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Characterization of new plant type core set of rice (*Oryza sativa* L.) using QTL/gene-linked markers

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ABSTRACT

Genetic diversity in New Plant Type core set of rice was studied at molecular level employing 52 yield related and 12 randomly chosen markers. 42 markers were polymorphic among the genotypes with a total of 84 alleles. The number of alleles per locus ranged from 2 to 4 with an average of 3.0 per locus. The PIC value ranged from 0.07 to 0.51 with an average of 0.31. Gene specific markers (SCM2-indel2, Gn1a-indel3, TGW6-1d and GS5-03SNP), functional genes (Ghd7-sel and DEP1-promoter), linked markers RM8080 and RM340 were found to be the most appropriate marker to discriminate among the rice genotypes owing to the highest PIC value of more than 0.5. The cluster analysis distinguished these accessions in to eight clusters based on the principle of Unweighted Pair Wise Method using Arithemetic Average (UPGMA) constructed by Jaccard's similarity Coefficient. The dendrogram showed that the genotypes with common phylogeny and geographical orientation tend to cluster together. The highest similarity coefficient value was observed between the IRGC 25510 and IRGC 10658 (0.67) whereas lowest value was observed for Swarnadhan (0.18) and Azucena (0.21), showing highly diverse genotypes. Thus, these accessions were genetically diverse and could be directly utilized in hybridization programme for improvement of yield and related traits.

Key words: *Culm strength, genetic diversity, gene specific markers, rice, yield*

INTRODUCTION

Rice (*Oryza sativa* L.), belonging to the family Gramineae, is the staple food for more than half of the world's population. Asia accounts for 90 percent of global rice consumption and the total rice demand continues to rise, which is insufficient to meet the food demand for the estimated nine billion people in 2050 (Khush, 2005 and Ray et al., 2013). To meet the demand of ever increasing population for rice, there is a need to exploit the genetic variability and diversity in plant breeding programmes, which basically depends on sustainable utilization of genetic resources. The rice accessions are a rich source of useful genes and the genetic variability available among the accessions could provide a wide scope for the crop improvement programme (Singh et al., 2015).

Genetic diversity is necessary for any crop improvement program as it helps in analyzing and establishing genetic relationship in accessions collection, its monitoring, identification of diverse parental combinations to create segregating progenies with high genetic variability and to obtain potential recombinations for further selection and introgression of desirable genes from these diverse accessions (Ramadan et al., 2015; Thompson et al., 1998; Islam et al., 2012). Exploitable genetic diversity is a key factor essential to enhance success rate in breeding by exploiting the variability present in the population. Unlike morphological traits used earlier to estimate genetic variability/relatedness, molecular markers have become quite handy in precisely understanding the extent of genetic divergence

among varieties. Evaluation of genetic diversity using DNA marker technology is non-destructive, not affected by environmental factors, requires small number of samples, and does not require large experimental setup and equipment for measuring physiological parameters (Kanawapee et al., 2011).

Yield improvement in rice requires identification of yield enhancing loci by using highly polymorphic molecular markers and characterization of genetic diversity, which is utilized effectively for mapping of genes/QTLs for yield contributing traits and their subsequent utilization in MAS. Several of the yield QTLs were identified in rice in the past and functional genes *viz*., (*Gn1a, OsSPL14, SCM2, Ghd7, DEP1, SPIKE, GS5* and *TGW6*) were cloned (Ashikari et al., 2005; Xue et al., 2008; Huang et al., 2009; Fan et al., 2006; Jiao et al., 2010). Further, allele specific markers were developed and validated for *Gn1a, OsSPL14, SCM2, Ghd7, DEP1, SPIKE, GS5* and *TGW6* facilitating the introduction of positive yield alleles from the donor lines for Marker Assisted Selection (MAS) of yield-enhancing traits/genes in rice (Kim et al., 2016).

In this context, our present study is focused on molecular marker characterization in new plant type core set genotypes of rice (Jyothi et al., 2018) along with checks using yield related markers.

MATERIALS AND METHODS

Plant material

The experimental material consisted of forty-one NPT core set (Jyothi et al., 2018), five checks. NPT core set comprised tropical japonica accessions (34), indica land races (6) and a cultivar from Sri Lanka (1). The field experiments were conducted at ICAR-Indian Institute of Rice Research (ICAR-IIRR) Ramachandrapuram farm, ICRISAT campus, Hyderabad, India during *kharif* 2016. Seeds were sown in nursery on raised beds and thirty days old seedlings of each genotype were transplanted in two rows with 20 plants per row following a spacing of 20 cm between rows and 15 cm between plants. The experiment was laid in Randomized Block Design (RBD) with three replications. Four popular local cultivars *viz*., BPT 5204, MTU 1010, Swarna and Swarnadhan and one early duration genotype 'Azucena' were used as checks. Recommended agronomic and plant protection measures were taken up for raising a healthy nursery and main crop.

DNA extraction and PCR amplification

Healthy leaf samples of 2-3 cm long were collected from young plants and DNA was extracted using cetyltrimethyl ammonium bromide (CTAB) protocol as per Doyle (1991). DNA concentration was estimated using agarose gel electrophoresis and λ DNA (Genaxy) and samples were diluted with 1x TE into an equal concentration of 25 ng/ul.

Amplification of the markers using polymerase chain reaction (PCR) was done with a 10 µl reaction volume with 3 µl of 25 ng DNA template, 1 µl of 10x PCR buffer and $MgCl_2$, 0.3 µl of each forward and reverse primers, 1 µl of 1 mMdNTP, 4.5 µl sterile distiled water and 0.2 µl of *Taq* DNA polymerase (Bangalore Genei, India). The following PCR profile was used for amplification in the thermocycler (Eppendorf, USA): initial denaturation at 94º C for 5 min and then 35 cycles of denaturation at 94º C for 45 s, annealing at 55º C for 45s and extension at 72º C for 1s; final extension at 72º C for 10 min and cooling at 4º C. Finally, 4 µl of 1x loading buffer for every 10 µl of PCR product was added to the PCR product prior to loading. The PCR products were resolved by electrophoresis using a 3.5% agarose gel electrophoresis (Thermofisher Scientific, USA). The gel was run in 1x TBE at 120 V for 2 hours depending on the product size of the marker and were visualized under the UV- transilluminator (Biorad) and documented and stored using GELSTAN.

Genetic diversity

Genetic diversity was estimated using 64 markers (SSRs, indels, STS, CAPS and SNPs) related to yield traits (Table 1). Polymorphic Information Content (PIC) was calculated as described by Botstein et al., 1980 using the below formula;

$$PIC = 1 - \left[\sum_{i=1}^{n} P_i^2\right] - \left[\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} 2P_i^2P_j^2\right]$$

Where p_i equals the frequency of the ith allele and p_j the frequency of the $(I+1)^{th}$ allele. For diversity analysis, only data from polymorphic loci was used. Genetic diversity was estimated by computing the mean

Table 1. List of markers used in the present study for genetic diversity.

S.No	Trait	QTL/gene	Chr.	Marker No	Forward sequence	Reverse sequence	No of alleles	PIC	Reference
1	M	*SCM2*	6	SCM2-indel1	GGAAATGATGAACACTGTCCA	GTTTGTCTCAGCTCTGATCTG	4	0.55	Kim et al. 2016
2	GN	*Gn1a*	1	Gn1a-indel3	GATCTAGATGCTCCAAAGTCC	CTGTACGTACGTGCACGTAG	3	0.5	
3	TW	*TGW6*	6	TGW6-1d	GCCAACTGATCAGACTGAG	CGTGGGGAGAGTCGATTCC	3	0.51	
4	GW	*GS5*	5	GS5-03SNP	ACTTTCAACTAAAGTGATATTACCTC	TCTATATATCCATCGTCCATGGTG	3	0.57	
5	GN	*Ghd7*	7	Sel	TGCATGCATATACATTAGCT	GAGTGATAAAGCATCTAAGG	2	0.23	Xue et al. 2008
6	GN	*Ghd7*	7	Insitu	TTATCCGTTCATGTCGATGG	ACCATCTCCTTGGGCATCGA	M		
7	GN	*Ghd7*	7	Hd3a	GCTCACTATCATCATCCAGCATG	CCTTGCTCAGCTATTTAATTGCATAA	M		
8	GN	*Ghd7*	7	Actin1	TGCTATGTACGTCGCCATCCAG	AATGAGTAACCACGCTCCGTCA	2	0.37	
9	GN	*Ghd7*	7	LHY	CAGATAAGGCCGACACCAAAC	GGTGTGTTGGAACCACATG	M		
10	GN	*Ghd7*	7	PRR	CTGCTGAACCTCTGGACCCA	GGTTCCGATAACGCCAACTC	2	0.22	
11	GN	*Ghd7*	7	Fusin	GGTACCTTATCCGTTCATGTCGATGG	GGATTCTCTGAACCATTGTCCAAGCTC	M		
12	GN	*SPL14*	8	M9	ACCAGTAGCAGTAGCATCATTG	CTGTTCTTTGATTCTCCTCTCG	2	0.34	Jiao et al. 2010
13	GN	*SPL14*	8	Os08g39880	CACAGTGAAAAAGAGTCGTGTC	CCATCATTGCATTGAAGATAGA	M		
14	GN	*SPL14*	8	Os08g39950	TCATAAAGATGGGAACTAGGCAC	TATTAGGGGAGAAAAAAAGAGAA	2	0.13	
15	GN	*SPL14*	8	Os08g39960	CTTATCAAGACAAACCTCACAGG	GAGAAGAGAATATTGGAACACCA	M		
16	GN	*Ghd8*	8	SEQ3-1	TCCACATCATGCATACACTTG	GTGACACGAAAGTACATAGAC	2	0.32	Yan et al. 2011
17	GN	*Ghd8*	8	SEQ5-1	ATCTCACTGTACTGTATTCC	GCTTCTTTGAAGCAAATGCTGTG	2	0.36	
18	GN	*Ghd8*	8	C8dsF/R	ACTAGTGGTACCCGTCAGGGAACA AGGCGTACTG	AGAGCTCGGATCCGATCACAACCGAA CTCCTACAG	M		
19	GN	*Ghd8*	8	OsMADS51	GAAATCAAAGAAGATGTTGGCAAA	CTTCCTCCTGCCCCCTAGAG	2	0.12	
20	GN	*Ghd8*	8	qRFT1	TGACCTAGATTCAAAGTCTAATCCTT	TGCCGGCCATGTCAAATTAATAAC	M		
21	GN	*Ghd8*	8	qMOC1FF/R	ACTGGCCTCGAGTTTCACCC	CATGGCCTTCACCCACTTCA	M		
22	GN	*Ghd8*	8	PGAP1	GCTCGATAACGACAACAGCATG	CGAAACTGCAAACCATGTGTAGG	M		
23	GN	*Ghd8*	8	PGAP3F/54	GTCGATGTAATGACTTGCTGG	GTCACACTCACACCATGGTCATC	M		
24	GN	*Ghd8*	8	PC-3	AGTGGCATTGATGCACTGC	CAGGCTTCTTATGTTTCAC	M		
25	GN	*DEP1*	9	S5	GAAAGCATACGGATGCCAAT	TCATGTTTGCTGGGTGACAT	2	0.37	Xu et al. 2016
26	GN	*DEP1*	9	Promoter of Dep1	GAATTCGTCTCTCAGTGAGCCGTTCC	GGATCCTCATGGGCATTATAGCAGCA	3	0.55	
27	M	*SCM2*	6	ApoI_InDel_9	ATGGTGCATGCATCGAGTTTGTT	TATAGGCCCAAAATGGTTCCGAG	2	0.32	Ookawa et al. 2010
28	GN	*COX*	1	SC1845	TGAGGATGCCGTGGAAGACG	TTCGTGTTCGCGCAGGACGT	M		
29	GW	*GS3*	3	RM 8080	CAGTTTCAGTTTCAGGTCAGT	CTGATGCTGATCACCTGA	3	0.55	Bai et al. 2012
30	GW	*GS3*	3	RM 156	GCCGCACCCTCACTCCCTCCTC	TCTTGCCGGAGCGCTTGAGGTG	2	0.37	
31	GN	Gn1a	1	RM 1220	TGCTTCCTGCAGGGGTATAG	GGCAATAGCTAGCAAGGCAG	2	0.34	

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32	GN	Gn1a	1	RM84	TAAGGGTCCATCCACAAGATG	TTGCAAATGCAGCTAGAGTAC	2	0.11
33	GN/TN	LRK1	2	RM53	ACGTCTCGACGCATCAATGG	CACAAGAACTTCCTCGGTAC	3	0.3
34	GW	GW2	2	RM555	TTGGATCAGCCAAAGGAGAC	CAGCATTGTGGCATGGATAC	M	
35	GN/TN	LRK1	2	RM279	GCGGGAGAGGGATCTCCT	GGCTAGGAGTTAACCTCGCG	2	0.34
36	GW/GF	FLO2	4	RM16742	GAACAGAATCCAGGAATGAACTGC	GTCAGATCAGTCTTCTGCAAATGG	2	0.37
37	GW/GF	FLO2	4	RM6997	CAACGCGGCAGTAAATTTGC	GGCCTTGTCAGTCTACATGC	2	0.13
38	GW/GF	GIF1	4	RM273	GAAGCCGTCGTGAAGTTACC	GTTTCCTACCTGATCGCGAC	M	
39	GW	GS5	5	RM574	GGCGAATTCTTTGCACTTGG	ACGGTTTGGTAGGGTGTCAC	M	
40	GW	GS6	5	RM13	TCCAACATGGCAAGAGAGAG	GGTGGCATTCGATTCCAG	3	0.45
41	HI/GN	APO1	6	RM340	GGTAAATGGACAATCCTATGGC	GACAAATATAAGGGCAGTGTGC	3	0.45
42	HI/GN	APO1	6	RM30	GGTTAGGCATCGTCACGG	TCACCTCACCACACGACACG	2	0.37
43	GN/TN	PROG1	7	RM21078	CAAGCTGCCGTGTTCTACTGG	GCACACAACAAGAGACAGTAACATGC	3	0.37
44	GW	DEP2	7	RM1132	ATCACCTGAGAAACATCCGG	CTCCTCCCACGTCAAGGTC	2	0.36
45	GW	DEP2	7	RM118	CCAATCGGAGCCACCGGAGAGC	CACATCCTCCAGCGACGCCGAG	2	0.12
46	GN	Ghd7	7	RM125	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC	3	0.43
47	GN	Ghd7	7	RM542	TGAATCAAGCCCCTCACTAC	CTGCAACGAGTAAGGCAGAG	2	0.24
48	GN/TN	WFP	8	RM502	GCGATCGATGGCTACGAC	ACAACCCAACAAGAAGGACG	2	0.37
49	GN/TN	WFP	8	RM149	GGAAGCCTTTCCTCGTAACACG	GAACCTAGGCCGTGTTCTTTGC	2	0.19
50	GN	*Ghd8*	8	RM25	GGAAAGAATGATCTTTTCATGG	CTACCATCAAAACCAATGTTC	M	
51	GN	*Ghd8*	8	RM137	GACATCGCCACCAGCCCACCAC	CGGGTGGTCCCCGAGGATCTTG	2	0.26
52	GN	DEP1	9	RM434	GCCTCATCCCTCTAACCCTC	CAAGAAAGATCAGTGCGTGG	2	0.35
53	-	-	1	RM490	ATCTGCACACTGCAAACACC	AGCAAGCAGTGCTTTCAGAG	M	-
54	-	-	2	RM475	CCTCACGATTTTCCTCCAAC	ACGGTGGGATTAGACTGTGC	2	0.17
55	-	-	3	RM15855	GGAGTTTAGAAATATGGGCTCTGG	TGGTTGATGTCTGAACCGTATAGC	2	0.15
56	-	-	3	RM55	CCGTCGCCGTAGTAGAGAAG	TCCCGGTTATTTTAAGGCG	M	
57	-	-	4	RM470	TCCTCATCGGCTTCTTCTTC	AGAACCCGTTCTACGTCACG	2	0.35
58	-	-	5	RM289	TTCCATGGCACACAAGCC	CTGTGCACGAACTTCCAAAG	3	0.39
59	-	-	6	RM540	GCCTTCTGGCTCATTTATGC	CTAGGCCTGCCAGATTGAAC	3	0.52
60	-	-	6	RM588	GTTGCTCTGCCTCACTCTTG	AACGAGCCAACGAAGCAG	2	0.3
61	-	-	7	RM6389	GACGAGGAGTTCGTCGCTAC	CCTTCTCCTTCGTCTCCTCC	M	
62	-	-	7	RM5436	CAAAGGGGGTGTCCTCTATG	GTTGCTCGTCCTACATGTGC	M	
63	-	-	7	RM21945	CTACACAAGTGAACGCCATCAGG	GTTCTAGGGTGTCCTTTCATGAGC	M	
64	-	-	7	RM3325	GGAGCCCTGAACTTTTTGTG	GGGGAATCCTACTTGCTTCC	2	0.35

number of pair wise differences over each locus among markers. Similarities between any two genotypes were estimated according to Nei and Li, 1979 as;

$$S_{ij} = 2\ N_{ij}\ (N_i + N_j),$$

Where N_{ij} is the number of bands in common accessions *i* and *j*, N_i and N_j are the total number of bands in common between any 2 accessions and may range from 0 (no common bands) to 1 (identical band profile for the 2 accessions). A dendrogram was constructed based on the S_{ij} values by adopting the Sequential Hierarchical Agglomerative Non-overlapping (SHAN) clustering on squared euclidean distance matrix and similarity matrix using Jaccard's coefficient utilizing Unweighted Pair Group Method with Arithmetic means (UPGMA) method. Data analysis was done using NTSYSpc version 2.02 (Rohlf, 1999).

RESULTS AND DISCUSSION

Number of alleles and PIC value

In the present study, considerable variability was found among different genotypes. The level of polymorphism among the rice accessions was evaluated by calculating the number of alleles and PIC values. A total of 64 markers related to yield QTLs and candidate gene based markers, allele specific markers and some randomly chosen markers that covered 10 chromosomes, were used to assess the extent of genetic diversity across 46 rice genotypes. A total of forty two markers produced reproducible polymorphic pattern while remaining twenty two primers were monomorphic. These 42 markers showed a total of 84 alleles and the number of alleles per locus ranged from 2 to 4 with an average of 3.0 per locus. Among the polymorphic markers, 28 produced 2 alleles each, 12 markers produced 3 alleles each and 1 marker SCM2 - indel1a maximum of 4 alleles. The overall size of amplified products varied from 50 (RM 156) to 500 bp (RM 21078). A genotype was assigned null allele for the locus, which shows no amplification for a particular genotype-marker combination.

The polymorphic information content (PIC) is the reflection of allele diversity and their frequency among genotypes. PIC values ranged from a 0.07 to 0.51 with an average of 0.31 (Table 1).The marker RM6997 showed low PIC value and high PIC value was obtained for RM340.

Jaccard's similarity coefficient

The Jaccard's similarity coefficient varied from 0.12-0.67 as revealed by UPGMA cluster analysis using sixty four markers. The genotypes close to the similarity coefficient of 0.12 were considered as more dissimilar, while the genotypes close to the similarity coefficient of 0.67 as similar. On the basis of dendrogram, the highest similarity coefficient value was observed between the cultivar IRGC 25510 and IRGC 10658 (0.67) followed by IRGC 1172456 and IRGC 56735 (0.66), IRGC 25239 and IRGC 18021 (0.66) and Azhoghi and Kaoyeng (0.65), whereas lowest value was observed for Swarnadhan (0.18) and Azucena (0.21). Thus, these accessions were genetically diverse and could be directly utilized in hybridization programme for improvement of yield and related traits.

Dendrogram analysis

A dendrogram based on Jaccard's similarity coefficient was constructed using UPGMA method. The forty-six genotypes were grouped in to eight main clusters (Fig. 1) at a cut-off similarity coefficient of 0.32. The accessions that are derivatives of genetically similar parents are dropped in one cluster. Each cluster distinguishes the genotypes clearly from the other. In the dendrogram, cluster IA-1 had maximum twenty five genotypes followed by cluster IB and cluster IA-2. Clusters IIA, IIB, III, V and VII have two genotypes each, whereas the clusters IV, VI and VIII have only one genotype each. Cluster I was the major cluster with 33 genotypes and divided in to two sub clusters IA and IB with similarity coefficient (0.37). The sub cluster IA with 28 genotypes, was divided further in to two sub clusters IA-1 and IA-2 at similarity coefficient (0.37), the sub cluster IA-1 has 25 genotypes and sub cluster IA-2 had three genotypes *viz*., IRGC 43741, IRGC 8192 and IRGC 7486. Sub cluster IB had five genotypes viz., Azhoghi, Kaoyeng, Thangmoi, BG-380-2 and BPT5204. Cluster II was divided in to two sub clusters IIA and IIB at similarity coefficient (0.33). Sub cluster IIA have two genotypes IRGC 117027, IRGC 78392 at similarity coefficient (0.47) and IIB has two genotypes IRGC 53089, IRGC 50448 at similarity coefficient (0.45). Cluster III has two genotypes viz., Solumpiket and Haoreimachang at similarity coefficient (0.43). Cluster IV has one genotype, IRGC 6309 at similarity coefficient (0.32). Cluster V has two

genotypes *viz*., Swarna and MTU1010 at similarity coefficient (0.38). Cluster VI has one genotype, Azucena at similarity coefficient (0.32). Cluster VII has two genotypes *viz*., IRGC 9147 and IRGC 1742 at similarity coefficient (0.33). Cluster VIII has one genotype, Swarnadhan at similarity coefficient (0.32). Clustering pattern is presented in the Table 2.

Knowledge of the genetic diversity and genetic relationships between germplasm accessions is the basic foundation for crop improvement programs (Thomson et al., 2008). Genetic diversity studies is required for effective incorporation into breeding strategies for the selection of diverse parents to obtain heterotic hybrids as well as for the conservation and characterization of germplasm and management of plant genetic resources. Classical methods of estimating the genetic diversity among groups of plants have relied upon morphological characters. However, these characters are influenced by environment factors. Molecular markers avoid many of the complications of environmental effects acting upon characters by directly looking at the variation controlled by genes. They are powerful tools in the assessment of genetic variation, elucidation of genetic relationships within and among species, with the potential to detect genetic diversity and to aid in the management of plant genetic resources.

The genotypes under evaluation in the present investigation are essentially selected with trait combinations of high grain number, strong culm, more number of productive tillers etc forming a core set for new plant type traits (Jyothi et al., 2018). Further characterization of such genotypes along with checks at molecular level employing yield related markers was done to ascertain their diversity and applicability in breeding programmes aimed at yield improvement. Number of alleles per marker obtained in the present study are comparable to the earlier findings by Rashmi et al. (2017) and Singh et al. (2015), however the extent of polymorphism vis-a-vis type of the marker employed indicate that since SNP/INDEL markers were deployed in the present study, the number of polymorphic alleles was less compared to abundant SSR markers. Similar to our findings, He et al. (2012) detected an average of 6.1 (SSR) and 2.0 (SNP) alleles per locus respectively in 168 hybrid rice parents.

The PIC value observed in the present study are also comparable to the earlier reports by Singh et al. (2015), Umadevi et al. (2014) and Hossain et al. (2012). The higher the PIC value of a locus, the higher the number of alleles detected. All the gene specific markers (SCM2-indel2, Gn1a-indel3, TGW6-1d and GS5-03SNP), functional genes (Ghd7-sel and DEP1-promoter), linked markers RM8080 and RM340 were found to be the most appropriate marker to discriminate among the rice genotypes used in the present study owing to the highest PIC value of 0.51. The frequencies of null alleles were not included in the genetic diversity calculation for each locus as they may influence the gene and genotypic frequencies in a population deviating genotypes from Hardy-Weinberg expectation.

Table 2. Clustering pattern of 46 genotypes based on molecular data analysis.

S.no.	Cluster	Number of genotypes	Genotype
1	Cluster IA-1	25	IRGC 1172456, IRGC 56735, IRGC 117255, IRGC 43343, IRGC 31063, IRGC 78629, IRGC 26998, IRGC 25510, IRGC 10658, IRGC 39111, IRGC 27435, IRGC 29772, IRGC 117028, IRGC 33130, SRAC 34997, IRGC 54201, IRGC 19144, IRGC 27129, IRGC 25239, IRGC 18021, IRGC 15147, IRGC 15046, IRGC 14694, IRGC 8269, IRGC 1723
2	Cluster IA-2	3	IRGC 43741, IRGC 8192, IRGC 7486
2	Cluster IB	5	Azhoghi, Kaoyeng, Thangmoi, BG-380-2, BPT5204
4	Cluster IIA	2	IRGC 117027, IRGC 78392
5	Cluster IIB	2	IRGC 53089, IRGC 50448
6	Cluster III	2	Solumpiket, Haoreimachang
7	Cluster IV	1	IRGC 6309
8	Cluster V	2	Swarna, MTU1010
9	Cluster VI	1	Azucena
10	Cluster VII	2	IRGC 9147, IRGC 1742
11	Cluster VIII	1	Swarnadhan

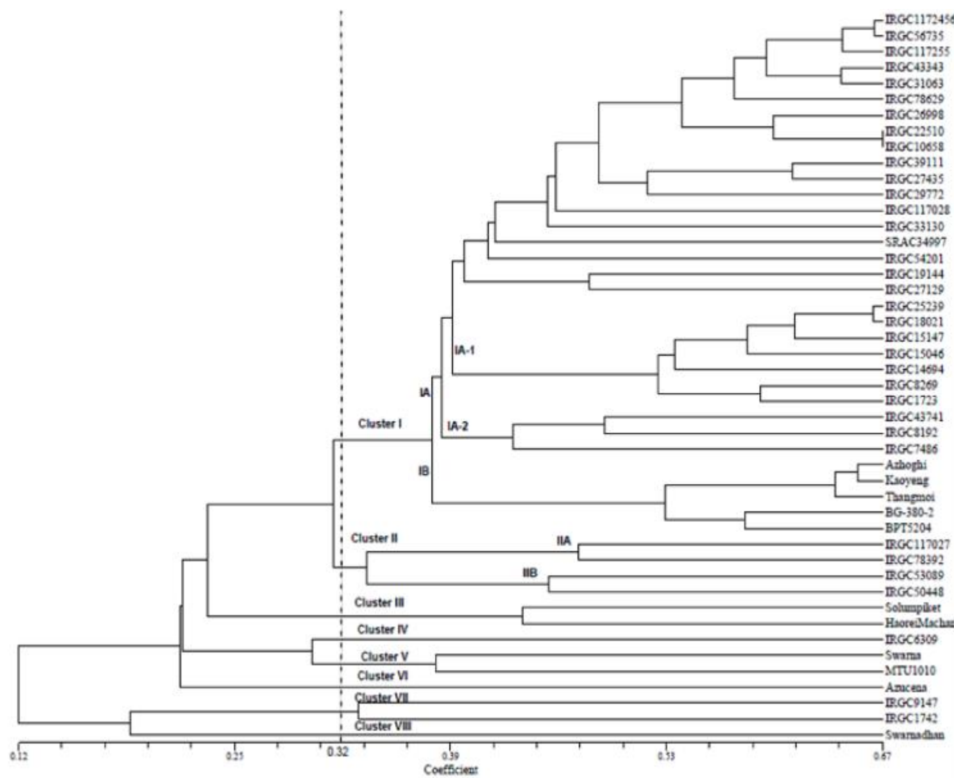

Fig. 1. An UPGMA cluster dendrogram showing the genetic relationships among 46 genotypes of rice.

Similarity coefficient ascertains the relatedness between the genotypes. When more clusters are obtained with few genotypes in each cluster, the significance in clustering is high because of the presence of higher genetic differences between the genotypes in a cluster (Nihar et al., 2016). We observed three single genotype clusters in the dendrogram. Among all the genotypes, 'Azucena' singled out as an independent cluster. Phenotypically, 'Azucena' is very early in flowering with days to 50% flowering of 61 days. Divergence of Azucena' as revealed in the dendrogram could be due to its very early heading date when compared to all other genotypes assessed. Another genotype 'Swarnadhan' also formed an independent cluster. Swarnadhan was the best genotype in terms of single plant yield (30.55g) and plot yield (2.08 kg/m^2). For these genotypes, marker based information supports the morphological diversity present in them. Similar results were reported by Vhora et al. (2013) in twenty aromatic and non-aromatic rice genotypes using twenty five SSR markers and the genotypes were grouped in to two major clusters. Rashmi et al. (2017) reported the grouping of sixty-five rice accessions using SSR markers in to nine clusters. Singh et al. (2015) reported the grouping of the 20 genotypes in to two main clusters at 0.23 similarity coefficient. The allelic diversity among the genotypes clearly emphasizes on the scope for introgression of genes from genotypes of different clusters and could be directly utilized in hybridization programme for improvement of yield and related traits.

CONCLUSION

The markers employed in the present study were able to distinguish the genotypes into clear clusters and assess the genetic variability/similarity of the genotypes under study. The information about the genetic diversity of these rice varieties will be useful for proper identification and selection of appropriate parents for use in the breeding programmes, including gene mapping and ultimately for emphasizing the importance of marker assisted selection in rice improvement.

Conflict of interest

The authors declare that they have no conflict of

interest.

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