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RESEARCH ARTICLE

Molecular Characterization and Genetic Divergence Analysis of Traditional and Improved Aromatic Rice Varieties Using Microsatellite Markers

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Microsatellite profiling was employed to analyze the nature and extent of differentiation and divergence among eighteen traditional and improved varieties of aromatic rice. Analysis of 18 primers dependent genetic polymorphism revealed 89 shared and 91 unique allelic variants generated in the form of amplified products amongst 18 varieties. Polymorphic information content of the primers ranged from 0.60 to 0.88 with an average of 0.80. Ample genetic differentiation and divergence amongst entries was deduced by a comparison of similarity coefficients. Broadly, the entries were distinctly divided into three multi-genotypic groups, each of which accommodated traditional and improved varieties. However, the first and second groups were dominated by traditional varieties, whereas the third group was dominated by improved varieties. With maximum similarity in respect of targeted genomic regions, Champaran Basmati and Sanwal Basmati (0.45) were accommodated into the first group along with Jeerabati and Rajendra Kasturi (0.45), in addition to Baharni, Birsamati and Kasturi. Spatial distribution pattern of two dimensional ordinations of microsatellite primers specific genetic profiles from traditional and improved varieties completely validated the hierarchical classification pattern exhibited by sequential agglomerative clustering based dendrogram. The varieties from different groups could be utilized in breeding programs for genetic enhancement of aromatic rice germplasm base and molecular profiles could be helpful in preserving the integrity of these valuable aromatic rice varieties.

Key Words: Allelic diversity, Aromatic rice, Genetic diversity, Genetic profile, Microsatellite.

Introduction

Rice is an important cereal crop grown under diverse eco-geographical conditions in various tropical and sub-tropical countries of the world and consumed as a staple food by about fifty per cent of the human population worldwide. Aromatic rice constitutes a small but special group of landraces and varieties of rice well known for its nut like flavour, specific aroma and superior grain quality (Priyadarshini *et al*., 2018). Presence of aroma in the grain is one of the most important attributes of high quality rice, especially for price determination in the domestic and international markets. Aroma is best developed when aromatic rice is grown in areas where temperature is cooler during maturity (Pachauri *et al*., 2010). Although majority of the aromatic rice varieties popular in the world market have long grains, many indigenous aromatic rice varieties have short and medium sized grains.

India is well known as a country having an immense wealth of aromatic rice. Unfortunately, many accessions of aromatic rice have been lost as an aftermath of the green revolution where emphasis was mainly placed on productivity rather than quality. Among the aromatic rice accessions, the highly valued rice accessions because of their superior grain, cooking and eating quality characteristics are collectively called as basmati rice (Amarawathi *et al*., 2008). As a result of properties like extra-long superfine slender grains with chalky endosperm, pleasant and exquisite aroma, sweet taste and soft texture when cooked, length-wise kernel elongation with least breadth-wise swelling on cooking and tenderness of cooked rice, basmati rice commands a premium price in the world market (Pachauri *et al*., 2010). However, many other indigenous varieties of scented rice excel equally as far as aroma and cooking qualities are concerned (Vhora *et al*., 2013). But, unfortunately, these have somehow not got the sincere attention of rice

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scientists and traders, including exporters (Anonymous, 2003; Pachauri *et al*., 2010; Roy *et al*., 2016). As a result, most of this valuable wealth has either already vanished or is on a decline.

Since recognizable variation in respect of the type and intensity of aroma exists in different groups of aromatic rice varieties (Widjaja *et al*., 1996; Singh *et al*., 2007; Pachauri *et al*., 2010), precise study of the nature and extent of genetic differentiation and divergence in basmati gene pool and other aromatic rice types is necessary for conservation of accessions, identification of duplicate accessions, proper purity maintenance and varietal identification. Therefore, increasing attention is being paid towards comprehensive characterization of elite basmati quality rice germplasm, supplementing the existing morphological descriptors with reliable and repeatable genomic sequences and markers based molecular profiles (Amrawathi *et al*., 2008; Rabbani *et al*., 2010; Shah *et al*., 2013). Genetic analysis at the molecular level is being considered as an important approach to assure the export quality of basmati rice in order to maintain the distinctiveness of basmati varieties and to differentiate between the various grades of basmati rice (Nagaraju *et al*., 2002; Pal *et al*., 2004). Genomic markers that are genetically linked to fragrance along with the advantage of being inexpensive, simple and reliable, requiring small amounts of tissue have been developed for their utilization in the breeding and selection of fragrant rice (Cordeiro *et al.,* 2002).

Morphological evaluation and characterization is an important methodology for analyzing the differentiation and diversity, but it is less efficient and cumbersome. Advantages offered by the genomic sequences based markers that they remain unaffected across different stages, seasons and locations, in addition to their abundance and genome wide coverage, project them as a powerful tool in the assessment of genetic variation and elucidation of the genetic relationships. Among genomic markers, microsatellites are known to be highly polymorphic, easily automated, more reproducible, co-dominant and distributed throughout the genome. Usefulness of microsatellite markers for germplasm characterization in rice is well established (Mia *et al*., 2010; Singh *et al*., 2011; Samal *et al*., 2014; Singh *et al.*, 2016, Priyadarshini *et al*., 2018; Kumari *et al*., 2018a). Keeping all above in view, the present study was conducted to evaluate the genetic polymorphism at the molecular level and to investigate the nature and pattern of genetic differentiation and divergence among some traditional and improved varieties of aromatic rice.

Materials and Methods

Eighteen rice accessions comprising, landraces, advanced breeding lines and improved varieties of aromatic rice, namely Champaran basmati, Sanwal basmati, Jeerabati, Birsamati, Rajendra Kasturi, Baharni, Kasturi, Jasmine, Marcha, Lalmati, Kalanamak, Rajendra Bhagwati, Ranbir Basmati, Sugandha, Basmati 370, Rajendra Suwasini, RAU 3055 and Pusa Basmati-1, were subjected to molecular characterization in the present study conducted in the Molecular Biology Laboratory of the Department of Agricultural Biotechnology & Molecular Biology, Dr. Rajendra Prasad Central Agricultural University, Pusa. Using modified cetyl-trimethyl-ammonium bromide (CTAB) method (Ferdous *et al*., 2012) with slight modifications (Shamim *et al*., 2016a), the genomic DNA was extracted from the leaves of fifteen days old seedlings of these entries. The amplification of targeted genomic regions in extracted DNA samples was carried out utilizing a panel of eighteen primer pairs specific to the unique flanking sequences of the microsatellites distributed among six chromosomes present in the genome of rice (Table 1). The amplification was performed in a total volume of 15µl containing 3.8 µl water (Protease and Nuclease free), 3.0 µl 5X PCR buffer, 0.3 µl 10 mM $MgCl_2$, 3.0 µl 200 µM dNTPs mixture, 1.2 µl (5 µM) Primer F, 1.2 µl (5 µM) Primer R, 1.0 µl Taq Polymerase (1 unit) and 1.5 µl DNA template. The reaction condition along with thermal profile cycling was optimized in the Master Cycler (Eppendorf) using initial denaturation at 94 ºC for 5 min; 35 cycles of denaturation at 94 ºC for 1 min, annealing at 53º-58 ºC for 1 min and extension at 72 ºC for 2 min followed by a final extension at 72 ºC for 7 min. The amplicons were loaded onto 2% agarose gel containing ethidium bromide (0.5µg/ml) in 0.5X TBE buffer and resolved at 100 volts and then visualized and documented under gel documentation system (Alpha Innotech, USA). The size of the most intensely amplified products was determined in relation to the size of markers in 50 bp DNA ladder with the help of gel reader (Alpha View Gel Reader).

Molecular level genetic polymorphism was recorded on the basis of presence or absence of bands in different entries under investigation. All the entries were scored for the presence and absence of bands and the data recorded in this respect were entered into binary matrix as discrete variables and then this data matrix was

Table 1. Microsatellite primers used for amplification of targeted genomic regions in traditional and improved aromatic rice varieties

Locus	Ch. No.	Primer Sequence (5'- 3')	Repeat Motif	An. Tp. (^{0}C)
RM 13	5	(F) TCCAACATGGCAAGAGAGAG (R) GGTGGCATTCGATTCCAG	$(GA)_6$-$(GA)_{16}$	53
RM 16	3	(F) CGCTAGGGCAGCATCTAAA (R) AACACAGCAGGTACGCGC	$(TCG)_5(GA)_{16}$	56
RM 42	8	(F) ATCCTACCGCTGACCATGAG (R) TTTGGTCTACGTGGCGTACA	$(AG)_6(AG)_2$ T$(GA)_5$	56
RM 44	8	(F) ACGGGCAATCCGAACAACC (R) TCGGGAAAACCTACCCTACC	$(GA)_{16}$	56
RM 72	8	(F) CCGGCGATAAAACAATGAG (R) GCATCGGTCCTAACTAAGGG	$(TAT)_5C(ATT)_{15}$	55
RM 80	8	(F) TTGAAGGCGCTGAAGGAG (R) CATCAACCTCGTCTTCACCG	$(TCT)_{25}$	58
RM 223	8	(F) GAGTGAGCTTGGGCTGAAAC (R) GAAGGCAAGTCTTGGCACTG	$(CT)_{25}$	54
RM 225	6	(F) TGCCCATATGGTCTGGATG (R) GAAAGTGGATCAGGAAGGC	$(CT)_{18}$	53
RM 252	4	(F) TTCGCTGACGTGATAGGTTG (R) ATGACTTGATCCCGAGAACG	$(CT)_{19}$	54
RM 256	8	(F) GACAGGGAGTGATTGAAGGC (R) GTTGATTTCGCCAAGGGC	$(CT)_{21}$	54
RM 284	8	(F)ATCTCTGATACTCCATCCATC (R) CCTGTACGTTGATCCGAAGC	$(GA)_8$	54
RM 330	8	(F) CAATGAAGTGGATCTCGGAG (R) CATCAATCAGCGAAGGTCC	$(CAT)_5$	56
RM 337	8	(F)GTAGGAAAGGAAGGGCAGAG (R)CGATAGATAGCTAGATGTGC	$(CTT)_{4-19}(CTT)_8$	54
RM 339	8	(F) GTAATCGATGCTGTGGGAAG (R)GAGTCATGTGATAGCCGATG	$(CTT)_8$ CCT $(CCT)_5$	53
RM 426	8	(F) ATGAGATGAGTTCAAGGCCC (R) AACTCTGTACCTCCATCGCC	$(CA)_{10}$	54
RM 444	9	(F) GCTCCACCTGCTTAAGCATC (R) TGAAGACCATGTTCTGCAGG	$(AT)_{12}$	54
RM 505	7	(F) AGAGTTATGAGCCGGGTGTG (R) GATTTGGCGATCTTAGCAGC	$(CT)_{12}$	54
RM 515	8	(F) TAGGACGACCAAAGGGTGAG (R) TGGCCTGCTCTCTCTCTCTC	$(GA)_{11}$	56

Ch. No.: Chromosome number; An. Tp.: Annealing temperature

subjected to further analysis. Suitability of the marker based polymorphism for molecular differentiation and characterization of the entries was assessed by computing the polymorphism per cent (Kumari *et al*., 2018b). Allelic diversity exhibited by the microsatellite marker based polymorphism for identification of polymorphic and informative markers to characterize and differentiate the aromatic rice entries was evaluated on the basis of comparison of polymorphism information content (Anderson *et al*., 1993) of the primer pairs. Efficacy of individual primers in differentiation of the genotypes was investigated by computing the discrimination coefficient (DC) and non-discrimination coefficient (NDC) of the primer pairs (Kumar *et al*., 2018). Similarity coefficient (Dice, 1945) was computed for pair-wise combinations of the entries on the basis of comparison of presence and absence of bands and proportion of shared bands produced by the primers as;

$$Similarity\ coefficient = 21/(2a + b + c),$$

where, a, b and c represent number of bands shared between J^{th} and K^{th} genotypes, number of bands present in J^{th} genotype but absent in K^{th} genotype and number of bands absent in J^{th} genotype but present in K^{th} genotype, respectively.

Using sequential agglomerative hierarchical non-overlapping (SAHN) clustering based on similarity coefficients as the method for tree building in the cluster analysis, the dendrogram was generated by un-weighted pair-group method using arithmetic mean (UPGMA). Numerical taxonomic approach based analysis of the similarity matrix was performed with the help of

Table 2. Analysis of polymorphism revealed by microsatellite primers used for the amplification of targeted genomic regions in traditional and improved aromatic rice varieties

Primer	Allele size range (bp)	Allele size difference (bp)	No. of alleles	No. of unique alleles	No. of shared alleles	PP	PIC	DC	NDC
RM 13	127 - 153	026	07	04	3	57.1	0.803	0.84	0.15
RM 16	166 - 185	019	05	02	3	40.0	0.717	0.76	0.23
RM 42	166 - 538	372	19	14	5	73.6	0.850	0.94	0.05
RM 44	104 - 417	313	13	04	9	30.7	0.686	0.81	0.18
RM 72	146 - 211	065	09	06	3	66.6	0.810	0.85	0.14
RM 80	141 - 187	046	10	06	4	60.0	0.885	0.93	0.06
RM 223	159 - 309	150	11	06	5	54.5	0.870	0.83	0.16
RM 225	128 - 179	051	09	02	7	22.2	0.857	0.93	0.06
RM 252	213 - 811	598	17	09	8	52.9	0.840	0.92	0.07
RM 256	109 - 162	053	06	03	3	50.0	0.754	0.81	0.18
RM 284	144 - 161	017	06	02	4	33.3	0.791	0.83	0.16
RM 330	170 - 340	170	09	05	4	55.5	0.600	0.66	0.33
RM 337	155 - 216	061	08	03	5	37.5	0.871	0.92	0.07
RM 339	157 - 207	050	08	03	5	37.5	0.847	0.89	0.10
RM 426	177 - 228	051	08	02	6	25.0	0.873	0.92	0.07
RM 444	178 - 350	172	11	09	2	81.8	0.872	0.93	0.06
RM 505	180 - 507	327	15	08	7	53.3	0.696	0.80	0.19
RM 515	240 - 294	054	09	03	6	33.3	0.871	0.92	0.07

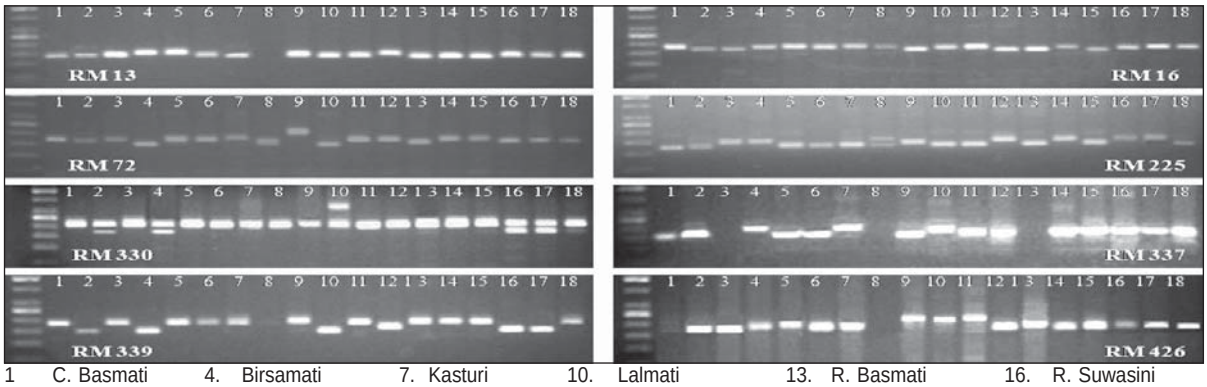
PP: Polymorphism per cent; PIC: Polymorphism information content; DC: Discrimination coefficient; NDC: Non Discrimination Coefficient

NTSYS-pc software (Rohlf, 1997). The information pertaining to the nature and magnitude of differentiation and divergence amongst the aromatic entries under evaluation was generated by identifying the clusters at appropriate phenon levels. Principal coordinate analysis of the microsatellite primers dependent genetic profiles of the entries was conducted and the two-dimensional spatial distribution pattern of the entries were compared with the results obtained from the cluster analysis and neighbour joining tree.

Results and Discussion

Differential banding patterns observed among the entries due to amplification of targeted genomic regions using eighteen microsatellite primers clearly indicated the existence of ample molecular level genetic polymorphism. The molecular level genetic polymorphism among the entries was recognized on the basis of presence or absence and size of bands, in addition to variation in respect of number and relative position of bands (Fig. 1). Strikingly different efficiency was exhibited by the primer pairs in terms of their ability to reveal variability amongst the entries. While some of the primer pairs generated several allelic variants as a result of variation in the length of microsatellite specific simple sequence repeats, others generated only a few allelic variants among the entries, corroborating the results of the several earlier researchers (Kumar *et al*., 2015; Shamim *et al*., 2016b; Kumari *et al*., 2018a; Priyadarshini *et al*., 2018). Further, all the primers utilized during molecular characterization generated unique allelic variants, but the number and proportion of unique alleles varied considerably among the primer pairs. Allele size range and allele size difference exposed the variability in the length of the simple sequence repeats at a microsatellite site, arising as a result of differences in the number of repeats existing in different entries and revealed by the primer pair involved in the amplification of targeted genomic region. Thus, microsatellite profiling clearly reflected the presence of plentiful genetic variability in terms of differences in the number of repeats at the primer specific microsatellite locus in evaluated aromatic rice entries.

Perusal of the data related to the analysis of polymorphism amongst traditional and improved aromatic rice varieties (Table 2) indicates that microsatellite primers used for the amplification of targeted genomic regions detected 180 allelic variants including 89 shared and 91 unique allelic variants. The number of alleles per primer ranged from six in the cases of RM 256 and RM 284 to nineteen in the case of RM 42 with an average of 10.0 alleles per primer. Experimental evidences using different panels of



1	C. Basmati	4.	Birsamati	7.	Kasturi	10.	Lalmati
2.	S. Basmati	5.	R. Kasturi	8.	Jasmine	11.	Kalanamak
3.	Jeerabati	6.	Baharni	9.	Marcha	12.	R. Bhagwati
13.	R. Basmati	16.	R. Suwasini				
14.	Sugandha	17.	RAU 3055				
15.	Basmati 370	18.	P. Basmati				

Fig. 1. Amplification patterns generated by microsatellite primers with eighteen traditional and improved varieities of aromatic rice

primer pairs and different set of genotypes of rice have documented considerably lower (<8.0) as well as higher (>12.0) number of allelic variants (Joshi and Behera, 2006; Brondani *et al*., 2006; Jayamani *et al*., 2007; Borba *et al*., 2009; Rabbani *et al*., 2010; Singh *et al*., 2011; Behera *et al*., 2012; Vhora *et al*., 2013; Kumar *et al*., 2015; Shamim *et al.,* 2016b; Priyadarshini *et al.*, 2018) than the allele number recorded in the present study. In general, the primer pairs yielding greater number of alleles per locus generated more number of unique alleles in accordance with the earlier reports (Joshi and Behera, 2006; Rabbani *et al*., 2010; Singh and Singh, 2012). Considerably greater percentage of unique alleles was generated by the primer pairs RM 444, RM 42, RM 72, RM 80, RM 223, RM 505, RM 330, RM 252, RM 13 and RM 256 in descending order of magnitude. Therefore, these ten primer pairs recorded remarkably greater polymorphism per cent.

Substantial differences were noticed in the polymorphism information content of the primer pairs, clearly reflecting ample variability in allelic diversity as well as allelic frequency among the entries under evaluation. The estimates, in general, reflected that the level of polymorphism revealed by the panel of primer pairs utilized during amplification was quite high but variable among the primers. Numerical value ranged from 0.600 in the case of RM 330 to 0.885 in the case of RM 80 with an average of 0.805 (Table 2). The values obtained in the present study are comparable to some of the earlier reports (Bansal *et al*., 2013; Priyadarshini *et al*., 2018; Kumari *et al*., 2018a; Kumar *et al*., 2018), but higher (Pal *et al*., 2004; Joshi and Behera, 2006, Brondani *et al*., 2006; Kibria *et al.*, 2009; Mia *et al.,* 2010; Das *et al*., 2012) or lower (Behera *et al.*, 2012) than the reports of other researchers in rice. Considering the number of alleles generated by different primer pairs in conjunction with the percentage of unique alleles and the level of polymorphism detected in the present study, the primers RM 42, 72, RM 80, RM 225, RM 252 and RM 444 appeared to be the more informative for the purpose of molecular characterization of aromatic rice entries under evaluation. Relatively greater efficiency of these primers was observed in discriminating the pair-wise combinations of entries evaluated.

Total repeat count of the di-nucleotide microsatellite loci was, in general, seemed to be more or less associated with the number of alleles detected per locus. Therefore, the results, in general, reflected that larger the repeat number involved in the microsatellite locus, the larger was the number of identified alleles. Further, the microsatellite loci with di-nucleotide repeat motifs, in general, tended to detect greater number of alleles than the repeat loci with tri-nucleotide repeat motifs in accordance with the earlier report (Kumar *et al*., 2018; Kumari *et al*., 2018a; Priyadarshini *et al*., 2018). Occurrence of null allele was inferred due to failure of amplification for a particular repeat locus specific to the unique flanking sequences of the microsatellite. The microsatellite locus associated with RM 13, RM 80, RM 337, RM 339, RM 426, RM 444 and RM 505 exhibited null allele ranging from one to three in the entries under evaluation. Similar results indicating the occurrence of null allele have also been reported earlier by (Pal *et al*., 2004; Shamim *et al*., 2016b; Priyadarshini *et al*., 2018; Kumari *et al*., 2018a). Appearance of more than one band in the same entry was noticed with the primer pairs RM 42, RM 44, RM

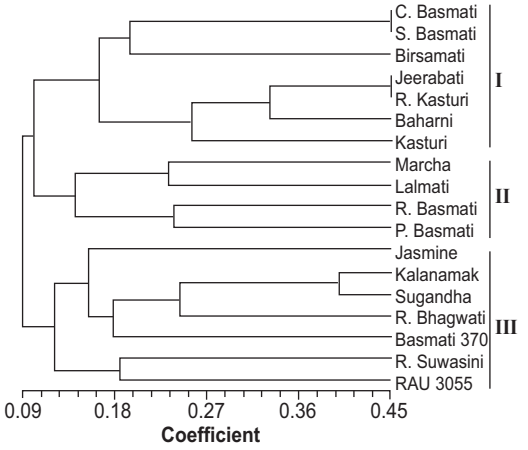


Fig. 2. Similarity indices based dendrogram showing interrelationship among traditional and improved aromatic rice varieties

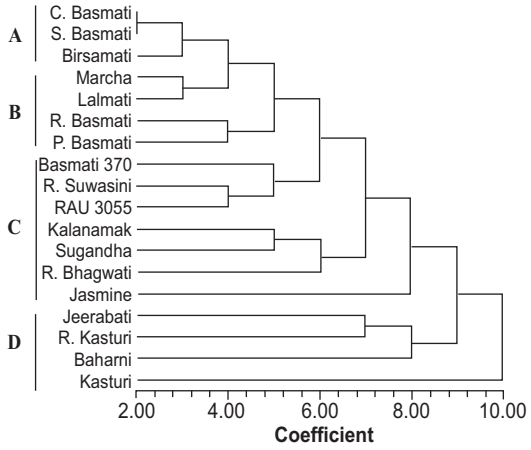


Fig. 3. Similarity indices based neighbour joining tree of traditional and improved aromatic rice varieties

223, RM 225, RM 252, RM 330 and RM 505, revealing most probably the existence of residual heterozygosity in genotypic background of some entries.

While assessing the genetic similarities and interrelationships, similarity coefficient between Sanwal Basmati and Champaran Basmati was found to be the maximum in magnitude (0.450) amongst pair-wise combinations of entries. Therefore, the molecular profiles of Sanwal Basmati and Champaran Basmati generated by using eighteen microsatellite specific primer pairs were relatively more similar with respect to the nucleotide sequence composition at primer binding sites and the molecular size of the genomic regions covered by the primer pairs. This was followed by remarkably higher magnitude of similarity coefficient between Jeerabati and Sanwal Basmati, Jasmine and Baharni, Kasturi and Baharni, Sugandha and Rajendra Bhagwati, Rajendra Kasturi and Sanwal Basmati, Lalmati and Kasturi, Kalanamak and Jasmine and Kasturi and Rajendra Kasturi. However, the magnitude of similarity coefficient between Jasmine and Champaran Basmati, Jasmine and Jeerabati, Jasmine and Birsamati, Marcha and Baharni, Lalmati and Rajendra Kasturi, Rajendra Bhagwati and Champaran Basmati, Rajendra Bhagwati and Sanwal Basmati, Ranbir Basmati and Kasturi, Basmati 370 and Kasturi, RAU 3055 and Champaran Basmati, RAU 3055 and Sanwal Basmati, RAU 3055 and Birsamati, Pusa Basmati 1 and Kasturi, Rajendra Bhagwati and Marcha, Ranbir Basmati and Jasmine, Basmati 370 and Lalmati, RAU 3055 and Marcha was equal to zero. Therefore, experimental results revealed enormous molecular level diversity amongst the eighteen aromatic entries evaluated in the present study.

Hierarchical classification pattern based cluster analysis of the traditional and improved rice varieties, as it is evident from a perusal of dendrogram (Fig. 2), basically yielded three multi-genotypic groups in which the entries were conveniently accommodated. All the multi-genotypic groups had a combination of traditional and improved varieties. However, the first and second multi-genotypic groups were dominated by traditional aromatic varieties, whereas, the third group predominantly consisted of improved aromatic varieties. The multi-genotypic groups were further divided into clusters, sub-clusters and sub-sub clusters at higher phenon levels, accommodating only the entries with increasingly similar pattern of markers together.

Neighbour joining tree also exhibited more or less similar type of overall clustering pattern but slightly different type of interrelationship amongst the entries (Fig. 3). As it is well established, this method is based on the idea of parsimony and hence considered as a method for estimating phylogenetic trees. Since it attempts to find a tree that is usually close to the true phylogenetic tree, it does yield relatively short estimated evolutionary trees rather than attempting to obtain the shortest possible tree for a set of data. Linking together the two objects that are the closest mutual neighbours, trees are constructed. Therefore, slight deviation in interrelationship and clustering pattern may be reflected as exhibited in the present study, but the inferences

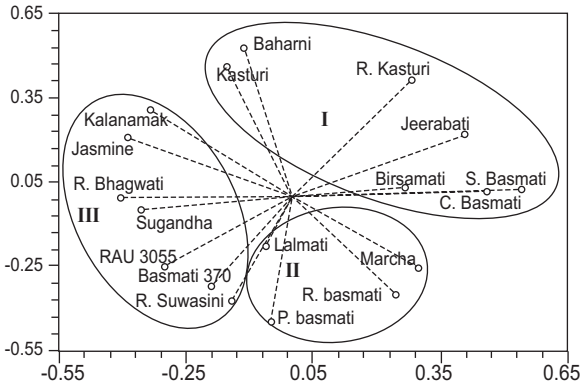


Fig. 4. Spatial distribution of the genetic profiles of traditional and improved aromatic rice varieties along principal coordinate axes

derived from neighbour joining tree were more or less in agreement with the results obtained from dendrogram.

Spatial distribution pattern of the traditional and improved aromatic varieties along the two principal axes in two dimensional plots of eighteen microsatellite primers dependent genetic profiles (Fig. 4), completely validated the numerical taxonomic approach based hierarchical classification pattern of the entries. Apparently it is recognizable that only some of the entries were placed around the centroid, while remaining entries were placed far away from the centroid, but the entries were distinctly divided into three major genotypic groups. The composition of these multi-genotypic groups is similar to that deduced from hierarchical classification approach based dendrogram of the entries.

Microsatellite scanning made it possible to discern adequate genetic variation at the molecular level amongst the aromatic rice entries and assisted in unique genotyping. Remarkably greater discrimination ability of microsatellite markers basically due to their polymorphic nature and allelic diversity, as reported earlier by several research workers (Rabbani *et al*., 2010; Behera *et al.*, 2012; Bansal *et al*., 2013; Shamim *et al*., 2016b; Singh *et al.*, 2016; Palanga *et al.*, 2016; Krupa *et al.*, 2017; Kumar *et al*., 2018; Kumari *et al*., 2018a; Priyadarshini *et al*., 2018) and also observed in the present study, indicates their usefulness and robustness in discrimination and unambiguous classification of aromatic rice varieties. Unique or genotype specific allele revealed by microsatellite based analysis of genetic polymorphism could be useful as molecular fingerprints in the identification and preservation of aromatic rice genotypes to benefit farmers, breeders and consumers. The varieties from different groups could be utilized in breeding programs for enhancement of genetic base of aromatic rice germplasm.

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