Received: 21 May 2020 | Revised: 06 May 2021 | Accepted: 07 May 2021

Electronic Journal of Plant Breeding

Research Note

Molecular characterization and genetic diversity analysis of aromatic rice (*Oryza sativa* L.) landraces using SSR markers

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Abstract
The present investigation was conducted at the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara, Thrissur and Regional Agricultural Research Station, Ambalavayal, Wayanad, The study was focused on genetic diversity among popular aromatic rice landraces using Simple Sequence Repeats (SSR) markers. Out of 86 SSR markers used for molecular characterization, 44 markers were polymorphic and remaining 42 were monomorphic. Maximum number of amplicons was exhibited by RM247 with five alleles, followed by RM85, RM251, RM248 and RM493 with four amplicons each. Among 86 SSR markers, 21 markers distinguished Basmati from traditional aromatic landraces of Wayanad viz., Gandhakasala and Jeerakasala. Seven SSR markers distinguished Gandhakasala from Jeerakasala, whereas 23 markers distinguished Basmati from Jeerakasala. Twenty-two markers distinguished Basmati from Gandhakasala and 23 markers distinguished aromatic group from non-aromatic group. Cluster analysis effectively differentiated Basmati, Jeerakasala, Gandhakasala, Uma and Aathira from each other. Among the five clusters formed, cluster III was the largest one comprising all the 12 Gandhakasala morphotypes, followed by cluster IV with all Jeerakasala morphotypes. Cluster I, Cluster II and Cluster V exhibited one genotype each namely Aathira, Uma and Basmati, respectively indicating their genetic distinctness.

Key words
Rice, Aromatic landraces, Genetic diversity, Simple sequence repeat, UPGMA.

Wayanad is a part of the Western Ghats and considered as a “hot-spot” of biodiversity. This district having the maximum tribal population in Kerala and as a part of their rituals, the tribal people conserve many rice landraces. It has been reported that, in Wayanad there were 106 traditional rice varieties, including scented (Jeerakasala and Gandhakasala) and medicinal varieties (Latha *et al.,* 2013). In the recent years, due to change in varietal spectrum and use of paddy fields for non-agricultural purposes, valuable rice germplasm of this region is disappearing fast (Latha *et al*., 2013). Hence, there is an urgent need for characterization and conservation of these traditional landraces.

In the evolution of rice and its genetic differentiation into distinct varietal groups, consumer quality preferences have played a significant role besides agroecological factors. One such varietal group comprising the aromatic/ scented rices of the India are highly priced rices in domestic as well as international markets. Wayanad Jeerakasala rice and Wayanad Gandhakasala rice are the two unique aromatic rices of Wayanad registered as Geographical Indications (GI) from Kerala (Elsy *et al*., 2010; Elsy, 2012). Identification of the above genotypes at molecular level is necessary for their commercial utilization. Characterization of these cultivars based on phenotype has limitations since most of the morphological

characters are greatly influenced by environmental factors and developmental stage of the plant. In contrast to morphological characters, molecular markers can reveal the abundant difference among genotypes at DNA level, providing a more direct, reliable and efficient tool for varietal characterization (Prabakaran *et al*., 2010). Thus molecular characterization can reveal the genetic identity of these rices registered as Geographical Indications in India. Characterization of aromatic genotypes at molecular level is more important for the commercial identification of their genuine goods.

Assessment of genetic diversity is very important in rice breeding from the standpoint of selection and conservation of different landraces for further utilization in crop improvement programmes (Patra, 2000). The landraces are valuable as they possess a huge treasure of genetic material which may prove important in future variety development programmes. Hence this study focussed on the collection and characterization of popular aromatic landraces of Wayanad at molecular level.

The experimental material comprised of 18 genotypes of rice, including the 12 Gandhakasala types, three Jeerakasala types collected from different parts of Wayanad district and three check varieties, including one aromatic variety (Basmati) and two non-aromatic varieties (Uma and Aathira). The experiment was conducted during 2027-18 and details of genotypes used in the study are given in **Table 1**.

All the landraces were grown in pots and 20-25 days older seedlings were used in order to get the sufficient leaf material for isolation of genomic DNA by following the protocol described for CTAB method (Dellaporta *et al*., 1983). A total of 86 SSR markers, including 64 hypervariable SSR markers available at www.gramene.org. (**Table 2**) and 22 aroma specific SSR markers were used for SSR profiling (**Table 3**). Clear DNA bands of various molecular weights were scored manually for the presence of band in a particular base pair position and scored as '1' (one) and the absence of band at that particular base pair position was scored as '0' (zero) respectively. Each marker was individually scored and binary matrix was prepared using Excel sheet. This data matrix was subjected to analysis using NTSYS (Numerical Taxonomy and Multivariate Analysis System) version 2.1 (Rohlf, 2000).

The genetic association among the genotypes were estimated by Jaccard's similarity coefficients (Jaccard, 1908) using NTSYS. The Dendrogram was constructed by using UPGA clustering method based on Jaccard's similarity coefficient values.

'Basmati' is a long grained fine aromatic rice grown in Indo-Gangetic plains. Agro-climatic conditions of the specific geographical area leads to its superior aroma making it unique among other aromatic rice varieties of the country. Molecular characterization of aromatic rice genotypes of Wayanad by SSR profiling revealed high level of genetic polymorphism among the genotypes studied. Out of 86 SSR markers used for molecular characterization, 44 markers were polymorphic and remaining 42 were monomorphic (**Fig 1**). The profiling with different markers revealed the presence of amplicons ranging from 63 bp (RM248) to 518 bp (RM18941) in size. Maximum number (5) of amplicons was exhibited by RM247, followed by RM85, RM251, RM248 and RM493 producing 4 amplicons each, indicating the informative nature of these SRR markers in polymorphism study. More number of amplicons in case of a few SSR markers indicated that, the genotypes under the study are genetically diverse at the particular marker locus. The various sized amplicons observed relate to the allelic diversity at the gene or marker locus. Sajib *et al.* (2012) studied polymorphism in 12 aromatic rice genotypes and reported similar results for SSR marker RM247. Ashraf*et al*. (2016) studied genetic diversity analysis of 18 aromatic rice genotypes using 24 SSR markers and reported variation in the number of amplicons ranging from 2 to 6. Diagramatic representation of polymorphism between 18 rice genotypes in SSR profiling are given in **Fig. 2.**

Table 1 List of rice genotypes used for molecular characterization

S. No.	Genotype	S. No.	Genotype
1	Gandhakasala-1	10	Gandhakasala-10
2	Gandhakasala-2	11	Gandhakasala-11
3	Gandhakasala-3	12	Gandhakasala-12
4	Gandhakasala-4	13	Jeerakasala-1
5	Gandhakasala-5	14	Jeerakasala-2
6	Gandhakasala-6	15	Jeerakasala-3
7	Gandhakasala-7	16	Basmati (aromatic check variety)
8	Gandhakasala-8	17	Uma (non-aromatic check variety)
9	Gandhakasala-9	18	Aathira (non-aromatic check variety)

Table 2. List of hypervariable SSR markers used for molecular characterization

SSR Primer	Chromosome number	Primer sequences	
		Forward sequence	Reverse sequence
RM1	1	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC
RM490	1	ATCTGCACACTGCAAACACC	AGCAAGCAGTGCTTTCAGAG
RM11069	1	GGTACAATGAAGCTTGGCAACG	CGGTGGAGTAGAACCACGAAGC
RM11313	1	TGAGGCTGATAGAAAGCAGAATGC	CCCGTTTCTTCCATATCATGTCG
RM233	1	CCAAATGAACCTACATGTTG	GCATTGCAGACAGCTATTGA
RM250	2	GTTCAAACCAAGCTGATCACAAGC	GGCGTCAGAGTCAGAGATGAAGG
RM482	2	TCTGAAAGCCTGACTCATCG	GTCAATTGCAGTGCCCTTTC
RM12941	2	TTATGCCATGTGGTCCAATCAGC	ATTTGAACCATTTGGGCCTTGG
RM13599	2	GTTCATGGCACTCCTCTCCTAGC	GAGGAATGAACAGTGCCTACACG
RM13910	2	GAGCGAGCTATACCACCGTGACC	ATCGCGTCCAAGAAAGGTGTCG
RM16	3	GTGCGCCAGGAGTAGTTGTCTCC	GACGTGTACACATAGCCAAATCATCC
RM60	3	CAAGTTCACCCGCCTTCTCG	TTTCCATCATTAGCAGGCAGTAGC
RM85	3	CCAAAGATGAAACCTGGATTG	GCACAAGGTGAGCAGTCC
RM251	3	GAATGGCAATGGCGCTAG	ATGCGGTTCAAGATTCGATC
RM411	3	ACACCAACTCTTGCCTGCAT	TGAAGCAAAAACATGGCTAGG
RM14723	3	GCAAAGTCCTTTGGACAGGTAGC	CGTCCCAGATCAAAGTACACTCTTCC
RM307	4	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC
RM5586	4	AGATGGCTGGCCAACAGACTGG	ACAATGCCCATCCACTGCTTCC
RM13	5	TCCAACATGGCAAGAGAGAG	GGTGGCATTCGATTCCAG
RM110	5	TCGAAGCCATCCACCAACGAAG	TCCGTACGCCGACGAGGTCGAG
RM163	5	ATCCATGTGCGCCTTTATGAGGA	CGCTACCTCCTTCACTTACTAGT
RM18622	5	GGCATGCATGTGTCTAACATTCG	AAGCAGAATTTGGCCGTGTTAGC
RM18941	5	GTGAAGTGCAGCCGAAGAGC	ATCGATCTCTCATCACGATCAACC
RM217	6	ATCGCAGCAATGCCTCGT	GGGTGTGAACAAAGACAC
RM238	6	GATGGAAAGCACGTGCACTA	ACAGGCAATCCGTAGACTCG
RM253	6	TCCTTCAAGAGTGCAAAACC	GCATTGTCATGTCGAAGCC
RM340	6	GGTAAATGGACAATCCTATGGC	GACAAATATAAGGGCAGTGTGC
RM402	6	GAGCCATGGAAAGATGCATG	TCAGCTGGCCTATGACAATG
RM541	6	TATAACCGACCTCAGTGCCC	CCTTACTCCCATGCCATGAG
RM18	7	TTCCCTCTCATGAGCTCCAT	GAGTGCCTGGCGCTGTAC
RM214	7	CTGATGATAGAAACCTCTTCTC	AAGAACAGCTGACTTCACAA
RM248	7	TCCTTGTGAAATCTGGTCCC	GTAGCCTAGCATGGTGCATG
RM295	7	CGAGACGAGCATCGGATAAG	GATCTGGTGGAGGGGAGG
RM25	8	GGAAAGAATGATCTTTTCATGG	CTACCATCAAAACCAATGTTC
RM72	8	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGGG
RM223	8	GAGTGAGCTTGGGCTGAAAC	GAAGGCAAGTCTTGGCACTG
RM264	8	GTTGCGTCCTACTGCTACTTC	GATCCGTGTCGATGATTAGC
RM5556	8	GTAAGCCATTTGCACGGACAAGG	GAGCTCAGGATCATCCCTACATGC
RM23087	8	GATATTAGCTAGACATGGCACTCTGC	GTACATCCGCATGAATAGAGTGG
RM205	9	CTGGTTCTGTATGGGAGCAG	CTGGCCCTTCACGTTTCAGTG
RM266	9	GATGGTAAAGGAAGAACGTGTGC	CACTCATAGACGCATCACATAGCC
RM257	9	CAGTTCCGAGCAAGAGTACTC	GGATCGGACGTGGCATATG
RM524	9	ATCATAGCCCAGACCAAGAATGC	AGATGAAGAGCAGGAACCGTAGG
RM23998	9	CTGCACGTACGGTCAAGTCTACC	GCATTGCAAGGGTTGAAGTGG
RM216	10	GATGGTAAAGGAAGAACGTGTGC	CACTCATAGACGCATCACATAGCC
RM222	10	CTTAAATGGGCCACATGCG	CAAAGCTTCCGGCCAAAAG
RM271	10	TCAGATCTACAATTCCATCC	TCGGTGAGACCTAGAGAGCC
RM304	10	TCAAACCGGCACATATAAGAC	GATAGGGAGCTGAAGGAGATG
RM333	10	GTACGACTACGAGTGTCACCAA	GTCTTCGCGATCACTCGC
RM24866	10	CCCTTTCATTTGCGCTTTATGG	GGGTTATTTCAGTCCGTGATTGC
RM25066	10	GTTGTTAGGTGTAGCCGTGTAGG	GTACACCAATAACTGTGGAAGAGC
RM21	11	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG
RM202	11	TGGAACACCCATAGACAAACAGC	TGGCAAGTGGTATTCTTCCTTCC
RM224	11	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG
RM254	11	AGCCCCGAATAAATCCACCT	CTGGAGGAGCATTTGGTAGC
RM332	11	GAAGGCGAAGGTGAAGAAGAAGC	CCTCCCTTGCATGATACCTTGG
RM26213	11	GCCACAGGAGACAGCAAGAACC	CGATCCAATTCCAGCCTAGATAGC
RM17	12	TGCCCTGTTATTTTCTTCTCTC	GGTGATCCTTTCCCATTTCA
RM19	12	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA
RM20	12	ATCTTGTCCCTGCAGGTCAT	GAAACAGAGGCACATTTCATTG
RM247	12	TAGTGCCGATCGATGTAACG	CATATGGTTTTGACAAAGCG
RM260	12	ACTCCACTATGACCCAGAG	GAACAATCCCTTCTACGATCG
RM27841	12	TAAATACCCGACAATGCCCTAGC	GGAAATCCCATCAATCACAAGAGC
RM28277	12	TGCACCACCTATTTCAATCCACTCC	CCTTCCTCAAGGGAAATCACAGAAGC

Table 3. List of aroma specific SSR markers used for molecular characterization

SSR Primer	Primer sequences	
	Forward sequence	Reverse sequence
RM9	GGTGCCATTGTCGTCCTC	ACGGCCCTCATCACCTTC
RM180	CTACATCGGCTTAGGTGTAGCAACACG	ACTTGCTCTACTTGTGGTGAGGGACTG
RM215	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG
RM228	CTGGCCATTAGTCCTTGG	GCTTGCGGCTCTGCTTAC
RM243	GATCTGCAGACTGCAGTTGC	AGCTGCAACGATGTTGTCC
RM245	ATGCCGCCAGTGAATAGC	CTGAGAATCCAATTATCTGGGG
RM249	GGCGTAAAGGTTTTGCATGT	ATGATGCCATGAAGGTCAGC
RM256	GACAGGGAGTGATTGAAGGC	GTTGATTTCGCCAAGGGC
RM288	CCGGTCAGTTCAAGCTCTG	ACGTACGGACGTGACGAC
RM302	TCATGTCATCTACCATCACAC	ATGGAGAAGATGGAATACTTGC
RM323	CAACGAGCAAATCAGGTCAG	GTTTTGATCCTAAGGCTGCTG
RM335	GTACACACCCACATCGAGAAG	GCTCTATGCGAGTATCCATGG
RM338	CACAGGAGCAGGAGAAGAGC	GGCAAACCGATCACTCAGTC
RM410	GCTCAACGTTTCGTTCCTG	GAAGATGCGTAAAGTGAACGG
RM433	TGCGCTGAACTAAACACAGC	AGACAAACCTGGCCATTCAC
RM444	GCTCCACCTGCTTAAGCATC	TGAAGACCATGTTCTGCAGG
RM484	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC
RM493	TAGCTCCAACAGGATCGACC	GTACGTAAACGCGGAAGGTG
RM510	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTC
RM535	ACTACATACACGGCCCTTGC	CTACGTGGACACCGTCACAC
RM566	ACCCAACTACGATCAGCTCG	CTCCAGGAACACGCTCTTTC
RM590	CATCTCCGCTCTCCATGC	GGAGTTGGGGTCTTGTTCG

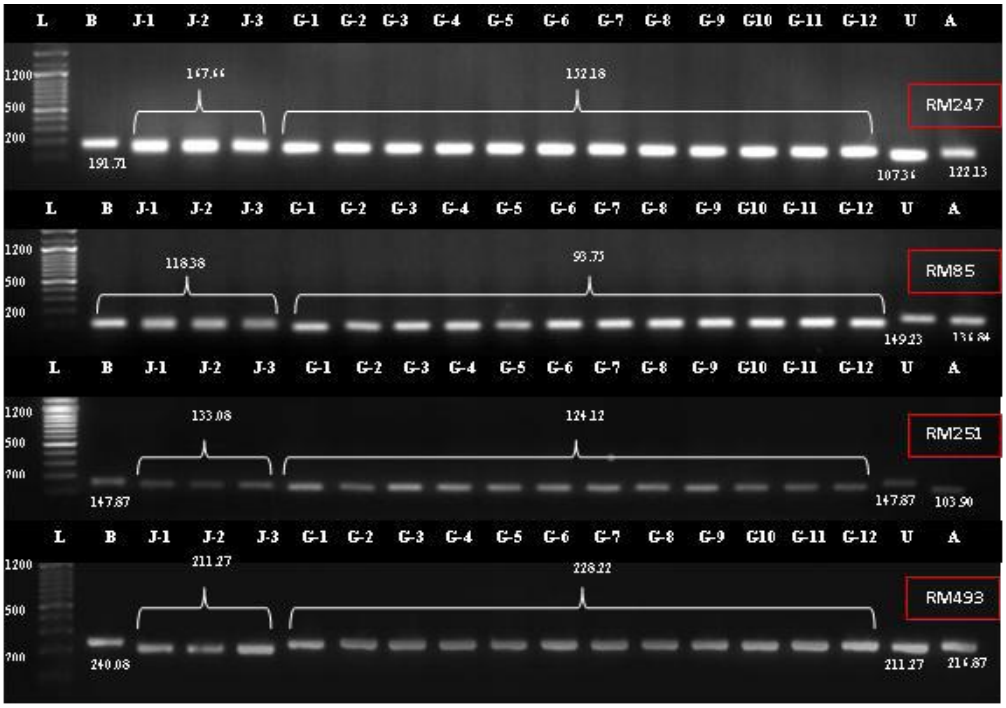

Fig.1. Allelic variation exhibited by polymorphic markers

B- Basmati, J- Jeerakasala, G- Gandhakasala, U- Uma, A- Aathira.

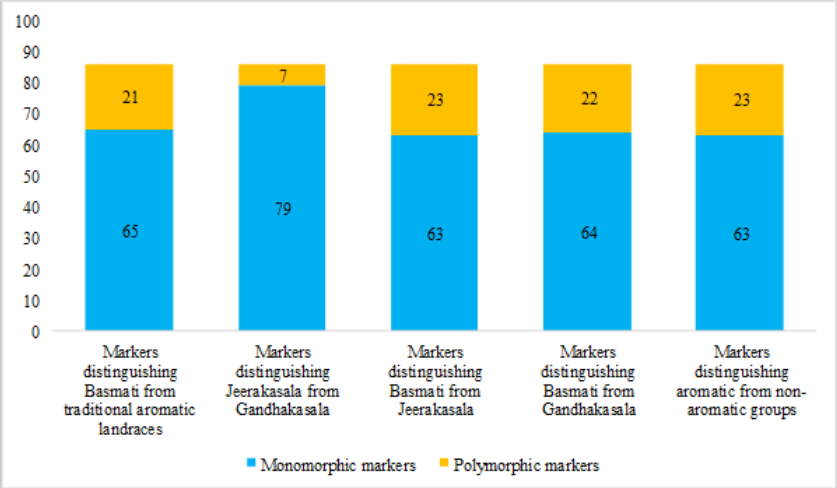

Fig. 2. Polymorphism between 18 genotypes in SSR profiling

The genetic association among the genotypes were estimated by Jaccard's similarity coefficients (Jaccard, 1908) using NTSYS (Numerical Taxonomy and Multivariate Analysis System) software version 2.1 (Rohlf, 2000). The Jaccard's similarity coefficient values obtained are presented in Table 4. The Dendrogram was constructed by using UPGA clustering method based on Jaccard's similarity coefficient values (**Fig.3**).

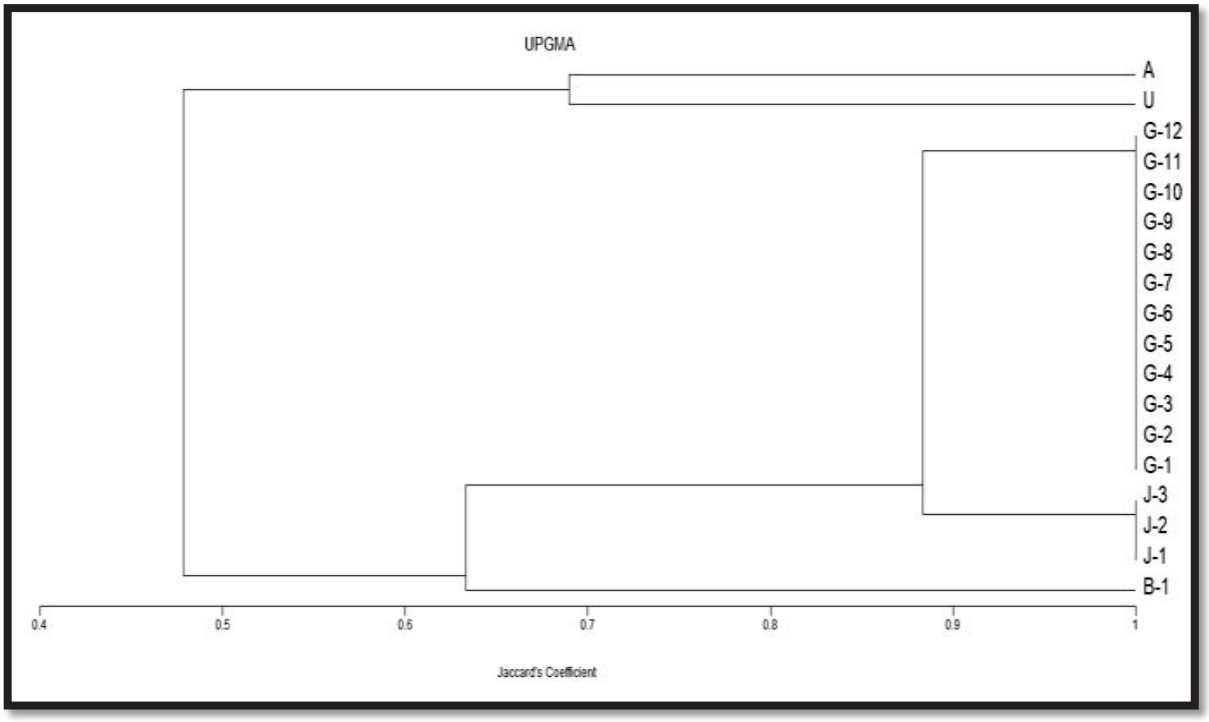

Fig. 3. Dendrogram based on similarity coefficients among 18 rice genotypes
(**A-**Aathira, **U**-Uma, **G**-Gandhakasala, **J**-Jeerakasala, **B**-Basmati).

Table 4. Jaccard's similarity coefficient matrix for 18 ricegenotypes

	B-1	J-1	J-2	J-3	G-1	G-2	G-3	G-4	G-5	G-6	G-7	G-8	G-9	G-10	G-11	G-12	U	A
B-1	1.00																	
J-1	0.63	1.00																
J-2	0.63	1.00	1.00															
J-3	0.63	1.00	1.00	1.00														
G-1	0.63	0.88	0.88	0.88	1.00													
G-2	0.63	0.88	0.88	0.88	1.00	1.00												
G-3	0.63	0.88	0.88	0.88	1.00	1.00	1.00											
G-4	0.63	0.88	0.88	0.88	1.00	1.00	1.00	1.00										
G-5	0.63	0.88	0.88	0.88	1.00	1.00	1.00	1.00	1.00									
G-6	0.63	0.88	0.88	0.88	1.00	1.00	1.00	1.00	1.00	1.00								
G-7	0.63	0.88	0.88	0.88	1.00	1.00	1.00	1.00	1.00	1.00	1.00							
G-8	0.63	0.88	0.88	0.88	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00						
G-9	0.63	0.88	0.88	0.88	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00					
G-10	0.63	0.88	0.88	0.88	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
G-11	0.63	0.88	0.88	0.88	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
G-12	0.63	0.88	0.88	0.88	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
U	0.60	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	1.00	
A	0.52	0.50	0.50	0.50	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.69	1.00

B- Basmati, **J-** Jeerakasala, **G-** Gandhakasala, **U-** Uma, **A-** Aathira.

Similarity coefficient ranged between 1.00 and 0.46. Maximum similarity coefficient (1.00) was exhibited within all the Jeerakasala morphotypes and all the Gandhakasala morphotypes. The lowest similarity coefficient (0.46) was exhibited between Uma and all other genotypes except Basmati. It indicated that, the selected morphotypes of all Gandhakasala were genetically similar and but distinct from the selected morphotypes of Jeerakasala, which in turn were genetically similar among themselves. The lowest similarity coefficient (0.49) was exhibited between Uma and all other genotypes except Basmati.

Among the aromatic genotypes (Basmati, morphotypes of Jeerakasala and morphotypes of Gandhakasala), maximum similarity coefficient (0.88) was recorded between morphotypes of Jeerakasala and morphotypes of Gandhakasala, whereas comparatively lower similarity coefficient (0.63) was recorded between Basmati and non-Basmati traditional landraces (morphotypes of Jeerakasala and morphotypes of Gandhakasala). It clearly indicated that, the traditional aromatic landraces of Wayanad were distinct from Basmati. The comparative proximity of Jeerakasala and Gandhakasala may be due to the same geographical origin of Jeerakasala and Gandhakasala. All the aromatic genotypes (Basmati, morphotypes of Jeerakasala and morphotypes of Gandhakasala) were distinct from non-aromatic genotypes (Uma and Aathira). Pervaiz *et al.* (2009) has done diversity analysis in aromatic and non-aromatic genotypes using SSR markers and reported that the similarity coefficient ranged between 0.19 to 0.90.

Cluster analysis based on UPGMA categorized 18 genotypes including three check varieties into five

Table 5. Clustering of rice genotypes based on molecular characterization

Cluster No.	No. of genotypes	Genotype
Cluster I	1	Aathira
Cluster II	1	Uma
Cluster III	12	G-1, G-2, G-3, G-4, G-5, G-6, G-7, G-8, G-9, G-10, G-11, G-12
Cluster IV	3	J-1, J-2, J-3
Cluster V	1	Basmati

(G- Gandhakasala, J- Jeerakasala)

clusters at 60 per cent similarity level (**Table 5**). Among the five clusters, cluster III was the largest comprising 12 Gandhakasala morphotypes namely G-1, G-2, G-3, G-4, G-5, G-6, G-7, G-8, G-9, G-10, G-11 and G-12, followed by cluster IV with three morphotypes of Jeerakasala (J-1, J-2 and J-3) and Cluster I, Cluster II and Cluster V exhibiting one genotype each namely Aathira, Uma and Basmati.

The results of cluster analysis effectively revealed the uniqueness of Basmati, Jeerakasala, Gandhakasala, Uma and Aathira from each other. Even though the fine grained aromatic variety Basmati exhibited 63 per cent similarity with Gandhakasala and Jeerakasala, it is still different from these traditional aromatic landraces, indicated by forming separate cluster. Hossain *et al.* (2007) studied genetic diversity in aromatic and non-aromatic landraces and reported separate cluster for Basmati type. All the Gandhakasala morphotypes were grouped under same cluster (cluster III); similarly all the Jeerakasala morphotypes were grouped under same cluster (cluster IV), indicating 100 per cent similarity within them. The non-aromatic genotypes (Uma and Aathira) were separated from all the aromatic genotypes, and grouped under separate clusters individually, indicating less similarity between these non-aromatic genotypes. Genetic diversity in Basmati and non-basmati aromatic rice genotypes using SSR markers revealed higher similarity coefficient in aromatic genotypes as compared to non-aromatic genotypes (Shah *et al*., 2013)

Genetic diversity is very important in rice breeding from the standpoint of selection and conservation of different landraces for further utilization in crop improvement programmes. The present investigation revealed that, SSR markers provide adequate power of resolution to distinguish Basmati from traditional aromatic rices of Wayanad (Gandhakasala and Jeerakasala) and it could also serve as a potential tool for the maintenance of purity of these traditional aromatic rices.

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