African Journal of Biotechnology Vol. 9 (26), pp. 3981-3987, 28 June, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB09.649
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Genetic diversity analysis of some exotic, Indian and mutant *Brassica* sp. through RAPD markers

S. N. Malode[1]*, S. M. Shingnapure[1], V. N. Waghmare[2] and S. Sutar[2]

[1]P.G. Department of Botany, Government Vidarbha
Institute of Science and Humanities, Amravati-444604, India.
[2]Biotechnology Division, C.I.C.R., P. B. No.2, Shankar Nagar, P.O., Nagpur-440010, India.

Accepted 2 July, 2009

Genetic make-up of *Brassica* crops has been playing a major contributory role towards its enhanced production. The exploitation of genetic variation among races and gene pools in oilseed *Brassica* is essential as considerable diversity exists in the set of plants. In the present investigation, the isolated DNA from 20 *Brassica* genotypes including exotic, Indian and few mutant varieties were subjected to 40 RAPD primers among which 31 detected polymorphism. A total of 240 DNA fragments were generated by the 31 primers. Cluster analysis of 1 - 0 bivariate data using UPGMA method delineated the genotypes into 4 groups and the data was further used for constructing a dendrogram.

Key words: *Brassica carinata, Brassica juncea, Brassica napus,* RAPD markers, polymorphism, genetic diversity.

INTRODUCTION

Brassica is cosmopolitan in distribution occupying a prominent place in world's agrarian economy as vegetables, oilseed, feed and fodder, green manure and condiment. The optimism for high placement on *Brassica* as contributors to the vegetable oil supply and to the national economy as well as in the research field is based on their amenability to the use of genetic engineering and biotechnological techniques for genetic modification (Chopra and Prakash, 1996).

The 3 important *Brassica* sp.; exotic *Brassica carinata,* Indian *Brassica napus* and *Brassica juncea* with their mutants undertaken for the study exhibit a number of morphological and biochemical characteristics. The presence of glucosinolates, erucic acid, protein and oil contents in these plants are those factors that can be estimated by biochemical analyses. But, there are some other factors existing within these plants such as seed colour, presence or absence of albugo and white rust resistance, shattering resistance, and so on that need different parameters that can evaluate them at genetic level.

The advent of molecular markers such as RAPD have proved their potential to measure such genetic variations with good coverage of entire genome and thereby have been used for diversity analysis in a vast array of field crops including *Brassica*. These PCR based markers reveal variations at DNA level, those variations that are obtained from the huge extent of genetic polymorphism generated by these markers, leading to evaluation of phenotypic variability.

RAPD analysis plays a key role in studying the DNA profile and thereby detecting the extent of polymorphism within species. The purpose of the present study was to characterize 20 exotic, Indian and mutant *Brassica* genotypes at molecular level using RAPD primers.

MATERIALS AND METHODS

DNA isolation

20 Indian, exotic and mutant *Brassica* genotypes utilized for the present study for RAPD analysis differ in their morphological and biochemical characters (Table 1). Genomic DNA isolation was carried out according to the protocol in the manual 'biochemical methods' by Sadasivam and Manickam (1996). 2 g of fresh leaf material was grind to fine powder using liquid nitrogen. The powder was homogenized with 30 ml extraction buffer (100 mM Tris-HCL, 10 mM EDTA Na_2, 500 mM NaCl, pH 7.8). The homogenate was thoroughly mixed with 2 ml of 20% sodium dodecyl sulphate using magnetic stirrer for 20 min followed by incubation of the contents at 65°C for 10 min. For precipitation of proteins and polysaccharides, 20 ml of 5 M potassium acetate solution was added to the mixture and was further incubated at 0°C for 30 min. The precipitate was then removed by centrifugation at 25,000 g for 15 min. 6 tenth volume of isopropanol was added to the supernatant and the con-

*Corresponding author. E-mail: satishmalode17@gmail.com.

Table 1. 20 *Brassica* genotypes with their morphological and biochemical characters.

S.No.	Genotypes	Characteristics
1	BCDB-1	Brownish yellow, waxy leaves, tall, Albugo and white rust resistant, high glucosinolate, high erucic acid and low protein.
2	BCDB-2	Yellow seeded, tall, Albugo and white rust resistant, high glucosinolate, high erucic acid and low protein.
3	BCDB-3	Small yellow brown seeded, tall, Albugo and white rust resistant, high glucosinolate, high erucic acid and low protein.
4	BJ-206	Brown black seeded, resistant to Albugo and susceptible to white rust, high glucosinolate and zero erucic acid, shattering resistant and high oil.
5	BJ-203	Yellow seeded, resistant to Albugo and susceptible to white rust, high glucosinolate and zero erucic acid.
6	BJ-200	Bright yellow seeded, resistant to Albugo and susceptible to white rust, high glucosinolate and zero erucic acid, high protein and low crude fibre.
7	BJ-202	Bold yellow seeded, resistant to Albugo and susceptible to white rust, low glucosinolate and zero erucic acid, high protein and low crude fibre.
8	BJ-217	Bold yellow seeded, resistant to Albugo and susceptible to white rust, high glucosinolate and zero erucic acid, low protein and low crude fibre.
9	BJ-207	Bold yellow seeded, resistant to Albugo and susceptible to white rust, low glucosinolate and zero erucic acid, low protein and low crude fibre.
10	BJ-PBold	Bold black seeded, susceptible to Albugo and white rust, high glucosinolate and high erucic acid.
11	BCDB-4	Yellow seeded, tall, white flower, insect and pest resistance, high glucosinolate and high erucic acid.
12	BJ-Varuna	Black small seeded, high glucosinolate, high erucic acid, low protein and low oil.
13	BJ-201	Small uniform black seeded, resistant to Albugo and susceptible to white rust, high glucosinolate and zero erucic acid, low protein and high oil.
14	BJ-352	Bold black seeded, resistant to Albugo and susceptible to white rust, high glucosinolate and high erucic acid, low protein and low oil, CMS line.
15	BN-317	Tall, late maturing, black seeded, yellow flowers, resistant to Albugo and susceptible to white rust, low glucosinolate, zero erucic acid and 34% oil.
17	BN-352	Tall, late maturing, black seeded, yellow flowers, resistant to Albugo and white rust, low glucosinolate, zero erucic acid and high oil.
18	BCDB-5	Tall, brownish yellow seeded, Albugo and white rust resistant, high erucic acid and low protein.
19	BCDB-6	Tall, brownish yellow seeded, less waxy, susceptible to Albugo and white rust, high glucosinolate, high erucic acid and low protein.
20	BCDB-7	Dwarf, small yellow seeded, less waxy, light yellow flowers, good plant type, early flowering, susceptible to Albugo and white rust, high glucosinolate, high erucic acid and low protein.

tents were then allowed to stand at -20°C for at least 30 min to precipitate DNA. DNA thus obtained was centrifuged at 20,000 g for 15 min. After discarding the supernatant the DNA pellets were redissolved in 1.2 ml of suspension buffer (50 mM Tris-HCL, 10 mM EDTA Na_2^+, pH 8.0) followed by addition of 0.72 ml of isopropanol and 72 µl of 3 M sodium acetate solution. The suspended DNA pellets were allowed to stand at -20°C for 1 h. The DNA was again pelleted by recentrifugation, washed with ice cold 80% ethanol and redissolved in 2 ml of TE buffer (10 mM Tris- HCL, 1mM EDTA Na_2, pH 7.5). The extracted DNA samples were run on 1% agarose gel for confirmation of isolated DNA followed by quantification of DNA for confirmation of its good quality.

Polymerase chain reaction (PCR)

The PCR was carried out with modification in thermal profile given in Table 2. The RAPD primers obtained from operon technologies, USA were employed for genetic diversity analyses. DNA amplification was carried out for 40 cycles in PCR tubes containing 25 µl reaction mixture (reagents of a single PCR reaction given in Table 3). Finally, the PCR tubes were subjected to the thermal profile given above. The reaction was carried out in a gradient Mastercycler for amplification program.

A 1.5% agarose gel in 1 X TAE buffer (Tris-base, glacial acetic acid, EDTA) with 30 µl EtBr (10 mg/ml) per 300 ml of gel volume

Table 2. PCR amplification program.

	Step	Temperature (°C)	Duration
1	Initial denaturation	94	60 min
2	Denaturation	94	45 s
3	Primer annealing	36	1 min
4	Primer extension	72	1 min
5	Final extension	72	10 min

Steps 2 to 4 were repeated for 40 cycles.

Table 3. Reagents for single PCR reaction.

Reagents	Quantity (µl)
Sterile Distilled water	18.8
Buffer	2.5
$MgCl_2$	1.0
dNTPs	1.0
Primer	0.5
Taq DNA polymerase	0.2
DNA (10 ng)	1.0
Total	**25.0**

was prepared. 2 µl of loading dye was added to each PCR tube for sample loading. Electrophoresis was carried out at 50V for 3 h and visualized under UV transilluminator.

Data analysis

For statistical analysis, the amplification products were scored across the lanes comparing their respective molecular weight. Presence of band was scored as "1" and "0" for absence. Homology of band was based on distance of migration in the gel. The SIMQUAL (Similarity for qualitative data) program was used to calculate the Jaccard's coefficient. Jaccard's similarity coefficient (J) was used to calculate similarity between genotypes by the formula,

J = a/n-d or J = a/a+b+c.

The genetic associations between genotypes were evaluated by calculating the Jaccard's Similarity coefficient for pairwise comparisons based on the proportion of shared band produced by the primers. Data analysis was performed using the NTSYS-PC (Numerical Taxonomy System, Version 2.02, Rohlf, 1990). Dendrogram was constructed using UPGMA (Unweighted pair group method for arithmetic mean) method.

RESULTS AND DISCUSSION

Optimization of various experimental steps involved during the present investigation, starting from DNA extraction protocol to the PCR amplification reaction and gel electrophoresis was done in order to overcome the reported sensitivity problem of RAPD technique. In total, 40 RAPD primers were screened out to amplify DNA from 20 *Brassica* genotypes of which 31 primers detected polymorphism (Table 4). The highest number of bands (14) was obtained with primer B10 (Figure 1a.) and B14. A total of 240 amplified DNA fragments ranging from 250 to 3500 bp were generated by 31 primers with 7.74 as the average number of bands amplified per primer (average polymorphism). The rare and unique banding pattern observed in the DNA profile exhibited by each species resulted in 100% polymorphism. In *Brassica*, generally lower level of polymorphism is expected since they have a lower level of out-crossing due to a weak and often non-existing self-incompatibility system (Uzunova et al., 1995; Cheung et al., 1997).

The 1-0 bivariate data similarity coefficient matrix for 20 *Brassica* genotypes based on the data of 31 primers using UPGMA method was utilized to construct a dendrogram using NTSYC-PC program. The similarity coefficient between each of the 2 genotypes was calculated based on Jaccard's Similarity coefficient (Table 5). Highest similarity coefficient of 73% among *B. carinata* was found between brown yellow seeded BCDB-1 (check) and yellow seeded mutant BCDB-2, both possessing albugo and white rust resistance character. Lowest similarity of 11% was reported between brown yellow seeded mutant BCDB-5 and small yellow seeded mutant BCDB-7, the latter largely differing from the former one in terms of less waxy leaf character, early flowering and in being dwarf in nature. Similarly within *B. juncea*, highest similarity coefficient of 84% was found between bold yellow seeded mutants BJ-217 and BJ-207, which is obvious as both belong to var. pusabold known for its bold seeded appearance. The minimum similarity of 45% was noted between brown black seeded mutant BJ-206 of var. varuna with shattering resistance and bright yellow seeded mutant BJ-200 of var. pusabold lacking the same character. Within *B. napus*, highest similarity coefficient of 81% was found between black seeded mutants BN-362, a white flowered variety and BN-352, a yellow flowered variety, both merely differing with each other in their flower colour and sharing maximum of the characteristic features as plant height, resistance to albugo and white rust. Lowest similarity of 29% was found between black seeded check BN-317 and mutant BN-352, which was unusual as both of them exhibit maximum similar characters such as tall plant height, late maturity, black seed colour, and resistance to albugo and white rust. From this, it can be stated that the morphological characters of these *Brassica* varieties cannot be considered as the true reflection of their genotypic characteristics and need further screening of more random primers to simplify their genetic relationships.

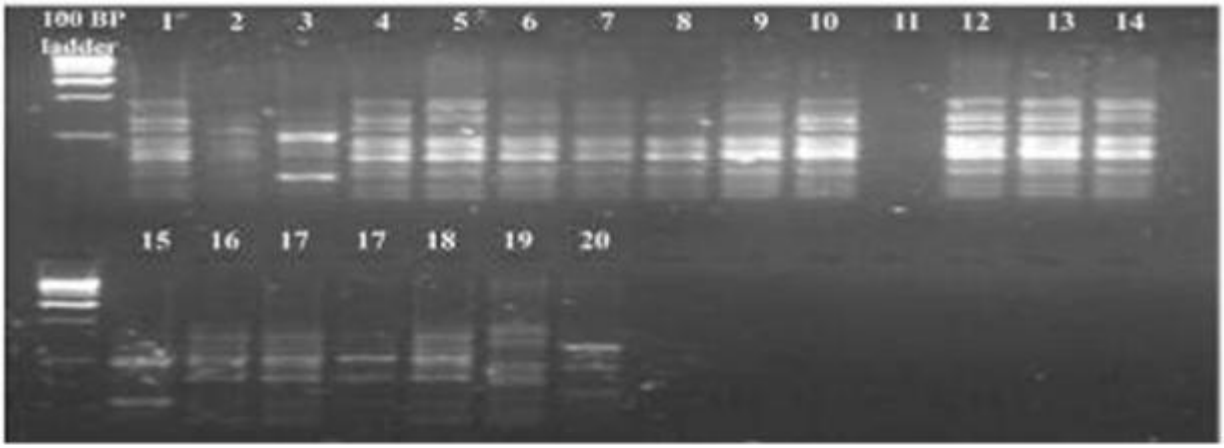
From dendrogram analysis (Figure 2), the 20 *Brassica* genotypes were delineated into four clusters; A, B, C and D using UPGMA cluster analysis. The 2 main clusters A and B were separated from each other at 23% similarity. Cluster A consisted of small yellow brown seeded mutant

Table 4. RAPD polymorphism among 20 *Brassica* genotypes with primer sequence information.

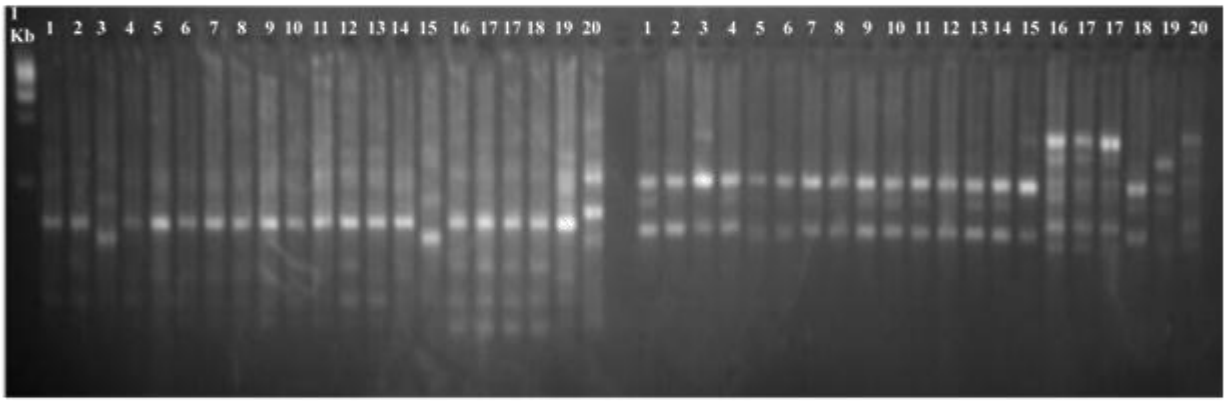
S/N	Primer	Sequence (5'-3')	Total no of bands amplified	Total no of polymorphic bands	% Polymorphim
1	B01	GTTTCGCTCC	05	05	100
2	B02	TGATCCCTGG	04	04	100
3	B04	GGACTGGAGT	02	02	100
4	B06	TCGTCTGCCC	03	03	100
5	B07	GGTGACGCAG	06	06	100
6	B08	GTCCACACGG	05	05	100
7	B10	CTGCTGGGAC	14	14	100
8	B11	GTAGACCCGT	09	09	100
9	B13	TTCCCCCGCT	03	03	100
10	B14	TCCGCTCTGG	14	14	100
11	B15	GGAGGGTGTT	08	08	100
12	B16	TTTGCCCGGA	02	02	100
13	B17	AGGGAACGAG	13	13	100
14	B18	CCACAGCAGT	12	12	100
15	B19	ACCCCGGAAG	11	11	100
16	B20	GGACCCTTAC	02	02	100
17	C05	GATGACCGCC	04	04	100
18	C06	GAACGGACTC	11	11	100
19	C07	GTCCCGACGA	08	08	100
20	C08	TGGACCGGTG	08	08	100
21	C09	CTCACCGTCC	08	08	100
22	C10	TGTCTGGGTG	09	09	100
23	C11	AAAGCTGCGG	04	04	100
24	C12	TGTCATCCCC	06	06	100
25	C13	AAGCCTCGTC	09	09	100
26	C14	TGCGTGCTTG	08	08	100
27	C15	GACGGATCAG	10	10	100
28	C16	CACACTCCAG	11	11	100
29	C18	TGAGTGGGTG	11	11	100
30	C19	GTTGCCAGCC	12	12	100
31	C20	ACTTGCCCAC	08	08	100
Total			240	240	100
Mean			7.74	7.74	

BCDB-3 of *B. carinata* and black seeded check BN-317 of *B. napus*. Both of these varieties differ in terms of their seed colour, resistance to albugo and white rust and flower colour and are still being grouped in one cluster. Cluster B consisted of 17 genotypes and was divided into 2 sub clusters C and D separated by 33% similarity. Cluster C included 3 genotypes; black seeded mutants BN-352, BN-362 of *B. napus* and brown yellow seeded mutant BCDB-5 of *B. carinata*; three of these genotypes are tall with a good withstanding capacity towards white rust and albugo. D cluster consisted of 14 genotypes of which 10 belong to *B. juncea*; brown black seeded BJ-206, yellow seeded BJ-203, bright yellow seeded BJ-200, bold yellow seeded BJ-202, BJ-217, BJ-207, bold black seeded BJ-PBold, BJ-352, black small seeded BJ-varuna, small uniform black seeded BJ-201 and 4 to *B. carinata*, brown yellow seeded BCDB-1(check) along with its yellow seeded mutants BCDB-2, BCDB-4, BCDB-5 and BCDB-6. Small yellow seeded mutant BCDB-7 of *B. carinata* has been considered as an outgroup because it differs from rest of the group. Consequences of reverse mutation in this mutant and its close affinity with *Arabidopsis thaliana* genome in terms of early flowering and a life span of only 29-30 days may be serving as the most diversifying factors of this genotype making it unique among other mutants of the respective species.

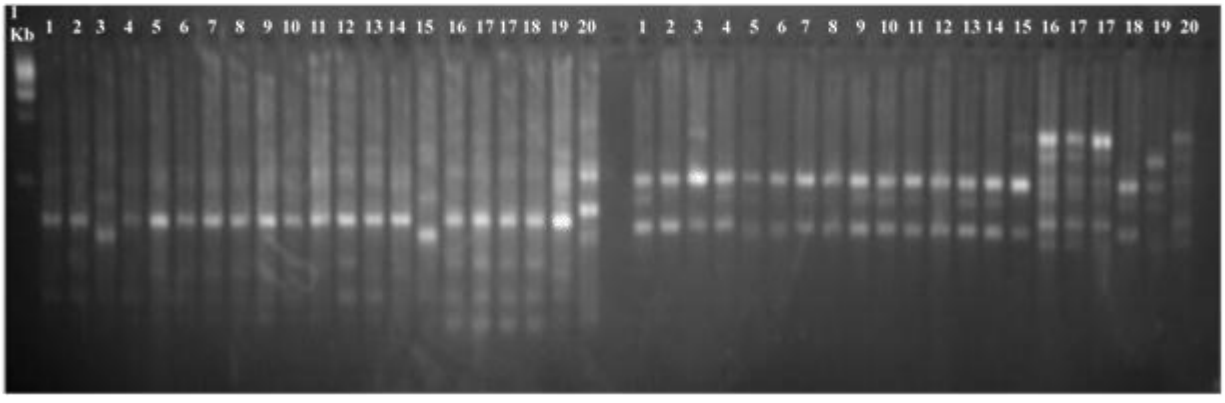
From the above, it is clear that the data proved insufficient in differentiating between *B. juncea* and *B. carinata* and between *B. napus* and *B. carinata* with few exceptions. Both *B. carinata* and *B. napus* show affinity towards each other as they are the closest groups in regards to their morphological features in comparison to



(a)



(b)



(c)

Figure 1. (a) Banding profile of 20 *Brassica* genotypes generated by primer OPB-10. Lane **Kb.** 100 bp ladder, lane **1**. BCDB-1, lane **2**. BCDB-2, lane **3**. BCDB-3, lane **4**. BJ-206, lane **5**. BJ-203. lane **6**. BJ-200, lane **7**.BJ-202, lane **8**. BJ-217, lane **9**. BJ-207, lane **10**. BJ-PBold, lane **11**. BCDB-4, lane **12**. BJ-Varuna, lane **13**. BJ-201, lane **14**. BJ-352, lane **15**. BN-317, lane **16**. BN-362, lane **17**. BN-352, lane **18**. BCDB-5, lane **19**. BCDB-6, lane **20**. BCDB-7. **(b).** Banding profile of 20 *Brassica* genotypes generated by primer OPC-9, OPC-10. **(c).** Banding profile of 20 *Brassica* genotypes generated by primer OPC-11 and OPC-12.

Table 5. Jaccard's similarity coefficient values of 20 *Brassica* genotypes.

	BCDB-1	BCDB-2	BCDB-3	BJ-206	BJ-203	BJ-200	BJ-202	BJ-217	BJ-207	BJ-PBold	BCDB-4	BJ-Varuna	BJ-201	BJ-352	BN-317	BN-362	BN-352	BCDB-5	BCDB-6	BCDB-7
BCDB-1	1.00																			
BCDB-2	0.73	1.00																		
BCDB-3	0.19	0.19	1.00																	
BJ-206	0.62	0.54	0.15	1.00																
BJ-203	0.60	0.57	0.17	0.55	1.00															
BJ-200	0.52	0.53	0.18	0.45	0.61	1.00														
BJ-202	0.56	0.55	0.18	0.56	0.65	0.64	1.00													
BJ-217	0.59	0.59	0.18	0.55	0.64	0.68	0.76	1.00												
BJ-207	0.63	0.63	0.18	0.56	0.62	0.69	0.75	0.84	1.00											
BJ-PBold	0.68	0.69	0.19	0.56	0.57	0.59	0.61	0.64	0.70	1.00										
BCDB-4	0.63	0.72	0.20	0.49	0.57	0.59	0.63	0.71	0.78	0.68	1.00									
BJ-Varuna	0.65	0.65	0.18	0.57	0.65	0.54	0.55	0.59	0.63	0.69	0.62	1.00								
BJ-201	0.65	0.59	0.18	0.62	0.50	0.51	0.58	0.58	0.63	0.67	0.57	0.72	1.00							
BJ-352	0.62	0.55	0.20	0.57	0.57	0.56	0.62	0.68	0.75	0.64	0.68	0.64	0.70	1.00						
BN-317	0.27	0.26	0.43	0.27	0.26	0.28	0.24	0.26	0.26	0.26	0.27	0.25	0.26	0.27	1.00					
BN-362	0.28	0.29	0.23	0.26	0.23	0.25	0.23	0.25	0.27	0.29	0.31	0.27	0.28	0.32	0.33	1.00				
BN-352	0.33	0.34	0.25	0.25	0.26	0.27	0.27	0.28	0.31	0.32	0.34	0.31	0.32	0.35	0.29	0.81	1.00			
BCDB-5	0.36	0.37	0.27	0.28	0.29	0.28	0.29	0.31	0.33	0.35	0.36	0.32	0.35	0.36	0.31	0.69	0.78	1.00		
BCDB-6	0.50	0.50	0.18	0.48	0.44	0.49	0.54	0.56	0.69	0.53	0.61	0.54	0.55	0.61	0.22	0.29	0.29	0.33	1.00	
BCDB-7	0.17	0.17	0.13	0.17	0.15	0.15	0.16	0.14	0.17	0.19	0.16	0.18	0.15	0.17	0.16	0.13	0.15	0.11	0.15	1.00

that of *B. juncea*. 69-78% similarity among the two *B. napus* and one *B. carinata* genotype in cluster D has done justice to the fact stated above. On the contrary, the dendrogram provides a satisfactory differentiation between *B. napus* and *B. juncea*. Placement of some mutants in other group by statistical analysis proves that clustering of these 20 genotypes into four groups has not strictly followed the morphological features exhibited by these mutants that group them in their respective species and needs further screening of these mutants with random primers to solve the query.

The genotypes undertaken for the present investigation exhibited a huge range of variation in their characteristics. Thus, understanding of the genetic relationships among these exotic, Indian and mutant *Brassica* varieties together is essential to exploit them at commercial level with the help of RAPD-like assays to explore large genomic portions and thereby giving a more accurate picture of the genetic diversity within the genus *Brassica*.

ACKNOWLEDGEMENT

The author acknowledges the Biotechnology Division, Central Institute for Cotton Research, Nagpur for providing necessary facilities to carry out RAPD analysis.

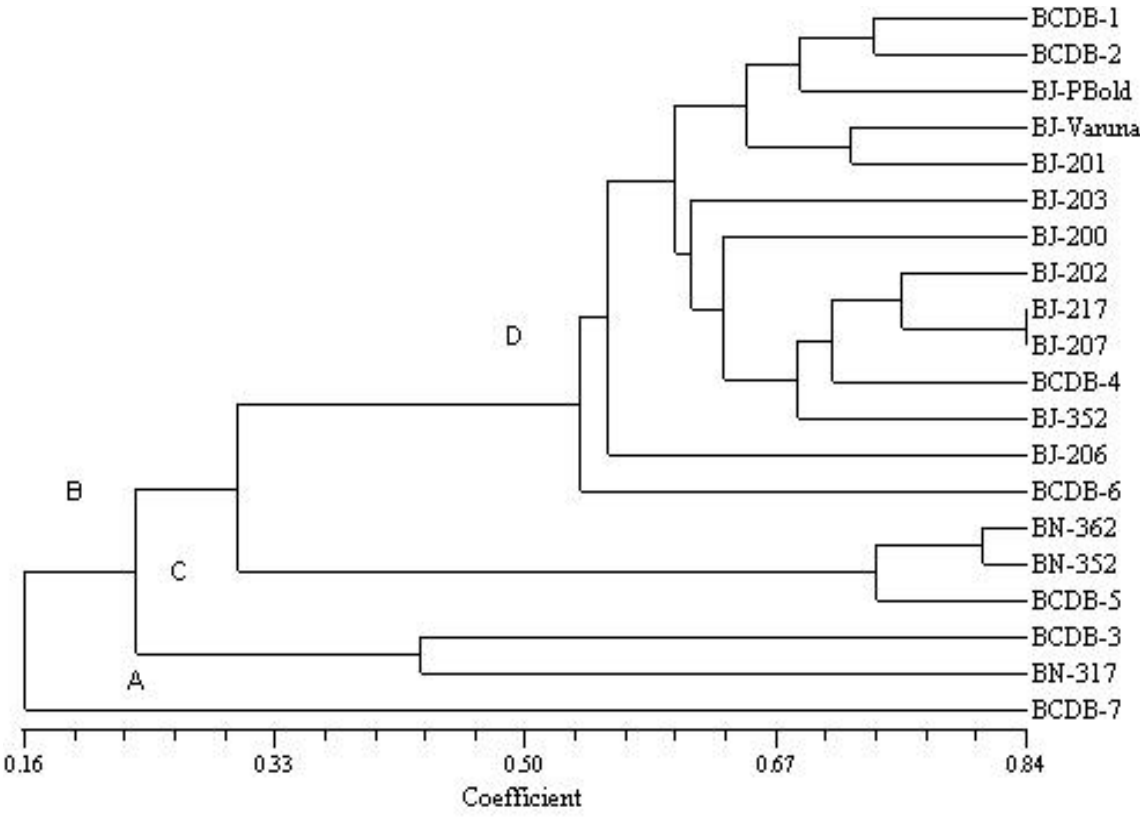


Figure 2. Dendrogram of 20 *Brassica* genotypes generated by UPGMA analysis of RAPD bands obtained with 31 random primers. Scale at the bottom depicts the similarity values obtained using Jaccard's Similarity Coefficient.

REFERENCES

Cheung WY, Friesen L, Rakow FFW, Seguin-Swartz G, Landry BS (1997). A RFLP-based linkage map of mustard [*Brassica juncea*.L.Czern and Coss] TAG.

Chopra VL, Prakash S (1996). Oilseed and vegetable *Brassicas*: Indian perspective. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi.

Sadasivam S, Manickam A (1996). Biochemical Methods. 2nd Edn. New Age International Publishers, New Delhi. pp. 154-155.

Uzunova M, Ecke W, Weissleder K, Robbelen G (1995). Mapping of the genome of rapeseed. *Brassica napus* Ll. Construction of an RFLP linkage map and localization of QTLs for seed glucosinolate content. Theor. Appl. Genet. 90: 194-204.