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GENETIC DIVERSITY IN BASMATI AND NON-BASMATI RICE VARIETIES BASED ON MICROSATELLITE MARKERS

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Abstract

Molecular markers are useful tools for evaluating genetic diversity and determining cultivar identity. The purpose of this study was to evaluate the genetic diversity within a diverse collection of rice (*Oryza sativa* L.) accessions and to determine differences in the patterns of diversity within the aromatic and non-aromatic rice varieties. Forty rice accessions were evaluated by means of 24 microsatellite markers distributed over the whole rice genome. A total of 66 alleles were detected at 24 SSR loci, and the number of alleles per marker ranged from 2 to 4, with an average of 2.75. Polymorphism information content (PIC) value ranged from 0.0476 (RM315) to 0.5993 (RM252), with an average of 0.3785 per marker. The average genic diversity over all SSR loci for the 40 genotypes was 0.4477, ranging from 0.0488 to 0.6638. Major allele frequency ranged from 0.4250 (RM252) to 0.9750 (RM315), with an average of 0.6472. The dendrogram based on the cluster analysis by microsatellite polymorphism, grouped 40 rice cultivars into three groups effectively differentiating basmati cultivars from non-basmati cultivars. These results could be useful for monitoring purity, genotype identification and for plant variety protection.

Introduction

Pakistan is unique in its diversity for agriculture and is separated into 10 agro ecological regions. These all consisted of diverse climate and has great potential for producing all types of food commodities. In Pakistan lot of scientist are working for the evaluation of genetic diversity of local agricultural commodities like wheat (Khan *et al.*, 2010a), cotton (Khan *et al.*, 2010b, Mumtaz *et al*., 2010), rice (Pervaiz *et al*., 2010; 2010a; Shah *et al*., 2011; Rabbani *et al*., 2010); Turmeric (Jan *et al*., 2011, 2012); Guar (Sultan *et al*., 2012); Sesame (Akbar *et al*., 2011); Sugar cane (Mumtaz *et al*., 2011); Brassica (Turi *et al*., 2012); Black gram(Ghafoor *et al.*, 2012), lentil (Sultana *et al.*, 2010), peach (Bakth *et al.*, 2012) and Sheesham (Ashraf *et al.*, 2010).Due to the population explosion the demand for the food production in the world is likely to increase in the coming decades. Among many other food crops, rice is the world's most important food crop feeding half of the world population. Compared with other crop species, the world rice germplasm genetic diversity is quite large. Rice subspecies, i.e., indica, japonica, and javanica, compose of large reservoir of rice germplasm including a variety of local landraces and cultivars (Khush, 1997; McCouch *et al.*, 2005; Garris *et al*., 2005). Landraces are valuable genetic resources because they contain enormous genetic variability which can be used to broaden the gene pool of advanced genotypes (Kobayashi *et al.*, 2006). Genetic diversity in a crop population is due to recombination, mutation, selection and random genetic drift. In Pakistan rice is used as staple food after wheat. Pakistan is an important rice exporter especially Basmati rice since 1970. Unfortunately green revolution resulted in eradication of many of Basmati landraces grown in Pakistan. In Pakistan a large number of varieties and improved cultivars have been released for cultivation in different regions but have a narrow genetic base. Particularly in case of 5 basmati varieties currently under cultivation, 5 have 'Basmati-370' as one of the parents (Rabbani *et al.*, 2008).

The intensity of the genetic diversity is essential for world food security and survival of human civilization on earth. In the past, breeders exploited plant species for their livelihoods that resulted in domestication of many of them as improved cultivars to produce food for the better supply of the human diet (Oka, 1988; Ross-Ibarra *et al*., 2007). Knowledge of the genetic diversity of germplasm collections is an important foundation for crop improvement. Due to the importance of rice as a major world crop, the diversity of *Oryza sativa* has attracted great interest. For thousands of years conventional breeding approaches were used to improve rice cultivars, but the progress was very slow due to the time-consuming process, quantitative nature of most agronomic traits and difficulties in genotype selection (www.fao.org/docrep/004/y3557e/y3557e09.htm).Exploring diversity in a landrace collection is very important for identifying new genes and further improvement of the germplasm (Brondani *et al.*, 2006; Oliveira *et al.*, 2007; Thomson *et al.*, 2007). In spite of the richness of genetic resources, only a small proportion of the world rice germplasm collections have been utilized in breeding programs, as a consequence a high genetic similarity is found within several commercial rice germplasms around the world (Ghneim *et al*., 2008). Sequencing of the indica and japonica genomes have made rice a model crop and provided breeders a quick approach to improve rice cultivars.

Molecular markers can have a number of applications in agriculture, and their application in rice improvement has been reviewed (Collard & Mackill, 2008; Jena & Mackill, 2008).Simple Sequence Repeat (SSR) markers are easily available for any region of the genome, and candidate gene markers are being developed rapidly. In rice, SSR markers have been effectively utilized for many purposes including (i) association mapping (Abdel khalik *et al.*, 2007; Jin *et al.*, 2010; Oliveira *et al.*, 2010); (ii) genetic diversity and relatedness (Pervaiz *et al*., 2010; Zhao *et al*., 2010); (iii) Qtl mapping (GUO *et al.*, 2010),(iv) mutation analysis (Li *et al.*, 2010; Wang *et al.*, 2009) and maker assisted selection (Thomson, 2009) and rice domestication (Sweeney *et al*, 2007). The present study has been conducted to estimate the pattern and level of genetic variability and relatedness among rice cultivars from Pakistan along with germplasm from other countries. DNA marker analysis will help to distinguish different accession by means of specific SSR alleles.

Materials and Methods

Plant material: Forty rice genotypes comprising land races, pure-lines, breeding lines and varieties specifically adapted to different ecological zone of Pakistan and exotic lines under field experiments were chosen in the study (Table 1).

DNA extraction and SSR marker analysis: DNA was extracted from five day old young leaves using CTAB method (Doyle & Doyle, 1987). Twenty five SSR markers covering different genomic regions of rice were selected in this study and are listed in Table 2. PCR reactions were carried out in Thermal cycler (Bio Rad Inc. USA) with the total reaction volume of 25µl containing, 5µl of genomic DNA, 1X assay buffer, 200µM of dNTPs, 2µM $MgCl_2$, 0.2µM of forward and reverse primer and 1 unit of Taq DNA polymerase (Fermentas Life Sciences). The PCR cycles were programmed as 95°C for 2 min, 94°C for 1 min, 55°C for 1 min, 72°C for 2 min for 35 cycles and an additional temperature of 72°C for 10 min for final extension. The amplified products were separated on 2.5 percent agarose gel prepared in 0.5X TAE buffer. The gel was run in 0.5X TAE buffer at constant voltage of 80 V for a period of 1h to 2 h and stained with ethidium bromide. The gel was then visualized under UV trans-illuminator and photographs were taken using gel documentation instrument. Clearly resolved, unambiguous bands were scored visually for their presence or absence with each primer. The scores were obtained in the form of matrix with '1' and '0', indicating the presence and absence of bands in each variety respectively.

Data analysis: Using the Power Marker version 3.25 software package (Liu *et al.,* 2005), the diversity of each accession was analysed on the basis of four statistical parameters: major alleles frequencies, alleles number, gene diversity and polymorphism information content (PIC), which measures the genic diversity (Botstein *et al.*, 1980). For the unrooted phylogenetic tree, genetic distance was calculated using the "C.S. Chord 1967" distance (Cavalli-Sforza & Edwards, 1967) followed by phylogeny reconstruction using neighbor-joining as implemented in POWER MARKER with the tree viewed using TREEVIEW. Bootstrapping of the NJ tree was performed using POWERMARKER with 1,000 iterations.

Table 1. List of rice accessions used to study microsatellite marker variation.

Varieties	Origin	Type	Varieties	Origin	Type
B.198	Pakistan	Basmati	IR 24	Philippines	Non-Basmati
B.2000	Pakistan	Basmati	APO	Philippines	Non-Basmati
B.370	Pakistan	Basmati	IR88021_B_86_3_4	Philippines	Non-Basmati
B.385	Pakistan	Basmati	IR64	Philippines	Non-Basmati
S.B.	Pakistan	Basmati	IR72	Philippines	Non-Basmati
B.Pak	Pakistan	Basmati	SALUMPIKIT	Philippines	Non-Basmati
Shaheen	Pakistan	Basmati	DT-48	Philippines	Non-Basmati
KSK133	Pakistan	Non-Basmati	IR29	Philippines	Non-Basmati
KS282	Pakistan	Non-Basmati	IR58	Philippines	Non-Basmati
PK386	Pakistan	Non-Basmati	IR8	Philippines	Non-Basmati
Supri	Pakistan	Non-Basmati	IR9	Philippines	Non-Basmati
Supra	Pakistan	Non-Basmati	IRBLta2-Pi	Philippines	Non-Basmati
S.fine	Pakistan	Non-Basmati	Vandana	India	Non-Basmati
IR 6	Pakistan	Non-Basmati	TKM6	India	Non-Basmati
DR-82	Pakistan	Non-Basmati	Nona Bokra	India	Non-Basmati
DR-83	Pakistan	Non-Basmati	Pokkali	India	Non-Basmati
DR_92	Pakistan	Non-Basmati	Brown Gora	India	Non-Basmati
Sufaid-86	Pakistan	Non-Basmati	Way Rarem	India	Non-Basmati
NIAB_IR_9	Pakistan	Non-Basmati	N_22	India	Non-Basmati
KS_427	Pakistan	Non-Basmati	W 1263	Malaysia	Non-Basmati

Table 2. List of microsatellite markers used in the study.

Marker	CH. No.	Repeat motif	Allele frequency	Allele No.	Gene diversity	PIC
RM1	1	(GA)26	0.4750	3.0000	0.6113	0.5313
RM212	1	(CT)24	0.7250	3.0000	0.4113	0.3448
RM315	1	(AT)4(GT)10	0.9750	2.0000	0.0488	0.0476
RM263	2	(CT)34	0.5250	3.0000	0.5738	0.4879
RM251	3	(CT)29	0.4595	3.0000	0.5902	0.5020
RM55	3	(GA)17	0.7750	2.0000	0.3488	0.2879
RM7	3	(GA)19	0.5641	2.0000	0.4918	0.3709
RM252	4	(CT)19	0.4250	4.0000	0.6638	0.5993
RM334	5	(CTT)20	0.5000	3.0000	0.5249	0.4121
RM162	6	(AC)20	0.5000	3.0000	0.5238	0.4103
RM190	6	(CT)11	0.9487	2.0000	0.0973	0.0926
RM253	6	(GA)25	0.5250	3.0000	0.5863	0.5063
RM11	7	(GA)17	0.5128	3.0000	0.5917	0.5111
RM234	7	(CT)25	0.7000	2.0000	0.4200	0.3318
RM223	8	(CT)25	0.9500	2.0000	0.0950	0.0905
RM339	8	(CTT)8CCT(CTT)5	0.7297	3.0000	0.4076	0.3437
RM342A	8	(CAT)12	0.8250	2.0000	0.2888	0.2471
RM72	8	(TAT)5C(ATT)15	0.5250	4.0000	0.6213	0.5593
RM201	9	(CT)17	0.6389	2.0000	0.4614	0.3550
RM242	9	(CT)26	0.5750	3.0000	0.5088	0.4023
RM202	11	(CT)30	0.7250	2.0000	0.3988	0.3192
RM206	11	(CT)21	0.5250	4.0000	0.6288	0.5717
RM229	11	(TC)11(CT)5C3(CT)5	0.8250	3.0000	0.2963	0.2647
Average			0.6526	2.6957	0.4402	0.3707

Result

Forty diverse rice accession from Pakistan, India and Philippines were analysed using 24 microsatellite (Table 2) covering 10 chromosomes. Only one SSR marker (RM42) was found monomorphic and showed only one allele among all varieties in all countries and was discarded. The level of divergence varied among different accession for 24 microsatellite loci. The allelic richness per locus varied from 2 to 4 alleles, with an average of 2.75 alleles. Maximum number of alleles were observed by RM72, RM252 and RM206.The average gene diversity was 0.4477, varied from 0.0488 (RM315) to 0.6638 (RM252). The level of polymorphism among the 40 genotypes was evaluated by calculating PIC values for each of the 24 SSR loci. PIC values fluctuated from 0.0476(RM315) to 0.5993(RM252), with an average of 0.3785. Results in accumulative form are shown in Table 3.

Maximum numbers of polymorphic alleles were observed in IR8, Superfine, PK386 and Sufaid86. Eight accessions show minimum number of alleles. Out of 8, 7 accessions were from Pakistan and one from Philippines. RM339 is marker of choice to differentiate Basmati and non-basmati varieties grown in Pakistan. RM11 is capable of differentiating KSK133 and KS282. Super Basmati can be easily differentiated from other Basmati under study with the help of RM252. RM190 is capable of differentiating PK386 and Supri from all other accession under analysis. Two non-aromatic long grain varieties PK386 and Superfine give different allele from all other varieties with RM206. In order to distinguish Basmati Pak and Shaheen from other Basmati RM206 is the marker of choice.

Rare alleles, defined as those alleles with a frequency less than 5%, were identified at two loci. In general, markers detecting a greater number of alleles per locus detected more rare alleles. The numbers of rare alleles were present in three cultivars Supri, IR29 and W1263 from Pakistan, Philippines and Malaysia respectively. Rare allele for Supri was detected by RM315 while in case of IR29 and W1263, rare allele was observed in RM223.

A dissimilarity matrix based on the C.S. Cord shared SSR fragments was used to establish the level of relatedness between the accession surveyed from different countries (Table 4). Pair-wise estimates of similarity ranged from 0.036 to 0.684 and the average similarity among all 40 accessions was 0.39. Maximum similarity was observed between KS282 and KSK133. On the other hand lowest similarity was observed between Basmati198 and Supri, Basmati385 and Pokkali, and for IR24 and Salumpikit. The similarity coefficient was higher in aromatic varieties as compared to non-aromatic accession.

Table 3. Summary of results.

Plant material	40 accession of rice
Markers	24 SSR marker
Alleles per marker	2 to 4, average of 2.75
Polymorphism information content (PIC)	Minimum 0.0476 (RM315), Maximum 0.5993 (RM252), Average of 0.3785
Genic diversity	Average 0.4477, Ranging from 0.0488 to 0.6638
Major allele frequency	Ranges from 0.4250 (RM252) to 0.9750 (RM315), Average of 0.6472.
The dendrogram	divides accession into three groups effectively differentiating basmati cultivars from non-basmati cultivars

A neighbor joining cluster analysis based on C.S. Cord dissimilarity matrix grouped the 40 accessions from 4 countries into 3 major groups. The cluster analysis effectively differentiated the aromatic rice cultivars from non-aromatic accession (Figs. 1 & 2; Table 5). Group-I consisted of 24 genotypes which were further subdivided into 5 sub-clusters. Sub-cluster 1-3 consisted of nine accessions each having 3 genotypes. The six accessions forming cluster 4 in group-I included, one accession DR-82 from Pakistan, while 2 accession from India (TKM 6 and Vandana) and 2 accession from Philippines (IR-29 and APO). The only one accession from Malaysia W1263 also included in this cluster. In Group-I cluster 5 comprised 9 accession representing 3 countries in close ancestry. Group-II consisted of 6 accessions and these all have Pakistani origin. The Group-III consisted of 7 accessions merges all aromatic accessions from Pakistan. Three accessions, 2 from Pakistan and 1 from Philippines showed mixed ancestry between aromatic and non-aromatic rice. Cluster analysis placed all aromatic traditional long-grained cultivars in group showing a high level of genetic relatedness and close parentage. Cluster analysis showed that the accessions that resulted from genetically similar parent clustered together.

Discussion

The DNA primers have the ability to differentiate different plant accession based on the difference in the representing genomic region and also depend upon the numbers of alleles. Genetic diversity is crucial for adaptation of rice on diverse agro ecological origins. Assessment of genetic diversity is key factor for germplasm conservation, characterization and breeding. Classical breeding affects genetic diversity by Selection of combination out comes from desirable allele frequencies and leads to favorable effects and loss of diversity. Little was known about the relationship between Pakistan rice cultivars to other countries on the basis of molecular analysis. In this study, 24 microsatellite markers were used to assess the genetic diversity of 40 genotypes of rice including varieties Super-basmati (aromatic) and IR6 (Indica type, Non-aromatic). The results indicated significant genetic variation among the rice accessions. Microsatellite assays identified a number of alleles that were shared among the basmati and non-basmati varieties. A close relationship between 'Super-basmati' and a group of 6 basmati blood genotypes was observed. That phenomenon could be a support towards strong bias power of some of the DNA markers. In Major group-II 'IR6' (Indica type and non-aromatic) and 5 genotype were cluster together and suggesting a close association.

Highest PIC values were observed for SSR primers RM252 (0.5993). PIC value is reflection of allele diversity and frequency among the cultivars. The higher the PIC value of a locus, the higher the number of alleles detected. This observed pattern was consistent with the findings of Lapitan *et al*., (2007) and Wang *et al*., (2009). The average PIC value for the remaining SSR loci was 0.3785.The number of alleles detected by microsatellite markers varied from 2 to 4 with an average of 2.6957 alleles per locus and were similar to those reported by Wang *et al*., (2009) and Jalaluddin *et al*., (2007) using a different set of rice germplasm. In contrast, the average numbers of alleles noticed in present study were lower than those reported previously (Siwach *et al*., 2004; Brondani *et al*., 2006; Oliveira *et al*., 2007; Thomson *et al*., 2007; Ghneim *et al*., 2008; Pervaiz *et al*., 2010 & 2011). Those reports had an average of 4.5, 14.6, 7.7, 13.0, 4.5, 4.5 and 4.4 alleles per locus. The contradiction among reports might be due to the genotypes used and selection of microsatellite primers with scorable alleles. The microsatellite markers used in this study were well distributed amongst the 10 chromosomes, and were located in both coding and non-coding segments of the genome. Only one marker was monomorphic, while remaining 23 gave polymorphic alleles. Nine makers yielded 2 alleles while 12 and 3 makers produce 3 and 4 band respectively.

Basmati varieties gain special place in rice and hence sold at 2-3 time higher price as compared to other rice varieties and subject to adulterations with other long grain non-approved basmati and non-basmati varieties. Various methods, based on morphological / anatomical characterization and organoleptic markers (odor, color, texture) or chemical testing, have been developed to authenticate and to check for adulterants (Shaw *et al.*, 2002). Varietal identification by means of molecular analysis is most authenticated as compared to all other method. Bligh (2000) reported use of fluorescent labeled sample sequence length polymorphism for basmati rice adulterations. Steele *et al.,* (2008) used In Del marker to distinguish basmati from other fragrant rice varieties. A comparison of calibration method for quantification of basmati and non-basmati rice using microsatellite analysis was performed by Colyer *et al*., (2008).

Table 4. SC. Cord coefficients of dissimilarity among pairs of 40 rice accession.

OTU	APO	B.198	B.2000	B.370	B.385	B.Pak	Brown Gora	DR_92	DR-82	DR-83	DT-48	IR 24	IR 6	IR29	IR58	IR64	IR72	IR8	IR88021_B_86_3_4	IR9
APO	0.00																			
B.198	0.55	0.00																		
B.2000	0.51	0.25	0.00																	
B.370	0.51	0.18	0.07	0.00																
B.385	0.55	0.22	0.25	0.25	0.00															
B.Pak	0.51	0.32	0.14	0.22	0.25	0.00														
Brown Gora	0.25	0.41	0.49	0.49	0.56	0.45	0.00													
DR_92	0.47	0.34	0.26	0.26	0.43	0.34	0.36	0.00												
DR-82	0.20	0.47	0.47	0.47	0.54	0.50	0.30	0.47	0.00											
DR-83	0.29	0.53	0.45	0.45	0.60	0.53	0.43	0.36	0.23	0.00										
DT-48	0.30	0.43	0.43	0.43	0.51	0.43	0.31	0.33	0.16	0.20	0.00									
IR 24	0.47	0.32	0.47	0.40	0.36	0.54	0.45	0.47	0.47	0.64	0.55	0.00								
IR 6	0.47	0.54	0.61	0.54	0.43	0.54	0.41	0.39	0.47	0.49	0.47	0.29	0.00							
IR29	0.27	0.58	0.61	0.61	0.65	0.65	0.45	0.51	0.29	0.34	0.31	0.50	0.50	0.00						
IR58	0.29	0.49	0.38	0.38	0.45	0.53	0.34	0.32	0.26	0.35	0.35	0.34	0.30	0.34	0.00					
IR64	0.16	0.58	0.47	0.47	0.54	0.50	0.45	0.34	0.36	0.19	0.31	0.54	0.47	0.32	0.30	0.00				
IR72	0.16	0.61	0.58	0.58	0.50	0.54	0.45	0.47	0.32	0.30	0.35	0.43	0.43	0.32	0.34	0.11	0.00			
IR8	0.31	0.54	0.43	0.43	0.54	0.50	0.34	0.30	0.29	0.30	0.23	0.50	0.36	0.36	0.19	0.29	0.32	0.00		
IR88021_B_86_3_4	0.20	0.64	0.53	0.53	0.53	0.56	0.31	0.36	0.23	0.27	0.25	0.45	0.26	0.38	0.20	0.19	0.15	0.15	0.00	
IR9	0.37	0.49	0.53	0.53	0.49	0.53	0.31	0.30	0.34	0.35	0.33	0.53	0.30	0.38	0.27	0.34	0.30	0.15	0.20	0.00
IRBLta2-Pi	0.31	0.32	0.43	0.43	0.47	0.54	0.34	0.43	0.29	0.34	0.35	0.36	0.58	0.40	0.38	0.36	0.40	0.40	0.38	0.45
KS_427	0.47	0.43	0.43	0.43	0.61	0.50	0.34	0.34	0.40	0.45	0.35	0.36	0.43	0.47	0.30	0.47	0.54	0.32	0.41	0.45
KS282	0.43	0.47	0.47	0.47	0.29	0.54	0.56	0.43	0.40	0.41	0.43	0.29	0.29	0.58	0.38	0.40	0.36	0.43	0.30	0.38
KSK133	0.47	0.50	0.50	0.50	0.32	0.58	0.60	0.43	0.43	0.41	0.43	0.32	0.25	0.58	0.41	0.40	0.36	0.40	0.26	0.34
N_22	0.25	0.43	0.47	0.47	0.51	0.59	0.23	0.33	0.27	0.33	0.29	0.39	0.31	0.35	0.23	0.31	0.35	0.23	0.20	0.33
NIAB_IR_9	0.39	0.43	0.58	0.58	0.50	0.50	0.26	0.47	0.25	0.41	0.23	0.47	0.32	0.36	0.34	0.50	0.40	0.32	0.30	0.23
Nona Bokra	0.25	0.49	0.53	0.53	0.49	0.41	0.23	0.47	0.23	0.35	0.33	0.41	0.30	0.34	0.35	0.41	0.38	0.41	0.31	0.39
PK386	0.39	0.54	0.54	0.47	0.50	0.61	0.56	0.34	0.47	0.41	0.51	0.43	0.36	0.58	0.49	0.36	0.43	0.43	0.38	0.41
Pokkali	0.20	0.65	0.61	0.61	0.68	0.58	0.26	0.39	0.25	0.30	0.27	0.50	0.36	0.36	0.34	0.29	0.29	0.22	0.19	0.23
S.B.	0.51	0.32	0.14	0.22	0.22	0.14	0.41	0.17	0.50	0.53	0.39	0.43	0.50	0.61	0.45	0.50	0.54	0.43	0.49	0.49
S.fine	0.39	0.54	0.47	0.47	0.58	0.43	0.41	0.26	0.29	0.38	0.31	0.43	0.29	0.47	0.38	0.40	0.36	0.25	0.23	0.26
S-86	0.23	0.50	0.47	0.47	0.50	0.47	0.45	0.39	0.22	0.34	0.23	0.47	0.47	0.36	0.34	0.22	0.25	0.25	0.23	0.34
SALUMPIKIT	0.39	0.47	0.40	0.40	0.50	0.36	0.49	0.30	0.36	0.23	0.31	0.68	0.43	0.50	0.38	0.25	0.36	0.29	0.30	0.26
Shaheen	0.47	0.32	0.18	0.25	0.22	0.18	0.60	0.39	0.43	0.49	0.43	0.43	0.58	0.61	0.41	0.43	0.40	0.47	0.49	0.49
Supra	0.47	0.25	0.32	0.25	0.22	0.32	0.41	0.30	0.47	0.56	0.47	0.22	0.29	0.61	0.34	0.43	0.47	0.43	0.41	0.45
Supri	0.47	0.68	0.61	0.61	0.58	0.65	0.68	0.43	0.43	0.41	0.51	0.43	0.32	0.50	0.41	0.40	0.43	0.47	0.34	0.49
TKM6	0.16	0.47	0.47	0.47	0.54	0.50	0.34	0.51	0.07	0.23	0.23	0.40	0.47	0.29	0.34	0.32	0.29	0.36	0.26	0.41
Vandana	0.16	0.50	0.50	0.50	0.54	0.43	0.26	0.47	0.25	0.38	0.23	0.47	0.54	0.36	0.41	0.32	0.29	0.32	0.30	0.41
W 1263	0.16	0.47	0.47	0.47	0.54	0.54	0.30	0.39	0.22	0.34	0.23	0.47	0.47	0.14	0.26	0.29	0.32	0.25	0.26	0.30
Way Rarem	0.29	0.45	0.49	0.49	0.49	0.34	0.39	0.36	0.26	0.34	0.20	0.56	0.41	0.38	0.43	0.26	0.30	0.30	0.27	0.27

Table 4. (Cont'd.).

OTU	IRBLta2-Pi	KS_427	KS282	KSK133	N_22	NIAB_IR_9	Nona Bokra	PK386	Pokkali	S.B.	S.fine	S-86	SALUMPIKIT	Shaheen	Supra	Supri	TKM6	Vandana	W 1263	Way Rarem
IRBLta2-Pi	0.00																			
KS_427	0.32	0.00																		
KS282	0.36	0.50	0.00																	
KSK133	0.40	0.54	0.04	0.00																
N_22	0.23	0.31	0.43	0.39	0.00															
NIAB_IR_9	0.47	0.43	0.50	0.47	0.27	0.00														
Nona Bokra	0.38	0.45	0.45	0.49	0.25	0.26	0.00													
PK386	0.50	0.58	0.29	0.25	0.47	0.61	0.53	0.00												
Pokkali	0.43	0.40	0.43	0.40	0.27	0.32	0.30	0.32	0.00											
S.B.	0.47	0.43	0.43	0.47	0.47	0.54	0.49	0.50	0.54	0.00										
S.fine	0.43	0.36	0.36	0.32	0.35	0.36	0.41	0.32	0.29	0.40	0.00									
S-86	0.40	0.47	0.40	0.36	0.35	0.36	0.38	0.32	0.22	0.47	0.32	0.00								
SALUMPIKIT	0.47	0.47	0.47	0.43	0.39	0.40	0.41	0.43	0.32	0.50	0.32	0.29	0.00							
Shaheen	0.47	0.54	0.36	0.40	0.55	0.50	0.49	0.50	0.54	0.25	0.47	0.36	0.36	0.00						
Supra	0.40	0.43	0.29	0.32	0.43	0.50	0.41	0.36	0.58	0.29	0.36	0.40	0.47	0.36	0.00					
Supri	0.47	0.54	0.32	0.29	0.47	0.58	0.45	0.25	0.43	0.58	0.36	0.36	0.47	0.54	0.43	0.00				
TKM6	0.32	0.43	0.40	0.43	0.31	0.32	0.19	0.40	0.25	0.50	0.36	0.22	0.43	0.43	0.47	0.40	0.00			
Vandana	0.36	0.43	0.54	0.58	0.31	0.40	0.30	0.50	0.25	0.40	0.43	0.29	0.47	0.43	0.50	0.58	0.22	0.00		
W 1263	0.36	0.40	0.47	0.50	0.23	0.32	0.26	0.50	0.29	0.47	0.40	0.29	0.43	0.54	0.47	0.54	0.22	0.25	0.00	
Way Rarem	0.49	0.45	0.49	0.45	0.41	0.26	0.31	0.41	0.23	0.45	0.30	0.11	0.19	0.38	0.41	0.45	0.26	0.26	0.30	0.00

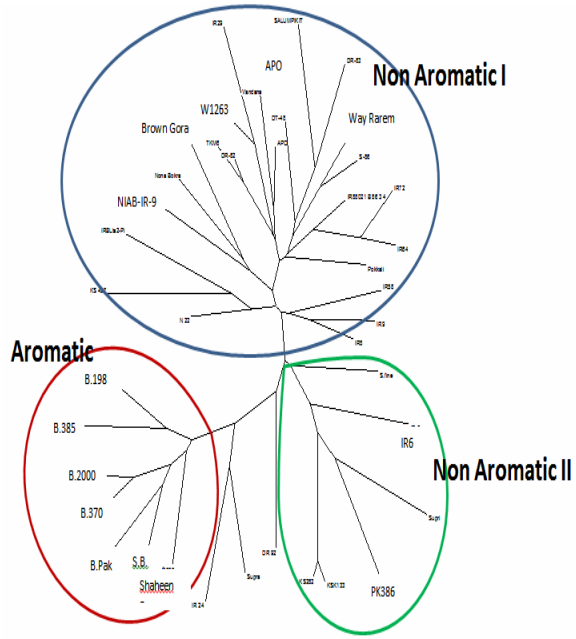

Fig. 1. An unrooted NJ tree showing the genetic relationships between the 40 rice accessions based on 24 microsatellite markers. The three major groups are labeled.

Amplification of microsatellite markers RM339 located on chromosome 8 results in 2 polymorphic alleles and clearly differentiates aromatic and non-aromatic rice cultivars. RM263 is a marker of choice for differentiation among aromatic rice cultivars Basmati 198, Basmati 2000 and Basmati 370 from Basmati Pak, Basmati 370 Shaheen and Super Basmati. Super Basmati purity can be checked by using RM252 among the Basmati group. Super basmati has a close parentage with Basmati Pak as compared to other basmati varieties. KS282 and KS133 have varied close parentage about 96% similarities and only one marker RM11could be used to differentiate between both accessions. Most suitable markers to differentiate one to 2 accessions from the rest are given in Table 6.

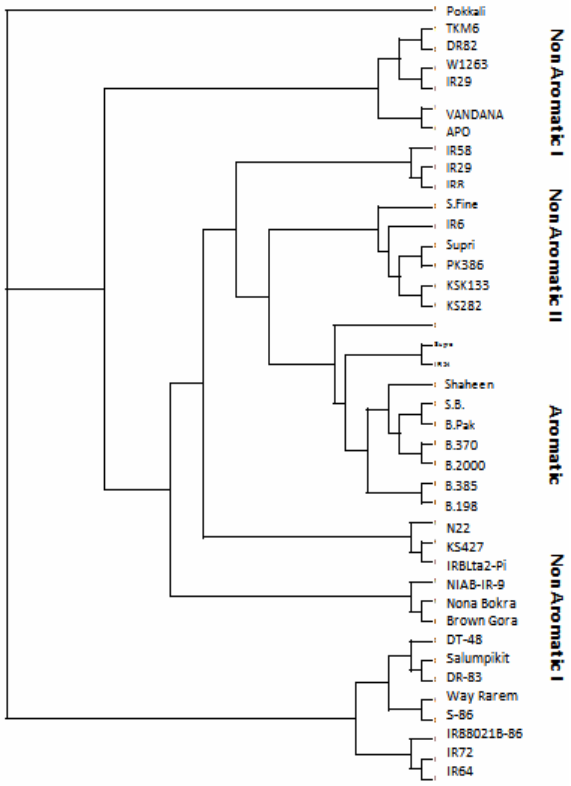

Fig. 2. Hierarchical cluster dendrogram of rice accessions.

Rice landraces had been studied for genetic diversity using several methods involving their morphological and physiological characters (Oka, 1988), isozymes (Glaszmann, 1987), RFLP markers (Chuan *et al*., 2001; Kojima, 2005) RAPD, (Qian *et al*., 2001) or microsatellite markers (Wang *et al*., 2009). However, such studies generally used each rice landrace or cultivar as one single sample and did not focus on the diversity within them. On the other hand, heterogeneous are reported in rice landraces within the population (Fukuoka *et al*., 2006). Microsatellite markers proved to be a useful tool for clarifying the genetic diversity among landrace genotypes.

Table 5. Classification of accessions in tabulated form.

Group	No. of accession	Accession	Country
Aromatic	7	B.198, B.385, B.2000, B.370, B.Pak, Super Basmati, B.Shaheen	Pakistan
Non Aromatic I	24	N-22, KS-427, IRBLta2-Pi, NIAB-IR-9, Nona Bokra, Brown Gora, TKM-6, DR-82,W-1263, IR-29, Vandana, APO, DT-48, Salumpikit, DR-83, Way Rarem, Sufaid-86, IR-88021-B-86-3-4, IR-72, IR-64, Pokkali, IR-58, IR-9, IR-8	Pakistan, India, Philippines, Malaysia
Non Aromatic II	6	Super fine, IR-6, Supri, PK-386, KSK-133, KSK-282	Pakistan
Transitional Group	3	DR-92, Supra, IR-24	Pakistan and Philippines

Table 6. Markers suitable for differentiation of one to two accessions from rest of accessions.

Marker	Differentiate between accession	
RM339	Pakistan Aromatic rice	Pakistan Non Aromatic Rice
RM11	KSK133	KS282
RM252	Aromatic	Non Aromatic
RM190	PK386 and Supri	All other Accession
RM206	B.Pak and Shaheen	Other Basmati Accession
RM206	PK386 and Superfine	All other Accession
RM315	Supri	All other Accession
RM223	IR29 and W1263	All other Accession

Conclusion

Microsatellite analysis was an efficient tool for diversity analysis, and to differentiation rice accession from diverse climate. Overall results show that accession from four countries Pakistan, Philippines, India and Malaysia share common alleles along with some which are specific to cultivar and can be used to differentiate varieties from different countries. The markers used in this study of value to construct a database for accession important in breeding programs and characterization of other rice cultivars. In present analysis aromatic and nonaromatic type shows a clear divergence from common ancestor. The microsatellite analysis results in some cultivar-specific alleles; that will be helpful for cultivar identification and DNA fingerprints.

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