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Research Article

Molecular genetic diversity analysis using SSR markers of basmati rice (*Oryza sativa* L.) genotypes of northern hill region, India

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Abstract

The genetic diversity of 30 rice accessions both of basmati and non-basmati including 2 red rices collected from Rice and Wheat Research Centre, Malan was performed on the basis of 36 genome wide SSR markers with the objectives to quantify the genetic divergence and to identify the potential accessions. Molecular characterization grouped them according to their varietal affiliations into five major clusters. Majority of basmati genotypes were clustered together. SSR markers detected a total of 83 alleles ranging from 2-4. The pairwise genetic similarity values among different genotypes ranged from 0.17 to 0.92 with an average of 0.48 indicating the presence of moderate genetic diversity among the genotypes analysed. The PIC values reflected the level of diversity and allelic frequency among the varieties which varied widely among SSR loci from 0.062 to 0.664. Two SSR markers namely, RM7173 and RM101 were identified as genotype specific, for genotype HPR 2761 and Vasumati, respectively. Two advanced breeding lines of basmati HPR 2852 and HPR 2855 were clustered together with their basmati parents in dendrogram indicating maximum recovery of basmati genotypes. The polymorphism detected among the genotypes will be helpful in selecting genetically diverse parents in the future breeding programme. The information generated will be used for advanced studies in germplasm, pureline selection and rice breeding in the future breeding programme.

Keywords: Basmati, cluster analysis, genetic diversity, rice, SSR markers

1. Introduction

Rice (*Oryza sativa* L.) occupies the premier place among the food crops cultivated around the world, thus its production and improvement are of interest to the Indian economy (Salgotra *et al.,* 2015). India is the second largest rice-growing country in the world after China. In India, rice is cultivated on about 43.99 million ha area with a production of 116.42 million tonnes (Anonymous, 2018). Himachal Pradesh occupies an area of 73.69 thousand hectares with production of 129.88 thousand tonnes and productivity 1.76 tonnes ha^{-1} (Anonymous 2016). Assessment of genetic diversity is inevitable for a plant breeder as its exploitation help in developing new varieties, identifying desirable traits, developing unique germplasm, estimating and establishing genetic relationship in germplasm collection, identifying diverse parental combinations to create segregating progenies with maximum genetic variability and superior recombination for further selection and introgressing desirable genes from diverse germplasm (Thompson *et al.,* 1998; Islam *et al.,* 2012; Ramadan *et al.,* 2015). An appraisal of genetic diversity at molecular level of basmati germplasm provides information to characterize and cluster genotypes on similarity index for exploitation either directly as a cultivar or indirectly through hybridization in breeding programme. Genetic diversity is mainly measured based on the morphological differences of important quantitative traits. Traditionally, the characterization of germplasm done on the basis of morphological descriptors, had some disadvantages in terms of time, space, and labour cost (Aljumaili *et al.,*

2018). For the assessment of genetic diversity molecular markers have been generally superior to morphological, pedigree and biochemical data (Melchinger *et al.,* 1991). The DNA markers have offered easy, cost-effective and environmentally-neutral means for deciphering genetic diversity in plants as it remains unaffected across different growth stages, seasons and environments leading to more reproducible results (Saini *et al.,* 2004; Rahman *et al.,* 2012). DNA markers are predominantly used in molecular characterization and diversity studies due to their abundance and repeatability (McCouch *et al.,* 1997). Different molecular markers *viz.* Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSRs), Amplified Fragment Length Polymorphism (AFLP) and Single Nucleotide Polymorphisms (SNPs) have been used to assess the genetic diversity of various rice cultivars throughout the world. Among the several classes of available DNA markers, microsatellite or simple sequence repeat (SSR) markers are considered the most suitable due to their ease of application, high reproducibility, codominant inheritance, rapid analysis, low cost, easy scoring patterns, greater allelic diversity and extensive genome coverage (Pérez-Jiménez *et al.* 2013; Phumichai *et al.,* 2015; Chen *et al.,* 1997). So screening of basmati genotypes using SSR markers would create a valuable database for varietal identification, measure the extent of genotypic differences, genetic relationship and assist in broadening of genetic bases of the cultivars. The present study was thus aimed to evaluate the morphological and molecular variation among the basmati genotypes so that genetic relationship could be determined for utilization and further improvement of the existing lines.

Table 1: List of 30 rice genotypes with pedigree details.

S.no	Variety	Parentage/Source
Basmati genotypes		
1	Pusa 1121	Pusa-751-87-7-1/IR8
2	Hasansarai	Introduction from Iranian Basmati
3	Vasumati	PR 109/Pakistani Basmati
4	Lakhamandal	Selection from local basmati collections from Lakhamandal village of Dehradun
5	Basmati-370	Pure line selection from Dehraduni basmati landraces
6	PB-1509	Improved version of Pusa 1121 derived from cross Pusa1301/Pusa 1121
7	T-23	Traditional basmati selection from Kala Sukhdas
8	Kasturi	Basmati 370/CR 88-17-1-5
9	HPR 2612	Hassan Sarai/T 23//IR 66295-36-2
Basmati type advanced breeding lines (ABL)		
10	HPR 2323	HPU 741× PR 72
11	HPR 2692	Hasansarai/T23//IR67011
12	HPR 2693	Hasansarai/T23//TR66295-36-2
13	HPR2746	Hasansarai/T23//IR670
14	HPR 2747	Hasansarai/T23//IR66295
15	HPR 2749	Hasansarai/T23//IR66295-36-2
16	HPR 2761	Hasansarai/Kasturi
17	HPR 2763	Hasansarai/Kasturi
18	HPR 2852	Hasansarai/T23//IR66295-36-2
19	HPR 2855	Hasansarai /T23//IR66295-36-2
20	HPR 2858	Kalizhini/HPR 2143//HPR 2143
21	HPR 2667	Palampur Purple/Kasturi
22	HPR 2861	Palampur purple/Kasturi
23	HPR 2862	Palampur Purple/Kasturi
24	HPR 2863	Palampur Purple/Kasturi
25	HPR 2864	Palampur Purple/Kasturi
Non-basmati		
26	Sharbati	*Indica* rice with long slender grains
27	PR-121	Non-basmati *indica* rice derived from cross PR116///PR108/IRRI76/ PR106-2
28	HPR 2880	HPU 2216 /Tetep
29	HPR 2795	Pure line selection from Sukara red
30	HPR 2720	Pure line selection from IC 455333I

2. Material and Methods

Material consisting of thirty rice genotypes comprising 9 basmati, 16 basmati type advanced breeding lines (ABL) and 5 non-basmati types collected from Rice and Wheat Research Centre, Malan were subjected to diversity analysis are listed in Table 1.

All the thirty genotypes were planted in pots in order to avoid any type of contaminations and admixtures. DNA was isolated from young leaf tissue using CTAB method (Murray and Thompson, 1980) and used for polymerase chain reaction (PCR) analysis. A total of 36 SSR markers (www.gramene.com) which were dispersed throughout the 12 chromosomes of rice were used to assess the extent of genetic diversity of the genotypes. Details of SSR markers are given in Table 2.

Amplified products from SSR markers analysis were scored qualitatively and the generated binary data matrix were subjected to further analysis to generate a similarity matrix using Jaccard's coefficient in SIMQUAL programme of NTSYS–pc package (Rohlf, 1993). Polymorphic Information Content (PIC) value was calculated using the online software PIC calculator available at www.agrihuji.ac based on the formula given by Anderson *et al.* (1993) to estimate the discriminatory power of the SSR marker.

3. Results and Discussion

Of the 36 SSR markers tested, 32 were found to be polymorphic and four monomorphic. A total of 83 alleles were detected across 30 rice genotypes by 32 polymorphic SSR markers. Banding pattern of SSR markers generated over 30 rice genotypes are presented in Fig. 1. The number of alleles generated per SSR locus ranged from 2 (RM312, OSR13, RM405, RM305, RM402, RM461, RM340, RM248, RM445, RM192, RM284, RM278, RM5629, RM7173, RM3428 and RM101 to 4 (RM495, RM3505 and RM85) with an average of 2.30 alleles per locus (Table 3). The level of allelic diversity observed in this investigation was low as compared to that reported by Joshi and Behra (2006) who recorded 2.6 alleles per locus. The low level of allelic diversity observed could be attributed to the fact that the majority of genotypes

Table 2: List of simple sequence repeats (SSR) used in the present study.

Marker	Forward Primer	Reverse Primer
RM1195	ATGGACCACAAACGACCTTC	CGACTCCCTTGTTCTTCTGG
RM495	AATCCAAGGTGCAGAGATGG	CAACGATGACGAACACAACC
RM312	GTATGCATATTTGATAAGAG	AAGTCACCGAGTTTACCTTC
RM3505	GATGAGGTGGGACGACGAC	TCTTCACAGTGACGAAACCG
RM498	AATCTGGGCCTGCTCTTTTC	TCCTAGGGTGAAGAAAGGGG
RM525	GGCCCGTCCAAGAAATATTG	CGGTGAGACAGAATCCTTACG
RM293	TCGTTGGGAGGTATGGTACC	CTTTATCTGATCCTTGGGAAGG
RM85	CCAAAGATGAAACCTGGATTG	GCACAAGGTGAGCAGTCC
OSR13	CATTTGTGCGTCACGGAGTA	AGCCACAGCGCCCATCTCTC
RM1155	AGGGAGTGTGGCAACTATGC	GGGAGGAGTGAGAAGGGATC
RM142	CTCGCTATCGCCATCGCCATCG	TCGAGCCATCGCTGGATGGAGG
RM280	ACACGATCCACTTTGCGC	TGTGTCTTGAGCAGCCAGG
RM153	GCCTCGAGCATCATCATCAG	ATCAACCTGCACTTGCCTGG
RM405	TCACACACTGACAGTCTGAC	AATGTGGCACGTGAGGTAAG
RM305	TACTGCCAAAGGCGAGCTTC	GTGAGAGGCTACAGCTAACC
RM402	GAGCCATGGAAAGATGCATG	TCAGCTGGCCTATGACAATG
RM461	GAGACCGGAGAGACAACTGC	TGATGCGGTTTGACTGCTAC
RM340	GGTAAATGGACAATCCTATGGC	GACAAATATAAGGGCAGTGTGC
RM248	TCCTTGTGAAATCTGGTCCC	GTAGCCTAGCATGGTGCATG
RM445	CGTAACATGCATATCACGCC	ATATGCCGATATGCGTAGCC
RM192	GCGGCGGATCATGAATTGCGAG	CTTGTTCCCCGGCGTCGAGTCC
RM3710	GGAGGAAAGATGCAAGTTGC	GATTGTTTCCTCCGCCATTC
RM330	CAATGAAGTGGATCTCGGAG	CATCAATCAGCGAAGGTCC
RM284	ATCTCTGATACTCCATCCATCC	CCTGTACGTTGATCCGAAGC
RM205	CTGGTTCTGTATGGGAGCAG	CTGGCCCTTCACGTTTCAGTG
RM278	GTAGTGAGCCTAACAATAATC	TCAACTCAGCATCTCTGTCC
RM288	CCGGTCAGTTCAAGCTCTG	ACGTACGGACGTGACGAC
RM484	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC
RM5629	AGCTCAACTCGACAACTCCC	CCATCTCCTCTTTCACCTCG
RM5147	GTTGAAAGTGTCCAAGCTCT	AATTTGTCACGACCCAAATA
RM7173	GAGCGTTTTTAGGATGCCAC	GTGATGTCGGATTCTTGGTG
RM457	CTCCAGCATGGCCTTTCTAC	ACCTGATGGTCAAAGATGGG
RM3428	ATTCATGCTTCCTTTCAGTG	GATTACTGGTTTGCCATTTG
RM20 A	ATCTTGTCCCTGCAGGTCAT	GAAACAGAGGCACATTTCATTG
RM270	GGCCGTTGGTTCTAAAATC	TGCGCAGTATCATCGGCGAG
RM101	GTGAATGGTCAAGTGACTTAGGTGGC	ACACAACATGTTCCCTCCCATGC

analyzed were closely related genotypes represented by Indian basmati varieties and the breeding lines emanating from the crosses of closely related basmati lines. Contrary the level of allelic diversity was high (3.8) in traditional and evolved basmati genotypes and also reported by several workers (Nagaraju *et al*., 2002; Rabbani *et al.,* 2010; Hossain *et al*., 2012; Krupa *et al.,* 2017). In addition, comparatively a very high level of allelic diversity with 4.4 to 7.8 alleles per locus has also been recorded in studies involving more diverse collections of rice accessions including cultivated and wild rice varieties, aromatic and quality basmati rice, *indica* and *japonica* varieties (Ni *et al.,* 2002; Ravi *et al.,* 2003; Jain *et al.,* 2004; Pervais *et al.,* 2010).

3.1. Allelic and loci variation within the lines PIC values

The polymorphic information content (PIC) values reflected the level of diversity and allelic frequency among the varieties and varied widely among SSR loci tested ranging from 0.062 (RM7173 and RM101) detected on chromosome 11 and 12 respectively to 0.664 (RM3505) detected on chromosome 2, with an average of 0.348 (Table 3). These results are in agreement with Singh *et al.* (2004) where an average PIC value of 0.34 was recorded. The level of diversity was however, slightly lower than the earlier observations reported by Seetharam *et al.* (2009), Shu-kun *et al.* (2010), Rahman *et al.* (2012), Sajib *et al.* (2012), Yadav *et al.* (2013) and Krupa *et al.* (2017) who have recorded the PIC value of 0.46, 0.487, 0.488, 0.48, 0.41 and 0.49 in that order. The apparent deviation of our findings from these studies could be attributed to the use of a great proportion of closely related genotypes. According to Akkaya and Buyukunal-Bal (2004), the differences in the level of inherent polymorphism of SSR markers used across different studies could be other reason for different levels of PIC values observed. Seven SSR markers namely, RM495, RM3505, RM525, RM85, RM1155, RM20A and RM280 exhibited PIC values greater than 0.5 (0.562 to 0.664). These microsatellite sequences may be useful tools in future genetic studies of closely related basmati varieties.

3.2. Genotype specific marker

In the present study, out of 32 polymorphic markers, 2 SSR markers were identified as genotype specific, namely, RM7173 and RM101 located on chromosome 11 and 12 which generated a specific banding pattern for genotype HPR 2761 and Vasumati, respectively Fig. 1. Jain *et al.* (2004) also observed similar findings and reported that specific marker RM252 could distinguish commercially important traditional basmati rice varieties *viz.,* Taraori Basmati and Basmati 370 from cross-bred varieties Pusa Basmati 1 and Haryana

Table 3: Number of scorable and polymorphic SSR alleles obtained in 30 rice genotypes using 32 polymorphic markers.

S.No	Polymorphic Marker	Number of polymorphic alleles	PIC value
1	RM1195	3	0.419
2	RM495	4	0.624
3	RM312	2	0.117
4	RM3505	4	0.664
5	RM498	3	0.445
6	RM525	3	0.562
7	RM293	4	0.451
8	RM85	3	0.629
9	OSR13	2	0.346
10	RM1155	3	0.516
11	RM142	3	0.407
12	RM280	3	0.576
13	RM153	3	0.347
14	RM405	2	0.164
15	RM305	2	0.239
16	RM402	2	0.234
17	RM461	2	0.315
18	RM340	2	0.346
19	RM248	2	0.332
20	RM445	2	0.332
21	RM192	2	0.164
22	RM3710	3	0.123
23	RM330	3	0.463
24	RM284	2	0.332
25	RM205	3	0.400
26	RM278	2	0.117
27	RM5629	2	0.117
28	RM7173	2	0.062
29	RM457	3	0.419
30	RM3428	2	0.239
31	RM20A	3	0.583
32	RM101	2	0.062
	Total	83	-
	Range	2-4	0.062-0.664
	Mean	2.30	0.348

Basmati 1. Similar results had also been reported earlier by Sivaranjeni *et al.* (2010) where RM577 on chromosome 1 was found to be a distinguishing marker for the genotypes *viz.,* Basmati 334, Knakjeer B and RAU 3014 while, RM3 and RM38 on chromosome 6 and 8, respectively were found to be the distinguishing markers for the genotype Lectimachi from the rest of the genotypes. Freeg *et al.* (2016) detected ten SSR markers which were able to discriminate the specific genotypes by generating unique allele. Such markers detecting specific banding pattern for the particular genotype could be used as a molecular ID for that particular accession or in varietal identification.

3.3. Genetic similarity

The pairwise genetic similarity values observed amongst different genotypes ranged from 0.17 (between Hasansarai and HPR 2720) to 0.92 (between HPR 2861 and HPR 2862) with an average value of 0.48. Highly diversified lines or dissimilar lines could be useful in a breeding programme to have potential genetic gains. These results suggest the presence of moderate degree of genetic diversity among the genotypes analyzed in the study. The comparable levels of genetic similarity (0.55) using SSR markers have also been observed in basmati rice germplasm lines from Jammu and Kashmir (Salgotra *et al.,* 2015). The cluster analysis grouped the genotypes based on their varietal affiliations into five major clusters *viz.,* I, II, III, IV and V comprising of 9, 17, 2, 1 and 1 genotypes, in that order in Figure 2. While all basmati genotypes (except Hasansarai) and basmati derived breeding lines were grouped in cluster I and II, majority of non-basmati varieties formed distinct clusters III and IV. The conspicuous differentiation between basmati and non-basmati genotypes was indicative of divergence and subsequent independent evolution of the basmati through artificial selection (Nagaraju *et al.,* 2002). Cluster I comprising of basmati rice varieties was

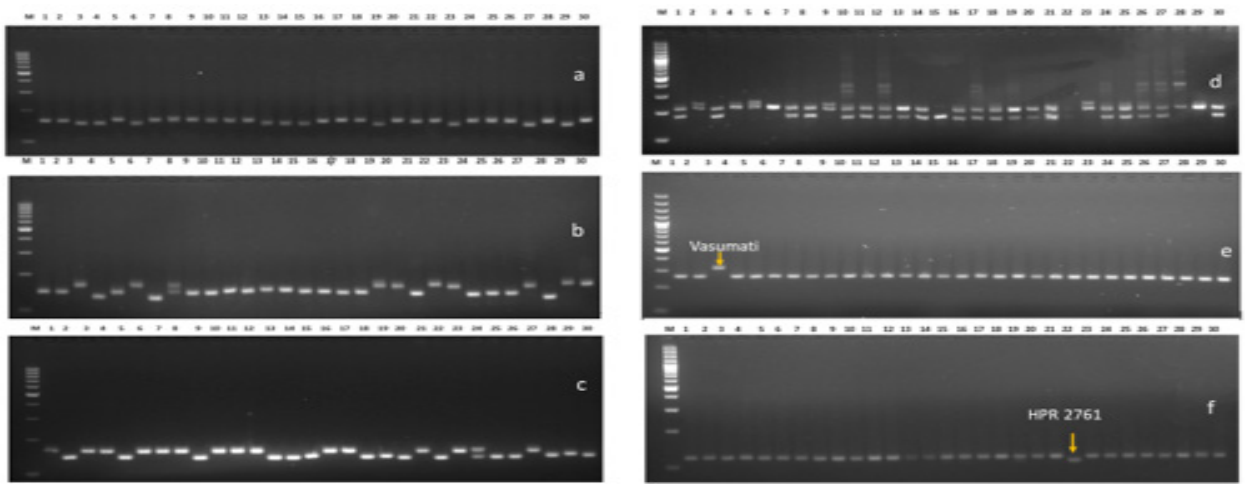

Fig. 1: Agarose gel electrophoresis of PCR amplified fragments for the SSR markers a) RM495; b) RM 3505; c) RM 1155; d) RM 330 and genotype specific markers e) RM 101 and f) RM 7173 Legend M is 100 bp DNA ladder1:Pusa 1121, 2:Hasansarai, 3:Vasumati, 4:Lakhamandal, 5:Basmati-370, 6:PR-121, 7:PB-1509, 8: Sharbati, 9:T-23, 10:HPR 2858, 11:HPR 2323, 12:HPR 2667, 13:HPR 2693, 14:HPR 2749, 15:HPR 2746, 16: HPR 2861, 17: HPR 2863, 18:HPR 2852, 19:HPR 2763, 20:HPR2747, 21: HPR2862, 22:HPR 2761, 23: HPR 2692, 24: HPR 2864, 25: HPR 2855, 26: Kasturi, 27: HPR 2612, 28: HPR 2880, 29: HPR 2795, 30: HPR 2720

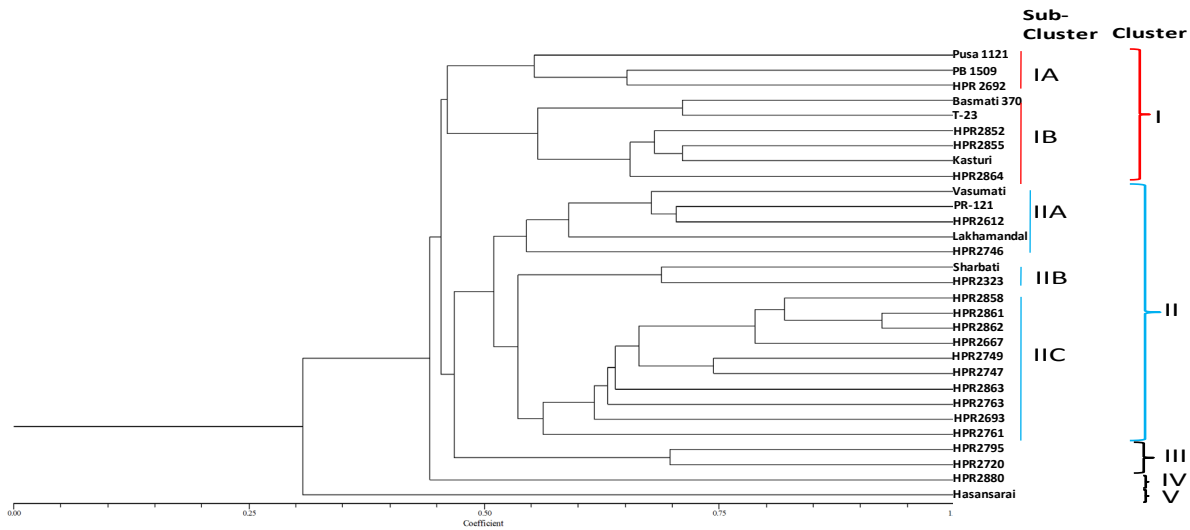

Fig. 2: Dendrogram of 30 rice genotypes generated by SSR data using the UPGMA method.

further divided into two sub-clusters *viz.,* IA and IB comprising of three and six genotypes, respectively. Sub-cluster IA included Pusa1121, PB-1509 and HPR 2692 of which Pusa 1121 and PB-1509 were sister lines differing for days to maturity and height. Other basmati genotypes i.e., Basmati-370, T-23, Kasturi, HPR 2852 and HPR 2855 (basmati ABL) were grouped in cluster IB showing 56% similarity amongst each other. A great majority of the basmati breeding lines developed at Rice and Wheat Research Centre, Malan were grouped together in cluster II (11 out of 16). While HPR 2612 and HPR 2746 were clustered together with basmati varieties Vasumati and Lakhamandal in sub-cluster IIA, nine basmati breeding lines grouped together in sub-cluster IIC. The grouping of a great majority of basmati breeding lines developed at RWRC, Malan in sub-cluster IIC reflects the narrow genetic base of these line as majority of them have been bred involving common basmati parents Kasturi, T-23 and Hasansarai. There is an urgent need for broadening the genetic base of these lines to improve their per se performance and degree of heterosis. Yadav *et al.* (2013) have also reported similar findings that clustering of lines originating from different rice breeding centres of the country including RWRC, Malan owing to the narrow genetic base of breeding material developed. The presence of high genetic similarity among landraces from other varieties has been also described earlier by Ram *et al.* (2007). Two non-basmati lines Sharbati and PR-121 that possess long slender grains similar to typical basmati rice varieties were also clustered together with basmati lines in cluster-II indicating their closer relationship with basmati rices albeit they lack aroma typical of basmati rices. Hasansarai, a basmati type variety introduced from Iran was distinct from all other genotypes studied including the typical basmati varieties. These results suggested divergent genealogies for the basmati lines originated in Indian-subcontinent and other parts of the world. Alternatively, the variety Hasansarai represents a relic of ancient diversity of basmati rices that migrated to Iran from Indian subcontinent during ancient times and thereafter diverged independently under different geographic and genetic bottlenecks.

Of the 16 breeding lines analysed, HPR 2852 and HPR 2855 were clustered together with their basmati parents indicating maximum recovery of basmati type alleles amongst these genotypes. These lines could be the potential lines for identification of basmati lines and for release as varieties. Two non-basmati genotypes *viz.,* Sharbati and PR 121 were clustered with basmati genotypes and the reason could be attributed to their possession of long slender grains and could be further utilised in rice breeding. The polymorphism detected among the genotypes will be helpful in selecting genetically diverse parents in the future breeding programme.

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