Evaluation of Genetic Diversity in Mustard (*Brassica juncea* L.) Using RAPD Analysis

Gowher A. Wani[1*], Mudasir A. Dar[1], Mudasir A.Mir[2], Mudaser A. Mir[1*]

[1]Department of Botany, University of Kashmir, Srinagar-190006, Jammu &Kashmir, India.

[2]Centre for Plant Biotechnology, Division of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, 191121, India.

ABSTRACT

In view of the importance of information about genetic diversity for the genetic improvement of crops, we evaluated the genetic diversity of 10 mustard varieties using 10 random primers. A total of 47 bands were scored corresponding to an average of 4.7 bands per primer. Level of genetic polyphorphism was in the range of 0 to 75 %. A dendrogram, constructed using the unweighted pair group method arthimetic averages (UPGMA), revealed the maximum similarity of variety Narendra Rai with Maya (EC-98) (similarity index 0.8577) while distantly related varieties were Rohini and GM-3 (similarity index 0.427). The RAPD cluster pattern showed four major clusters, cluster-1 comprised of Rohini and Varuna, cluster – II Narendra Rai and Maya (EC-98) , cluster III Kanti and Urvashi. Similarly cluster IV including Pusa jaikisan and Pusa Agrani. The varieties Pusa Tarak (EJ9912-13) and GM-3 occupied distinct places in the dendrogram, thereby indicating its distinctiveness from other varieties.

Keywords: Mustard; Genetic diversity; Varieties; RAPD; UPGMA

I. INTRODUCTION

Estimates of genetic relatedness are important in designing crop improvement programmes. Different types of marker systems have been used for biodiversity analysis like restriction fragment length polymorphic pattern (RFLP), simple sequence repeats (SSR), amplified fragment length polymorphism (AFLP), sequence tagged sites (STS), sequence characterized amplified region (SCAR), allele specific associated primers (ASAP), single primer amplification reaction (SPARs), SSR- anchored PCR, cleaved amplified polymorphic Sequences (CAPs), microsatellitic repeat polymorphisms allele specific PCR, allele specific ligation, single strand conformational polymorphism and DNA amplification fingerprinting (DAF) {10}. These techniques differ in their principles and generate varying amounts of data. RFLPs are studied for the construction of linkage maps because of their high specificity {11}.RFLPs are the first class of genetic markers which allow construction of highly saturated linkage maps. RFLPs are codominant markers that enable the heterozygotes to be differentiated from homozygotes at the species population level (single locus probes) or individual level (multi locus probes). Because of their codominant nature they have also been

used for analysis of genetic diversity {12,13}. While, RFLP analysis is labour intensive, time consuming and expensive, the RAPD has been shown to be non reproducible as it is highly influenced by environmental conditions{14}.

There is increasing number of reports where RAPD has been used to estimate genetic variability in common wheat {1, 2}, *Brassica* {3,4} and barley {5}.RAPD markers have been used successfully for identification and phylogentic relationship among and within the species{6}. Random amplified polymorphic DNA (RAPD) markers are used as a powerful tool for the identification of species or strains, the estimation of genetic variability between isolates, and the construction of dendrograms out of the computed distances{7,8,9}.The objective of present study was to characterize *Brassica juncea* varieties at molecular level so that the information thus yielded can be potentially utilized for selection of better parents for effective breeding programmes. In view of the paucity of such data for *Brassica juncea*, the study assumes importance not only for researchers of agricultural sciences but also breeders and farmers interested in this crop. For this purpose ten *Brassica* varieties was analyzed at molecular level using random amplified polymorphic DNA (RAPD) primers.

II. MATERIAL AND METHODS

2.1. PLANT MATERIAL

Ten Mustard varieties viz. Pusa jaikisan, Pusa Tarak (EJ9912-13), Rohini, Varuna, Kanti, GM-3, Urvashi, Narendra Rai, Maya (EC-98) and Pusa Agrani collected from Chandrasekha, Azad Agricultural university; Kanpur (U.P) used in present study (Table 1). Plants were grown in pots and leaf samples pooled from all plants of each variety were collected into labelled bags and stored at -69ºC in liquid N_2 prior to DNA isolation.

2.2. DNA ISOLATION AND POLYMERASE CHAIN REACTION

Genomic DNA was isolated by grinding 0.5 g of fresh leaf tissue in liquid nitrogen and by using a prewarmed cetyltrimethylammonium bromide (CTAB) extraction protocol (Doyle and Doyle, 1987). PCR amplifications were carried out in total reaction volumes of 25 µL containing 1 µl (50 ng) of template DNA, 1 µl primers, 1 mM dNTPs (Applied Biosystems/ Life Technologies, Grand Island, New York, USA), 1 × PCR buffer including $MgCl_2$ (10 mM Tris [pH 8.0], and 1 unit of Taq DNA polymerase (Sigma Aldrich). The thermal cycling profile was 5 min at 94 ^{0}C; followed by 35 cycles of 94 ^{0}C for 1 min, 37 ^{0}C annealing for 1 min, and 72 ^{0}C for 2 min; followed by a final extension of 72 °C for 10 min. The PCR products were separated by electrophoresis in 1.5% agarose gels in 1 × Tris-borate/EDTA (TBE) buffer and visualized by ethidium bromide staining.

Table 1. Description of 10 *Brassica juncea* varieties used in the current Study.

Genotype	Sample name
Pusa jaikisan	G1
Pusa Tarak (EJ9912-13)	G2
Rohini	G3
Varuna	G4
Kanti	G5
GM-3	G6
Urvashi	G7
Narendra Rai	G8
Maya (EC-98)	G9
Pusa Agrani	G10

Table 2: Total number of amplified bands and number of polymorphic bands generated by PCR, using ten randomly selected primers

Primer name	Primer sequence	% G+C Content	Total bands	Monomorphic bands	Polymorphic bands	% polymorphism
Oligo 338	CTGTGGCGGT	70	5	2	3	60
Oligo 339	CTCACTTGGG	60	4	1	3	75
Oligo 340	GAGAGGCACC	70	6	2	4	66.66
Oligo 341	CTGGGGCCGT	80	4	3	1	25
Oligo 342	GAGATCCCTC	60	5	2	3	60
Oligo 343	TGTTAGGCTC	50	6	2	4	66.66
Oligo 346	GCGTGACCCG	80	3	3	0	0
Oligo 347	TTGCTTGGCG	60	4	1	3	75
Oligo 348	CACGGCTGCG	80	6	3	3	50
Oligo 349	GGAGCCCCCT	80	4	2	2	50

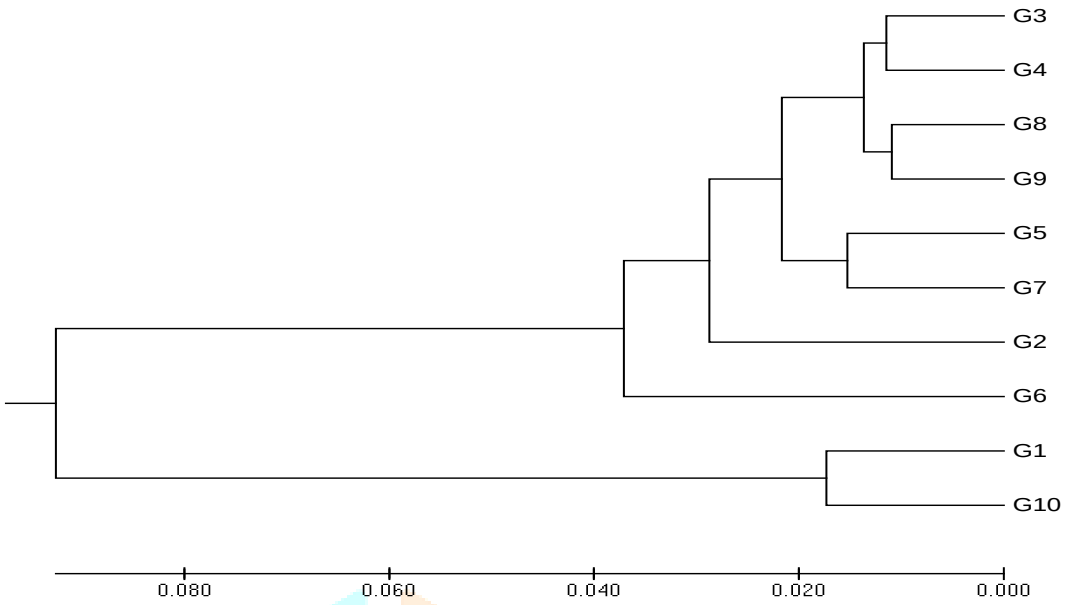

Fig 1: UPGMA- based dendrogram showing genetic relationship among ten *Brassica* genotypes based on Dice's similarity estimates for RAPD data.

III. RESULT AND DISCUSSION

All ten random primers (G1, G2, G3, G4, G5, G6, G7, G8, G9 and G10) generated a total of 47 bands in all ten varieties corresponding to an average of 4.7 bands per primer. Primers Oligo 338 and Oligo 34 generated a total of 5 bands each of which 3 (60%) were scored as polymorphic. Similarly Oligo 339 and Oligo 347 primers generated a total of 4 bands each of which 3 (75%) were scored as polymorphic. Oligo 340 and Oligo 343 primers each generated 6 bands of which 4 (66.66%) were polymorphic. Oligo 341 primer generated 4 bands of which 1 (25%) were polymorphic. Primer Oligo 346 generated 3 bands in total out of which no one was polymorphic. Primers Oligo 348 and Oligo 349 generated 4 and 6 bands of which 3 (50 %) and 2 (50%) were polymorphic. For individual RAPD primers, higher level of genetic polymorphism among the *B. Juncea* varieties was found in case of Oligo 339 and Oligo 347 primers, where higher levels of genetic polymorphism was detected, indicating its power for the identification of individual genotypes (Table 2). Further the similarity index revealed the maximum similarity of variety Narendra Rai with Maya (EC-98) (similarity index 0.8577) while distantly related varieties were Rohini and GM-3 (similarity index 0.427).

The dendrogram that resulted from hierarchical cluster analysis of the molecular data is shown in Fig. 1. Four major clusters were clearly distinguished from the dendrogram, namely cluster-1 to cluster IV representing 2 varieties each. Cluster -1 comprised of Rohini and Varuna, cluster –II Narendra Rai and Maya (EC-98), cluster -III Kanti and Urvashi. Similarly cluster- IV including Pusa jaikisan and Pusa

Agrani. The varieties Pusa Tarak (EJ9912-13) and GM-3 occupied distinct places in the dendrogram, thereby indicating its distinctiveness from other varieties. Such a cluster pattern can be compared to study the genetic relationships among some *Hesperis* L. species assessed by RAPD analysis carried out earlier {15}.

Random amplified polymorphic DNA analyses (RAPDs) have widely used for detecting genetic polymorphism between genotypes at molecular level in many crop species. RAPD markers have not only been used to study taxonomic relationships {3}, but they have been also shown to detect higher polymorphism than RFLP markers {15}. In *Brassica* and its related genera, RAPD markers have been successfully used earlier for identification and phylogenetic relationship among and within the species {6}.Our results are in agreement with the earlier reports of genetic variation in *Brassica* using RAPD analysis {16,17,18}. RAPD markers revealed high degree of polymorphism (75%) among the 10 experimental *Brassica* varieties, indicating thereby the possibility of polymorphic nature of most of the genetic loci with respect to target varieties.

IV. CONCLUSION

In conclusion, the present investigations using RAPD markers revealed high degree of polymorphism (75%) among the 10 experimental *Brassica* varieties. Moreover, both morphological and genetic variations exist among 10 investigated varieties of *Brassica*, and morphological variations of the varieties corroborated with the molecular level variations in the present study. However, the preliminary work carried out with 10 random primers selected revealing the genetic diversity among 10 mustard varieties could be exploited further by increasing the number of random primers and by validating it with other available DNA marker. It is recommended that genetically distant varieties observed among 10 *B. Juncea* genotypes should be used in future breeding programme for improving yield and quality characteristics of *Brassica.* In view of the paucity of such data for *Brassica juncea*, the results are of pivotal importance for researchers of agricultural sciences and breeders and farmers interested in this crop.

List of Abbrevations:

CTAB: Cetyltrimethylammonium bromide

RAPD: Random amplified polymorphic DNA

RFLP: Restriction fragment length polymorphism

UPGMA: Unweighted pair group method arthimetic average.

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REFERENCES

1. Liu, Z.Q., Pei, Y. and Pu, Z.J. (1999). Relationship between hybrid performance and genetic diversity based on RAPD markers in wheat, *Triticum aestivum* L. *Plant Breeding*. 118: 119-123.
2. Sivolap ,Y. M., Chebotar ,S. V., Topchieva, E.A., Korzun, V. N. and Totskiy, V. N (1999). RAPD and SSRP analyses of molecular-genetic polymorphism in *Triticum aestivum* L. cultivars. *Russian J. Genet.* 35: 1433- 1440.
3. Demeke, T., Adams, R.P. and Chibbar, R. (1992). Potential taxonomic use of random amplified polymorphic DNA (RAPD): A case study in *Brassica*. *Theor. Appl. Genet*. 84: 990-994.
4. Dulson, J., Kott, L. S. and Ripley, V.L. (1998). Efficacy of bulked DNA samples for RAPD DNA fingerprinting of genetically complex *Brassica napus* cultivars. *Euphytica*., 102: 65-70
5. Hamza, S., Hamida ,W.B., Rebai, A. and Harrabi, M. (2004). SSR based genetic diversity assessment among Tunisian winter barley and relationship with morphological traits. *Euphytica*.. 135: 107-118.
6. Ren, J., McFerson, J.R., Li, R., Kresovich, S. and Lamboy, W.F. (1995). Identities and relationships among Chinese vegetable *Brassica*s as determined by random amplified polymorphic DNA markers. *Amer. Soc. Hort. Sci*..120: 548-555
7. Welsh J., and McClelland M. (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res.* 18: 7213–7218.
8. Williams, G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V. (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18: 6531–6535
9. Bruns, T.D., White, T.J. and Taylor, J.W.(1991). Fungal molecular systematics. *Annu. Rev. Ecol. Syst*. 22: 525–564.
10. Vos, P., Hogers, R., Bleeker, M., Reijans, M., Lee, T.V.D., Hornes, M., Frejters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. (1995). AFLP a new technique for DNA fingerprinting. *Nucleic Acid. Res.* 23: 4407-4414.
11. Chyi, Y.S.W., Hoencke, M.E. and Sernyk,J.L. (1992). A genetic linkae map of restriction fragment length polymorphism loci for *Brassica rapa* (syn. *campestris*).*Genome*. 35. 746-757.
12. Song ,K. M., Obsorn, T. C. and Williams, P. H. 1988b *Brassica* taxonomy based on nuclear restriction fragment length polymorphism (RFLPs). 2. Preliminary analysis of sub-species within *B. rapa* (syn*campestris*) and*B. oleracea*;*Theor. Appl. Genet.* 76: 593–600

13. Song, K. M., Obsorn, T. C. and Williams, P. H. 1988a *Brassica* taxonomy based on nuclear restriction fragment length polymorphism (RFLPs). 1. Genome evolution of diploid and amphidiploid species. *Theor. Appl. Genet.* 75: 784–794
14. Karp, A., Kresovich, S.,Bhat, K. V., Ayad,W. G. and Hodgkin T (1997).Molecular tools in plant genetic resources conservation: a guide to the technologies; in *IPGRI Technical Bulletin No. 2. International Plant Genetic Resources Institute, Rome, Italy*.
15. Thormann, C.E., Ferreira, M.E., Camargo, L.E.A., Tivang G.J. and Osborn T.C. (1994). ,Comparison of RELP and RAPD markers to estimating genetics relationship within and among cruciferous species. *TAG Theoretical and Applied Genetics*. 88 (8): 973-980.
16. Zhu, L. Li, R.G. and Wu, X.M. (1988). RAPD analysis in part of Chinese *B. compestris*. *Biology diversity*. 6: 99-104.
17. Jain, A., Bhatia ,S., Banga , S., Prakash, S.and 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
18. Bhatia, S., Das, S., Jain, A. and Lakshmikumaran, M. (1995). DNA fingerprinting of the *B. juncea* cultivars using the microsatellitle probes. *Electro-phoresis.* 16: 1750-1754.
19. Aras, S., Duran, A., Yenilmez,G.and Duman, D.C. (2009). Genetic relationships among some *Hesperis* L. (Brassicaceae) species from Turkey assessed by RAPD analysis. *African journal of Biotechnology*.8 (14): 3128-3134.