African Journal of Biotechnology Vol. 6 (14), pp. 1634-1638, 18 July 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Molecular characterization of some local and exotic *Brassica juncea* germplasm

Waqar Ali[1], Iqbal Munir[1], Mian Afaq Ahmad[1], Wisal Muhammad[1], Nisar Ahmed[1], Durrishahwar[2], Shahid Ali[1] and Zahoor A. Swati[1]

[1]Institute of Biotechnology and Genetic Engineering (IBGE) NWFP Agricultural University, Peshawar, Pakistan.
[2]Department of Plant Breeding and Genetics, NWFP Agricultural University Peshawar, Pakistan.

Accepted 14 June, 2007

The production of *Brassica* germplasm with a wider genetic base is essential for using them properly in the genetic improvement of rapeseed/mustard. During the present study, different RAPD (Random Amplified Polymorphic DNA) primers were used to estimate the genetic distances among thirty different genotypes in all possible combinations. Level of genetic polymorphism at DNA level observed during present study varied, but in general it was in the range of 21.54 to 59.36%. The dissimilarity coefficient matrix of these lines based on the data of four RAPD markers using UPGMA method was also used to construct a dendrogram. The dendrogram analysis indicated that the lines UCD-319/2, UCD-310/2 and P34/R3 were genetically apart from other lines that can be exploited in a synergistic way to create wider genetic base in local *Brassica* germplasm thus augmenting the *Brassica* breeding program.

Key words: Rapeseed, *Brassica juncea*, genetic diversity, RAPD, molecular markers.

INTRODUCTION

Genetic variability is of prime importance for the improvement of many crop species including *Brassica.* There is increasing number of reports where Random Amplified Polymorphic DNAs (RAPDs) have been successfully used to estimate genetic variability in *Brassica* (Demeke et al., 1992; Wang et al., 2002, Dulson et al., 1998; Divaret et al., 1999), common wheat (Liu et al., 1999; Sivolap et al., 1999), maize (Zhang et al., 1998; Bernardo et al., 1997) and barley (Hamza et al., 2004). In *Brassica* and its related genera, RAPD markers have been used successfully for identification and phylogenetic relationship among and within the species (Ren et al., 1995). In general, polymorphism in amphidiploids is less than that observed in diploid species. For example, the level of polymorphism reported for *B. napus* is less than amphidiploids 45% (Ferreira et al., 1994; Uzunova et al., 1995; Cheung et al., 1997), whereas in *B. oleracea* it can be higher than 80% (Cheung et al., 1997). Similarly, in *B. juncea* a polymorphism of approximately 60% has been reported by Cheung et al. (1997). Lower levels of polymorphism in are expected since they have also a lower level of out-crossing due to a weak and often non-existing self-incompatibility system.

Molecular markers are the best tools for determining genetic relationships. A variety of molecular markers have been used to study the extent of genetic variation among the diverse group of important crops in the genus *Brassica*. These include restriction fragment length polymorphism (RFLP), simple sequence repeats (SSR), random amplification of polymorphic DNA (RAPD) (Os-born and Lukens, 2003; Karp et al., 1997) and amplified fragment length polymorphism (AFLP) (Negi et al., 2001; Vos et al., 1995). These techniques differ in their principles and generate varying amounts of data. RFLP analysis is labour intensive, time consuming and expensive.

The purpose of present study was to characterize *Brassica juncea* lines from local and exotic sources at molecular level. Such analyses, which are not previously available for these *Brassica* lines, can be utilized for selecting better parents for breeding programs. For this purpose thirty *Brassica* lines were analyzed at molecular

*Corresponding author. E-mail: iqmunir@hotmail.com, waqarali04@yahoo.com.

Table 1. Description of all 30 *B. juncea* lines used in the current study.

S. No	Genotypes	S. No	Genotypes
1	UCD – 635	16	RL – 16
2	UCD - 310/2	17	T – 42
3	UCD - 310/2	18	500 – 2
4	UCD - 6/15	19	PR 79 R6
5	UCD - 10/8	20	PR – 78
6	UCD - 5/12	21	P34 – R3
7	UCD - 8/10	22	P105 - R7
8	UCD - 40/1	23	P118 - R8
9	UCD - ¾	24	P 27 - R 2
10	UCD - 46/1	25	P107 - R8
11	UCD - 6/24	26	P – 35
12	UCD - 319/2	27	P 269 – 1
13	K – 424	28	PR57/72
14	CV - 54 – 102	29	Raya Anmol
15	SONG – 86	30	BSA

Table 2. PCR thermal profile.

Step	Temperature	Duration
Hot Start	94°C	4 min.
Denaturation	94°C	1 min.
Primer Annealing	36°C	1 min.
Extension	72°C	2 min
Total Cycles (40)		
Final Extension	72°C	10 min

level using random amplified polymorphic DNA (RAPD) primers.

MATERIALS AND METHODS

DNA isolation

Thirty *B. juncea* genotypes of local and exotic origin were characterized during the present study using molecular approach (Table 1). For genomic DNA isolation, about 10 cm of fresh leaf material from 3 - 4 weeks old seedling was grind to fine powder in a 2 ml eppendorf tube under liquid nitrogen, following the method of Weining and Langridge (1991). The powder was homogenized with 500 µL of DNA extraction buffer (4% SDS, 0.1 M Tris-Hcl, 10 mM EDTA, pH 8.0) and an equal volume of phenol:chloroform:isoamyl alcohol in the ratio of 25:24:1 respectively, was added to it. The whole mixture was vigorously shaken for 20 - 30 s and aqueous phase was recovered by centrifugation at 5000 rpm for 5 min. The supernatant was transferred to a fresh tube and the DNA was precipitated from it by adding $1/10^{th}$ volume of 3 M sodium acetate (pH 5.0) with an equal volume of isopropanol. The DNA was pelleted by centrifugation for 7 min, washed twice with ice cold 70% ethanol, dried at 37°C and dissolved in 40 - 45 µl of TE buffer (10 mM Tris-Hcl, 1mM EDTA, pH 8.0) containing 40 µg/ml RNase A. The concentration of DNA was estimated by comparing its intensity with that of the λ DNA of known concentration on a 0.8% agarose gel ith Tris borate EDTA (TBE) buffer. The DNA was diluted with double distilled, autoclaved and de-ionized water at the ratio of 1:5 concentrations for use in PCR.

Polymerase chain reaction

The Polymerase Chain Reaction was carried out using protocols of Prasad et al. (2000) with modifications in thermal profile given in Table 2. The Random Amplified Polymorphic DNA markers synthesized from the Gene Link Technology, USA were employed for genetic diversity analyses (sequence information for the primers are given in Table 3).

DNA amplification was carried out in PCR tubes containing 17 µl reaction mixtures, having 1 µl template DNA, 0.5 µl RAPD primer, 9 µl of dd H_2O and 5 µl of PCR mix (composed of 460 µl H_2O, 500 µl buffer, 10 µl each of dNTPs and 300 µl $MgCl_2$) (Table 5).

The PCR reaction mixture, except DNA and primer in the above mentioned volume was pooled to sterilized PCR tubes and was mixed thoroughly by gentle pipetting. DNA and primer were added to the reaction mixture and centrifuged mildly to collect all constituents and were subjected to the thermal profile given above. The reaction was carried out in the Thermocycler (GeneAmp 2700) (Table 4).

Data analysis

For statistical analysis, all the scorable bands were considered as single locus/allel. The loci were scored as present or abscent. Bivariate 1-0 data matrix was generated. Genetic distances were calculated using Unweighted Pair Group of Arithmetic Means (UPGMA) procedure as follows (Nei and Li, 1979):

$$GD= (1- dxy)/(dx+dy-dxy)$$

where GD = Genetic distance among two genotypes, dxy = total number of common loci (bands) in two genotypes, dx = Total number of loci (bands) in genotype 1, and dy = total number of loci (bands) in genotype 2. The 1-0 bivariate data matrix for each set of wheat lines based on the data of four RAPD primers was used to construct a dendrogram using computer program “Popgene32” version 1.31 (http://www.ualberta.ca./~fyeh/fyeh)

RESULTS AND DISCUSSION

Random Amplified Polymorphic DNAs (RAPDs) analyses are widely used for detecting genetic polymorphism between genotypes at molecular level in many crop species. During the present study four RAPD primers (GLA05, GLA07, GLA09 and GLA10) were used to estimate genetic diversity in *B. juncea* genotypes. Level of genetic polymorphism (estimated as percent genetic distance) observed during present study varied, but in general it was in the range of 21.54 to 59.36%. Size range of scorable bands (alleles) was from 100 -7000 bp range of scorable bands (alleles) was from 100 -7000 bp (an example of PCR amplification using RAPD primer GLA10 is presented in Figure 1). Maximum genetic distance (86%) was observed between UCD-6/15 and P269-1, closely followed by UCD-6/15 and P118-R8 (84%). Minimum genetic distance (8.3%) was observed between P107-R8 and Raya Anmol. Similar results were reported by Das et al. (1999) and Cansian and Echeverrigaray (2000), who obs-

Table 3. Detailed description and Sequence information of primers used.

Primer	Sequence	Size	TM (°C)	Mol. Wt. (Da)
A05 primer	(AGGGGTCTTG)	10 bp	29.5	2987.9
A07 primer	(GAAACGGGTG)	10 bp	29.5	3037.0
A09 primer	(GGGTAACGCC)	10 bp	33.6	3012.9
A10 primer	(GTGATCGCAG)	10 bp	33.6	3012.9

TM = Melting temperature of primer, Mol. Wt = Molecular weight of the primer.

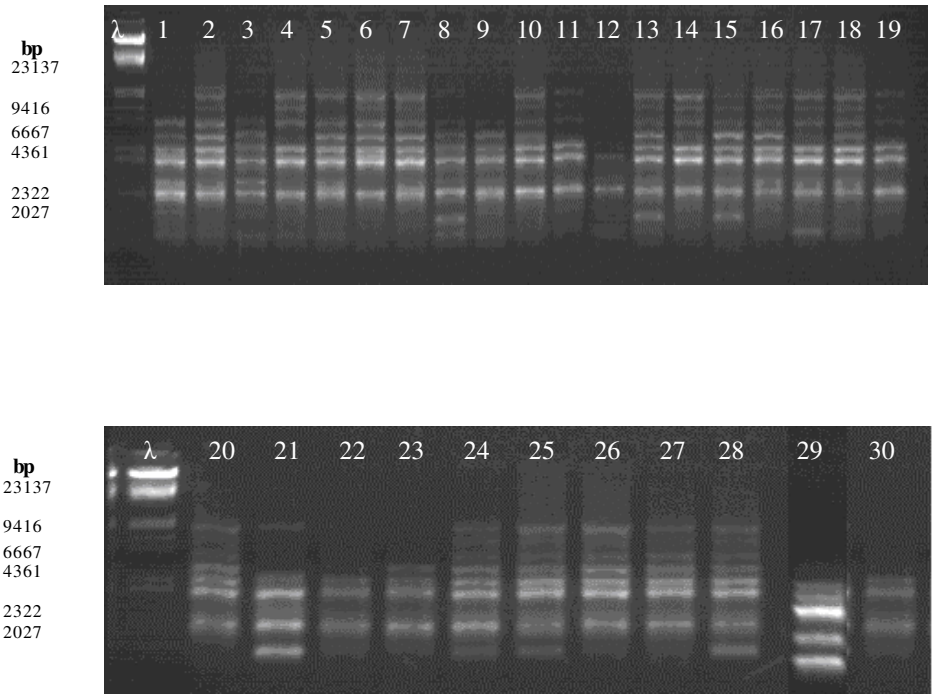

Figure 1. PCR profile of 30 genotypes of *Brassica juncea* using RAPD primer GLA10. (λ=marker, 1.UCD – 635, 2. UCD - 310/2, 3. UCD - 310/2, 4. UCD - 6/15, 5. UCD - 10/8, 6. UCD - 5/12, 7. UCD - 8/10, 8. UCD - 40/1, 9. UCD - ¾, 10. UCD - 46/1, 11. UCD - 6/24, 12. UCD - 319/2, 13. K – 424, 14. CV - 54 – 102, 15. SONG – 86, 16. RL – 16, 17. T – 42, 18. 500 – 2, 19. PR 79 R6, 20. PR – 78, 21. P34 - R3, 22. P105 - R7, 23. P118 - R8, 24. P 27 - R2, 25. P107 - R8, 26. P- 35, 27. P 269 – 1. 28. PR57/72, 29. Raya Anmol, 30. BSA).

Table 4. PCR reagents.

Reagents	Volume
H_20	460 µl
Buffer	500 µl
dATP (100 mM)	10 µl
dCTP (100 mM)`	10 µl
dGTP (100 mM)	10 µl
dATP (100 mM)	10 µl
$MgCl_2$ (25 mM)	300 µl
Total	**1.3 ml**

Table 5. Reagents for single reaction.

Reagents	Volume
H2O	9 µl
PCR Mix	5 µl
Primer	1 µl
Taq Polymerase	0.5 µl
DNA	1 µl
Total	**16.5 µl**

erved more or less similar ranges of genetic dissimilarities in different *Brassica* lines.

For individual RAPD primers, higher level of genetic polymorphism among the *B. juncea* lines was found in case of RAPD primer GLA07 (not shown), where higher levels of genetic variability were observed among differ-

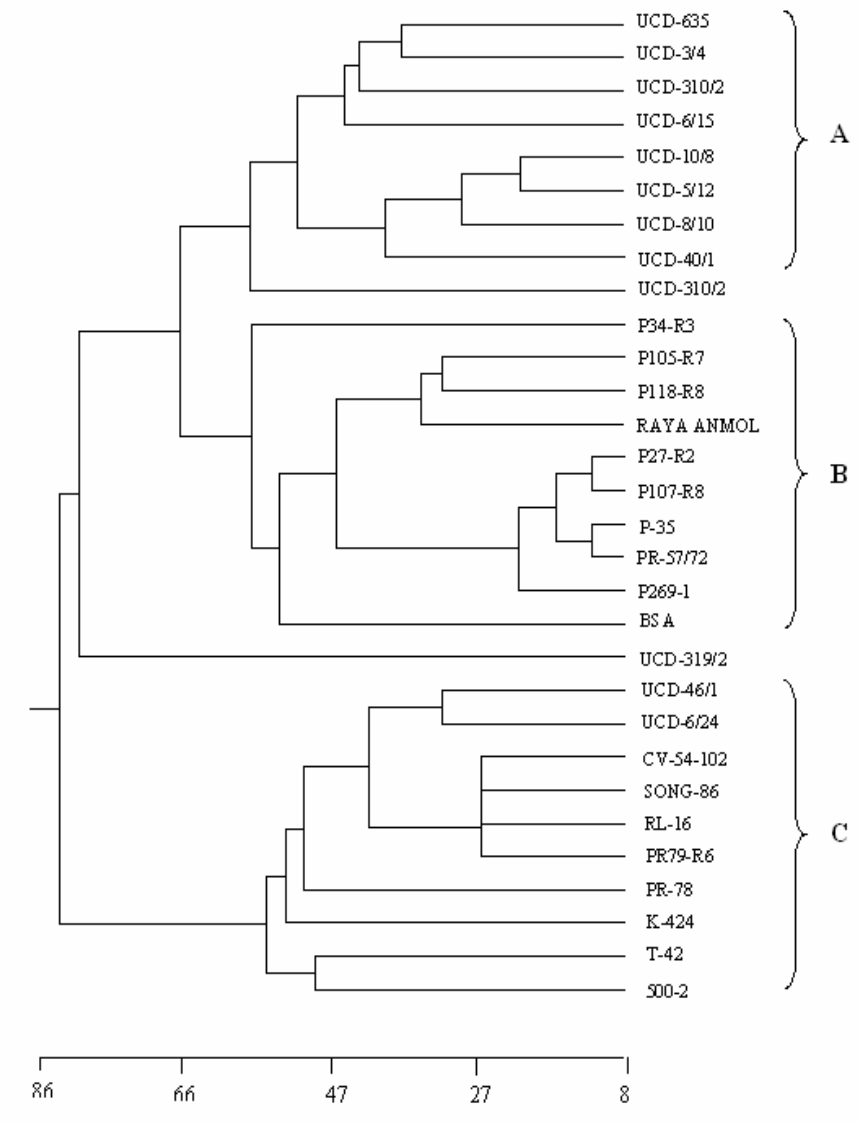

Figure 2. Dendogram constructed for 30 *Brassica juncea* lines based on genetic distances using four RAPD primers.

ent comparisons, indicating its power for the identification of individual genotypes. In *Brassica* and its related genera, RAPD markers have been used successfully for identification and phylogenetic relationship among and within the species (Ren et al., 1995). RAPD analysis has also been extensively used to document the genetic variation in *Brassicas* (Zhu and Wu, 1998; Demeke et al., 1992; Jain et al., 1994; Bhatia et al., 1995; Thormann et al., 1994). PCR-based fingerprinting techniques, is very efficient both in cost and time to identify RAPD markers associated with a trait. The RAPD markers are easier and quicker to use and are preferred in applications where the relationships between closely-related breeding lines are of interest (Hallden et al., 1994).

The 1-0 bivariate data dissimilarity coefficient matrix for thirty *B. juncea* lines based on the data of four RAPD primers using UPGMA method was used to construct a dendrogram (Figure 2). Based on the dendrogram analysis the thirty *B. juncea* lines can be categorized into 3 major groups i.e. A, B and C. *Brassica* lines UCD-319/2 was most distantly related among the 30 lines used in the study. The other two lines, showing higher genetic dissimilarities, were UCD-310/2 and P34/R3.

It is recommended that genetically distant lines observed among the 30 *B. juncea* genotypes should be used in future breeding program for improving yield and quality characteristics of *Brassica*. Further it was observed that PCR based assays like RAPD can be used effectively to estimate genetic variability in *B. juncea* and considering easy handling of the technique, they are especially suitable for breeding programs where large number of lines / accession have to be analyzed.

REFERENCES

Bernardo RA, Murigneux JP, Maisonneuve C, Johnsso Z, Karaman (1997). RFLP-based estimates of parental contribution to F2- and BC1-derived maize inbreds. Theo. App. Gen., 94: 652-656.

Bhatia S, Das S, Jain A, Lakshmikumaran M (1995). DNA fingerprinting of the *B. juncea* cultivars using the microsatellite probes. Electrophoresis., 16: 1750–1754.

Cansian RL, Echeverrigaray S (2000). Discrimination among cultivars of cabbage using randomly amplified polymorphic DNA markers. Hort. Sci., 35: 1155-1158.

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.

Das SJ, Rajagopal , Bhatia PS, Srivastava, Lakshmikumaran M (1999). Assessment of genetic variation within *Brassica* campestris cultivars using AFLP and RAPD markers. J. Biosci., 24: 433-440.

Demeke T, Adams RP, Chibbar R (1992). Potential taxonomic use of random amplified polymorphic DNA (RAPD): A case study in *Brassica*. Theor. Appl. Genet., 84: 990-994.

Divaret I, Margale E, Thomas G (1999). RAPD markers on seed bulks efficiently assess the genetic diversity of a *Brassica oleracea* L. collection. Theor. Appl. Genet., 98: 1029-1035.

Dulson J, Kott LS, Ripley VL (1998). Efficacy of bulked DNA samples for RAPD DNA fingerprinting of genetically complex *Brassica napus* cultivars. Euphytica., 102: 65-70.

Hallden C, Nilsson NO, Ebding LM, Sdl T (1994). Evaluation of RFLP and RAPD markers in a comparison of *Brassica napus* breeding lines. Theor. Appl. Genet., 88: 123-128.

Hamza S, Hamida WB, Rebai A, Harrabi M (2004). SSR based genetic diversity assessment among Tunisian winter barley and relationship with morphological traits. Euphytica., 135: 107-118.

Jain A, Bhatia S, Banga SS, Prakash S, Lakshmikumaran M (1994). Potential use of random amplified polymorphic DNA (RAPD) technique to study the genetic diversity in Indian mustard. *Brassica* juncea. and its relationship to heterosis. Theor. Appl. Genet., 88: 116–122.

Karp A (1997). Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. Mol. Breed., 3: 381–390.

Liu ZQ, Pei Y, Pu ZJ (1999). Relationship between hybrid performance and genetic diversity based on RAPD markers in wheat, Triticum aestivum L. Plant Breeding., 118: 119-123.

Negi MS, Prakash S, Lakshmikumaran M (2001)**.** Molecular markers and its utility for hybrid analysis, hylogenetic studies and tagging of important traits in *Brassica species*. In Proceedings Golden Jubilee Symposium on "Biotechnological Innovations in Conservation and Analysis of Plant Diversity" Feb. 7-9, Delhi University, Delhi.

Nei N, Li W (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci., 76: 5269-5273.

Osborn TC, Lukens L, (2003). The molecular genetic basis of flowering time variation in *Brassica* species. Biotechnol. Agric. For*.,* 52**:** 69–86.

Ren J, McFerson JR, Li R, Kresovich S, Lamboy WF (1995). Identities and relationships among Chinese vegetable *Brassica*s as determined by random amplified polymorphic DNA markers. J. Amer. Soc. Hort. Sci., 120: 548-555.

Sambrook F, Fritsch T, Maniatis (1989). Molecular Cloning: A Laboratory Manual.' (Cold Spring Harbor Laboratory Press, New York)

Sivolap YM, Chebotar SV, Topchieva EA, Korzun VN, Totskiy VN (1999). RAPD and SSRP analyses of molecular-genetic polymerphism in Triticum aestivum L. cultivars. Russian J. Genet. 35: 1433-1440.

Thormann CE, Ferreira ME, Carmago LEA, Tivang JG, Osborn TC (1994). Comparison of RFLP and RAPD markers to estimate genetic relationships within and among cruciferous species. *Theor. Appl. Genet.* 88: 973–980.

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

Vos P, Hogers R, Bleeker M, Reijans M, Lee TVD, Hornes M, Freijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995). AFLP a new technique for DNA fingerprinting. Nucleic Acids Res., 23:4407–4414.

Wang X, Fang Z, Huang S, Sun P, Liu Y, Yang L, Zhuang M, Qu D (2000). An extended random primer amplified region .ERPAR. marker linked to a dominant male sterility gene in cabbage (*Brassica oleracea* var. capitata). Euphytica., 112(3): 267-273.

Weining S, Leingridge P (1991). Identification and mapping of polymerphism in cereals based on Polymerase chain reaction. Theor. Appl. Genet., 82: 209-216.

Zhang C, ShiMeng S, DeMin J, ZhiLiang S, Tai GB, Bin W, Zhang CL, Sun SM, Jin DM, Sun ZL, Guo BT, Wang B (1998). Rapid identification of twelve elite maize inbred lines using RAPD markers Acta-Agronomica-Sinica., 24: 718-722.

Zhu L, Li RG, Wu XM (1998). RAPD analysis in part of Chinese *B. campestris*. Biology diversity. 6:99-104.