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GENETIC CHARACTERIZATION OF LOCAL RICE (*Oryza sativa* L.) GENOTYPES AT MORPHOLOGICAL AND MOLECULAR LEVEL USING SSR MARKERS

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ABSTRACT

Current study was conducted to estimate the genetic diversity of 29 local rice cultivars including 3 checks at both morphological and molecular level during *Kharif* 2017 in an augmented design. Significant results obtained from ANOVA of 29 genotypes for 16 quantitative traits; Mahalanobis' D^2 grouped the total genotypes into 6 clusters. Highest inter-cluster distance was found between clusters III and VI indicating the genotypes in these clusters are most diverse. The SSR banding pattern revealed a total of 65 alleles from 21 polymorphic markers across 29 rice genotypes with an average of 3.09 alleles. The polymorphism information content (PIC) values ranged from 0.701 (RM 277) to 0.346 (RM237) with a mean value of 0.571 showing the marker RM277 as best based on the above study. The dendrogram analysis revealed all the 29 genotypes were grouped into two main clusters *i.e.* cluster I and cluster II with dissimilarity coefficient 0.36. Both the clusters were further divided into two groups each of which are further divide into two sub-groups each. Based on the genetic distances and the dissimilarity coefficient obtained from both morphological and molecular analysis, genotypes like Bahubali, Golden 105, Pusa 1121, HUR-1301, RK-2 Lal kasturi and Pan 815 can be selected and used as parents due to their greater diversity. Knowledge of genetic diversity available within a population at both morphological and molecular level helps the breeder to formulate a successful hybridization programme and gain good results.

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1 Introduction

Rice (*Oryza sativa* L.) is an annual grass of the family Poaceae (graminae) with chromosome number $2n=24$. Rice is being consumed by more than half of the world's population and serves as a major source of carbohydrates. Having more amounts of carbohydrate, it provides instant energy, and is a staple food that is consumed by the majority of India's population. Rice grain contains about 75-80% starch, 12% water and 7% protein (Hossain et al., 2015). It is also an important source of vitamins like thiamine, riboflavin and niacin and minerals like magnesium, phosphorus and calcium. It is enormously diverse both in the way it is cultivated and its usage by the humans.

Having the smallest genome of all cultivated cereals, being diploid and self pollinating, it is the most extensively studied species among cereals (Pooja et al., 2019). Among very few options for increasing yield potential of rice, improvement of the genetic potential of the crop cultivars is one of the best options. Rice has endowed with rich natural genetic diversity and there is tremendous scope to exploit diversity for improvement of the genetic base.

To encounter the existing food requirements, genetic diversity is a natural source for rice breeding (Reig-Valiente et al., 2016). The higher level of genetic variation present in a population, the more valuable resource, is used for enlarging the genetic base of the breeding program (Nachimuthu et al., 2015). This would help in development of transgressive segregants which will perform better than both the parents. In addition, Haritha et al., 2016 postulated that genetic diversity obtained precious information for both basic studies and practical applications.

Mahalanobis's D^2 statistic is a powerful tool in measuring the degree of divergence between biological populations at genetic level and provides a quantitative measure of association between geographic and genetic diversity based on generalized distance. For the assessment of genetic diversity, microsatellite or simple sequence repeat (SSR)

markers are considered as most amenable, as they are multi-allelic in nature, highly informative, highly reproducible, have co-dominant inheritance and provide extensive genomic coverage (McCouch et al., 2002). SSR markers are able to detect great level of allelic diversity and they have been extensively used to identify genetic variation among rice subspecies. Keeping this in view, the present experiment was conducted using 29 local rice genotypes to characterize the morphological and molecular genetic diversity.

2 Materials and Methods

2.1 Plant material:

A total of twenty nine local rice genotypes including 3 checks were grown in an augmented block design with three blocks having repeated checks during Kharif 2017. The list of genotypes used in this experiment is presented in table 1.

2.2 Morphological observations:

Data was recorded on sixteen quantitative traits viz. days to first flowering, days to 50% flowering, tillers per plant, panicle length, plant height (cm), fertile spikelets, sterile spikelets, total grains per panicle, kernel length(cm), kernel breadth(cm), L/B ratio, 1000 grain weight(g), plot yield(kg/ha), grain yield (kg/ha), biomass(kg/ha) and Harvest Index. The data for days to first flowering, days to 50% flowering and plot yield was collected on plot basis whereas for other yield and yield attributing traits, data was recorded on five randomly selected plants of each genotype in the respective block.

2.3 Molecular analysis:

DNA was extracted following CTAB extraction method according to Doyle & Doyle, (1987) with few modifications and the DNA quality was estimated using Biophotometer plus. 21 simple sequence repeat (SSR) markers were used for molecular analysis. The details of SSRs used are listed in table 2 along with the forward and reverse sequence.

Table 1 List of rice (*Oryza sativa* L.) genotypes used in the present study

1.Kalanamak-3119	11. Red long	21. Sambha Red
2.RK-2 Lal Kasturi	12. Vishnu Bhog black	22. HUR-97PB-1-S
3. Damni	13.Lal Basmati	23.Govind Bhog
4.Golden G.R.32 White	14. Golden 105	24.Kalanamak-11
5.Vijay	15. Tulsi Manjari	25. Red Mahsuri
6.BHULC-13	16.Kudrat 5	26. HUR-1301
7. Divya	17. NDR 118	27. HUR-3022
8. Bahubali	18.RK-8 Gold	28. BPT5204
9. Pan 815	19.Kudrat 5-17	29.Pusa 1121
10.Red Basmati	20. BHULC-5	

Polymerase chain reaction (PCR) was performed using a thermal cycler (SureCycler 8800) *in vitro* to amplify a specific segment of the total genomic DNA to a billion fold (Mullis et al., 1986). The amplified DNA products generated through SSR primers were resolved through electrophoresis in 2.5 per cent agarose gel prepared in TAE buffer and the gels were visualized under a UV light source in a gel documentation system (Gel Doc™ XR+, BIO-RAD, USA). The images of amplified products were used for the evaluation of genetic diversity between the genotypes. A binary data matrix was utilized to generate genetic similarity data among the 29 lines of rice genotypes.

2.4 Statistical analysis

The quantitative trait mean values computed based on data on five randomly tagged plants in each genotype and checks were used for

statistical analysis. Analysis of variance was performed to partition the total variation among the genotypes and check entries into sources attributable to 'genotypes+ checks entries', 'genotype check entries' and 'genotype vs. check entries' following Augmented design (Federer, 1956) WINDOSTAT version 9.0. Adjusted trait mean of each of the genotype accession was estimated (Federer, 1956) and the same were used for all subsequent statistical analysis.

In the present study, Mahalanobis' generalized distance as described by Rao (1952) was used to estimate the genetic divergence. Data Analysis for the SSRs used in the present study was done using the software NTSYSpc version 2.02 (Rohlf, 1998). The binary data matrix generated by polymorphic SSR markers were subjected to further analysis using NTSYS-pc version 2.11W (Rohlf, 1997). The SIMQUAL programme was used to calculate

Table 2 Details of the SSR primers used in the present study along with No. of Alleles and PIC value obtained

S.No.	Marker name	Chromosome no.	Forward/Reverse	Sequence	T _a	No. of alleles obtained	PIC value obtained
1	RM431	1	Forward Reverse	TCCTGCGAACTGAAGAGTTC AGAGCAAAACCCCTGGTTCAC	55 55	3	0.567
2	RM433	1	Forward Reverse	TGCGCTGAACTAAACACAGC AGACAAACCTGGCCATTCAC	52 52	3	0.585
3	RM5	1	Forward Reverse	TGCAACTCTAGCTGCTCGA GCATCCGATCTTGATGGG	52 51	3	0.588
4	RM259	1	Forward Reverse	TGGAGTTTGAGAGGAGGG CTTGTTGCATGGTGCCATGT	50 52	3	0.591
5	RM11943	1	Forward Reverse	CTTGTTTCGAGGACGAAGATAGGG CCAGTTTACCAGGGTCGAAACC	57 57	3	0.527
6	RM237	1	Forward Reverse	CAAAATCCCGACTGCTGTCC TGGAAGAGAGCACTACAGC	53 54	2	0.346
7	RM514	3	Forward Reverse	AGATTTCATCTCCATTCCCC CACGAGCATATTATAGTGG	52 50	4	0.693
8	RM489	3	Forward Reverse	ACTTGAGACGATCGGACACC TCACCCATGGATGTTGTACAG	54 52	3	0.532
9	RM60	3	Forward Reverse	AGTCCCATGTTCCACTTCCG ATGGCTACTGCCTGTACTAC	54 52	3	0.586
10	RM334	5	Forward Reverse	GTTTCAGTGTTCAGTGCCACC CACGAGCATATTACTAGTGG	54 52	3	0.527
11	RM510	6	Forward Reverse	AACCGGATTAGTTTCTCGCC TGAGGACGACGAGCAGATTC	52 54	3	0.501
12	RM455	7	Forward Reverse	AACAACCCACCACCTGTCTC AGAAGGAAAAGGGCTCGATC	54 52	3	0.563
13	RM11	7	Forward Reverse	TCTCTCTTCCCCGATC ATAGCGGGCGAGGCTTAG	53 53	4	0.652
14	RM44	8	Forward Reverse	ACGGCAATCCGAACAACC TCGGGAAAACCTACCCTACC	53 54	3	0.580
15	RM284	8	Forward Reverse	ATCTCTGATACTCCATCCATCC CCTGTACGTTGATCCGAAGC	53 54	3	0.574
16	RM215	9	Forward Reverse	CAAAATGGAGCAGCAAGAGC TGAGCACCTCCTTCTCTGTAG	52 54	3	0.559
17	RM474	10	Forward Reverse	AAGATGTACGGGTGGCATTTC TATGAGCTGGTGAGCAATGG	52 52	3	0.576
18	RM171	10	Forward Reverse	AACGCGAGGACACGTTACTAC ACGAGATACGTACGCCTTTG	54 52	3	0.576
19	RM536	11	Forward Reverse	TCTCTCTCTTGTGTTGGCTC ACACACCAACACGACCACAC	52 54	3	0.589
20	RM552	11	Forward Reverse	CGCAGTTGTGGATTTCAGTG TGCTCAACGTTTGACTGTCC	52 52	3	0.585
21	RM277	12	Forward Reverse	CAAAATCCCGACTGCTGTCC TGGAAGAGAGAGCACTACAGC	51 52	4	0.701

Table 3 Grouping of twenty nine genotype of rice into six clusters by Tocher method

S.No.	Cluster Name	Number of Genotypes	Name of genotypes
1.	Cluster I	17	Vijay, Lal Basmati, Red Basmati, Divya, BPT5204, Kudrat 5, RK-2 Lal kasturi, Vishnu bhog, black Damni, Pusa 1121, Red long, Kalanamak 3119, Kudrat 5-17, BHULC-13, HUR-3022, HUR 1301, BHULC-5
2	Cluster II	8	RK-8 Gold, HUR 97 PB-1-S, Red Mahsuri, Tulsi manjari, NDR 118, Sambha red, Govind bhog, Kalanamak-11
3	Cluster III	1	Bahubali
4	Cluster IV	1	Golden GR-32 white
5	Cluster V	1	Pan 815
6	Cluster VI	1	Golden 105

Table 4 Average inter and intra cluster distance (D^2 values) among six clusters of twenty nine genotypes of rice by Tocher Method.

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	22.431	22.445	43.537	40.379	38.969	63.138,
Cluster II		11.657	41.122	28.715	23.371	42.910
Cluster III			0	61.741	57.887	99.217
Cluster IV				0	24.592	43.000
Cluster V					0	34.659
Cluster VI						0

the Jackard's dissimilarity coefficient. To measure the informativeness of the markers, the polymorphism information content (PIC) for each SSR marker was calculated according to the formula suggested by (Weir, 1996) and it is written as

$$PIC=1-(P_i^2)$$

Where, ' i ' is the total number of alleles detected for each SSR marker, ' P_i ' is the frequency of the i^{th} plus allele in the set of 29 genotypes studied. PIC value is used to estimate the discriminatory power of the SSR marker.

3 Results and Discussion

The result of ANOVA revealed significant difference among the genotypes with respect to all the characters under study and this indicates that there is an inherent genetic difference among the genotypes for all the traits studied.

3.1 Diversity analysis of morphological data

Mahalanobis' D^2 analysis grouped the total genotypes into 6 clusters (table 3) on the basis of inter-cluster genetic distances. Clustering pattern indicated that 17 out of 29 genotypes belong to

cluster I indicating their close relationship among themselves as compared to others. Genotypes grouped in one cluster are less diverse than the genotypes located in a different cluster. Further, 8 genotypes belong to the cluster II and 1 genotype each in cluster III, IV, V, & VI. The intra and inter cluster distances *i.e.* D^2 values are presented in table 4 and the diagrammatic representation of clusters with inter and intra cluster D^2 values have been presented in figure 1. Highest intra-cluster distance was observed in the cluster I (22.431) with 17 genotypes followed by cluster II (11.657) with 8 genotypes. Highest inter-cluster distance was found between clusters III and VI (99.21), indicating that the hybridization between the genotypes of these clusters would yield desirable segregates with the accumulation of favorable genes in the segregating generations. This is followed by cluster I and VI (63.13) and cluster III and IV (61.74). The smallest inter-cluster distance (22.44) was observed between cluster I and II followed by cluster II and V (23.37). Similar results were found by Awasthi et al. (2005), Rajesh et al. (2010) and Devi et al., (2019). Information about genetically diverse genotypes would help in selection of parents while planning for a hybridization programme which would yield useful segregants. However, while selecting parents for hybridization programme their yield potential should not be overlooked (Ramya & Kumar 2008).

Figure 1 Cluster diagram obtained from Mahalanobis D^2 analysis using Tocher method

Based on the results of Mahalanobis D^2 analysis, the genotypes grouped in cluster III are having maximum genetic distance with the genotypes placed in the cluster VI. This indicates that crosses between the genotypes present in these two clusters are expected to be much heterotic. It should be kept in mind that, along with the genetic distance, *per se* yield and yield contributing characters are to be taken into consideration while selecting the genotypes. In this respect, genotypes like Bahubali and Golden 105 are having highest inter cluster distance and high *per se* yield can be considered suitable for hybridization programme.

3.2 Diversity analysis of molecular data:

Molecular diversity analysis with the help of markers is based on the naturally occurring polymorphism of the individuals which is not affected by the environment. In the present study, all the 29 genotypes were analysed with 21 SSR markers and the results obtained are explained in the following subheads.

3.2.1 Scoring of SSR band and PIC value:

The presence or absence of each band in all genotype was scored manually by binary data matrix with 1 and 0 for presence and absence respectively. Level of polymorphism in rice genotypes was evaluated by calculating allelic number and PIC values for each of twenty one polymorphic SSR markers. 21 polymorphic markers run across 29 rice genotypes obtained a total of 65 alleles with an average of 3 alleles. Among the 21 SSRs, 1 marker produced 2 alleles, 3 markers have produced 4 alleles each and 17 markers have produced 3 alleles each. Similar results were shown by Pachauri et al. (2013) and Anh et al. (2018).

The PIC (Polymorphic information content) was assessed for each locus to evaluate the information of each marker and this PIC value is an evidence of allele diversity and frequency among genotypes and its values ranged from 0.701 (RM 277) followed by 0.693 (RM514) to 0.346 (RM237), with a mean value of 0.571. Hossain et al. (2012) reported similar range of PIC value (0.239 to 0.765) with an average of 0.508. The PIC values obtained for all the markers are shown in table 2. In another study conducted by Singh et al. (2015), PIC values ranged from 0.265 to 0.65 with an average of 0.47. Similar results were further obtained by Roy et al. (2015), Krupa et al. (2017) and Anh et al. (2018). Higher PIC value of a locus detected higher alleles value. From this data, it is shown that, RM 277 was found to be the most appropriate marker among the rice genotypes owing to the highest PIC value of 0.701 can be employed to enlarge the genetic foundation of the current genotypes (Anupam et al., 2017). The other markers with PIC value above 0.5 also play a significant role in studying the genetic divergence of the rice genotypes (Anh et al., 2018) Gel pictures obtained from analysis of 29 genotypes with some of the SSR markers is shown in figure 2 (figure 2a to 2c).

3.2.2 Dendrogram analysis

A dendrogram (figure 3) based on Jackard's dissimilarity coefficient was constructed using UPGMA (Unweighted Pair Group Method with Arithmetic Averages) method and the 29 rice genotype were grouped into two main clusters *i.e.* cluster I and cluster II with dissimilarity coefficient 0.36. Cluster I was further divided into two groups IA and IB with dissimilarity coefficient 0.46. Cluster IA was further divided into two sub-groups IA-1 and IA-2 with dissimilarity coefficient 0.56. Cluster IA-1 contains 6 genotypes and IA-2 contains 3 genotypes. Cluster IB further divided into IB-1 and IB-2 with dissimilarity coefficient 0.58. Cluster IB-1 consists of 8 genotypes and I B-2 contains 2 genotypes. Cluster II was divided into two main sub-groups II-A and II-B with dissimilarity coefficient 0.50. Cluster IIA had 6 and II B had 4 genotypes. Grouping of the clusters along with the name of genotypes included in each cluster is shown in table 5. Similar results were obtained by Sonkar et al., 2016, Vengadessan et al., 2016, Anh et al., 2018 and Pooja et al., 2019.

3.2.3 Jackard dissimilarity coefficient

To determine the level of relatedness among the genotypes the dissimilarity coefficient was used. The dissimilarity coefficient varies from zero to one, closer to one shows a higher dissimilarity, whereas, closer to zero shows a higher similarity. The average dissimilarity ranged from 0.6536 to 0.7262. The total average of dissimilarity coefficient of all the 29 genotypes is 0.714. The dissimilarity coefficient varied from the largest value 0.88 between the cultivar Pusa 1121 and HUR-1301 followed by RK-2 Lal kasturi and RK-8 Gold (0.86) and pan 815 and RK-8 Gold (0.86)

Figure 2a Gel image showing banding profile obtained by RM 334.

Figure 2b Gel image showing banding profile obtained by RM 227.

Figure 2c Gel image showing banding profile obtained by RM 11.

Figure 2 Gel images of 29 local rice genotypes analysed using SSR markers. Lane 1-29 represented the rice genotypes as listed in table 1 and L is the Ladder

which shows high dissimilarity between them showing that they are highly dissimilar with each other. The lowest value (0.48) was found between Red long and HUR-97 PB 1-S followed by 0.50 between Vishnu bhog black and Pusa 1121. Similar results were obtained by Pooja et al., 2019.

Finally, according to the dendrogram and Jackard dissimilarity coefficient values, the most diverse cultivars among the 29 local

rice genotypes studied are Pusa 1121 and HUR-1301 followed by RK-2 Lal kasturi and RK-8 Gold, Pan815 and RK-8 Gold as they showed greater genetic distances which means a greater chance of getting useful segregants when the above said genotypes used in a hybridization programme and having lower relatedness making them genetically diverse which is of prime importance for any breeding programme. Similar results were obtained and explained by Ahn et al., 2018 and Pooja et al., 2019.

Figure 3 Dendrogram based on Jackard's dissimilarity coefficient using UPGMA

Table 5 Grouping of Twenty Rice Genotypes into different clusters based on Jackard IJ coefficient

S. No	Cluster Name	Number of genotypes	Name of genotypes
1.	I A-1	6	Kalanamak 3119, Golden 105, GR.32, White Vijay, BHULC-5, NDR 118
2	I A-2	3	Red long, HUR-97, PB-1-S, HUR-3022
3	I B-1	8	Damni, BHULC13, Lal Basmati, RK-8 Gold, Bahubali, Red Basmati, Divya, Red Mahsuri
4	I B-2	2	Vishnu bhog black, PUSA 1121
5	II-A	6	RK-2 Lal Kasturi, BPT5204, Pan 815, Tulsi manjari, Kalanamak 11, Sambha red
6	II-B	4	Kudrat 5, Govind bhog Kudrat-5-17, HUR 1301

Conclusion

Study of genetic diversity at both morphological and molecular level gives a reliable information regarding the genotypes included in the study. From the above study, It is observed that both morphological and molecular variations exists among the 29 local rice genotypes evaluated and differences in the grouping of the genotypes in different clusters was observed. Considering all the aspects, the genotypes, Bahubali, Golden 105, Pusa 1121, HUR-1301, RK-2 Lal kasturi and Pan 815 have the advantage of both greater genetic distance from each other and have higher *per se* yield. Hence, these genotypes can be used by the breeders for planning a successful hybridization programme and for creation of more variability in rice.

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