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Assessment of genetic diversity of Basmati rice of Jammu Province using RAPD markers

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ABSTRACT:

Basmati rice is considered best because of its aroma and excellent cooking qualities. Phenotypic variability and genetic diversity was studied in twelve basmati rice genotypes collected from Jammu region. The length of the seeds varied from 0.72 cms to 0.90 cms and the seed width in uncooked seeds varied from 0.28 cms to 0.40 cms. A total of fifteen RAPD primers were used. The nine polymorphic primers produced a total of 48 bands out of which 26 bands were polymorphic, with an average of 2.6 bands per primer. The analysis and dendrogram construction was performed using the DARWin 5.0 software. The genetic dissimilarity index calculated between samples ranged from 0.09 to 0.68.The generated dendrogram based on the dissimilarity matrix using the neighbor-joining approach of the unweighted pair group with arithmetic mean method showed three distinct clusters.

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

INTRODUCTION:

Rice (*Oryza sativa* L.) is one of the oldest domesticated crop species in the world belonging to the genus O*ryza* of gramineae family. Most of the world's rice which is cultivated and consumed in Asia, constitutes more than half of the global population. It is one of the very few crop species endowed with rich genetic diversity as it is grown under diverse cultural conditions and over wide geographical ranges.

Basmati rice constitute a small but special group of rice, which is considered best because of its aroma and excellent cooking qualities [1, 2] Basmati rice has its origin in the foothills of Himalayas which then spread to eastern western regions of Indian sub-continent. Basmati consumed today is the result of centuries of selection and cultivation by farmers [3]. The variations present in rice are due to different genetic makeup and their preference for certain environmental conditions. Jammu is known for its high yielding basmati rice which is exported to different European countries. Therefore, molecular characterization of the genotypes gives precise information about the extent of genetic diversity, which is an important

issue for rice breeding programs. Many DNA based markers are used for assessing the genetic diversity but use of RAPD primers [4] is frequently applied to study the genetic variation, divergence and biography [5]. It has been used in studying variations in plants due to non-necessity of prior information of DNA sequence, simplicity of use, low cost and requirement of small quantity of DNA [6].

The assessment of diversity is an essential component to characterize and identify potential parents. It tells about the variation present within the species and is based on the total number of genetic characters contributing to it. Molecular markers which not stressed by environmental factors provide a good and informative approach to estimate the genetic diversity and genetic relationships.

MATERIALS AND METHODS

Plant material and growth experiment:

The experimental material consisted of 12 cultivated Basmati varieties of rice (*O. sativa*) growing in different habitats of Jammu region. Seeds of these varieties were collected from local farmers and SKUAST, Jammu. The varieties collected were from Jammu, Rajouri, Udhampur and Kathua (Table 1). 50 seeds from each collected rice sample were soaked in water overnight for 24 h. Then the seeds were placed in sterilized Petri plates (50 seeds/per plate) on a 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 and then the germinated seedlings were transferred from the Petri plates to the small plastic pots for their further growth to young plantlets (Fig 1).

S.No.	Variety name	Collection site	Code
1.	Basmati Kathua	Kathua	Bas-Kat
2.	Ranbir Basmati	Jammu	Ran Bas
3.	Basmati 11/21	Kathua	Bas 11/21
4.	Basmati 370	Rajouri	Bas 370
5.	Ratna Basmati (Udhampur)	Udhampur	Bas Rat Udm
6.	Pusa Sugandha-5	Rajouri	PS-5
7.	Pusa Basmati 1460	Rajouri	PB-1460
8.	Old Basmati	Jammu	OB
9.	Ranbir- Basmati	Jammu	Ran-Bas
10.	Ratna Basmati (R S Pura)	Jammu	Bas Rat
11.	Pusa Sugandha-2	Jammu	PS-2
12.	Pusa Basmati-1121	Jammu	PB-1121

Table 1: Basmati genotypes and their codes

Phenotypic evaluation of rice grain :

Twenty rice seeds taken from twelve basmati samples were dehulled and their average length and width (both cooked and uncooked), weight, ratio of length: width and variation in color was calculated.

Molecular Marker analysis:

Leaves were collected from young plants grown in pots and were surface sterilized and then dried on the filter paper. The genomic DNA was isolated from each rice sample by using the CTAB based method [7] which was modified by adding β mercaptoethnol and 1% PVP to improve the quantity and concentration of DNA. RNA was eliminated from the isolated DNA by adding 5U of RNase and incubating the vials at 37^{o}C for 50-60 minutes. The DNA was finally suspended in TE (10mm Tris HCl and 1mM EDTA, pH 8.0) and quantified by UV spectrophotometer and also on 0.8% (w/v) agarose gel and was stored in -20^{o}C till further use.

In vitro amplification using polymerase chain reaction (PCR) [8] was performed in 0.2ml PCR tubes (Tarsons) using 50-75ng of genomic DNA of each sample in a final volume of 25µl reaction mixture. A total of fifteen different 10 mer primers (Promega) were used for RAPD analysis (Table 2). The PCR reaction mixture contained 5.0 µl template DNA, 12.2 µL ddH_2O, 2.5 µl 10X PCR buffer, 3.5 µl of 100 mM dNTPs, 1.5 µl of 5 µM primer and 0.3 µL Taq polymerase (5 U/µl).

Amplification was done with Bio Rad "My cycler" machine. Each reaction was performed using initial denaturation of Template DNA at 94°C for 4 min followed by 45 cycles of PCR amplification following: 1 min of denaturation at 94°C, 1 min of primer annealing at 37°C and 2 min of primer extension at 72°C. Final incubation was at 72°C for 7 min so as to complete primer extension. The amplified products were electrophoretically resolved on a 1.5% agarose gel in 0.5X Tris-acetate-EDTA (TAE) and visualized under UV light after staining with 0.1% ethidium bromide. A negative control lacking DNA was included for each primer.

Data collection and diversity analysis

All the gels were scored manually for monomorphic and polymorphic bands The dissimilarity matrix used for clustering of genotypes was based on the unweighted neighbor-joining method and the analysis was performed using DARWin 5.0 (http://darwin.cirad.fr), [9]. The genetic dissimilarity was calculated for all the basmati rice samples under study. Confidence limits of different clades were tested by bootstrapping 500 times to assess the repetitiveness of genotype clustering [10].

S.No	Primer	Sequence (5'⟶ 3')
1	OPA-04	AATCGGGCTG
2	OPA-19	CAAACGTCGG
3	OPH-07	CAAACGTCGG
4	OPH-08	GAAACACCCC
5	OPH-03	TCTGTGCTGG
6	OPY-06	AAGGCTCACC
7	OPY-13	GGGTCTCGGT
8	OPBA-03	GTGCGAGAAC
9	OPBA-06	GGACGACCGT
10	OPBB-08	TCGTCGAAGG
11	OPBB-09	AGGCCGGTCA
12	OPBB-10	ACTTGCCTGG
13	OPBD-17	GTTCGCTCCC
14	OPX-19	TTTCCCACGG
15	OPU-20	ACAGCCCCCA

Table 2: List of RAPD primers and their sequence

RESULTS AND DISCUSSION

Fifty seeds from each 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 they showed little germination. And the best germination was observed at 30°C after 3 days of incubation.

Fig 1: Germination of seeds of Pusa Basmati after 48 hrs at 28ºC

Phenotypic Evaluation:

The genetic resources of Jammu showed a great diversity for all the measured seed morphological characters. The existence of great diversity in the seed morphology i.e. length, width and weight, indicates the presence of other related agronomic, physiological, cooking, nutritional traits or cultural aspects for their selection and adoption. Grain length in basmati rice is due to change in the consumer preference for better quality rice as a result of changed lifestyle of the consumers and India's emergence as one of the major exporter of rice in the international markets. Sano [11] had stated that phenotypic diversity increases during domestication as a general trend in crop plants and in general long grain type basmati is now the preferred trait by the consumers. In the present study five basmati rice seeds from each sample were evaluated phenotypically for both cooked and uncooked grains because grain quality is a very wide subject encompassing diverse characters that

are directly or indirectly related one quality type. Twelve different varieties of seeds were morphologically examined for different morphological characters. Some of the important grain characteristics studied, that constitute the grain quality were Color, Shape (Length/Width ratio), Size (Length/ Width) and Weight.

The length of the seeds varied from 0.72 cms to 0.90 cms (Table 3; Fig 2). The longest seed was from Basmati line Basmati 11/21 which was 0.90cms long. The seed length (uncooked) for Pusa Sugandha-5 and Basmati 1121 was same (0.88 cms) while the average length of seeds after cooking in both Pusa Sugandha-5 and Basmati 1121 was 0.98 cms and 1.24 cms respectively. The seed width in uncooked seeds varied from 0.28cms to 0.40 cms. Basmati sample with the highest seed width was Ratna basmati collected from Udhampur (0.40 cms) (Fig-2). The ratio of seed length to width varied between 1.80 cms to 3.80 cms. The lowest ratio was 1.76 cms for sample Pusa basmati 1460 (PB-1460) collected from Rajouri and the highest was 3.80 cms for old basmati (OB) (Table 2). The weight of the seeds varied from 0.09 gms to 0.96 gms (Table 3). Amarawathi *et al.* [12] also studied the seven important quality traits namely grain length (GL), grain breadth (GB), grain length to breadth ratio (LBR), cooked kernel elongation ratio (ELR), amylose content (AC) and aroma using a set of 209 recombinant inbred lines and mapped QTLs for these traits.

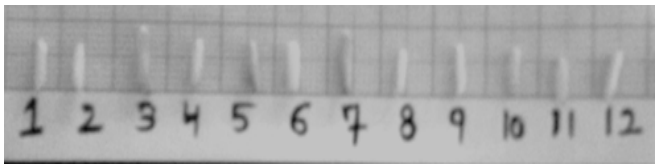

Fig 2: Length and width of different Basmati rice genotypes (1=Bas-Kat, 2=Ran Bas, 3=Bas11/21, 4=Bas 370, 5=Bas Rat Udm, 6=PS-5, 7= PB-1460, 8=OB, 9=Ran-Bas, 10=Bas Rat, 11=PS-2, 12=PB-1121)

Marker analysis:

A total of fifteen RAPD primers were used for DNA amplification in the twelve basmati rice samples (Table 2). The gel was scored on the basis

S.No	Genotypes	Color	Average length uncooked (cm)	Average width uncooked (cm)	Ratio L/W	Average weight (gm)	Average length cooked (cm)	Average width Cooked (cm)
1	Bas-Kat	Creamy white	0.70	0.28	2.50	0.21	0.90	0.36
2	Bas RB	Creamy white	0.74	0.30	2.40	0.95	0.74	0.38
3	Bas 11/21	Dark yellow	0.90	0.30	3.00	0.96	1.14	0.32
4	Bas-370	Creamy white	0.72	0.30	2.40	0.10	0.80	0.30
5	Rat Bas Udm	white	0.72	0.40	1.80	0.11	0.96	0.40
6	PS-5	Pale yellow	0.88	0.30	2.93	0.19	0.98	0.38
7	PB-1460	White	0.74	0.42	1.76	0.12	0.62	0.22
8	OB	Pale yellow	0.76	0.20	3.80	0.16	0.88	0.38
9	Ran-Bas	White	0.72	0.20	3.60	0.09	0.94	0.22
10	Bas- Rat	White	0.76	0.30	2.50	0.11	0.96	0.30
11	PS-2	Creamy white	0.73	0.30	2.40	0.11	0.86	0.30
12	Bas 1121	Pale yellow	0.88	0.30	2.93	0.21	1.24	0.30

Table 3: Morphological characteristics of 12 different basmati rice genotypes

of bands present. The presence of band was scored as 1 and absence as 0. Only the clear bands and ambiguous bands of RAPD markers were scored.

Out of fifteen primers, the number of RAPD loci generated was higher for the primer OPU-20 (9 loci) followed by primers OPBB-10 and OPY-06 (7 each). The lowest number of fragments was generated by the primer OPBD-17 and OPA-03. Out of fifteen RAPD primers, nine primers showed amplification and were polymorphic (Table 4) whereas four primer (OPX-19, OPBA-06, OPA-19, OPY-13) were monomorphic.The two primers (OPBB-09 and OPH-08) did not show any amplification. This may be due to insufficient attachment sites of the primer on template DNA.

Similar results were also observed by Verma *et al.* [13] in basmati rice with primer OPF-17. The nine polymorphic primers produced a total of 48 bands

S. No	Primer	Total number of bands	Number of polymorphic bands	Polymorphic percentage
1.	OPA-04	4	2	50.0
2.	OPBB-10	7	4	57.1
3.	OPBD-17	3	1	33.3
4.	OPH-07	4	3	75.0
5.	OPY-06	7	4	57.1
6.	OPBB-08	6	5	83.3
7.	OPU-20	9	2	22.2
8.	OPBA-03	5	1	20.0
9.	OPA-03	3	2	66.6
Total		48	24.0	-
Mean		5.3	2.6	51.6

Table 4: List of polymorphic primers and the degree of polymorphism obtained in twelve basmati rice samples

out of which 26 bands were polymorphic, with an average of 2.6 bands per primer. Primer OPU-20 produced highest number of 9 bands out of which 2 were polymorphic. A polymorphism percentage of 57.1 was observed with two primers OPBB-06 and OPBB-10, both producing a total of 7 bands out of which 4 bands were polymorphic (Table 4). Primer OPBD-17 produced three bands and only one was polymorphic thereby showing 33.3% polymorphism (Fig 3). Thus the polymorphism percentage ranged from 20% (Primer OPBB-10) to 83.3% (Primer OPBB-08). The oveall polymorphic percentage was 51.6% (Table 4).

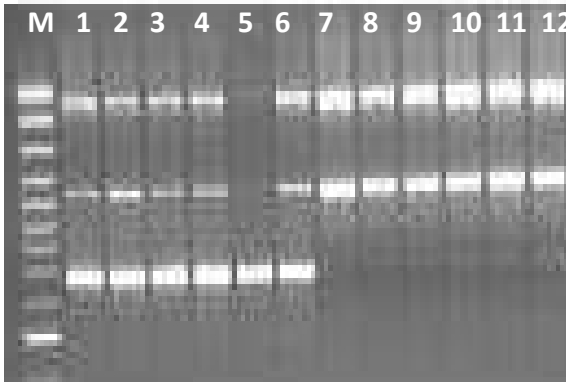

Fig 3: PCR Amplification of twelve basmati genotypes with RAPD primer OPBD-17 (M= Marker (100bp, Fermentas), (1=Bas-Kat, 2=Ran Bas, 3=Bas11/21, 4=Bas 370, 5=Bas Rat Udm, 6=PS-5, 7= PB-1460, 8=OB, 9=Ran-Bas, 10=Bas Rat, 11=PS-2, 12=PB-1121)

Analysis of genetic dissimilarity

Genetic dissimilarity was calculated using software DARWin 5.0, where "0" and "1" were standardized as the least and maximum of dissimilarity respectively. Genetic dissimilarity was calculated from the matrix of binary data using Dice index of similarity [14]. The genetic dissimilarity index calculated between accessions ranged from 0.09 to 0.68. The lowest value of genetic dissimilarity was 0.09, obtained between Basmati-1121 collected from Jammu and Pusa sugandha-2 (PS-2) collected from Rajouri, and Ranbir-Basmati (Ran-Bas) collected from Kathua and Basmati 11/21 (Bas 11/21) collected from Jammu while the highest dissimilarity value calculated was 0.68 between the basmati samples collected from Rajouri (PB-1460) and Rat-Bas collected from Udhampur (Table 5).

	Bas-Kat	Ran-Bas	Bas 11/21	Bas-370	Rat Bas-Udm	PS-5	PB-1460	Old Bas	Ran370	Rat Bas-RS	PS-2
Ran-Bas	0.24										
Bas11/21	0.15	0.18									
Bas-370	0.25	0.28	0.17								
Rat Bas-Udm	0.42	0.34	0.36	0.46							
PS-5	0.24	0.27	0.16	0.24	0.46						
PB-1460	0.46	0.50	0.38	0.32	0.68	0.46					
Old-Bas	0.33	0.37	0.25	0.19	0.55	0.33	0.23				
Ran-Bas	0.18	0.21	0.09	0.16	0.39	0.17	0.37	0.25			
Rat Bas- RS	0.41	0.33	0.35	0.45	0.42	0.44	0.66	0.54	0.38		
PS-2	0.25	0.29	0.17	0.11	0.47	0.25	0.23	0.10	0.16	0.45	
Bas-1121	0.23	0.26	0.15	0.09	0.44	0.22	0.30	0.17	0.14	0.43	0.09

Table 5: Diversity matrix based on genetic dissimilarity coefficient between twelve basmati genotypes as revealed by RAPD primers

The samples collected from Kathua and Jammu do not show much divergence. The dissimilarity between Bas 11/21 and Bas-Kat was only 0.15. Distance wise these two places are just 60 kms apart and temperature conditions are almost same in both the places.

The tree generated showed no divergence between samples collected from Rajouri and Jammu (Fig 4). The generated dendrogram based on the dissimilarity matrix using the neighbor-joining approach of the UPGMA method showed three distinct clusters (Fig 5):

1. Cluster I contains samples collected from Jammu, Kathua and Rajouri.
2. Cluster II and Cluster III contains samples collected from Jammu and Rajouri.

Basmati Kathua (Bas-Kat) and Basmati 11/21 (Bas11/21) both collected from Kathua are present in cluster I and show a genetic dissimilarity of 0.15 which means that both these basmati samples are not genetically much different from each other. Similarly Pusa Sugandha-2 (PS-2) and Pusa Basmati 1121 (PB1121) both collected from Jammu region are present in cluster II and show very less divergence. Ratna Basmati (Bas Rat Udm) collected from Udhampur and Ranbir Basmati (Ran Bas) collected from Jammu show a genetic distance of 0.34 (Fig 4).

In cluster II Pusa Basmati (PB 1460) and Old Basmati (OB) both collected from Jammu show a genetic dissimilarity of 0.23 whereas in cluster III Pusa Sugandha-5 (PS-5) collected from Rajouri and Ranbir Basmati (Ran-Bas) collected from Jammu show a less genetic dissimilarity of 0.17. This clearly indicated that the samples may have same genetic makeup and with no genetic difference and they may be spread to different areas because of human interference.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.

Rahman *et al.* [15] observed 53.85% polymorphism in six different rice cultivars using fifteen RAPDs as molecular markers. He grouped six cultivars of rice into three clusters following geographical proximity where Basmati 370 grouped with the DM 25 with genetic distance of 0.12. Similarly Arif *et al.* [16] grouped 19 genotypes of Pakistan rice into two main clusters with all the basmati genotypes in one cluster and the genetic similarity coefficients ranged from 0.42 to 0.85. Ashfaq and Khan [17] used SSR primers to study the genetic diversity among rice basmati

genotypes in India and observed that the genetic distance between rice genotypes ranged from 0.07 to 0.95. The dendrogram based on cluster analysis grouped the 20 genotypes of rice in to five clusters based on their genetic similarity. The lowest genetic diversity among traditional Basmati varieties has also been observed by Choudury *et al.* [18] for the classification of aromatic rices and by Cao and Oard [19] for the genetic diversity and quantitative variation in rice germplasm.

CONCLUSION

The characterization and quantification of genetic diversity within closely related crop germplasm has long been a major goal, as it is essential avoid the chance of use of genetically similar landrace/genotypes in future breeding programme. It can help us to select genetically diverse parents for basmati breeding. Above and beyond, the analysis of genetic variation contributes immensely to selection, monitoring of germplasm and also helps in prediction of potential genetic gains. Although a large number of aromatic and course 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 extense of genetic variability and relatedness among the varieties was conducted using RAPD markers. The information generated from this study will be used in identifying efficient strategies for the sustainable management of the different genetic resources in Basmati rice crops. In addition, marker-based identification and differentiation of different Basmati rice may help to maintain the reliability of this high quality product which is of benefit to both farmers and consumers.

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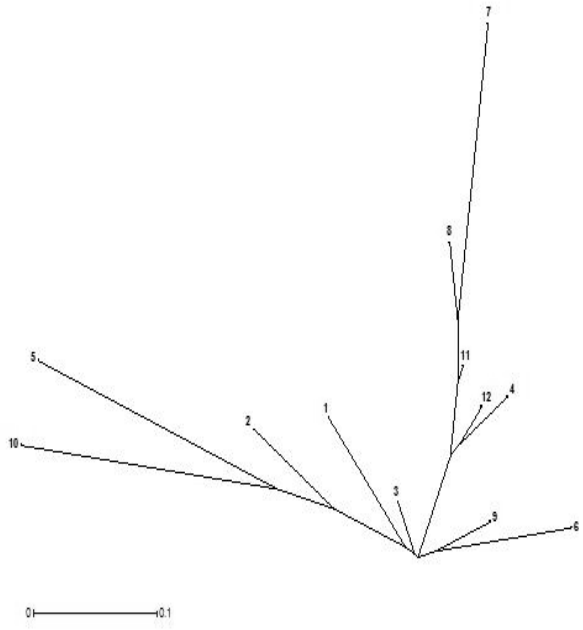

Fig 4: Tree based on neighbourhood joining method showing genetic dissimilarity between twelve basmati rice genotypes (1=Bas-Kat, 2=Ran Bas, 3=Bas11/21, 4=Bas 370, 5=Bas Rat Udm, 6=PS-5, 7= PB-1460, 8=OB, 9=Ran-Bas, 10=Bas Rat, 11=PS-2, 12=PB-1121)

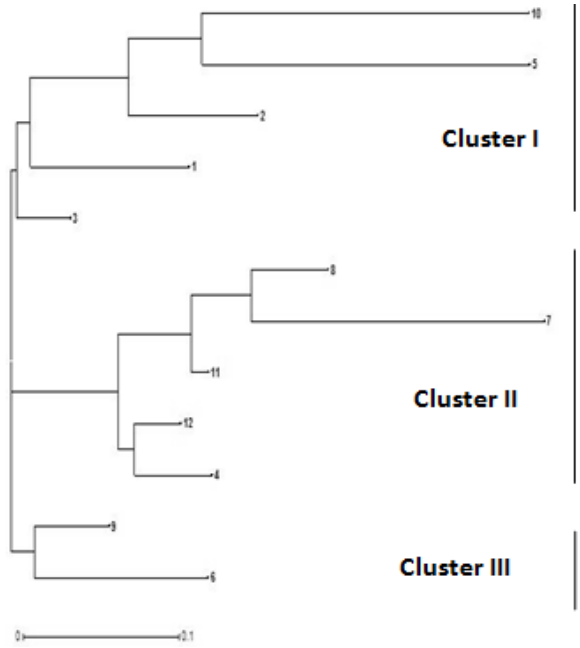

Fig 5: UPGMA based dendrogram of twelve basmati genotypes showing three clusters (1=Bas Kat, 2=Ran Bas, 3=Bas11/21, 4=Bas 370, 5=Bas Rat Udm, 6=PS-5, 7= PB-1460, 8=OB, 9=Ran-Bas, 10=Bas Rat, 11=PS-2, 12=PB-1121)

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