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Full Length Research Paper

Assessment of genetic diversity of non-basmati rice of Jammu and Kashmir using microsatellite markers

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Rice (*Oryza sativa* L.) is one of the oldest domesticated crop species in the world and is widely recognized as an ideal model plant for the study of grass genetics and genome organization. Rice improvement depends on the conserved use of genetic variability and diversity in plant breeding programmes. Phenotypic variability and genetic diversity was studied in 13 non basmati rice samples collected from Jammu and Kashmir. The length of the seeds varied from 0.5 to 0.8 cm and the seed width varied from 0.1 to 0.3 cm. The weight of the seed varied from 0.36 to 0.77 g. A total of 94 alleles were amplified in 13 rice samples using six simple sequence repeat (SSR) markers. The analysis and dendrogram construction was performed using the DARWin 5.0 software. The genetic dissimilarity index calculated between samples ranged from 0.00 to 0.63. The generated dendrogram based on the dissimilarity matrix using the neighbor-joining approach of the unweighted pair group with arithmetic mean (UPGMA) method showed three distinct clusters.

Key words: *Oryza sativa*, diversity, characterization, molecular markers.

INTRODUCTION

Rice (*Oryza sativa*), one of the agronomically and nutritionally important cereal crop in the grass family (Poaceae), is the principal staple food in developing countries. It is no longer a luxury food, but has become the cereal that constitutes a major source of calories for the urban and the rural dwellers (Sasaki and Burr, 2000). In the mid-1960s, the Green revolution resulted in a dramatic increase in food production as a result of a combination of advances in plant breeding and production-oriented intensification of agriculture, stimulated by the implementtation of enabling policies. This technological revolution integrated the development and use of modern high-yielding varieties and led to an increase in both level and efficiency of agricultural production.

Rice plays a pivotal role in Indian economy, being the staple food for two third of the population. However, its productivity per unit area compared to the world average is low. This may possibly be due to narrow range of genetic variability and lack of adequate genetic information regarding the inheritance of quantitative traits controlling important economic characters. Molecular markers are powerful tools in the assessment of genetic variation and in the elucidation of genetic relationships within and among species (Thomson et al., 2007). Among different polymerase chain reaction (PCR) based markers, microsatellites are highly polymorphic, more reproducible, co-dominant and distributed throughout the genome. More than 2200 microsatellite markers have been mapped to specific locations in rice genome (McCouch et al., 2002). These markers have been utilized for many purposes, including genome mapping, gene tagging, estimation of genetic diversity, varietal differentiation and purity testing (Nagaraju et al., 2002).

In order to increase rice productivity, high yielding and disease resistant varieties should be developed. Unlike high-yielding varieties (whose variability is limited due to homozygosity), the landraces maintained by farmers are endowed with tremendous genetic variability as they are not subjected to subtle selection over a long period of time. Further crop improvement depends on the conserved

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Table 1. List of SSR markers of rice.

Oligomers	Molecular weight	Size range	Tm	Sequence 5′ - 3′
RM 223F	6222	139-163	63.9	GAGTGAGCTTGGGCTGAAAC
RM 223R	6182		64.9	GAAGGCAAGTCTTGGCACTG
RM 210F	5505	141-165	64.4	TCACATTCGGTGGCATTG
RM 210R	6179		63.1	CGAGGATGGTTGTTCACTTG
RM 284F	6565	141-149	60.7	ATCTCTGATACTCCATCCATCC
RM 284R	6093		64.5	CCTGTACGTTGATCCGAAGC
RM 85F	6463	85-107	63.1	CCAAAGATGAAACCTGGATTG
RM 85R	5534		62.2	GCACAAGGTGAGCAGTCC
RM 42F	6062	161-171	64.0	ATCCTACCGCTGACCATGAG
RM 42R	6139		64.0	TTTGGTCTACGTGGCGTACA
RM515F	6240	205-231	63.9	TAGGACGACCAAAGGGTGAG
RM515R	5962		64.0	TGGCCTCCTCTCTCTCTCTCTC

use of genetic variability and diversity in plant breeding programmes and the use of new biotechnological tools. There is a wide genetic variability in varieties leaving a wide scope for future crop improvement.

MATERIALS AND METHODS

Plant material and growth experiment

The experimental material consisted of 13 cultivated non-basmati varieties of rice (*O. sativa*). Seeds of these varieties were collected from different areas of Jammu and Kashmir. The varieties collected were from Jammu (KJ-1, KJ-2 and JJ); Baderwah (Chi and Jap); Rajouri (Ro and Gi), Samba (SS); Udhampur (S-1 and S-2), Kathua (Kat), Mera Sahib (MS) and Kashmir (Kas). Since the temperature conditions were unfavorable in January (9 - 10°C) for the growth of rice plants in field conditions, the plants were therefore grown in laboratory conditions. For this, 50 seeds from each collected rice samples were taken and soaked in water overnight for 24 h. Afterward, the seeds were placed in sterilized Petri plates (50 seeds/per plate) on a wet sterilized filter paper soaked in distilled water. The Petri plates were then kept in an incubator at 25°C for 3 days till the seeds germinated. Subsequently, the germinated seedlings were transferred from the Petri plates to the small plastic pots for their further growth to young plantlets.

Phenotypic evaluation of rice grain

Twenty rice seeds taken from thirteen non-basmati samples were dehulled and their length and width, weight, ratio of length: width and variation in colour were calculated.

Molecular marker analysis

DNA isolation was carried out using cetyltrimethylammonium bromide (CTAB) method as modified by Saghai-Maroof et al. (1984). Six simple sequence repeat (SSR) markers RM223, RM210, RM284, RM42, RM85 and RM515 were selected from the linkage map of Temnykh et al. (2000) to study the diversity (Table 1). The PCR reaction mixture contained 75 ng template DNA, 12.3 µL ddH2O, 2.5 µl 10X PCR buffer, 2.5 µl of 100 mM dNTPs, 1 µl of 5 µM forward and reverse primer each and 0.3 µL Taq polymerase (5 U/µl). Template DNA was initially denatured at 94°C for 4 min followed by 35 cycles of PCR amplification following: 1 min of denaturation at 94°C, 1 min of primer annealing at 55°C and 2 min of primer extension at 72°C. Final 7 min incubation at 72°C was allowed to complete primer extension. The amplified products were electrophoretically resolved on a 2.5% agarose gel in 0.5X Tris-acetate-EDTA (TAE) and visualized under UV light after staining with 0.1% ethidium bromide. The bands representing particular alleles at the microsatellite loci were scored manually.

Data collection and diversity analysis

To measure the allelic diversity of the SSR markers, the polymorphism information content (PIC) for each marker was calculated according to the formula: $PIC = 1 - \Sigma(Pi\ j)^2$, where Pi j is the frequency of the ith allele in the jth population for each SSR locus (Botstein et al., 1980). The dissimilarity matrix was used for clustering of genotypes based on the unweighted neighbor-joining method and the analysis was performed using DARWin 5.0 (http://darwin.cirad.fr), (Perrier et al., 2003). Confidence limits of different clades were tested by bootstrapping 500 times to assess the repetitiveness of genotype clustering (Felsenstein, 1985).

RESULTS AND DISCUSSION

Fifty seeds from each non basmati rice sample were kept in plates with moist sterilized filter paper at different growth conditions. The seeds showed no germination when grown at room temperature (9 to 10°C in January) at 24 h, however, when grown at 25°C it showed growth of varying degrees after 48 h. The samples showed best

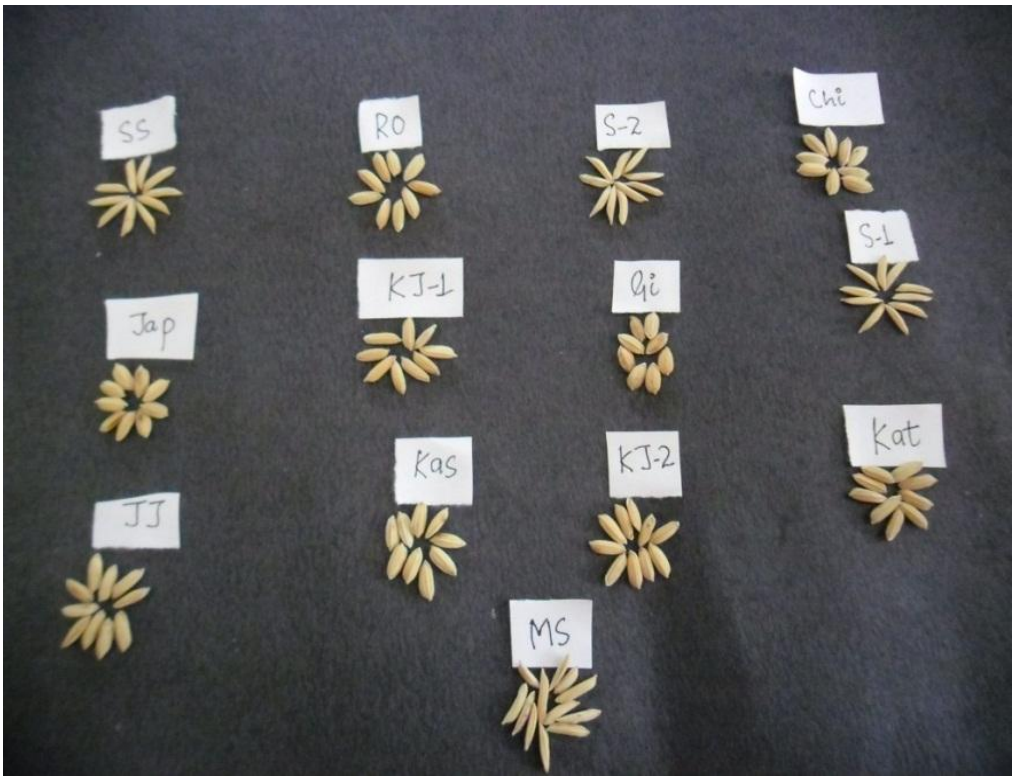

Figure 1. Shapes and sizes of different rice samples collected from Jammu and Kashmir.

germination at 30°C after 3 days of incubation.

Phenotypic evaluation of rice seeds

The rice genetic resources showed a great diversity for all the measured grain morphological characters (Figure 1). The variation for grain length ranged from 0.5 to 0.8 cm (Table 2 and Figure 2). The longest grain size was for sample SS and smallest was for Jap and Gi. The variation for grain width ranged from 0.1 to 0.3 cm, with the largest grain width for sample Jap and smallest for SS and S-1. The length to width ratio varied from 8.0 (for SS and S-1) to 1.83 (for Jap and Gi). The grain weight varied from 0.36 to 0.77 g for Kat and SS, respectively (Table 2). The long grain types were present in few locations (on the basis of seed length), whereas maximum medium length grain types were recorded while, the short grain types were not found.

On the basis of length to width ratio, long and medium grain types were found distributed in samples of all locations. However, the short grain types were only recorded in samples from the region of Bhaderwah, Rajouri and Kashmir. Samples from Udhampur and Samba showed the highest grain length and length to width ratio. Samples collected from Jammu showed comparatively little shorter grain length than those collected from Udhampur, but the grain size was larger than the rice samples collected from Kathua (Figure 1). It was observed that the longer grains had less width. The J&K rice cultivars with more width (0.3 cm or more) were shorter in length and samples with less width (0.1 cm) were longer in length. Similar results in seed shape variation were also found by Siddiqui et al. (2007).

Molecular analysis

Six microsatellite markers were chosen from Temynk et al. (2000) for diversity analysis of 13 non-basmati rice samples. The PIC value of each marker ranged from 0.6 to 0.9. The amplified product was scored on the basis of presence and absence of bands (Figure 3). A total of 94 alleles were amplified in 13 rice samples using 6 SSRs. Out of 94 alleles, 26 were monomorphic and the rest 68 were polymorphic. SSR markers RM 85 and RM 210 amplified all monomorphic alleles and marker RM 515, RM 223, RM42, RM 284 resulted in polymorphic alleles. The number of alleles generated by each marker varied from two (RM 284, RM 42 and RM 515) to three (RM 223). The number of alleles observed in the present study corresponded well with the earlier report of Siwach et al. (2004) among basmati and non-basmati rice varieties from India.

PIC is the reflection of allele diversity and frequency among the varieties and varied greatly for all SSR loci

Table 2. Phenotypic variation in average length, width, ratio of length: width, weight and seed colour in 13 non-basmati samples.

S/N	Variety code	Place of collection	Average weight (g)	Average length (cm)	Average width (cm)	Ratio length/ width (cm)	Colour
1	Kat	Kathua	0.77	0.60	0.20	3.0	Dark brown
2	(RO)	Rajouri	0.54	0.60	0.20	3.0	Light greenish
3	(Gi)	Rajouri	0.66	0.55	0.30	1.8	Golden brown
4	(KJ-1)	Jammu	0.62	0.70	0.20	3.5	Golden brown
5	Jap	Bhaderwah	0.71	0.55	0.30	1.8	Straw golden
6	Kas	Kashmir	0.68	0.70	0.25	2.8	Golden brown
7	S-1	Udhampur	0.58	0.80	0.10	8.0	Bright golden
8	Chi	Bhaderwah	0.76	0.60	0.25	2.4	Light brown
9	S-2	Udhampur	0.62	0.75	0.15	5.0	Light Golden
10	KJ-2	Jammu	0.50	0.65	0.20	3.2	Golden brown
11	SS	Samba	0.36	0.80	0.10	8.0	Straw golden
12	JJ	Jammu	0.59	0.60	0.20	3.0	Reddish brown
13	MS	Mera Sahib	0.40	0.65	0.15	4.3	Light golden

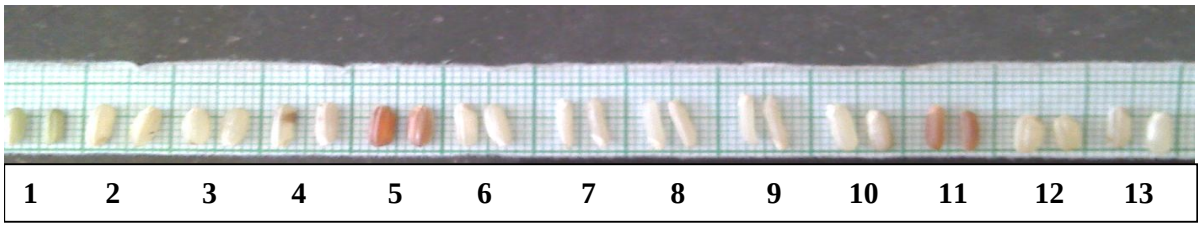

Figure 2. Length and width of different rice samples (1= Ro, 2= Kas, 3=Gi, 4=JJ, 5=KJ-2, 6=KJ-1, 7=SS, 8=S-2, 9=S-1, 10=Kat, 11=MS, 12=Jap, 13=Chi).

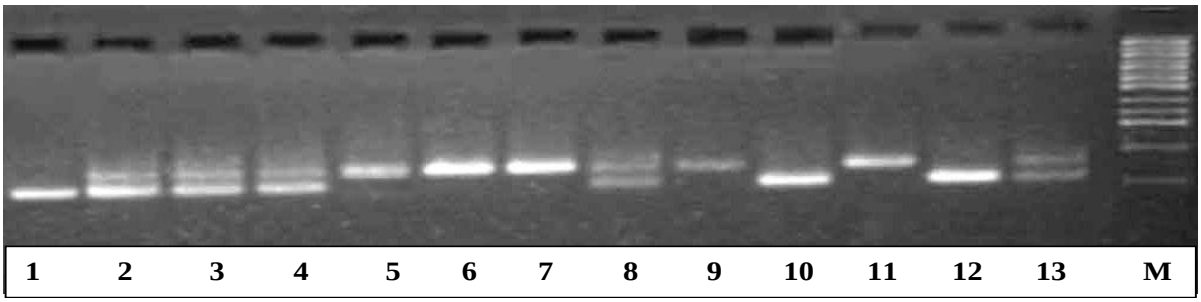

Figure 3. PCR amplification of thirteen non Basmati Rice samples with SSR marker RM 284. 1=Kat, 2= Ro, 3= Gi, 4= KJ-1, 5= Jap, 6= Kas, 7= S-1, 8= Chi, 9= S-2, 10= KJ-2, 11= SS, 12= JJ, 13=MS, M= Marker (250 bp).

tested. The PIC values varied from 0.34 (RM 42) to 0.72 (RM 223), whereas PIC values varied from 0.259 to 0.782 with an average of 0.571 in the studies conducted in basmati and non basmati rice of Pakistan by Rabbani et al. (2010). Similarly, the PIC values ranged from 0.09 to 0.60 and the overall frequency of biallelic markers was high in the rice landraces collected from Tamil Nadu (Vanniarajan et al., 2012). The lower level of polymorphism may be attributed to a narrow genetic diversity as some of the local varieties are product of the same location Ablett et al. (2006) stated that though SSRs have a potentially high polymorphic frequency, the level of polymorphism was found to be 26% when tested in 10 wheat varieties using 126 markers.

Analysis of genetic dissimilarity

Genetic dissimilarity was calculated from the matrix of binary data using software DARWin 5.0, where “0” and “1” were standardized as the least and maximum of dissimilarity respectively. To ascertain the statistical

Table 3. Diversity matrix between 13 different non-Basmati rice samples as revealed by SSR markers.

	1	2	3	4	5	6	7	8	9	10	11	12
2	0.45											
3	0.27	0.16										
4	0.63	0.16	0.33									
5	0.38	0.14	0.14	0.28								
6	0.45	0.00	0.16	0.16	0.14							
7	0.50	0.07	0.23	0.23	0.06	0.07						
8	0.00	0.45	0.27	0.63	0.38	0.45	0.50					
9	0.33	0.23	0.38	0.38	0.20	0.23	0.14	0.33				
10	0.40	0.09	0.27	0.27	0.23	0.09	0.16	0.40	0.33			
11	0.63	0.16	0.33	0.00	0.28	0.16	0.23	0.63	0.38	0.27		
12	0.63	0.16	0.33	0.00	0.28	0.16	0.23	0.63	0.38	0.27	0.00	
13	0.45	0.00	0.16	0.16	1.14	0.00	0.07	0.45	0.23	0.09	0.16	0.1

1=Kat, 2= Ro, 3= Gi, 4= KJ-1, 5= Jap, 6= Kas, 7= S-1, 8= Chi, 9= S-2, 10= KJ-2, 11= SS, 12= JJ, 13=MS.

strength of genetic relationships identified through this analysis, bootstrapping of the data (500 permutations) was performed. The dissimilarity coefficients were used for cluster analysis based on the unweighted neighbor-joining method and a dendrogram was generated with the aim of analyzing the relationship between rice samples. The genetic dissimilarity index calculated between samples ranged from 0.00 to 0.63 (Table 3). The lowest value 0.1 was obtained between Jaya (JJ) collected from Jammu and MS collected from Mera Sahib, while the highest dissimilarity value calculated was 0.63 between the samples collected from Jammu (K -448) and China (Chi) from Bhaderwah.

Cluster analysis

The tree generated showed no divergence between samples collected from Samba and Jammu (Figure 4). A dendrogram was generated by unweighted pair group with arithmetic mean (UPGMA) to show the genetic relationships of the samples studied and is presented in Figure 5. The dendrogram indicated that the samples collected from the same region clustered together and were clearly separated into three distinct clusters:

1. Cluster I contained samples collected from Kashmir, Jammu and Mera Sahib
2. Cluster 2 contained samples from Rajouri, Udhampur (Sharbati-1) and Bhaderwah
3. Cluster 3 contained a single rice sample collected from Udhampur (Sharbati-2)

Though both the samples of Sharbati were collected from Udhampur, the difference could be due to mixing of seeds or because it is a new or different variety (Figure 5). Adéoti et al. (2011) also reported the genetic diversity based on UPGMA cluster analysis of dissimilarity data in different accessions of leafy vegetables and observed the coefficient of genetic dissimilarity between 0.01 and 0.63. Similarly, Sharma et al. (2010) reported cluster analysis using Rogers' genetic distance in different maize accessions. Their results revealed distinct genetic identity of the 'Sikkim Primitives' from the rest of the accessions in India, including Sikkim.

Conclusion

The characterization and quantification of genetic diversity within closely related crop germplasm has long been a major goal, as it is essential for a rational use of genetic resources.

In all, the analysis of genetic variation among breeding materials is of fundamental interest to plant breeders as it contributes immensely to selection, monitoring of germplasm, and also prediction of potential genetic gains (Chakravarti and Rambabu, 2006).

Although a large number of aromatic and coarse cultivars of rice are available in Jammu and Kashmir, no systematic analysis has been carried out so far for genetic diversity, identification and discrimination. In the present study, the evaluation of the pattern and the extent of genetic variability and relatedness among traditional varieties to improved cultivars of rice were conducted using SSR markers.

The results obtained would help in the identification and differentiation of various cultivars being cultivated and/or for exports and will also contribute to maximize the selection of diverse parent cultivar and broaden the germplasm base in the future of rice breeding programs. In addition, it will help in identifying efficient strategies for the sustainable management of the genetic resources of rice crops.

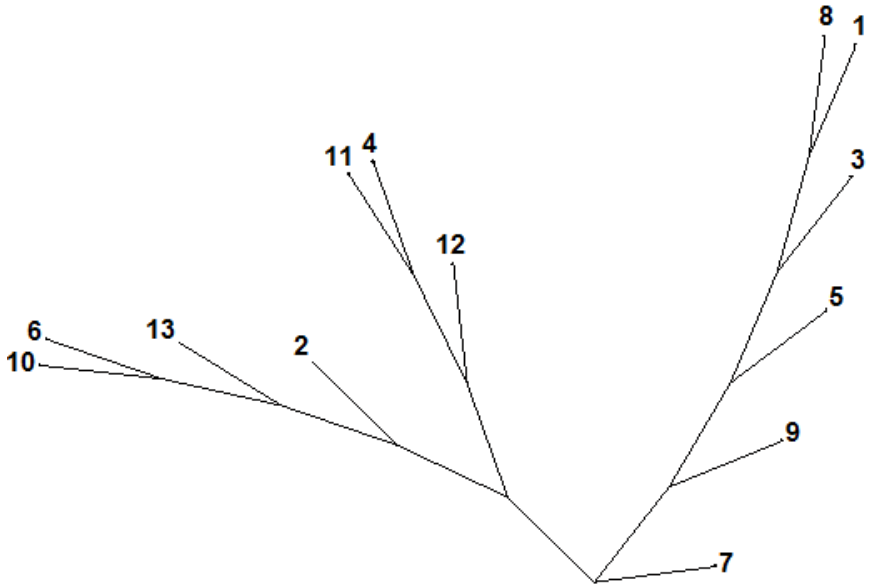

Figure 4. Tree based on neighborhood joining method showing genetic dissimilarity between (1=Kat, 2= Ro, 3= Gi, 4= KJ-1, 5= Jap, 6= Kas, 7= S-1, 8= Chi, 9= S-2, 10= KJ-2, 11= SS, 12= JJ, 13=MS).

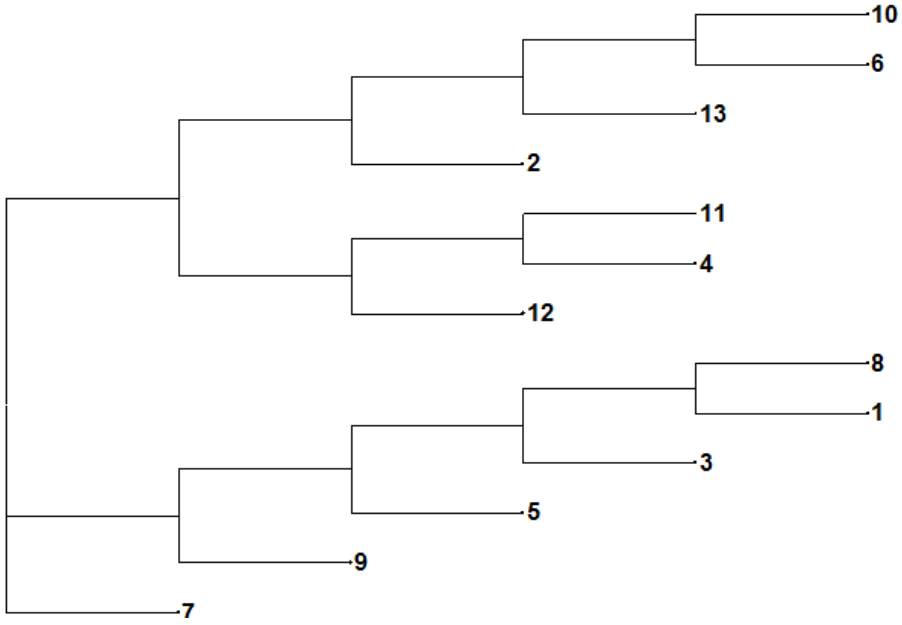

Figure 5. UPGMA based dendrogram for all the thirteen non basmati rice samples (1=Kat, 2= Ro, 3= Gi, 4= KJ-1, 5= Jap, 6= Kas, 7= S-1, 8= Chi, 9= S-2, 10= KJ-2, 11= SS, 12= JJ, 13=MS).

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