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MOLECULAR CHARACTERIZATION AND GENETIC DIVERSITY ANALYSIS IN FOUR *BRASSICA* SPECIES USING MICROSATELLITE MARKERS

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ABSTRACT
The genus *Brassica* consists of a broad range of oilseed, vegetable and condiment crops with high degree of genetic diversity and is one of the most important oilseed crops in India with little information on genetic diversity. Therefore, genetic diversity analysis of 30 *Brassica* genotypes belonging to four cultivated species was assessed using 24 SSR markers. With a 72% polymorphism, a total of 84 alleles varied from 1 to 8 (BRMS 14) with a mean of 2.79 alleles were observed. Nine, out of 24 SSRs produced 100% polymorphism. The amplicon size ranged from 99bp (BRMS-26) to 383bp (BRMS-31). The highest allele frequency of 0.933 was for BRMS-03 and BRMS-17 whereas PIC ranged from 0.79 (BRMS-31) to 0.12 (BRMS-003). BRMS-17 gave specific bands for *B. carinata*. In four clusters, all 30 accessions were grouped into their respective clusters on the basis of species. Analysis revealed 2.11, 0.38, and 0.56 effective allelic numbers (Ae), the observed heterozygosity (Ho) and expected heterozygosity (He), respectively. The study emphasized that species specific primers can be employed to discriminate between the species and that SSR markers can be a useful tool in the germplasm characterization of *Brassica* species as well as in various breeding programs.

INTRODUCTION

The angiosperm family Brassicaceae or commonly termed the mustard family contains the majority of Brassicaceae crop species, the most significant being, species such as *Brassica rapa*, *B. oleracea*, *B. napus* and *B. juncea*. Because of their agricultural importance, the genomes of several *Brassica* crop species have been characterized in detail over the past few years. *Brassica* crops consist of three primary species, namely *Brassica rapa* or chinese cabbage (n = 10), *Brassica oleracea* or Cole (n = 9), and *Brassica nigra* Koch (n = 8) and three amphidiploids, *Brassica juncea* (n = 2x = 18), *Brassica carinata* (n = 2x17) and *Brassica napus* (n = 2x = 19) (Ren *et al.*, 1995).

The maximum utilization of any species for breeding and its adaptation to different environments depend on the level of genetic diversity it holds. Genetic variation is a pre-requirement of crop-breeding program. Since its development, molecular markers have been used to study the genetic diversity and evolutionary relationships in *brassicas*. Among various markers available for genetic analysis in plants, molecular markers are more efficient, precise and reliable in discriminating closely related species and cultivars, even then, many breeding groups emphasize in morphological traits than molecular markers (Hu *et al.*, 2007).

In recent years, microsatellites or simple sequence repeats (SSRs), have been recognized as useful molecular markers in marker-assisted selection (MAS), the analysis of genetic diversity, population analysis and other purposes in various species (Gupta and Varshney, 2000). Microsatellites are short, tandemly repeated nucleotide motifs (1-6 bp) existing throughout the whole genome of an organism, especially in eukaryotes (Tautz and Renz, 1984, Dib *et al.*, 1996 and Dietrich *et al.*, 1996). They are abundant in most species and highly polymorphic, owing largely to variations in the number of repeat units (Tautz, 1989, Weber and May, 1989 and Hancock, 1995). They are inherited in a co-dominant manner (Morgante and Olivieri, 1993) and can be analyzed by a convenient PCR-based method, which makes it easy to screen a large number of individuals. Above all, microsatellites are preferable to other molecular markers such as RFLPs and RAPDs.

Microsatellite of *Brassica* species have been previously studied by Batley *et al.*, 2003, Hopkins *et al.*, 2006, Yadava *et al.*, 2009, Chen *et al.*, 2011 and Chandra *et al.*, 2013. The morphological studies also carried out by Kumar *et al.*, 2013 and Shekhawat *et al.*, 2014 in *brassicas*. However limited number of markers seems to be insufficient for detailed genetic diversity studies, particularly in advanced breeding material and among the species. In *Brassica*, RFLP and RAPD have been extensively used for phylogenetic studies and genetic

mapping (Hu and Quiros 1991). However, the utilization of RAPDs and RFLPs for genome analysis seems to be restricted because of the dominant character and the low specificity of RAPDs and the cumbersome technique of RFLPs.

The amphidiploid nature of the cultivated *brassica* species, the ambiguity in the manner of pollination and existence of self-incompatibility and male sterility make these crops highly diverse. The paper deals with investigation on genetic diversity of *Brassica* species and identification of species specific markers.

MATERIALS AND METHODS

Plant material

All plants used in this study were grown in pot. Thirty genotypes belonging to four different species were undertaken in the present study (Table 1). These genotypes are currently being cultivated in Gujarat and some of these are also cultivated in different agro-climatic zones of India. Fresh and young leaves of 15-20 days old seedlings were used as the sources of genomic DNA.

Extraction of genomic DNA

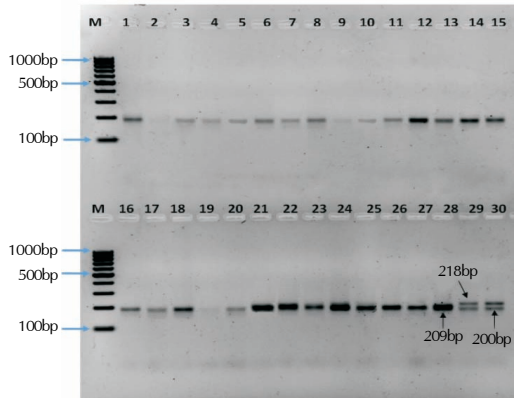
Genomic DNA was isolated from freeze-dried young leaves of a single plant of each line or accession by the CTAB method initially given by Murray and Thomson (1980) and later on modified by Doyle and Doyle (1990) with some minor modification.

PCR amplification

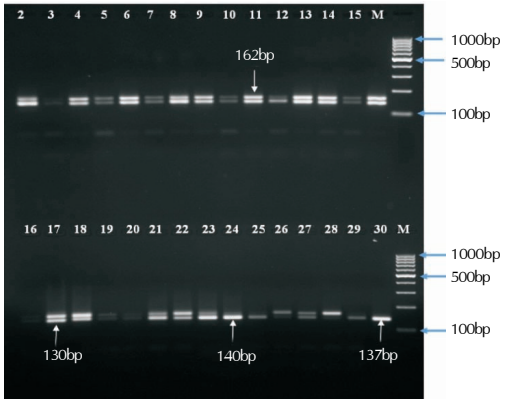
Twenty four pairs of SSR primers were synthesized according to the published common primers of *Brassica* (*http://www.brassica.info*). The primer is selected on the basis of their polymorphism information content. The polymerase chain reaction was carried out following the protocol of Suwabe *et al*. (2002) with some modification. PCR was performed in a 25-μL vol. containing 40ng/μL of genomic DNA, 250 nM of each primer, 0.25 mM of dNTPs, 1× reaction buffer (Thermo scientific, USA), and 1 unit of Taq polymerase (Thermo scientific, USA). The reaction mixture was initially denatured at 94ºC for 5 min, followed by 35 cycles of amplification at 94ºC for 1 min, 60ºC for 1 min and 72ºC for 1 min, and final extension at 72ºC for 4 min in a Veriti Thermal Cycler (Applied Bio-systems).

Agarose gel electrophoresis

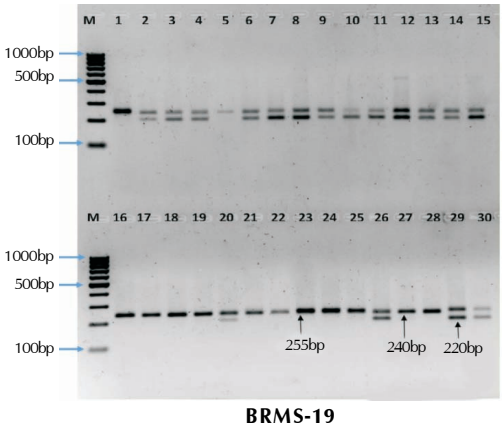
The PCR products were fractionated in a 2.5% agarose gel run at 80V for about 1.5h in 1X tris-borate-EDTA buffer. Molecular size of the amplified product was estimated using a



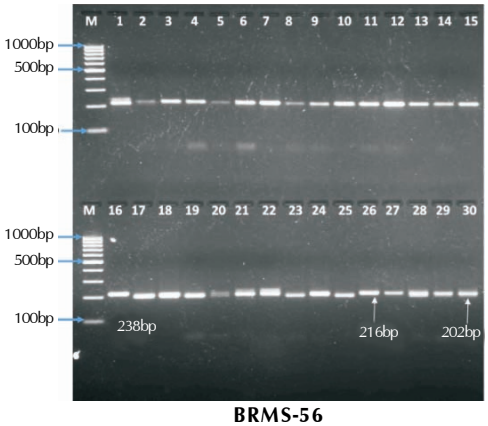
BRMS-17



BRMS-05



BRMS-19



BRMS-56

Figure 1: PCR profile of 30 genotypes using SSR primers. Lane M is 100 bp DNA ladder and Lanes 1-30 represent genotypes, viz. HNS-004, HYLO-401, PM-67, ZEM-1, GM-1, GM-2, GM-3, Varuna, BIO-34192, Kranti, Vardan, Laxmi, IC560696, IC 491446, IC 3999797, NRCYS-05-02, GT-1, IC363714, IC363710, IC363713, GSL-1, Neelam, IC399790, IC399819, IC560699, IC346013, IC365684, IC398101, Pusa Swarnim and Kiran

Table 1: List of thirty mustard genotypes and their species

Sr.No.	Genotypes	Species	Sr.No.	Genotypes	Species
1	GM-1	*Brassica juncea*	16	IC363713	*Brassica rapa*
2	GM-2	*Brassica juncea*	17	JT-1(toria)	*Brassica rapa*
3	GM-3	*Brassica juncea*	18	NRCYS	*Brassica rapa*
4	Laxmi	*Brassica juncea*	19	IC346013	*Brassica rapa*
5	Vardan	*Brassica juncea*	20	IC365684	*Brassica rapa*
6	PM-67	*Brassica juncea*	21	IC398101	*Brassica rapa*
7	ZEM-1	*Brassica juncea*	22	IC399790	*Brassica napus*
8	IC399797	*Brassica juncea*	23	IC399819	*Brassica napus*
9	IC491446	*Brassica juncea*	24	IC560699	*Brassica napus*
10	IC560696	*Brassica juncea*	25	Neelam	*Brassica napus*
11	Varuna	*Brassica juncea*	26	Hyola-401	*Brassica napus*
12	Bio-34192	*Brassica juncea*	27	H.N.S-0004	*Brassica napus*
13	Kranti	*Brassica juncea*	28	GSL-1	*Brassica napus*
14	IC363714	*Brassica rapa*	29	Kiran	*Brassica carinata*
15	IC363710	*Brassica rapa*	30	Pusa Swarnim	*Brassica carinata*

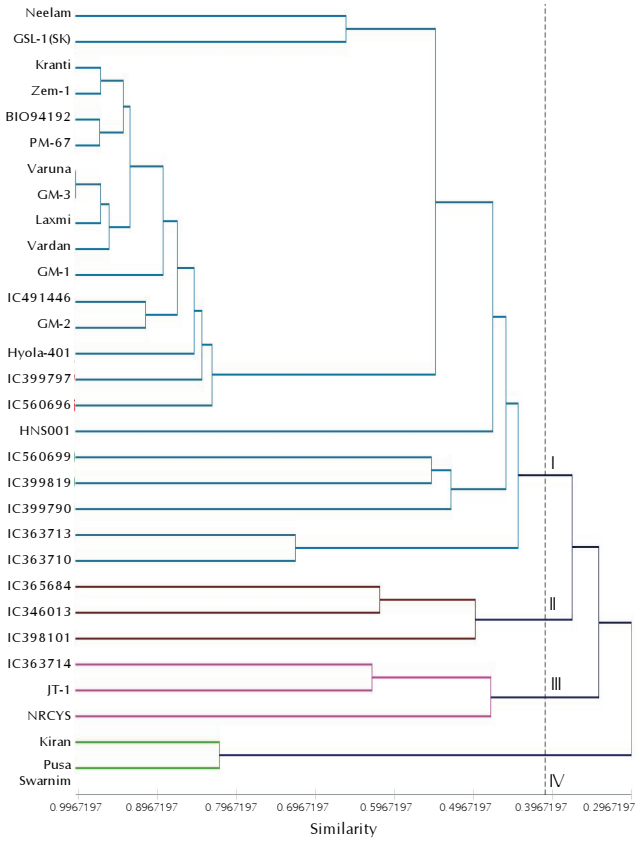


Figure 2: Dendrogram showing clustering of 30 mustard genotypes constructed using UPGMA based on Jaccard's coefficient obtained from SSR analysis

known molecular marker DNA (100 bp DNA ladder). After electrophoresis, the gel was stained with ethidium bromide and viewed with a UV illuminator.

Data analysis

Differences in the DNA banding patterns were qualitatively scored from gel photographs for presence (1) and absence (0) of bands assuming that each band represents a unique genetic locus. Homology of bands among samples was based on the distance of migration in gel. Scoring was done for clear, unambiguous amplicons and their sizes were determined by comparing with 100 bp DNA ladder. Based on the presence or absence of amplicons, a binary 1-0 data matrix was created and used to calculate Jaccard's similarity coefficient (Jaccard, 1908). Polymorphism Information content (PIC) was calculated according to formula described by Garcia *et al.* (2004). Clustering pattern in 30 mustard genotypes was constructed using the computer software "XLSTAT" Version 2012.3, based on UPGMA following the numerical taxonomic techniques and methods of Sneath and Sokal (1973).

RESULTS AND DISCUSSION

In the present Study, 30 primers were initially screened for their ability to produce polymorphic patterns and only 24 of them were selected which gave reproducible and distinct polymorphic amplified products (Table 2). A total of 84 alleles produced. The average number of alleles per locus was 3.50 with a range from 1 to 8, revealing a high level of genetic diversity of *Brassicas* (mustards). Therefore, SSR markers were able to effectively detect genetic variation in mustards. The maximum numbers of alleles were recorded for markers *viz.,* BRMS-005, BRMS-8, BRMS-14 and BRMS-31 produced 6, 6, 8 and 6 alleles respectively. Markers *viz.,* BRMS-002, BRMS-003, BRMS-30, BRMS-40 and BRMS-42 which produced minimum two alleles. The highest allele frequency (Table 3) was given by BRMS-003 and BRMS-17 marker was 0.933. The highest PIC value was recorded for BRMS-31 (0.79) and the lowest for BRMS-003 (0.12). The mean PIC value from all tested microsatellites was 0.45. The molecular size of the amplified PCR products ranged from 99bp (BRMS-26) to 383bp (BRMS-31). Nine primers out of 24 analyzed successfully produced 100% polymorphism. Total polymorphism (average) was 72.22%, whereas the level of polymorphism reported by Celucia *et al.*, 2009, was 71.08 in *Brassicas* and also level of polymorphism reported by Chandra *et al.*, 2013 was found higher 97.56. The reason might be that the *Brassica* varieties used in the present study are different in morphology, ploidy level and genome constituents. The SSR marker, BRMS-17 (Fig. 1.) gave specific bands for species *Brassica carinata*, which could be used for species identification in breeding programme. The highest similarity index value of 0.98 (Table 4) was found between Varuna and

Table 2: List of SSR primers and their sequences

Sr.No.	SSR Locus	Repeat motif	Primer	Sequence (5′-3′)	Expected size	AnnealingTm (°C)
1	BRMS01	$(GA)_{25}$	F	GGTGGCTCTAATTCCTCTGA	139	54.5
			R	ATCTTTCTCTCACCAACCCC		54.5
2	BRMS02	$(CT)_{22}$	F	GATCTTCTCTCCAAAA	168	50.2
			R	TCCAAGCTAAATTACG		50.2
3	BRMS03	$(CT)_{19}$	F	ACGAATTGAATTGGACAGAG	192	53.2
			R	CAGATGGGAGTCAAGTCAAC		57.3
4	BRMS05	$(GA)_{13}$	F	ACCTCCTGCAGATTCGTGTC	162	59.4
			R	GCTGACCTTTCTTACCGCTC		59.4
5	BRMS06	$(GA)_{34}$	F	CAGATGGGAGTCAAGTCAAC	193	55.3
			R	ACTCGAAGCCTAATGAAAAG		53.2
6	BRMS07	$(CT)_{24}$	F	AAATTGTTTCTCTTCCCCAT	152	57.3
			R	GTGTTAGGGAGCTGGAGAAT		57.3
7	BRMS08	$(TC)_{30}$	F	AGGACACCAGGCACCATATA	145	57.3
			R	CATTGTTGTCTTGGGAGAGC		57.3
8	BRMS14	$(TC)_{15}$	F	CCGTAAGGAATATTGAGGCA	156	53.2
			R	TTCCCAATTCTCAAACGGTA		55.3
9	BRMS16	$(TC)_{20}$	F	TCCCGTATCAATGGCGTAACAG	144	60.3
			R	CGATGGTGACATTATTGTGGCG		60.3
10	BRMS17	$(CA)_{33}$	F	GGAAAGGGAAGCTTCATATC	209	55.3
			R	CTGGAAAGCATACACTTTGG		55.3
11	BRMS19	$(GT)_{10}$	F	CCCAAACGCTTTTGACACAT	220	55.3
			R	GGCACAATCCACTCAGCTTT		55.3
12	BRMS26	$(CT)_{26}$	F	CCTATCCTCGGACTAATCAGAA	122	58.4
			R	GTGCTTGATGAGTTTCACATTG		56.5
13	BRMS27	$(GA)_{17}$	F	GTGCTTGATGAGTTTCACATTG	205	58.0
			R	GCAGGCGTTGCCTTTATGTA		58.0
14	BRMS30	$(CT)_{14}$	F	TCAGCCTACCAACGAGTCATAA	212	59.2
			R	AAGGTCTCATACGATGGGAGTG		59.0
15	BRMS31	$(TC)_{33}$	F	TGCCACCAATGACAATGACACTATC	238	61.5
			R	GATGCACTGGGACCACTTACATTTT		60.0
16	BRMS33	$(CA)_{11}$	F	GCGGAAACGAACACTCCTCCCATGT	225	66.3
			R	CCTCCTTGTGCTTTCCCTGGAGACG		67.9
17	BRMS34	$(GA)_{18}$	F	GATCAAATAACGAACGGAGAGA	145	56.5
			R	GAGCCAAGAAAGGACCTAAGAT		58.4
18	BRMS37	$(CA)_{10}$	F	CTGCTCGCATTTTTTATCATAC	154	58.5
			R	TACGCTTGGGAGAGAAAACTAT		59.5
19	BRMS40	$(GA)_{49,}(GT)_4$	F	GAGCCAAGAAAGGACCTAAGAT	283	58.4
			R	CCGATACACAACCAGCCAACTC		62.1
20	BRMS42	$((GA)_4, (CT)_{26}$	F	GGATCAGTTATCTGCACCACAA	220	58.4
			R	TCGGAATTGGATAAGAATTCAA		52.8
21	BRMS44	$(GA)_{27}$	F	AGGCGAGGAGAAGACAACACAA	355	60.1
			R	TACGGGTGGTTTGAATCAGCAG		61.2
22	BRMS50	$(AAT)_{4,}(TC)_{19}$	F	AACTTTGCTTCCACTGATTTTT	186	57.5
			R	TTGCTTAACGCTAAATCCATAT		57.5
23	BRMS51	$(TC)_{15}$	F	GGCCAAGCCACTACTGCTCAGA	265	64.0
			R	GCGGAGAGTGAGGGAGTTATGG		64.0
24	BRMS56	$(GA)_{13}$	F	GATCAAGGCTACGGAGAGAGAG	216	62.1
			R	CGTGACGCTAGAGTAATCGAGT		60.3

GM-3, while the least similarity index value of 0.15 was found between JT-1 and Pusa Swarnim. The average similarity coefficient among genotypes was 0.50.The genetic similarities of SSR markers among the thirty-four landraces ranged from 0.47 to 0.73, with an average of 0.62. Comparison of the landraces within the same region indicated that most landraces were grouped together and had similarity coefficients of over 0.70.

Correlation study was carried out to compare the correlation of original similarity matrix of SSR results with the dendrogram clustering pattern. High correlation between the similarity matrix and dendrogram pattern was justified by the r value which was found to be 0.96 which is very good to fit.

On the basis of cluster study, the total accessions were distributed into four main clusters at a similarity coefficient of 0.40 (Fig. 2.). All the genotypes mostly clustered according to their species, clearly distinguished the grouping among genera and species. The reason for this might be that they shared the common genomes (AA) which have been used in this study.

Within the species, the genotypes *viz.,* ZEM-1 and GM-2 were found to be most diverse in species *B. juncea,* Hyola-401 and HNS001 in species *B. napus* and IC363714 and IC346013 in species *B. rapa*.

Cluster I was divided into two sub clusters IA and IB. Sub cluster IA included only genotypes of species *B. napus* and *B. juncea viz.*, Neelam, GSL-1, Kranti, ZEM-1, PM-67, BIO94192,

Table 3: Results of SSR analysis

Sr.No	Markers	No. of bands amplified	Molecular size range (bp)	Total no. loci	No. of polymorphic loci	Percent Polymorphism (%)	PIC	Na	Ne	Ho	He
1	BRMS-01	52	115-190	5	5	100	0.75	5.000	4.138	0.767	0.758
2	BRMS-02	30	168-175	2	1	50	0.23	2.000	1.301	0.000	0.231
3	BRMS-03	30	180-193	2	1	50	0.12	2.000	1.142	0.000	0.124
4	BRMS-05	58	130-167	6	6	100	0.62	6.000	2.696	0.933	0.629
5	BRMS-06	30	193-200	2	1	50	0.27	2.000	1.385	0.000	0.278
6	BRMS-07	57	120-150	4	4	100	0.59	4.000	2.510	0.900	0.602
7	BRMS-08	52	125-238	6	6	100	0.73	6.000	4.063	0.733	0.754
8	BRMS-14	51	190-382	8	8	100	0.75	8.000	3.947	0.667	0.747
9	BRMS-16	53	108-144	3	2	66.66	0.51	3.000	2.125	0.767	0.529
10	BRMS-17	32	190-210	3	2	66.66	0.22	3.000	1.145	0.067	0.127
11	BRMS-19	47	220-255	3	1	33.33	0.57	3.000	2.281	0.633	0.562
12	BRMS-26	30	99-134	3	1	33.33	0.34	3.000	1.515	0.000	0.340
13	BRMS-27	44	195-240	3	3	100	0.48	3.000	1.839	0.467	0.456
14	BRMS-30	32	200-215	2	2	100	0.34	2.000	1.427	0.033	0.299
15	BRMS-31	45	267-383	6	6	100	0.79	6.000	4.215	0.533	0.763
16	BRMS-33	31	117-368	3	2	66.66	0.36	3.000	1.575	0.033	0.365
17	BRMS-34	30	145-168	3	2	66.66	0.38	3.000	1.613	0.000	0.380
18	BRMS-37	43	137-176	5	5	100	0.66	5.000	2.683	0.467	0.627
19	BRMS-40	33	196-218	2	1	50	0.18	2.000	1.220	0.000	0.180
20	BRMS-42	30	112-122	2	1	50	0.43	2.000	1.301	0.000	0.231
21	BRMS-44	30	155-156	2	1	50	0.27	2.000	1.385	0.000	0.278
22	BRMS-50	30	175-191	3	2	66.66	0.38	3.000	1.625	0.000	3.384
23	BRMS-51	39	250-265	3	2	66.66	0.44	3.000	1.638	0.300	0.389
24	BRMS-56	33	202-238	3	2	66.66	0.51	3.000	2.025	0.100	0.560
Total	-	926		84	67	1733.28	10.92	84	50.794	7.4	13.593
Average	-	38.58	179-207bp	3.50	2.79	72.22	0.45	3.5	2.11	0.308	0.566

Na = No. of Different Alleles, Ne = No. of Effective Alleles, Ho = Observed Heterozygosity, He = Expected Heterozygosity.

Table 4: Genetic similarity matrix of pooled SSR data based on jaccard's similarity coefficient.

	HNS001	Hyola-401	PM-67	ZEM-1	GM-1	GM-2	GM-3	Varuna	BIO34192	Kranti	Vardan	Laxmi	IC560696	IC491446	IC399797
HNS001	1														
Hyola-401	0.52	1													
PM-67	0.44	0.85	1												
ZEM-1	0.48	0.91	0.94	1											
GM-1	0.48	0.81	0.9	0.9	1										
GM-2	0.44	0.85	0.88	0.94	0.84	1									
GM-3	0.49	0.85	0.94	0.94	0.85	0.88	1								
Varuna	0.44	0.85	1	0.94	0.9	0.88	0.98	1							
BIO34192	0.47	0.88	0.97	0.97	0.88	0.91	0.97	0.97	1						
Kranti	0.47	0.88	0.91	0.97	0.88	0.91	0.91	0.91	0.94	1					
Vardan	0.45	0.88	0.97	0.97	0.93	0.91	0.91	0.97	0.94	0.94	1				
Laxmi	0.47	0.82	0.97	0.91	0.88	0.85	0.91	0.97	0.94	0.88	0.94	1			
IC560696	0.53	0.77	0.85	0.85	0.82	0.8	0.81	0.85	0.83	0.83	0.88	0.88	1		
IC491446	0.48	0.78	0.86	0.86	0.77	0.91	0.91	0.86	0.89	0.83	0.83	0.89	0.78	1	
IC399797	0.51	0.79	0.88	0.82	0.79	0.77	0.88	0.88	0.85	0.8	0.85	0.91	0.8	0.86	1
NRCYS	0.23	0.4	0.39	0.42	0.38	0.42	0.43	0.39	0.41	0.41	0.4	0.38	0.35	0.42	0.39
JT-1	0.25	0.35	0.31	0.34	0.3	0.34	0.35	0.31	0.33	0.33	0.32	0.31	0.28	0.35	0.31
IC363714	0.34	0.34	0.36	0.36	0.36	0.36	0.38	0.36	0.35	0.35	0.37	0.35	0.38	0.37	0.36
IC363710	0.42	0.43	0.42	0.42	0.41	0.39	0.4	0.42	0.41	0.41	0.43	0.41	0.41	0.36	0.42
IC363713	0.35	0.48	0.5	0.5	0.46	0.47	0.48	0.5	0.49	0.49	0.51	0.49	0.45	0.43	0.5
GSL1	0.44	0.53	0.59	0.59	0.59	0.55	0.56	0.59	0.58	0.58	0.61	0.58	0.62	0.51	0.51
Neelam	0.43	0.55	0.54	0.54	0.54	0.5	0.51	0.54	0.52	0.52	0.55	0.52	0.49	0.47	0.5
IC399790	0.36	0.33	0.36	0.36	0.35	0.33	0.34	0.36	0.35	0.35	0.36	0.35	0.41	0.31	0.36
IC399819	0.39	0.5	0.56	0.53	0.53	0.49	0.54	0.56	0.55	0.51	0.54	0.59	0.51	0.52	0.56
IC560699	0.43	0.44	0.5	0.47	0.46	0.43	0.51	0.5	0.49	0.45	0.48	0.52	0.45	0.5	0.58
IC346013	0.35	0.38	0.43	0.4	0.36	0.43	0.44	0.43	0.42	0.39	0.41	0.45	0.42	0.5	0.5
IC365684	0.35	0.39	0.44	0.41	0.4	0.38	0.45	0.44	0.43	0.4	0.42	0.47	0.4	0.44	0.51
IC398101	0.35	0.29	0.34	0.31	0.33	0.29	0.33	0.34	0.33	0.31	0.32	0.36	0.36	0.32	0.34
Pusa swarnim	0.26	0.37	0.33	0.36	0.33	0.33	0.35	0.33	0.36	0.36	0.34	0.33	0.33	0.31	0.3
Kiran	0.3	0.36	0.32	0.35	0.31	0.32	0.36	0.32	0.34	0.34	0.33	0.34	0.34	0.35	0.35

GM-1, GM-2, Laxmi, Vardan, Varuna, IC491446, Hyola-401, IC399797, IC560696, HNS001, IC560699, IC399819, IC399790 and GM-3. Sub cluster IB included only two genotypes *viz.*, IC363713, IC363710. Cluster II includes the genotypes *viz.*, IC365684, IC346013 and IC398101. Cluster III includes the genotypes IC363714, JT-1 and NRCYS-05-02.

Table 4: Cont.....

	NRCYS	JT-1	IC363714	IC363710	IC363713	GSL1	Neelam	IC399790	IC399819	IC560699	IC346013	IC365684	IC398101	Pusa	Kiran
HNS001															
Hyola-401															
PM-67															
ZEM-1															
GM-1															
GM-2															
GM-3															
Varuna															
BIO34192															
Kranti															
Vardan															
Laxmi															
IC560696															
IC491446															
IC399797															
NRCYS	1														
JT-1	0.55	1													
IC363714	0.4	0.63	1												
IC363710	0.22	0.38	0.54	1											
IC363713	0.27	0.39	0.51	0.72	1										
GSL1	0.36	0.29	0.42	0.39	0.47	1									
Neelam	0.27	0.28	0.33	0.41	0.49	0.66	1								
IC399790	0.18	0.24	0.4	0.46	0.44	0.49	0.55	1							
IC399819	0.3	0.27	0.37	0.43	0.44	0.49	0.55	0.54	1						
IC560699	0.24	0.31	0.41	0.38	0.45	0.4	0.39	0.51	0.55	1					
IC346013	0.27	0.21	0.3	0.22	0.31	0.31	0.28	0.27	0.38	0.56	1				
IC365684	0.33	0.26	0.28	0.22	0.26	0.29	0.29	0.22	0.39	0.47	0.62	1			
IC398101	0.19	0.23	0.35	0.29	0.39	0.31	0.31	0.38	0.38	0.45	0.52	0.47	1		
Pusa swarnim	0.23	**0.15**	0.22	0.26	0.27	0.22	0.2	0.23	0.28	0.27	0.3	0.3	0.36	1	
Kiran	0.27	0.19	0.23	0.24	0.26	0.22	0.19	0.22	0.3	0.31	0.29	0.29	0.31	0.82	1

Cluster IV includes only two genotypes of species *B. carinata viz.,* Pusa Swarnim and Kiran.

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