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Potential use of random amplified polymorphic DNA (RAPD) technique to study the genetic diversity in Indian mustard (*Brassica juncea*) and its relationship to heterosis

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Abstract RAPD assays were performed, using 34 arbitrary decamer oligonucleotide primers and six combinations of two primers, to detect inherent variations and genetic relationships among 12 Indian and 11 exotic *B. juncea* genotypes. Of 595 amplification products identified, 500 of them were polymorphic across all genotypes. A low level of genetic variability was detected among the Indian genotypes, while considerable polymorphism was present among the exotic ones. Based on the pair-wise comparisons of amplification products the genetic similarity was calculated using Jaccard's similarity coefficients and a dendrogram was constructed using an unweighted pair group method was arithmetical averages (UPGMA). On the basis of this analysis the genotypes were clustered into two groups, A and B. Group A comprised only exotic genotypes, whereas all the Indian genotypes and four of the exotic genotypes were clustered in group B. Almost similar genotypic rankings could also be established by computing as few as 200 amplification products. In general, a high per cent of heterosis was recorded in crosses involving Indian × exotic genotypes. On the other hand, when crosses were made amongst Indian or exotic genotypes, about 80% of them exhibited negative heterosis. Results from this study indicate that, despite the lack of direct correlation between the genetic distance and the degree of heterosis, genetic diversity forms a very useful guide not only for investigating the relationships among *Brassica* genotypes but also in the selection of parents for heterotic hybrid combinations.

Key words *Brassica juncea* · RAPD · DNA polymorphism · Genetic distance · Heterosis breeding

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Introduction

Indian mustard [*Brassica juncea* (L.) Czern and Coss] is the predominant oilseed brassica in the Indian subcontinent and East Europe. Although predominantly autogamous, 5–18% outcrossing has been reported in this species (Labana and Banga 1984).

Traditionally, the cultivars are developed through pure-line selection. However, the yield increment using this approach has reached a plateau. Of late, it has been realised that heterosis breeding is one of the potential approaches to overcome the existing yield plateau. Worldwide attempts are now underway to develop F_1 hybrids in this crop as up to 200% yield heterosis has been reported under a range of test conditions in intervarietal hybrids of *B. juncea* (Banga and Labana 1984). Generally, hybrids between the genotypes of different geographic origins have been more productive than those involving genotypes of the same origin (Banga and Labana 1984). Likewise, in the related crop species *B. napus* F_1 hybrids between European and Asiatic groups were more productive than among European or Asiatic ones (Lefort-Buson et al. 1987).

Different heterotic groups have been assigned on the basis of morphological traits and biochemical parameters such as isozyme analysis and seed storage proteins (Nucca et al. 1978; Arus et al. 1985). However, these characters reveal only a limited amount of polymorphism among closely-related genotypes and are also influenced by the prevailing environmental conditions. As an alternative, RFLP analysis has been employed to study genome organization, phylogeny, and polymorphism in the genus *Brassica* by using a number of single-copy probes (Landry et al. 1991; Song et al. 1991) and specific oligonucleotide repeats (Weising et al. 1991). Although RFLP analysis reveals extensive polymorphism, it is labour-intensive, time consuming and requires the use of hazardous radioactive chemicals. To circumvent many of these problems, the RAPD (random amplified polymorphic DNA) assay has been proposed as an alternative (Welsh and McClelland 1990; Williams et al. 1990; Caetano-Anolles et al. 1991). In *Brassica*, the RAPD assay has been employed to develop genome-specific markers (Quiros et al. 1991) and to resolve taxonomic relationships (Demeke et al. 1992). It has also been used for the identification of *B. oleracea* cultivars (Hu and Quiros 1991; Kresovich et al. 1992). A potentially more important use of this technique would be the allocation of genotypes to specific heterotic groups which would reduce both cost and labour by eliminating intra-group crossings.

This paper presents our results on a RAPD assay of 24 *B. juncea* genotypes of diverse geographic origin and explores its potential to evaluate the relationships of these genotypes and to predict the performance of hybrids between them.

Materials and methods

Plant material

The plant material used in this study comprised 12 Indian and 11 exotic genotypes of *B. juncea* (L.) Czern and Coss (2n = 36; AABB) (Table 1). A majority of the Indian accessions are the products of directional selection for higher yield and/or oil content. All the genotypes, along with 24 selected hybrids (see Table 3), were field tested at Punjab Agricultural University, Ludhiana. Genotypes used in the present study were maintained through selfing.

Field evaluation

In the hybrid evaluation block, RLM 619 (local commerical check) and Varuna (national commercial check) were also included. Each plot in a varietal block consisted of single rows, 3 m long and spaced 30 cm apart. In the hybrid test block, each block comprised three rows, 3 m long and spaced 30 cm apart. Plant-to-plant distance was maintained at 15 cm.

DNA isolation and PCR amplification

Genomic DNA was extracted from young leaves following the procedure of Dellaporta et al. (1983). Amplification reactions (25 µl final volume) contained 50 ng genomic DNA, 240 µM each of dATP, dCTP, dGTP and dTTP, 30 ng primer (Operon), 1 × *Taq* polymerase buffer, 0.5 units of *Taq*DNA polymerase (Genei, India) and 2.5 mM $MgCl_2$. Each reaction mixture was overlaid with 25 µl of mineral oil. DNA amplification was performed in a DNA thermal cycler (Perkin Elmer Cetus, Norwalk, Conn, USA). The thermal cycles used were: 1 cycle of 3.5 min at 92 °C, 1 min at 37 °C and 2 min at 72 °C, then 44 cycles of 1 min at 92 °C, 1 min at 37 °C and 2 min at 72 °C. One additional cycle of 7 min at 72 °C was used for final extension. Amplification products were analysed by electrophoresis on 1.5% agarose gels run in 1 × TBE.

Table 1 *B. juncea* accessions and their source

Genotype	Source
Pusa bold[a]	India
Pusa barani[a]	India
Varuna[a]	India
Kranti[a]	India
Rohini[a]	India
RLM 514[a]	India
RC 135	India
RC 493	India
RC 781	India
SRM 147	India
B 328	India
CSR 1110	India
Donskaja	Russia
Skorospieka	Russia
Vniimk	Russia
Malopolska	Poland
SJ 11[b]	United Kingdom
EJ 10[b]	Germany
Pakistan collection	Pakistan
ZemI	Australia
Ekla	Australia
Draznaya	Australia
IC 41734	USA

[a] Commercial varieties
[b] Original name not available

Data analysis

Results of the RAPD assay represented a consensus of two replicates. Amplification products were scored on the basis of their presence or absence. Amplification products below 500 bp were not reproducible and thus were not scored. Although there was considerable variation in the intensity of the same band across the genotypes, these differences were not considered in the analysis. Pair-wise comparisons of varieties, based on both unique and shared amplification products, were employed to calculate similarity coefficients (Jaccard 1908). Genetic distance (GD) was calculated as: GD = 1 – GS. the data was subsequently used to construct dendrograms using the unweighted pair-group method with arithmetical averages (UPGMA) and the computation for multivariate analysis was done using the NTSYS-pc programme (Rohlf 1989).

For hybrid yield determination, the yield from only the central row was used for analysis to avoid a border row effect. Heterosis values for 24 hybrids over the local check (RLM 619) and the national check (Varuna) were calculated.

The GD values between the parents used in each of the 24 hybrid combinations were considered as independent variables for developing the linear regression equation by using per-se hybrid performance as the dependent variable.

Results

Single-primer PCR

The number of amplification products produced by each primer (Table 2) varied from as few as two (with A06) to as many as 27 (with A10). The size of the amplified DNA fragments ranged from 500 bp to 3 kb. Out of 34 single primers tested, 32 revealed polymorphism ranging from 33.3% (with A14) to 100.0% (with C04). Primers A06 and C16 failed to reveal any polymorphism. In all, 500 amplification products were obtained out of which 378 showed polymorphism; the remaining products were monomorphic across both the Indian and the exotic cultivars.

Figure 1 shows tha amplification profiles generated by primer A10 across the Indian and exotic *B. juncea* accessions. Out of the 27 scorable bands, 18 of them were polymorphic across the 23 genotypes. The level of polymorphism was much higher among the exotic cultivars as compared to the Indian cultivars. Interestingly, many primers revealed characteristic fragments in RC 781 which were not produced in any of the other Indian varieties but were found in some of the exotic genotypes. For example, primer C13 produced three specific fragments (1.40 kb, 1.50 kb and 1.55 kb) in RC 781 which were also identified in many exotic accessions.

Many primers also generated variety-specific amplification products. Six unique products with different primers were produced in the Pakistan collection, three of which were generated by A13 alone and one each by A08, C10 and C13.

Two-primer PCR

To generate additional polymorphism, RAPD assay was also conducted with six primer combinations (in a 1:1 ratio) of the decamer oligonucleotides that had been used in the single-primer PCR (Table 2). Although primers A10 and A11 failed to generate distinct profiles for four closely related Indian genotypes (Pusa bold, Pusa barani, Varuna and Kranti), when

Table 2 Details of randomly-selected decamer oligonucleotides

Primer	Nucleotide sequence (5′ to 3′)	No. of amplification products (a)	No. of polymorphic products (b)	% Polymorphism (b/a × 100)
Single-primer RAPD				
A01	C A G G C C C T T C	24	18	75.00
A02	T G C C G A G C T G	18	12	66.66
A03	A G T C A G C C A C	15	8	53.33
A04	A A T C G G G C T G	17	14	82.35
A05	A G G G G T C T T G	16	14	87.50
A06	G G T C C C T G A C	2	0	0.00
A07	G A A A C G G G T G	19	15	78.94
A08	G T G A C G T A G G	19	17	89.47
A09	G G G T A A C G C C	23	18	78.26
A10	G T G A T C G C A G	27	18	66.66
A11	C A A T C G C C G T	15	13	86.66
A12	T C G G C G A T A G	10	8	80.00
A13	C A G C A C C C A C	12	9	75.00
A14	T C T G T G C T G G	6	2	33.33
A15	T T C C G A A C C C	9	7	77.77
A16	A G C C A G C G A A	13	12	92.30
A17	G A C C G C T T G T	15	12	80.00
A18	A G G T G A C C G T	13	12	92.30
A19	C A A A C G T C G G	6	4	66.66
A20	G T T G C G A T C C	13	12	92.30
C01	T T C G A G C C A G	13	10	76.92
C02	G T G A G G C G T C	14	14	100.00
C04	C C G C A T C T A C	9	6	66.66
C06	G A A C G G A C T C	16	9	56.25
C07	G T C C C G A C G A	16	10	62.50
C08	T G G A C C G G T G	26	17	65.38
C09	C T C A C C G T C C	12	9	75.00
C10	T G T C T G G G T G	14	11	78.57
C11	A A A G C T G C G G	10	6	60.00
C12	T G T C A T C C C C	16	12	75.00
C13	A A G C C T C G T C	18	17	94.40
C14	T G C G T G C T T G	13	9	69.20
C15	G A C G G A T C A G	28	22	78.57
C16	C A C A C T C C A G	2	0	0.00
Two-primer RAPD				
A4 + A5	A A T C G G G C T G + A G G G G T C T T G	11	8	72.70
C7 + C9	G T C C C G A C G A + C T C A C C G T C C	15	10	66.60
A1 + A8	C A G G C C C T T C + G T G A C G T A G G	15	14	93.30
A10 + A11	G T G A T C G C A G + C A A T C G C C G T	22	15	68.18
A11 + C09	C A A T C G C C G T + C T C A C C G T C C	19	13	68.42
C15 + A9	G A C G G A T C A G + G G G T A A C G C C	14	8	57.14

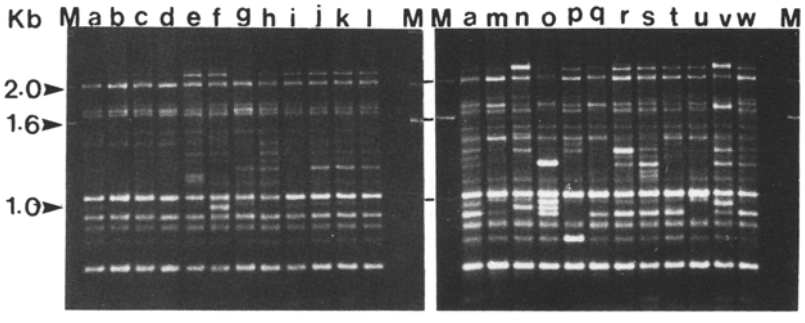


Fig. 1 RAPD profiles generated by primer A10. *M* represents a 1-kb ladder as a molecular weight marker. All the samples were analysed on two gels. To check for variation, if any, in the amplification profile of a sample from one gel to the other, Pusa bold (*lane a*) was loaded on both the gels. *Lanes a through w* contain amplified DNA products from the following accessions: Pusa bold (*a*), Pusa barani (*b*), Varuna (*c*), Kranti (*d*), Rohini (*e*), RC135 (*f*), RC781 (*g*), RLM514 (*h*), SRM147 (*i*), B328 (*j*), CSR1110 (*k*), RC493 (*l*), Donskaja (*m*), ZemI (*n*), Malopolska (*o*), Skorospieka (*p*), Vniimk (*q*), SJ11 (*r*), EJ10 (*s*), Ekla (*t*), Draznaya (*u*), IC41734 (*v*), Pakistan collection (*w*)

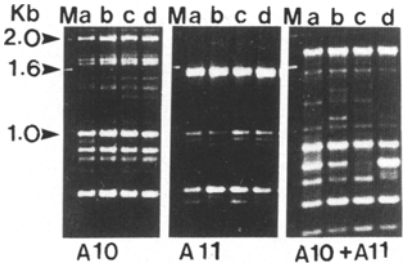


Fig. 2 RAPD profiles generated by the two primers A10 and A11 which were used separately and in combination. *M* represents a 1-kb ladder as a molecular weight marker. *Lanes a through d* contain amplification products of Pusa bold (*a*), Pusa barani (*b*), Varuna (*c*) and Kranti (*d*)

tried individually, when tested in combination these same primers generated distinct amplification profiles for these varieties (Fig. 2). The two-primer PCR reaction with A04 and A05 resulted in the appearance of many new and relatively small amplification products (< 1 kb) which concurred with the disappearance of larger fragments (> 1 kb) produced in a single-primer reaction with these component primers. On the other hand, the combination of primers A11 and C09 disclosed many fragments across all the genotypes that were not detected with either of the two primers individually.

Cluster analysis of RAPD data

The twenty-three genotypes analysed clustered into two groups, A and B (Fig. 3). Group A comprised seven exotic accessions with a similarity coefficient of 0.67. Donskaja, Draznaya, Zem I, Skorospieka and Vniimk formed one cohesive subgroup (A′) distinct from the other exotic accessions, i.e., Ekla (subgroup A″) and Malopolska with GS estimates of 0.72 and 0.67, respectively. In subgroup A′, the Russian accession Donskaja and the Australian accession Draznaya were the most closely associated exotic genotypes with a similarity coefficient of 0.79.

Group B comprised 12 Indian and four exotic accessions with a similarity coefficient of 0.69. All the Indian accessions, such as Pusa bold, Varuna, Kranti, Pusa barani, RLM 514, Rohini, RC 135, SRM 147, B 328, CSR 1110 and RC 493, formed a cohesive subgroup, B′, that was significantly distinct from the exotic genotype IC 41734 (subgroup B″). In subgroup B′, Pusa bold and Varuna exhibited the highest genetic similarity of 0.9 but these two varieties could still be differentiated by primers A01 and A08. The only discrepancy in these groupings was the placement of one of the Indian genotypes, RC 781, which was distinctly separated from the others (subgroup B′) and was closely allied to some of the exotic genotypes.

Dendrograms based on a lesser number of amplification products

One pertinent question related to our analysis was whether the same general observations could be made if a smaller number of amplification products were used. The question was addressed by constructing the dendrogram based on the analysis of 200 and 300 amplification products (data not shown). These two dendrograms were then compared to that shown in Fig. 3 (arbitrarily designated as the standard dendrogram). With both 200 and 300 amplification products, two main clusters (A and B) were formed as was the case with the standard dendrogram. Also, cluster A was further divided into two subgroups (A′ and A″) but there was slight variation between two dendrograms in the composition of the subgroup A′. Compared with the standard dendrogram, in the one with fewer amplification products, Ekla was grouped in subgroup A′ along with Skorospieka, Zem I, Donskaja and Draznaya, whereas, Vniimk was differentiated from that subgroup. Similarly, two exotic accessions, SJ 11 and EJ 10, were grouped along with Indian genotypes in the B′ subgroup of the dendrograms constructed from lesser amplification products. It appears that both these accessions are of Indian origin. In both dendrograms RC 781 remained well separated from the B′ subgroup. This observation suggested that there were no significant differences among the dendrograms obtained using

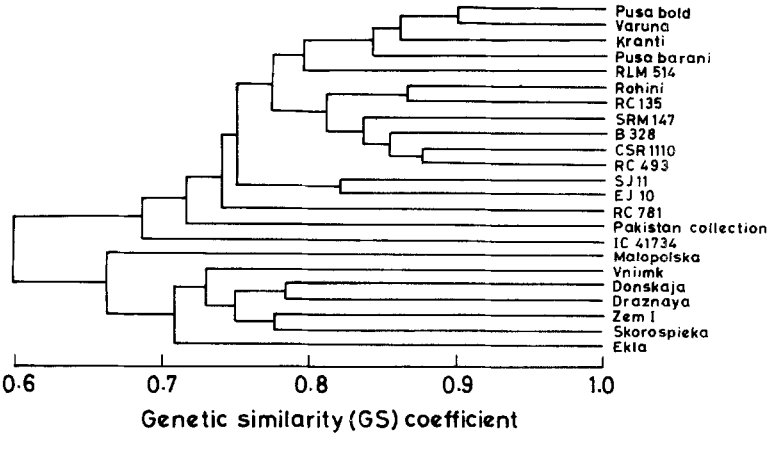


Fig. 3 Associations among *B. juncea* genotypes revealed by UPGMA cluster analysis of Jaccard genetic similarity (GS) coefficients calculated from RAPD data of 595 amplification products generated by 34 primers and six primer combinations

200, 300 and 595 amplification products thereby indicating that genotypes could be assigned to different heterotic groups by using only a limited number of amplification products.

Correlations between genetic distance and hybrid performance

The genetic distance (GD), the yield of the F_1 hybrids and the commercial yield heterosis in 24 hybrids are presented in Table 3. GDs between Indian accessions averaged 0.214, ranging from 0.183 to 0.241. The GD between Indian and exotic genotypes averaged 0.342 and ranged from 0.253 to 0.456. GDs between exotic genotypes showed a much wider range (0.179–0.441) with a high average of 0.35. The low GD of 0.183 between Indian genotypes Varuna and RLM 514 was correlated with low heterosis (7.5%) and the high GD of 0.441 between exotic accessions EJ 10 and Ekla with high heterosis (96.12%). However, the correlation between GD and heterosis was not consistent. For example, the genetic distances between Varuna and Ekla and between Ekla and the Pakistan collection were identical (0.42) but there were appreciable differences in their yield performance (Table 3).

Table 3 Heterosis in the intra- and inter-group hybrids of *B. juncea*. GD indicates the genetic diversity between the parents

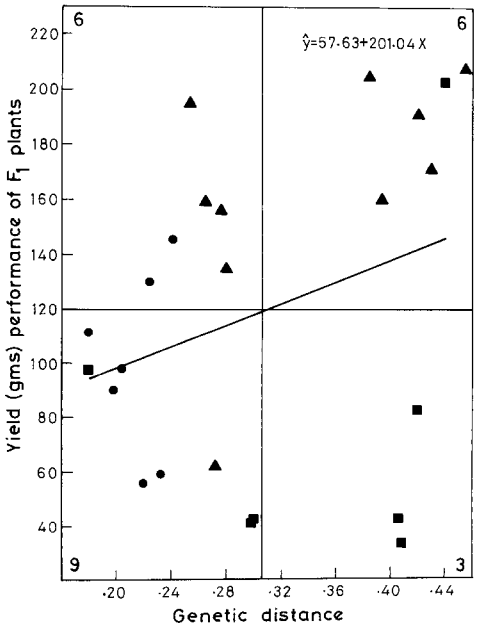
Cross combination	GD	Yield (gms)	Heterosis (%) over Varuna	Heterosis (%) over RLM 619
Indian × Indian				
Varuna × RLM 514	0.183	111.1	7.5	−12.4
Kranti × RLM 514	0.197	89.7	−13.2	−29.2
Varuna × RC 493	0.208	97.9	−5.2	−22.8
CSR 1110 × RLM 514	0.219	55.9	−45.9	−55.9
RLM 514 × SRM 147	0.224	129.5	25.4*	2.1
RLM 514 × B 328	0.232	58.5	−43.4	−53.9
Kranti × B 328	0.241	144.4	39.8**	13.9*
Indian × Exotic				
Varuna × RC 781	0.253	194.6	88.4**	53.5**
EJ 10 × RLM 514	0.263	159.4	54.3**	25.7*
RLM 514 × SJ 11	0.272	62.1	−39.9	−51.0
Varuna × SJ 11	0.276	156.2	51.3**	23.1*
EJ 10 × Kranti	0.279	135.0	30.6**	6.4
Varuna × Zem I	0.383	204.7	98.1**	61.6**
Kranti × Draznaya	0.393	150.4	45.6**	18.6*
Varuna × Ekla	0.420	190.6	84.5**	50.3**
RLM 514 × Draznaya	0.430	171.4	65.9**	35.1**
RLM 514 × Ekla	0.456	207.7	98.2**	63.8**
Exotic × Exotic				
EJ 10 × SJ 11	0.179	97.8	−5.3	−22.9
EJ 10 × RC 781	0.299	43.1	−58.2	−66.0
SJ 11 × RC 781	0.300	41.8	−59.5	−67.0
EJ 10 × Zem I	0.406	43.1	−58.3	−66.0
EJ 10 × Draznaya	0.408	34.1	−67.0	−73.1
Ekla × Pakistan collection	0.420	82.9	−19.7	−34.6
EJ 10 × Ekla	0.441	202.6	96.12**	59.8**
Checks				
RLM 619 (local check)	—	126.8	—	—
Varuna (national check)	—	103.3	—	—

CD = P (0.05) = 13.46*, P (0.01) = 28.08**

In general, the Indian × exotic combinations were more productive than the Indian × Indian and the exotic × exotic combinations. High heterosis over the national check (Varuna) was recorded in many of the Indian × exotic crosses such as RLM 514 × Ekla (98.2%), Varuna × Zem I (98.1%), Varuna × RC 781 (88.4%), and Varuna × Ekla (84.5%). However, one of the Indian × exotic crosses, RLM 514 × SJ 11, exhibited negative heterosis (−39.9%). Over 57% of the Indian × Indian crosses and 85% of the exotic × exotic crosses showed negative heterosis. EJ 10 × Ekla was the only cross which showed high heterosis (96.12%). In contrast, EJ 10 × Draznaya was the lowest yielding cross.

The plot of the yield performance of F_1 plants versus genetic distance was divided into quadrants by horizontal and vertical lines located at the mean values for the respective axes (Fig. 4). Out of the 24 crosses, 15 were located in the lower right and the upper left quadrants, implying a relationship between an increase in yield and an increase in GD for these crosses. Five of the six crosses placed in the upper right quadrant belong to Indian × exotic crosses. None of the Indian × Indian crosses were present in this quadrant. Among exotics also, only one of the seven crosses (EJ 10 × Ekla) was placed in the upper right quadrant. A majority of the Indian × Indian crosses (71%) were found in the lower left quadrant. All the crosses in the lower right quadrant represented exotic × exotic types which had below-average yield performance and above-average genetic distance values. The upper left quadrant had above-average yield performance and below-average genetic distance values. This quadrant was represented by Indian × Indian crosses (33.33%) and Indian × exotic crosses (66.66%). The plot clearly indicated that

Fig. 4 Genetic distance (GD) versus yield (gms) performance of F_1 plants in 24 crosses. Quadrants are divided along mean values for the respective axes with numbers showing the number of crosses located in the respective quadrant. ● Indian × Indian crosses, ■ exotic × exotic crosses, ▲ Indian × exotic crosses



90% of the Indian × exotic crosses had an above-average yield performance.

Discussion

This is the first report on *B. juncea* which deals with the molecular basis of genetic diversity using RAPD markers. The genetic variability in Indian mustard has previously been characterised using isozyme analysis (Kumar and Gupta 1985) and morphological and yield-related traits (Gupta et al. 1991). However, these parameters are labour-intensive and time consuming. Furthermore, plants cannot be fully analysed until harvest and morphological evaluation is subject to genotype × environment interaction. The present studies clearly showed that most of the allopatric accessions could be clearly differentiated by the single-primer RAPD assay. However, some of the Indian genotypes, such as Pusa bold, Pusa barani, Varuna and Kranti, revealed only a moderate degree of polymorphism which could be attributed to the autogamous nature of *B. juncea*. In addition, the close parentage of these genotypes and their repeated selfing for five to six generations further contributed towards their modest genetic variation. Increased genetic diversity has in many systems been correlated with higher outcrossing rates (Miller and Tanksley 1990). The allogamous nature of *B. oleracea* has also been correlated with the abundant polymorphism which could be easily detected by the RAPD assay (Hu and Quiros, 1991; Kresovich et al. 1992).

A combination of primers generated additional polymorphism even in those Indian genotypes which could not be resolved by single-primer RAPD assay. The amplification profiles produced by the two-primer assays were not simply the sum of the two profiles generated by the component primers. This clearly indicated that the two-primer assay further increases the number of potential amplification profiles. The potential has also been demonstrated in tomato (Klein-Lankhorst et al. 1991).

The RAPD assay generated variety-specific products in some of the accessions screened; these can be used as DNA fingerprints for variety identification. This would be of immense use for the establishment of proprietary rights and the determination of cultivar purity in both pure lines and F_1 hybrids. Kresovich et al. (1992) has reported numerous unique markers in *B. oleracea* cultivars and suggested their application in DNA typing and accession identification. It would be especially valuable if some of the RAPD markers could be linked to agronomically-important traits. Penner et al. (1993) has reported the linkage of RAPD markers with disease resistance genes in oats by using a pair of near-isogenic oat lines.

Despite the known advantages of RAPD assay, a problem generally encountered is to decide the number of primers to be tested for the proper evaluation of the germplasm. In our study the number of primers tested was sufficient to generate discrete amplification profiles for each of the genotypes analysed. However, a large number of amplification products are required when there is very little genetic diversity among the selected genotypes as was observed with some of the closely-related Indian accessions such as Pusa bold, Varuna and Kranti. On the other hand, if the objective is simply to cluster the genotypes into different heterotic groups based on genetic diversity, the number of primers required would be far less. We have demonstrated that this objective could be well achieved by using only 200 amplification products (about 15–20 primers). RAPD assay thus provides a simple and easily automated technique for cultivar identification and for assigning genotypes to different heterotic groups in comparison to operationally more difficult and time consuming RFLP-based DNA fingerprinting (Weising et al. 1991).

The grouping of genotypes on the basis of geographic diversity is understandable given that all the evidence, cytological, biochemical and molecular, points to a polyphyletic rather than a monophyletic origin of *B. juncea* (Prakash 1973; Vaughan 1977; Song et al. 1988). According to the dendrogram, the majority of the exotic accessions were clustered together, with Ekla being the most diverse. Notable exceptions are the two exotic accessions SJ 11 and EJ 10 which have morphological features similar to the Indian genotypes and are placed between the Indian and the exotic clusters. Another interesting observation was the closeness of an Indian genotype, RC 781, to the exotics rather than its local counterparts. This observation, based on RAPD assay, was in agreement with agronomic traits such as late flowering, tall stature, large-sized leaves and resistance of white rust which are all characteristic features of some of the exotic accessions, namely Zem I, Donskaja, Draznaya, Skorospieka and Malopolska. Based on these observations RC 781 is believed to be an exotic accession.

Our study does not suggest the possibilty of employing RAPD-based genetic distance measures alone for predicting hybrid performance. However, in general, most of the crosses made amongst Indian or exotic genotypes exhibited negative heterosis. Exotic × exotic hybrids, in general, were late in flowering and thus could not express their yield potential as flowering was terminated by rising temperatures during the end of March. This could be one of the reasons that crosses made amongst exotic genotypes did not show high heterosis in spite of having high GD mean values between the parents. In view of the above observations we believe that the information generated through RAPDs can be utilised in allocating genotypes of unknown origin to known heterotic groups. Such an allocation of genotypes would reduce the breeding cost by eliminating the evaluation of intra-group hybrids.

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