DNA Marker Based Diversity Across Rice Genotypes and Advanced Breeding Lines Bred for Temperate India

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Abstract

Background: Characterization and evaluation of plant genetic resources play an important role for their utilization in the crop improvement programmes.

Methods and results: This study entails the agro-morphological, cooking quality and molecular characterization of 51 genotypes / advance breeding lines of rice from Kashmir Himalayas. Significant variability was observed for all agro-morphological and cooking quality traits among all the studied genotypes. Cluster analysis using UPGMA method divided the genotypes into two major clusters having 15 and 36 genotypes. Thirty eight genotypes screened using 24 SSR markers detected 48 alleles with 2.0 alleles per locus and an average polymorphism information content (PIC) of 0.37. High polymorphism information content (PIC) values was observed for the primers RM263 (0.67), RM159 (0.59) and RM333 (0.50). Furthermore, out of 38 SSR markers screened on 192 temperate rice germplasm lines, RM17 accurately differentiated indica and temperate japonica genotypes amplifying 220 bp and 169bp, respectively. Accordingly, 15 genotypes were reported as indica and 28 temperate japonica in addition to 149 genotypes as intermediate types.

Conclusion: The information on marker-based diversity and performance based on cooking quality and agronomic traits helped to select the most divergent lines for crossing and also the analysis was useful to generate information on indica - japonica classification of our germplasm.

Introduction

Rice (*Oryza sativa* L.) is a staple crop of Kashmir Himalayas and covers an area of 150 thousand hectares grown above 1550msl. The cultivars grown include landraces and formally bred varieties which belong to cold tolerant indica and temperate japonica. Genetic improvement in any crop species is inexorable and a continuous process to meet the future challenges regarding food security. Evaluation, characterization and identification of plant genetic resources play an important role for their utilization in the crop improvement programmes [1, 2]. The landraces, traditional and improved cultivars together represent repositories of genetic diversity and can serve as resources for improving yield and resistance to pests and diseases [3-5] or else can be used as parents in development of superior recombinants [6]. Conventionally, morphological traits have been used to determine the genetic diversity and for classification of the germplasm into different groups. Alternately, the genetic diversity can be estimated through the use of molecular markers which offer a high polymorphism range and reproducibility [7, 8]. The PCR based markers such as [microsatellites](#) or simple sequence repeats (SSRs) are highly polymorphic, reproducible, co-dominant and widely distributed throughout the rice genome [9]. The two of the sub species viz., indica and temperate japonica are being grown across irrigated ecologies of Kashmir valley with 80% and 20% of the total (1.4 lakh ha) rice grown area, respectively [10]. The present study was undertaken to generate an information on genetic divergence and sub-species allocation of a set of elite rice genotypes which are targeted for different mountainous ecological niches across Kashmir valley.

Materials And Methods

Evaluation for agronomic and quality related traits

Fifty one temperate rice accessions were grown under irrigated field conditions at MRCFC, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Khudwani Campus (34°N latitude and 74°E longitude at 1690 msl) during the *Kharif* season of 2018-19 (Table 1). The materials were raised in a randomized complete block design with three replications. Thirty days old seedlings of each accession were transplanted in the field with the standard spacing of 20cm x 15cm and net plot size of 5 m² per replication. Recommended package of practices were followed to raise a healthy crop. Five randomly selected plants from each replication were selected for recording the observations on various agro-morphological traits. However, flowering, maturity and yield data was recorded on whole plot basis. The description of various agro-morphological traits along with grain quality features was carried out using Standard Evaluation System of IRRI [11]. Amylose content and gel consistency were determined by the method developed by Juliano [12] and Cagampang [13], respectively. Alkali spreading value and Aroma were determined as per the procedure described by Jennings [14].

Similarly the list of germplasm lines (192 genotypes) for their classification into indica and temperate japonica groups are given in the Table 2.

DNA Marker analysis

The SSR markers used for the characterization of 38 varieties/advanced breeding lines were randomly selected from www.gramene.org. The selected microsatellite markers (two each for one chromosome) along with their chromosomal location and annealing temperature (T_m) are presented in the Table 3. Genomic DNA was extracted following the CTAB (Cetyl-Tri Methyl Ammonium Bromide) method with some modifications [15]. Quantification of DNA samples was done spectrophotometrically and quality was estimated by using 0.8 % agarose gel electrophoresis. High concentration of DNA samples was further diluted in 10:1 Tris-EDTA to a working concentration of 50ng/ μ L and stored at 40C for PCR based marker analysis. PCR reaction was prepared with 50 ng of rice genomic DNA, 0.2 μ g of 3 ϕ and 5 ϕ end primers, 200 mM of each dNTP, 1X PCR buffer containing 50 mM KCL, 10 mM Tris HCl (pH 8.9), 2.0 mM MgCl₂ and one unit of *Taq* Polymerase in a total of 25 μ L solution individually for all 24 primer pairs. PCR thermal cyclers were programmed for 1 min at 94° C, 1 min and 30 seconds at 55°C, 1 min at 72°C and a final cycle of 10 min at 72°C. Amplification product was separated on 3.5% of agarose gel in 1X TBE buffer followed by staining with ethidium bromide. The SSR allele sizes were determined by the position of bands relative to the DNA ladder. Number 1 was given to the allele having highest molecular weight. The amplified bands were recorded as 1 (band present) and 0 (band absent) in a binary matrix.

Statistical analysis

The set of observations recorded for was subjected to statistical analysis. Analysis of variance was carried out for various agro-morphological traits as per Gomez and Gomez [16]. Analysis of molecular variance was performed using software GenAlEx version 6.5. Euclidian distance and Jaccards coefficients were calculated from morphological and marker data, respectively. Unweighted Pair Group Method using Arithmetic Averages (UPGMA) method was used to obtain the dissimilarity trees from the two matrices, respectively, with the help of DARwin software (version 6.0.21). PIC values for each of the 24 primers were estimated using the equation proposed by Anderson [17]:

$$PIC = \sum_{i=1}^n (p_{ij})^2$$

Where p_{ij} is the frequency of j^{th} allele in i^{th} primer and summation extends over 'n' patterns.

Furthermore, the list of markers used in indica-temperate japonica differentiation is given in Table 4.

Results

The mean squares due to present set of genotypes were highly significant for all the characters studied (S. Table 1). ANOVA of some important agronomic and cooking quality traits are given in Table 5. Among the various quality features, amylose content and presence of aroma influence the consumer preferences and thus drive the breeding strategy. In the present study, amylose content ranged from 15.8% to 25.5% with minimum in Aromatic Zag and maximum in SKUA-420 (Fig. 1). Aromatic rices are highly demanded in the national and global markets. Among the rice varieties examined, SKUA-485, Mushk Budji, SKUA-494 and Pusa Sugandh 3 showed strong aroma. SKUA-420 and Aromatic Zag revealed the presence of mild aroma. Kamad, Nunbeoul, Shalimar Rice-4 and Koshihikari showed low aroma and the remaining 41 genotypes were completely non-aromatic (Fig. 2).

Cluster analysis using morphological traits

Cluster analysis was carried out to assess the extent of divergence using UPGMA method. The set of genotypes got grouped into two major clusters. Cluster I had 15 genotypes, and was further sub grouped into cluster Ia, Ib, Ic and Id with four, five, one

and five genotypes, respectively. The remaining 36 genotypes marked the cluster II and were sub grouped into IIa, IIb, IIc and IId with seven, eight, seven and 14, genotypes, respectively (Fig. 3).

Molecular characterization

PCR assay carried out on 38 genotypes using 24 SSR markers revealed three of them (RM1, RM60 and RM308) as monomorphic across all genotypes. A total of 48 alleles were amplified and the number of alleles per locus generated by each marker ranged from 1 (RM60, RM130, RM159, RM236, RM308 and RM332) to 3 (RM1, RM105, RM204, RM216, RM224, RM226, RM295, RM335 and RM340) with an average number of 2.0 per locus (Table 3) (Fig. 4).

The polymorphism information content (PIC) value ranged from 0 to 0.665 with an average of 0.37. High PIC values were observed for the primers RM263 (0.67), RM159 (0.59) and RM333 (0.50), while primers RM1, RM60 and RM308 (0) and RM105 (0.05) showed lower values of PIC (Table 3). PIC provides an estimate of discriminatory power of a marker by taking into account the relative frequency of the alleles. PIC values exceeding 0.5 reflects a better polymorphism range [18]. Therefore, the markers RM 263, RM159 and RM333 can be effectively used for determining the genetic differences among the rice genotypes and to study their phylogenetic relationship.

The trend line of number of effective alleles (N_e) versus expected heterozygosity (H_e), was plotted and marked a maximum value of $N_e = 2.5$ that indicated the high discriminatory power of the markers. Ideally the number of effective alleles should approximate to the number of actual alleles (Fig. 5).

Cluster analysis and genetic divergence pattern

The cluster analysis carried out through Unweighted Pair Group Method using Arithmetic Averages (UPGMA) helped to classify 38 genotypes into 2 clusters at an average dissimilarity of 30%. Cluster I consisted of 24 genotypes and got further divided into two sub clusters; sub-cluster Ia consisted of 9 genotypes and sub cluster Ib of 15 genotypes. Similarly, cluster II contained 13 genotypes and is divided into two sub clusters viz; IIa which consisted of 10 genotypes and IIb with 3 genotypes (Fig. 6).

Indica-japonica differentiation

The 192 rice germplasm lines got differentiated into indica and temperate japonica while using 38 SSR markers. Some of these markers included R1M30, R1M37, R1M47, R3M30, R4M17, R5M13, R5M20, R6M44, R7M7, R7M37, R9M10, R10M17, R10M30, R10M40 and R11M23 (Table 4). Out of these markers R4M17 accurately categorized indica and temperate japonica rice with 220 bp and 169bp, respectively. Besides another marker R5M13 amplified 175bp in japonica and 207bp in indica. Another set of markers (S01022, S01160, S02057B, S02085, S03027, S03048, S03136, S03145, S04060, S04077B, S04087A, S04097B, S04129B, S07048, S07050A, S09000A, S09026B, S09040B, S09058, S09065, S09073, S10001 and S10003A) was validated across some known varieties such as Pusa Sugandh 3, Pusa Basmati 1509, SKUA-420, SKUA-495, K-332, Mushk Budji, Kamad, IR-64, K-39, IR-70, and Shalimar Rice-2. Of these, only S04077B and S09026B were found to clearly differentiate between indica and temperate japonica rice. For marker S04077B, 174bp allele was found to be associated with temperate japonica and 201bp with indica. The marker S09026B produced 207bp allele in japonica and 182 bp allele in indica. Therefore, out of 38 SSR markers only four SSR markers (R4M17, R5M13, S04077B and S09026B) perfectly categorized indica and temperate japonica rice. These four markers were further screened on 194 genotypes for the purpose of indica-japonica classification. Among the 192 genotypes 15 were indica and 28 temperate japonicas in addition to 149 varieties that belonged to intermediate type (Table 6). With respect to the fingerprint devised based on the four markers, genotypes which amplified 220, 182, 207 and 201 bp fragments were regarded as indica while 169, 207, 175, 174 bp as temperate japonica (S. Table 2). Any genotype with mixed combination of these alleles was categorized as intermediate type.

Discussion

Characterization of germplasm accessions establishes distinctiveness among rice genotypes. It is not only important for utilizing the appropriate attribute based donors in breeding programmes, but also essential in the present era for protecting the

uniqueness of germplasm collections. In the present investigation 51 rice genotypes were evaluated for different agro-morphological and physiochemical parameters and 38 genotypes were evaluated for molecular characterization. Further, 192 rice germplasm lines were used for indica-japonica differentiation. The mean sums of squares due to genotype were significant for all agro-morphological and quality traits. The high variability for plant height is in agreement with Chakravorty and Priyanka [19, 20]. Significant variability for days to 50% flowering in the present study supports the findings of Sajid [21]. Similarly, significant variability for grain yield as observed in this study was supported by the findings of Vanisree and Tuhina-Khatun [22, 23]. Nascimento [24] observed significant differences and high variability for flag leaf length, number of tillers per plant, panicle length, panicle fertility, 1000 grain weight which is similar to that in the present study. Similar results were reported by Sravan [25] for number of tillers, flag leaf length, plant height and panicle length. Richa [26] observed highly significant differences for the characters *viz.*, plant height, tillers per plant, days to flowering, days to maturity, grain yield per plant, effective tillers per plant, grain length, grain breadth, length-breadth ratio and 1000 grain weight. Significant variability for days to 50% flowering, days to maturity, plant height, number of effective tillers per plant, panicle length, spikelet fertility percent, test weight, grain yield was reported by Kumari and Umesh [27–28]. Similarly, Waza and Jaiswal [29] reported significant variability for grain length, grain breadth, grain length/breadth ratio, 100 grain weight, kernel length, kernel breadth, kernel length/breadth ratio, kernel length after cooking, kernel elongation ratio, alkali spreading value, amylose content and aroma. These findings are in conformation with the results of present study.

Pusa Sugandh 3 revealed 25.3% amylose content, which is similar to the previous reports by Bano and Majid [30, 31]. Rice with low amylose content (10-20%) tends to be sticky and soft on cooking and becomes firmer as amylose content increases. Rice with intermediate amylose content (20-24%) cooks moist and remains soft on cooling. High amylose content (≥25%) rice is known to cook dry and fluffy, and becomes hard on cooling [32]. Rice with intermediate amylose is usually preferred in Indian Sub-continent. In general, the germplasm with medium amylose content coupled with other desirable quality traits can be the ideal source for use in rice breeding programs. Similarly, presence of aroma is another important trait in rice. Sensory evaluation of rice grain aroma revealed the range of sensory scores between 0 and 3. Pachauri [33] reported the similar results.

In the present study, based on morphological diversity analysis, the set of genotypes were grouped into two major clusters (Fig. 3). Similar results were also reported by Madhubabu and Sruthi [34, 35].

In the present investigation, the level of polymorphism among the genotypes of rice was evaluated from the number of alleles and PIC value for each of the 24 SSR loci. Each of loci differed significantly in their ability to determine variability among the genotypes. A total of 48 alleles were amplified and the number of alleles per locus generated by each marker ranged from 1 to 3 with an average number of 2.0 per locus (Table 3). The polymorphism information content (PIC) value ranged from 0 to 0.665 with an average of 0.37. McCouch [36] observed an average value of 2.08 alleles per locus among 48 traditional indigenous aromatic genotypes of rice using SSR markers. SSR markers are considered to be most amenable for genetic divergence studies due to their multiallelic nature, high reproducibility, co-dominant inheritance, extensive genomic coverage [37], exhibition of high degree of allelic variation [38] and ability to detect genetic variation within and between the accessions [39]. Saba [40] in their study observed the PIC value ranging from 0.25 (RM3872) to 0.98 (RM321) with an average value of 0.63.

In the molecular analysis, the 38 genotypes got grouped into 2 clusters (Fig. 6). Clustering of genotypes based on marker data has been reported in earlier studies too. Rashmi [41] grouped 65 genotypes into 9 clusters by UPGMA mean clustering method. Similarly, Richa [26] grouped the genotypes into 4 major clusters based on UPGMA clustering method with Jaccard's similarity coefficient ranging from 0.38 to 0.92. Maliha, Exonam and Prasad [42–44] also reported the similar results.

Genetic diversity and differentiation in indica and japonica groups of the 192 rice germplasm was studied by assaying 38 markers (InDel and STS) that clustered them into 15 indica and 28 temperate japonicas in addition to 149 varieties that belonged to intermediate type (Table 6). The studies on Indica-japonica differentiation was previously carried out by Yong, Bao and Zhiyuan [45–47].

Conclusions

The genotypes which preserve the significant amount of genetic variability for the important agro-morphological traits were broadly grouped into two clusters. The information on marker-based diversity and performance based on cooking quality and agronomic traits can help with regard to the effective utilization of the germplasm. Further, the indica-japonica classification of the germplasm lines shall be helpful to devise a strategy for inter-sub species hybridization to breed for improved indicalinous and japonicalinous types that can fit in temperate climatic conditions.

Declarations

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Competing interests All the authors declare no relevant financial or non-financial interests.

Authors contribution

Najeebul R. Sofi and Asif Bashir Shikari conceived the experiment. Nakeeb-Un-Nisa, Sofora Jan, Reyaz R. Mir and Najeebul R. Sofi conducted the DNA marker analysis for estimation of genetic diversity. Aafreen Sakina, Gazala H. Khan and Asif Bashir Shikari carried molecular marker work on indica-japonica differentiation. Materials were maintained by Najeebul R. Sofi and morphological data was generated by Nakeeb-Un-Nisa, Sumira Rafiqee and Shabir H. Wani. Analysis was carried out by Nakeeb-Un-Nisa, Asif Bashir Shikari, Reyaz R. Mir and Showkat A. Waza.

Ethical approval Not applicable

Consent to participate Not applicable

Consent to publish All the authors have read and agreed to published version of the manuscript

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Tables

Table 1 Experimental material used in agro-morphological and quality characterization

China-1007 [‡] , China-1039 [‡] , Chenab [‡] , Jhelum, Shalimar Rice-1, Shalimar Rice-2 [‡] , Shalimar Rice-3 [‡] , Shalimar Rice-4 [‡] , SKUA-292, SKUA-403 [‡] , SKUA-406 [‡] , SKUA-410 [‡] , SKUA-412 [‡] , SKUA-415 [‡] , SKUA-478 [‡] , SKUA-483 [‡] , SKUA-484 [‡] , SKUA-525 [‡] , SKUA-526 [‡] , Shalimar Rice-5 [‡] , Mushk Budji, Purple rice, Heera [‡] , Koshihikari [‡] , K-332 [‡] , Kohsar, SKUA-485, SKUA-486 [‡] , SKUA-487 [‡] , SKUA-488 [‡] , SKUA-491 [‡] , SKUA-494 [‡] ,

SKUA-495[‡], SKUA-496, SKUA-497, SKUA-499[‡], SKUA-500[‡], SKUA-501, SKUA-502, SKUA-524[‡], Kamad[‡], SKUA-420[‡], Pusa Sugandh 3[‡], SKUA-514[‡], SKUA-505[‡], Nunbeoul[‡], Tangdar Zag[‡], Aromatic Zag[‡], SKUA-521, SKUA-522[‡], SKUA-523

[‡]Genotypes used in molecular analysis

Table 2 Experimental material used for classification into indica and temperate japonica

GS-88, GS-152, GS-183, GS-184, GS-189, GS-7, GS-22, GS-33, GS-34, GS-35, GS-36, GS-37, GS-59, GS-61, GS-63, GS-81, GS-103, GS-106, GS-112, GS-116, GS-118, GS-125, GS-126, GS-128, GS-129, GS-130, GS-133, GS-135, GS-139, GS-142, GS-145, GS-147, GS-155, GS-161, GS-163, GS-164, GS-166, GS-177, GS-181, GS-188, GS-195, GS-199, GS-205, GS-208, GS-214, GS-231, GS-234, GS-237, GS-245, GS-246, GS-248, GS-255, GS-258, GS-263, GS-269, GS-272, GS-275, GS-276, GS-282, GS-290, GS-291, GS-305, GS-306, GS-318, GS-321, GS-322, GS-324, GS-326, GS-331, GS-332, GS-337, GS-344, GS-350, GS-351, GS-358, GS-363, GS-367, GS-368, GS-370, GS-372, GS-376, GS-381, GS-404, GS-410, GS-421, GS-435, GS-436, GS-434, GS-443, GS-444, GS-446, GS-450, GS-453, GS-461, GS-464, GS-467, GS-468, GS-471, GS-474, GS-476, GS-477, GS-481, GS-487, GS-492, GS-499, GS-503, GS-504, GS-514, GS-518, GS-520, GS-531, GS-537, GS-539, GS-542, GS-543, GS-554, GS-574, GS-580, GS-581, GS-584, GS-585, GS-590, GS-592, GS-595, GS-597, GS-596, GS-598, GS-601, GS-602, GS-603, GS-617, GS-628, GS-634, GS-639, GS-662, SKUA-412, AVT-V2, AVT-FG-V5, AVT-IV1, SKUA-478, SKUA-496, Purple rice, AVT-FG-V10, AVT-FG-V2, , AVT-FG-V4, SKUA-404, AVT-FG-V7, SKUA-500, Egyptian rice, SKUA-415, SKUA-499, SKUA-491, AVT-IV3, GS-203, GS-206, GS-307, GS-336, GS-337, GS-338, GS-340, GS-342, GS-416, GS-432, GS-455, GS-484, GS-490, GS-497, GS-530, GS-582, GS-583, GS-587, GS-588, GS-623, GS-624, Koshihikari, K-332GS-157, GS-162,GS-182, GS-185, GS-273, GS-373, GS-541, GS-572, SKUA-339, SKUA-406, AVT-FG-V6,SKUA-483, Shalimar Rice-2,China-1007, Chenab, China-988,

Table 3 List of microsatellite markers along with their primer sequence and annealing temperature, number of alleles and PIC value

S. No	Marker ID	Chr. Location	Annealing temperature	Forward Primer (5' to 3')	Reverse Primer (5' to 3')	No. of alleles	PIC
1.	RM1	1	54	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC	1	0.00
2.	RM60	3	55	AGTCCCATGTTCCACTTCCG	ATGGCTACTGCCTGTACTAC	1	0.00
3.	RM72	8	53	CCGGCGATAAAACAATGAG	GCATCGGTCCCTAACTAAGGG	2	0.45
4.	RM105	9	65	GTCGTCGACCCATCGGAGCCAC	TGGTCGAGGTGGGGATCGGGTC	3	0.05
5.	RM117	12	63	CGATCCATTCTGCTGCTCGCG	CGCCCCCATGCATGAGAAGACG	2	0.48
6.	RM130	3	62	TGTTGCTTGCCCTCACGCGAAG	GGTCGCGTGCTTGGTTTGGTTC	1	0.50
7.	RM159	5	63	GGGGCACTGGCAAGGGTGAAGG	GCTTGTGCTTCTCTCTCTCTCTCTCTC	1	0.59
8.	RM173	5	64	TCGCGCTTCTTCTCTGTCGACG	CCCCTTGCAGAGGAAGCAGCC	2	0.48
9.	RM204	6	56	GTGACTGACTTGATCATAGGG	GCTAGCCATGCTCTCGTACC	2	0.45
10.	RM216	10	54	GCATGGCCGATGGTAAAG	TGTATAAAACCACACGGCCA	3	0.43
11.	RM224	11	54	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTTCGGG	3	0.39
12.	RM226	1	58	AGCTAAGGTCTGGGAGAAACC	AAGTAGGATGGGGCACAAGCTC	3	0.43
13.	RM235	12	56	AGAAGCTAGGGCTAACGAAC	TCACCTGGTCAGCCTCTTTTC	2	0.48
14.	RM236	2	55	GCGCTGGTGGAAAATGAG	GGCATCCCTCTTTGATTCTT	1	0.30
15.	RM242	9	55	GGCCAACGTGTGTATGTCTC	TATATGCCAAGACGGATGGG	2	0.27
16.	RM248	7	55	TCCTTGTGAAATCTGGTCCC	GTAGCCTAGCATGGTGCATG	2	0.45
17.	RM252	4	55	TTCGCTGACGTGATAGGTTG	ATGACTTGATCCCAGAACG	2	0.30
18.	RM263	2	57	CCCAGGCTAGTCATGAACC	GCTACGTTTGAGCTACCACG	2	0.67
19.	RM295	7	56	CGAGACGAGCATCGGATAAG	GATCTGGTGGAGGGGAGG	3	0.30
20.	RM308	8	56	GGCTGCACACGCACACTATA	TTACGCATATGGTGAGTAGGC	1	0.00
21.	RM332	11	56	GCGAAGGCGAAGGTGAAG	CATGAGTGATCTCACTACCCC	1	0.50
22.	RM333	10	56	GTACGACTACGAGTGTACACAA	GTCTTCGCGATCACTCGC	2	0.50
23.	RM335	4	55	GTACACACCCACATCGAGAAG	GCTCTATGCGAGTATCCATGG	3	0.48
24.	RM340	6	54	GGTAAATGGACAATCCTATGGC	GACAAATATAAGGGCAGTGTGC	3	0.50

Table 4 Markers used in indica-japonica differentiation

S.No	Primer	Chromosome	Forward primer (5¢ to 3¢)	Reverse primer (5¢ to 3¢)
1.	R1M30	1	AAGGGGCCCTAATTTATCTAG	TGTTTACTTTGTTCTTGGACTG
2.	R1M37	1	ATAGTTCGCCATCGTCAT	ACACGCCATAGCAAGGAA
3.	R1M47	1	AATAGAATTACTGATGAAACCTTA	GCCCGTTACCGCTTATGT
4.	R3M30	3	AGGCTAAGTGAAGAAATAATAAG	CTCCGTATTCATTACTGGTTG
5.	R4M17	4	AGTGCTCGGTTTTGTTC	GTCAGATATAATTGATGGATGTA
6.	R5M13	5	GAGAAAGAGTGGAAGGAG	AGTATCGTCAGGAGGGTC
7.	R5M20	5	CTCGCTGTTTACTGACTGG	TTTGATGTACTGCCTGCTCT
8.	R6M44	6	TTAGGAATAAAGGCTGGATA	TTACCGTTAATAGGTGGAA
9.	R7M7	7	ACCTTCCCTCCCCTTTTGAT	AACTTGGTCTTCCTGTTTTATTG
10.	R7M37	7	CAGCCCTAAATCTAAATACCC	ACGTTGAGACAGGCGAGC
11.	R9M10	9	CTTTGGATTACAGGGGGA	AACTTGAAACGGAGGCAG
12.	R10M17	10	TGAACAATAAACCACAGAAGCA	CCCTTTATTCCCTCCTTTG
13.	R10M30	10	CCCTAAAAATAGAGCAACCT	ACCCATAATACTACCAATCAAC
14.	R10M40	10	GTCCCTAGGCCATCTCTTG	GCGAATAGGGGTGGACAG
15.	R11M23	11	AAGGTTGACAAGGACAGAAG	TCGCAGGAATGGATAAAA
16.	S01022	1	CATGGATGATGCTTCCCTCT	TTGACAGTGGCTCCACAAAG
17.	S01160	1	TTGCGATTTATTTGCCAGTG	CCAGGCATCCAATGCTTATT
18.	S02057B	2	AGCCTCTTCTCCCTCCTCAC	TGCAAACACCATAACAACCAA
19.	S02085	2	GCGAGAGTGTACCCCTTTGA	TGTGTACCTTGACCCCTGAA
20.	S03027	2	TGAACATTTTGGTCGTCTGG	TTGACGAAGTCACCATAGACG
21.	S03048	3	GGGATGGGAGAAGGGAATAA	GCCAGCTAGGATGTTGAAGG
22.	S03136	3	GCATTAAGGCACACAAAGCA	TGTTTGTAATCCGCATGGAA
23.	S03145	3	TCACCTACAGGAAGCAGCAG	GCCGTCGTTGAAGAAGTAGC
24.	S04060	3	TATGGTTTTATCCGCCAACC	GCTACAATAAAACAAGAAACGTGA
25.	S04077B	4	TCCCAGGTGAACTACGGACT	CAGCATTTTCAGTGAAGCA
26.	S04087A	4	ATGTTTGGCAATCCGCTAAG	AAAGATGGTTGAGCGGAAGA
27.	S04097B	4	TCCACAGTCTGTCCGTGAAA	CTCCTTGTGCTGCAGAATTG
28.	S04129B	4	AATCGATTCAATCGCACAAA	CTTTCATGCTCTGCCATTGA
29.	S07048	6	CATGGCACCTTGAGAGTTGA	ACACATGGAGCTGGCTTCTC
30.	S07050A	7	CTCCACTTATGGCAGCGAAT	CAAGTGAAGTGGGAGCAGGT
31.	S09000A	8	CCAATTCACGGTTTAAACAAGG	GCCATGAAGCTTCGTTAGGA
32.	S09026B	8	GGGAGGCAGAGGGAACACT	TTATCAGGCCAGGTCCTTTG
33.	S09040B	8	TAATATCGCATGGCAAGACG	ACTTTCAGAGGCGACAAAC
34.	S09058	8	CGTGAGAAGTCCAGTCCACA	ATTGATCGATTGGGGGATTT
35.	S09065	9	TGTGTTTCGACGTTTGACCAT	GGGCCAGGGTACATTGAATA
36.	S09073	9	ACCACCCTGAACCACAACAT	TCACTGGGTTCTGTGTCCAA

37.	S10001	9	ATCGTGGTCGGGATTATGAG	GCATCATGGCTTTTGTGTTG
38.	S10003A	9	ATAAGACGGACGGTCAAACG	ATCTCTTGTGGGCTTTGTGG

Table 5 ANOVA of important agronomic and cooking quality traits

Source of variation	d.f	DF	Mean Squares						
			GY q/ha	KLBC (mm)	L/B ratio BC	KLAC (mm)	L/B ratio AC	KER	ASV
Replication	2	3.0653	104.4675**	0.0224	0.0010	0.0628	0.0005	0.0010	0.4567
Treatments	50	130.2732**	1518.4569**	1.4691**	1.0833**	4.3403**	2.2071**	0.0328**	3.7513**
Error	100	3.2053	16.3088	0.0080	0.0009	0.0106	0.0009	0.0008	0.4474

** significant at 0.01% level of significance

** P <0.01

Table 6 Classification of germplasm based on the markers differentiating indica and japonica

Indica	Japonica	Intermediate
GS-157, GS-162, GS-182, GS-185, GS-273, GS-337, GS-373, GS-541, GS-572, SKUA-339, SKUA-406, AVT-FG-V6, SKUA-483, Shalimar Rice-2, China-1007,	GS-88, GS-152, GS-183, GS-184, GS-189, GS-203, GS-206, GS-307, GS-336, GS-337, GS-338, GS-340, GS-342, GS-416, GS-432, GS-455, GS-484, GS-490, GS-497, GS-530, GS-582, GS-583, GS-587, GS-588, GS-623, GS-624, Koshihikari, K-332	GS-7, GS-22, GS-33, GS-34, GS-35, GS-36, GS-37, GS-59, GS-61, GS-63, GS-81, GS-103, GS-106, GS-112, GS-116, GS118, GS-125, GS-126, GS-128, GS-129, GS-130, GS-133, GS-135, GS-139, GS-142, GS-145, GS-147, GS-155, GS-161, GS-163, GS-164, GS-166, GS-177, GS-181, GS-188, GS-195, GS-199, GS-205, GS-208, GS-214, GS-231, GS-234, GS-237, GS-245, GS-246, GS-248, GS-255, GS-258, GS-263, GS-269, GS-272, GS-275, GS-276, GS-282, GS-290, GS-291, GS-305, GS-306, GS-318, GS-321, GS-322, GS-324, GS-326, GS-331, GS-332, GS-344, GS-350, GS-351, GS-358, GS-363, GS-367, GS-368, GS-370, GS-372, GS-376, GS-381, GS-404, GS-410, GS-421, GS-434, GS-435, GS-436, GS-443, GS-444, GS-446, GS-450, GS-453, GS-461, GS-464, GS-467, GS-468, GS-471, GS-474, GS-476, GS-477, GS-481, GS-487, GS-492, GS-499, GS-503, GS-504, GS-514, GS-518, GS-520, GS-531, GS-537, GS-539, GS-542, GS-543, GS-554, GS-574, GS-580, GS-581, GS-584, GS-585, GS-590, GS-592, GS-595, GS-596, GS-597, GS-598, GS-601, GS-602, GS-603, GS-617, GS-628, GS-634, GS-639, GS-662, SKUA-412, AVT-V2, AVT-FG-V5, AVT-IV1, SKUA-478, SKUA-496, Purple rice, AVT-FG-V10, AVT-FG-V2, AVT-FG-V4, SKUA-404, AVT-FG-V7, SKUA-500, Egyptian rice, SKUA-415, SKUA-499, SKUA-491, AVT-IV3, Chenab, China-988

Figures

Figure 1

Frequency distribution of Amylose content among rice genotypes

AC: Amylose content where, 9.1-20% = low amylose content, 20.1-25% = medium amylose content, 25.1-33% = high amylose content

Figure 2

Frequency distribution of aroma among rice genotypes

Figure 3

Dendrogram of 51 rice genotypes generated through DARwin software using unweighted neighbor joining method based on agro-morphological traits

Cluster I: [Kamad, Tangdar Zag, Mushkbudji and Aromatic Zag]; [Shalimar Rice-5, K-332, SKUA-505, Kohsar and SKUA-514]; [SKUA-485]; [Purple rice, SKUA-420, Heera, SKUA-494 and Pusa Sugandh 3]

Cluster II: [Nunbeoul, SKUA-491, Koshihikari, SKUA-495, SKUA-488, SKUA-406 and China-1039]; [Shalimar Rice-2, Shalimar Rice-4, SKUA-501, SKUA-412, SKUA-500, Shalimar Rice-1, SKUA-415 and SKUA-487]; [SKUA-478, SKUA-496, SKUA-502, Jhelum, SKUA-410, SKUA-488 and SKUA-499]; [SKUA-524, SKUA-483, Chenab, SKUA-486, SKUA-523, SKUA-526, SKUA-521, SKUA-292, SKUA-522, Shalimar Rice-3, China-1007, SKUA-403, SKUA-403, SKUA-525, SKUA-497].

□ designates sub-cluster

Figure 4

Agarose Gel image depicting SSR marker (RM252) amplification profile of 38 rice genotypes

L; Ladder, 1; SKUA-484, 2; Aromatic Zag, 3; SKUA-487, 4; Nunbeoul, 5; SKUA-499, 6; Koshihikari, 7; SKUA-488, 8; Shalimar Rice-2, 9; SKUA-403, 10; Chenab, 11; SKUA-406, 12; SKUA-500, 13; SR-4, 14; SKUA-483, 15; Shalimar Rice-3, 16; SKUA-410, 17; SKUA-412, 18; SKUA-494, 19; Pusa Sughandh 3 20; SKUA-495, 21; SKUA-486, 22; SKUA-491, 23; SKUA-420, 24; Kamad, 25; Heera, 26; SKUA-415, 27; Tangdar Zag, 28; SKUA-478, 29; China-1007, 30; SKUA-522, 31; SKUA-524, 32; SKUA-525, 33; SKUA-526, 34; Shalimar Rice-5, 35; K-332, 36; SKUA-514, 37; SKUA-505 and 38; China-1039.

Figure 5

Number of effective alleles in relation with gene diversity

Figure 6

UPGMA based clustering pattern showing genetic relationship among 38 genotypes based on genetic distance matrix using 24 SSR markers

Cluster I: [SKUA-415, SKUA-412, SKUA-486, Pusa Sugandh 3, SKUA-410, SKUA-500, SKUA-524, China-1007 and SKUA-406]; [China-1039, SKUA-494, SKUA-505, Shalimar Rice-3, Shalimar Rice-4, SKUA-403, SKUA-525, SKUA-522, SKUA-478, SKUA-491, SKUA-483, Shalimar Rice-2, SKUA-488, SKUA-499 and SKUA-487].

Cluster II: [Tangdar Zag, Kamad, Shalimar Rice-5, Koshihikari, SKUA-514, K-332, Nunbeoul, SKUA-526, Heera and SKUA-420]; [Chenab, Aromatic Zag and SKUA-484]. SKUA-495 did not fall in any cluster and appear as distinct one. [] indicates sub-clusters

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