generated by unweighted pair group with arithmetic mean (UPGMA) to illustrate the genetic relationships of the samples studied. The indicated in the dendrogram, the 94 genotypes were clustered into three distinct clusters (Table 2) with two sub clusters in each cluster. Cluster I was the smallest with seven genotypes in sub group I a and eight in I b. Cluster II was the largest and comprised of ten genotypes in sub group IIa and thirty two in sub group IIb. On the other hand, cluster III contained six genotypes in sub group IIIa and thirty one in IIIb. The desirable heat tolerant genotypes identified on the basis of various morpho-physiological traits by Kaur (2016) were scattered in all clusters but the maximum desirable genotypes like Teqing, Ai Lan Ke 1110 and Tsipala Fotsywere present in cluster III. As such relationship cannot be correlated with heat tolerance but if primers associated with heat tolerance traits are used then more information can be generated. The genotypes which are clustered together are assumed to have high genetic similarity while those that are far away from each other are considered to be divergent. The genotypes present in the same cluster could also be traced back to some common ancestral or geographic origin.

DNA-based molecular markers have proven to be powerful tools in the assessment of genetic variation and in the elucidation of genetic relationships within and among species, characterized by abundance and not affected by environmental influence demonstrated the remarkable potential of micro satellite markers to discriminate between rice genotypes as compared to other molecular markers (Powell *et al* 1996). However, the genetic diversity analysis with SSR markers will contribute to maximize the selection of diverse parents and broaden the germplasm base in the future rice breeding program or development of heat tolerant cultivars. In addition, it will help in identifying efficient strategies for the sustainable management of genetic resources of rice crops to cope with the climate change. According to Singh *et al* (2016) the trait based improvement program in the last decades have forced breeders to rely on few parents, which resulted in loss of gene diversity and they stressed upon the need for broadening the genetic base of Indian rice varieties through the use of diverse parents in the current breeding program.

Thus present study provided an overview of the genetic diversity in a set of genotypes evaluated for yield components under high temperature conditions. Forty eight polymorphic SSR markers were used to assess

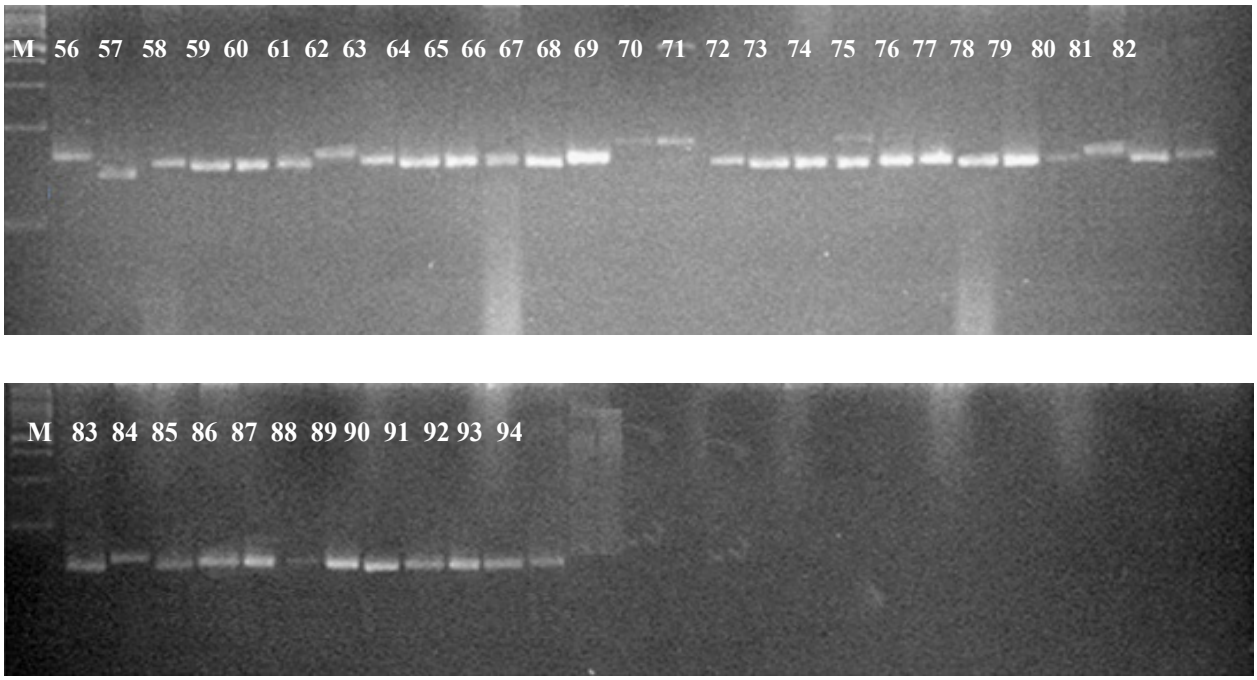

Fig. 1. Amplification profile with primer pair RM 547 using 4%Agarose gel electrophoresis for a set of rice genotypes

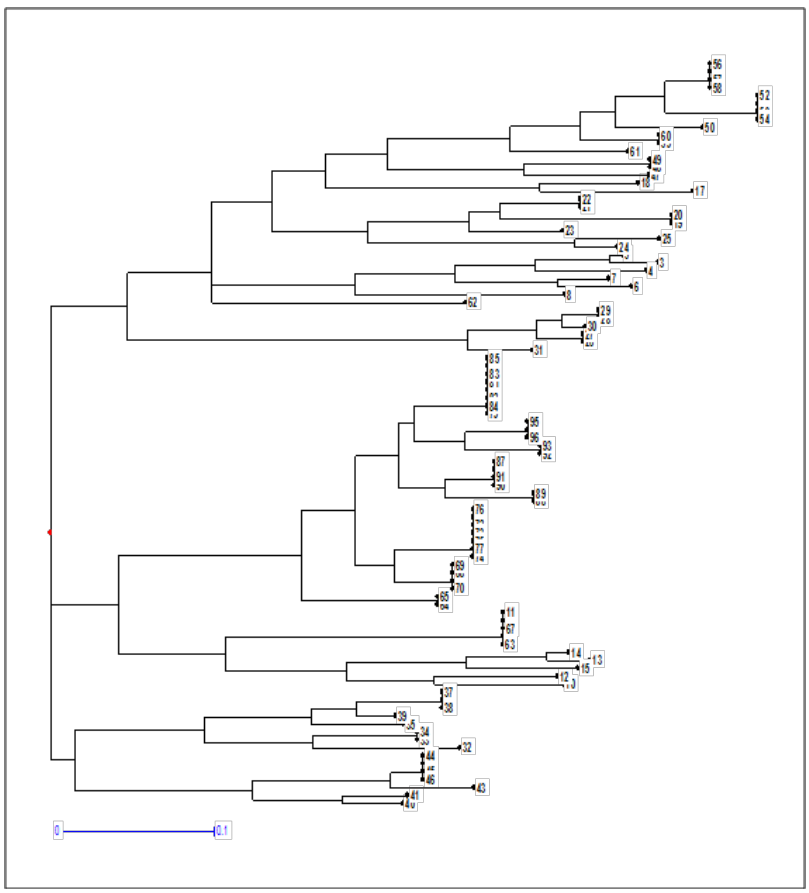

Fig. 2: UPGMA based dendrogram for all the 94 rice genotypes based on SSR primers

Table 1. Primers with their chromosome number and PIC values

Sr. no.	Primer name	No. of alleles	Chr	PIC value	Sr. no.	Primer name	No. of alleles	Chr	PIC value
1	RM 486	3	1	0.32	25	RM 295	3	7	0.39
2	RM 151	3	1	0.60	26	RM 542	3	7	0.49
3	RM 472	3	1	0.48	27	RM 11	3	7	0.47
4	RM 212	3	1	0.52	28	RM 70	-	7	0.52
5	RM 4355	3	2	0.28	29	RM 6193	1	8	0.51
6	RM 13170	3	2	0.34	30	RM 502	2	8	0.29
7	RM 6307	3	2	0.47	31	RM 408	3	8	0.22
8	RM 221	3	2	0.51	32	RM 547	4	8	0.53
9	RM 426	3	3	0.39	33	RM 1026	3	9	0.46
10	RM 227	3	3	0.47	34	RM 296	3	9	0.23
11	RM 554	3	3	0.43	35	RM 410	2	9	0.54
12	RM 545	3	3	0.25	36	RM 23654	3	9	0.36
13	RM 16493	2	4	0.19	37	RM 474	3	10	0.27
14	RM 142	2	4	0.50	38	RM 216	3	10	0.56
15	RM 1018	3	4	0.44	39	RM 6673	3	10	0.38
16	RM 551	3	4	0.24	40	RM 147	2	10	0.50
17	RM 507	2	5	0.55	41	RM 536	3	11	0.38
18	RM 169	3	5	0.41	42	RM 26105	2	11	0.45
19	RM 3664	3	5	0.27	43	RM 209	2	11	0.48
20	RM 18614	3	5	0.51	44	RM 2136	3	11	0.52
21	RM 20069	3	6	0.36	45	RM 6306	3	12	0.28
22	RM 103	3	6	0.29	46	RM 519	2	12	0.45
23	RM 3431	3	6	0.45	47	RM 28570	2	12	0.48
24	RM 7434	3	6	0.52	48	RM 5568	3	12	0.34

Table 2. Clustering of genotypes based on DARwin 5.0 software program

Cluster No.	Sub Cluster No.	No. of genotypes	Genotypes
I	I a	7	NS 113, Patchaiperumal, Qing Shui Zhao, Race, Ray Jazaykayz, RR 166-645, Piconegro
	I b	8	Karayal, Lua Tau Duc, Norunkan, J 104, IR 77298-14-1-2, IR 31917-45-3-2, Icta Polochic
II	II a	10	Chiem Chanh, Safari, Pao Tou Hung, Jamajigi, MTU 9, IR 8, O-Luen-Cheung, IR 36, Chang Ch'sang Hsu Tao
	II b	32	WAS 170-B-B-1-1, Sona, Mamoriaka 114, Sahel 108, UPL-R1 7, WAS 30-11-4-6-2-2-1, Way Rarem, IR 57920-AC-25-2-B, Pusa Basmati 1509, Punjab Basmati 3, WAS 63-22-5-9-10-1, WAS 33-B-B-15-1-4-5, WAS 174-B-3-5, WAS 21-B-B20-4-3-3, WAS 206-B-B-2-2-1, WAS 203-B-B-2-4-1, WAS 173-B-6-2-2, WAS 62-B-B-17-1-1-3, WAS 169-B-B-4-2-1, WAS 30-11-4-6-2-2-1, WAS 197-B-6-3-11, Eloni, CT 6510-24-1-2, WAS 20-B-B-1-2-2, Tokambany 669, Telimani, Mngavava Fotsilanstsika 1177, Vary Madinika 3494, Lohambitro 224, Kalinga III
III	III a	6	Hong Mi Dong Mao Zhan, E 5168, Deng Deng Qi, Djogolon Djogolon, Ea Houm, E Zi 124
	III b	31	BH 2, Bodomano, C 21, Zao Shao Zhan, Zao Shou 691-11, 91-385, Tsipala Fotsy, IR 52, IR 55419-04, Ti ku, Telovolana, Sokou Malsira, Peh Kuh Tsao Tu, Peh Kuh, Bandiourou, Anayansi, Ai Lan Ke 1110, Teqing, BG 34-11, Cimmarron, Chi Tou Huang, Pin Kaeo, Zhenshan 97 B, CO 39, RTS 14, TD 25, BR 24, IR 72, IR 52

diversity across ninety four rice genotypes with an average of 2.7 alleles per polymorphic marker. PIC value ranged from 0.19 to 0.60 and markers RM151 and RM 507 were found to be the most appropriate markers for discriminating different rice genotypes because of their higher PIC value. The genetic divergence study grouped ninety four rice genotypes into three clusters. On the basis of dendrogram the genotypes MTU 9 and IR 8 while

Teqing and Pao Tou Hung were found to be most diverse. The study provided a platform for identifying appropriate parents that can be used in the hybridization programme for improvement of yield related traits.

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