Assessment of genetic diversity in some Iranian populations of *Bunium persicum* using RAPD and AFLP markers

Maryam Pezhmanmehr[1], Mohammad Esmaeil Hassani[1], Fatemeh Jahansooz[*2], Ali Akbar Najafi[2], Fatemeh Sefidkon[3], Mohsen Mardi[4], Mostafa Pirseiedi[4]

[1]Department of Horticultural Sciences, Faculty of Agriculture, University of Tehran, P.O. Box 31587-77871, Karaj, I.R. Iran [2]Department of Science, Faculty of Sciences, University of Tehran, P.O. Box 14415514174, Tehran, I.R. Iran [3]Research Institute of Forests and Rangelands, P.O. Box 13185-116,Tehran, I.R. Iran [4]Department of Genomics, Agricultural Biotechnology Research Institute, P.O. Box 31535-1897, Karaj, I.R. Iran

Archive of SID

Abstract

The genetic diversity of 20 Iranian populations of *Bunium persicum* has been evaluated with random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers. Fresh leaves of seedlings from each population were used for genomic DNA extraction. Analysis of banding patterns of 15 RAPD primers and 17 AFLP primer combinations, revealed 192 (86%) and 228 (75%) polymorphic bands, respectively. The range of similarity coefficients within populations were 0.4-0.82 for RAPD and 0.39-0.96 for AFLP markers. No association was observed between similarity matrices of both the DNA markers. Genetic distance patterns between *B. persicum* populations, expressed by the RAPD and AFLP cluster analyses were relatively different. The resulting dendrograms based on AFLP and RAPD + AFLP markers were more similar when compared to that derived from RAPD analysis. The AFLP generated dendrogram was supported with the highest bootstrap values. The measures of relative genetic distances among populations did not completely correlate with geographical distances of places of their origin. Several populations of black cummin were represented as independent groups in the clusters, showing a high level of genetic diversity and unique genetic background. Knowledge of wide genetic diversity observed in the *B. persicum* populations provides important information for management of germplasm resources with regard to future domestication and breeding programs.

Keywords: *Bunium persicum*; Genetic diversity; RAPD; AFLP

[*]*Correspondence to:* **Fatemeh Jahansooz,** *M.Sc.*
Tel/ Fax: +98 261 2248721
Email: jahansooz@khayam.ut.ac.ir

INTRODUCTION

Bunium persicum (Boiss.) B. Fedtsch is an important medicinal and spice plant belonging to the Apiaceae family with a diploid chromosomal number of 2n = 14 (Sheidai *et al*., 1996). It is a small, grassy and perennial plant, which produces white or pink compound umbel of flowers on the terminal and lateral stems during the third year of its life (Omidbeigi, 1997). Essential oil from the ripe seeds of black cummin contains p-mentha-1, 4-diene-7-al, gamma-terpinene, beta-pinene and cuminaldehyde (Baser *et al*., 1997). Several therapeutic effects including those on digestive disorders, urinary tract disorders, diuretic, gynaecologic, anti-convulsion, anti-helmetic, anti-asthma and dyspnea have been described for the seeds of *B. persicum* (Boskabady and Moghaddas, 2004). This plant is native to central and southern parts of Asia, with a wide geographical distribution in Iran. At the present time, seeds of this valuable medicinal spice plant are extensively collected from natural habitats (Khosravi, 2005). Due to increasing demands of the food and pharmaceutical industries, domestication and registration of high quality genotypes of *B. persicum* are necessary. Although, some attempts have been started in recent years for domestication of this plant in Iran (Khosravi, 2005), no effort has been made to analyze variability among different populations of this plant. Information on genetic variation of the available germplasm is fundamental to its domestication, improvement and management. This could also provide information on the evolving process and distribu-

www.SID.ir

tion of the germplasm in different isolated regions (Heywood, 2002).

Today, DNA marker systems play an important role in many aspects of plant breeding, ranging from the evolutionary process and genetic relationship of plant species to germplasm management. Random amplified polymorphic DNA (RAPD) (Williams *et al*., 1990) and amplified fragment length polymorphism (AFLP) (Vos *et al*., 1995) are two DNA marker systems that can be used for investigation of genetic diversity in plants. These marker systems are very useful due to their rapidity, efficiency, and non-requirement of sequence information. Although both techniques are frequently used, they can not distinguish homozygous loci from heterozygous loci (Yonemoto *et al*., 2007). RAPD markers have been extensively used to assess genetic diversity in members of the Apiaceae family such as *Apium graveolens* (Yang and Quiros, 1993), *Daucus carota* (Briard *et al*., 2000) and *Angelica gigas*, *Angelica acutiloba*, *Angelica dahurica*, *Angelica decursiva*, *Bupleurum falcatum* and *Peucedanum japonicum* (Lee and Rasmussen, 2000) revealing high levels of genetic variability among evaluated genotypes.

Genetic diversity has also been investigated by AFLP markers on *Daucus carota*, *Apium graveolens*, *Cuminum cyminum* from the Apiaceae family demonstrating a considerable degree of genetic variation in all populations (Kermani *et al*., 2006; Muminović *et al*., 2004; Bradeen *et al*., 2002). Because of the importance of these two different marker systems (RAPD and AFLP markers), some researches have applied both markers for genetic diversity analyses. Assessment of genetic differences and relatedness among *Ficus carica*, *Lupinus* spp., *Mentha arvensis* and *Mentha spicata* have been carried out using RAPD and AFLP markers, revealing a high level of genetic diversity among the genotypes (Shasany *et al*., 2005; Talhinhas *et al*., 2003; Cabrita *et al*., 2001).

There is little study on the genetic diversity of *B. persicum* using DNA markers. Majeed (2005) has assessed the genetic diversity of *B. persicum* by employing morphological and RAPD markers on populations collected from Himachal Pradesh, Jammu and Kashmir, revealing a high level of genetic variability among the investigated populations. The present study was undertaken to assess genetic diversity within some *B. persicum* populations of Iran using RAPD and AFLP markers, and to compare the results obtained with the two DNA marker systems with respect to their relative efficiencies of germplasm analyses.

MATERIALS AND METHODS

Plant material and DNA extraction: Seeds from 20 populations of *B. persicum* were collected from wild habitats in different regions of Iran (Table 1). A few seeds of each population were placed at 4ºC for the purpose of cold treatment for a period of 11 weeks and the resulting germinated seeds were transferred into pots under greenhouse conditions. Fresh leaves were collected from 7-10 seedlings and used for genomic DNA extraction, according to Sharp *et al*. (1988).

Assay of DNA markers

RAPD analysis: In order to select primers with a high level of polymorphism, 38 RAPD primers (obtained from TIB MOLBIOL, Germany) were analyzed for polymorphism in 4 populations,. DNA of all populations was tested with 15 selected random primers (Table 2). PCR was carried out in a 15 μl reaction volume containing 1 μl of genomic DNA (10 ng), 7.5 μl of ready master mix (*Taq* DNA polymerase master mix consisting of 1.5 mM $MgCl_2$) (Aria Teb Gene, Iran), 1 μl of each individual primer and 5.5 μl of distilled water. Amplification was performed in a Perkin-Elmer 9600 thermocycler (Applied Biosystem, Boston, MA, USA) as follows: 94ºC/4 min for pre-heating, followed

Table 1. Information on *B. persicum* populations.

Population number	Province of origin	Collection site	Latitude, N	Longitude, E
1	Kerman	Bidooeie	28°95'N	56°60'E
2	Kerman	Khebr	28°80'N	56°38'E
3	Kerman	Orzooeie	28°45'N	56°40'E
4	Khorasan	Mashhad	36°40'N	59°60'E
5	Kerman	Margiry	30°55'N	57°70'E
6	Khorasan	Ghoochan	37°15'N	58°50'E
7	Kerman	Koohbanan	31°20'N	56°20'E
8	Kerman	Hank	-	-
9	Khorasan	Saride-Bajestan	34°45'N	58°05'E
10	Kerman	Joopar	30°10'N	57°04'E
11	Kerman	Sardooeie	29°25'N	56°25'E
12	Kerman	Firoozabad	28°10'N	53°15'E
13	Kerman	Bam	29°10'N	58°33'E
14	Esfahan	Saghafy	33°50'N	55°02'E
15	Ghazvin	Alamoot	36°45'N	50°80'E
16	Semnan	Semnan	35°55'N	53°38'E
17	Kerman	Kooshk	28°90'N	56°65'E
18	Kerman	Jiroft	28°60'N	57°75'E
19	Kerman	Khomroot	36°15'N	51°45'E
20	Kerman	Circh	30°25'N	57°50'E

Archive of SID

www.SID.ir

Table 2. List of RAPD primers and AFLP primer combinations used.

RAPD primer	sequences	RAPD primer	sequences
TIBMBA-02	5'TGCTCGGCTC3'	TIBMBC-03	5'GGCTTGACCT3'
TIBMBA-03	5'GTGCGAGAAC3'	TIBMBC-05	5'GAGGCGATTG3'
TIBMBA-06	5'GGACGACCGT3'	TIBMBC-06	5'GAAGGCGAGA3'
TIBMBA-09	5'GGAACTCCAC3'	TIBMBC-08	5'GGTCTTCCCT3'
TIBMBB-13	5'CTTCGGTGTG3'	TIBMBC-10	5'AACGTCGAGG3'
TIBMBB-15	5'AAGTGCCCTG3'	TIBMBC-12	5'CCTCCACCAG5'
TIBMBB-16	5'TCGGCACCGT3'	TIBMBC-16	5'CTGGTGCTCA3'
TIBMBB-17	5'ACACCGTGCC3'		

AFLP primer combinations and sequences	
Ecs+GTC/Mcs+CAG	Ecs+TC/Mcs+GA
Ecs+ GTC/Mcs+TA	Ecs+TC/Mcs+GT
Ecs+TG/Mcs+CAG	Ecs+ACT/Mcs+GAG
Ecs+AGG/Mcs+AGA	Ecs+GTC/Mcs+GAG
Ecs+AGG/Mcs+CAG	Ecs+TG/Mcs+GC
Ecs+ AGG/Mcs+AG	Ecs+TG/Mcs+GAG
Ecs+AGG/Mcs+TG	Ecs+AGG/Mcs+CAT
Ecs+AGG/Mcs+TC	Ecs+AGG/Mcs+GA
Ecs+AGG/Mcs+GC	

by 35 cycles of amplification (94ºC/1 min for denaturing, 37ºC/1 min for annealing, 72ºC/2 min for extension) and a 10 min final extension at 72ºC. After amplification, the PCR products were resolved by electrophoresis on 1.5% (w/v) agarose gels (Cinagene Company, Iran). Electrophoresis was carried out for 140 min at 70 V and DNA fragments were visualized by ethidium bromide staining and photographed with a UVP Bio Doc-It™ system CCD camera (UVP Inc, Upland, USA). RAPD analysis was repeated twice in order to ensure the reliability of this marker.

AFLP (*amplified fragment length polymorphism*) *analysis*: The AFLP analysis was performed as described by Vos *et al*. (1995) with minor modifications. Genomic DNA (250 ng) was double digested with two restriction enzymes *Eco*RI / *Mse*I and the resulting digested DNA was ligated to *Eco*RI (5 pmol) and *Mse*I (50 pmol) adapters. The ligated DNA was pre-amplified using two primers without any selective nucleotides. For the pre-amplification procedure, the following cycle profile was used: 2 min at 72°C, 20 cycles: 30 sec at 94°C for denaturing, 1 min at 60°C for annealing and 2 min at 72°C for extension. Selective amplification was carried out in a reaction mixture volume of 20 μl containing 50 ng of PCR product from the pre-amplification step, 1x PCR buffer, 200 μM of each dNTP, 1 Unit of *Taq* DNA polymerase, 1.5 mM $MgCl_2$ and 0.4 μM of each primer with two or three selective nucleotides at the 3´ end of the primers. Seventeen primer combinations were selected for the analysis of genetic variability (Table 2). The PCR reactions were performed in the abovementioned thermocycler. The following cycle profile was used: 2 min at 94°C for pre-heating, 10 cycles: 30 sec at 94°C for denaturing, 30 sec at 63°C (touchdown 1°C per cycle to 54°C) for annealing, 2 min at 72°C for extension and 23 cycles: 30 sec at 94°C for denaturing, 30 sec at 54°C for annealing, 2 min at 72°C for extension.

The amplified DNA products were separated on a 6% (w/v) denaturing polyacrylamide gel and detected by using the silver staining method as described by Bassam *et al*. (1991).

Data analysis: For subsequent statistical analysis, in order to obtain a binary matrix, polymorphic bands amplified by both RAPD and AFLP markers were scored as present (1) or absent (0). The generated data matrices were subjected to statistical analysis using the NTSYS-pc analytical software (Applied Biostatistics, Setauket, USA) (Rohlf, 1998). Genetic similarities for RAPD, AFLP and RAPD +AFLP data were calculated by using the Dice similarity index, according to Nei and Li (1979).

Dendrograms showing genetic relationships of the

Archive of SID
www.SID.ir

Table 3. Effectiveness of RAPD and AFLP markers in detecting polymorphism of *B. persicum* populations.

Parameter	RAPD	AFLP
Number of assay units	15	17
Total bands scored	221	303
Polymorphic fragments scored	192	228
Percentage of polymorphism	86	75
Minimum polymorphism scored per assay	8	5
Maximum polymorphism scored per assay	20	40
Average polymorphism scored per assay	12.8	13.4

Table 4. Average, minimum and maximum values of Dice similarity coefficients (RAPD, AFLP and RAPD+ AFLP) among 20 populations of *B. persicum* (190 pairwise comparisons).

	Marker systems		
	RAPD	AFLP	RAPD+ AFLP
Average	0.64	0.69	0.68
Minimum	0.40	0.39	0.50
Maximum	0.82	0.96	0.85

20 populations were constructed using the unweighted pair-group method with arithmetic averages (UPGMA). For each dendrogram the cophenetic coefficients between the matrix of genetic similarities and the matrix of cophenetic values were computed and the significance of cophenetic correlation was tested by using the Mantel matrix correspondence test (Mantel, 1967). The support values for the degree of confidence at the nodes of the dendrogram were analyzed by bootstrap re-sampling 1000 times using the Free Tree computer software (available at http://www.natur.cuni.cz/~flegr/programs/freetree) (Hampl, *et al*., 2001).

RESULTS

Variation of DNA markers: A total of 221 bands with an average of 12.8 polymorphic bands per primer were amplified by RAPD analysis. Among these, 192 were polymorphic (86%) across the 20 populations. The highest and the lowest number of polymorphic bands per assay were 20 and 8 bands, respectively. On the other hand, 17 AFLP primer combinations generated a total of 303 fragments, from which 228 fragments (75%) were polymorphic. The number of polymorphic bands per assay ranged from 5 to 40 bands with an average of 13.4 (Table 3). Examples of RAPD and AFLP banding patterns are shown in Figures 1 and 2.

Estimation of genetic similarity: A summary of the genetic similarity estimates between pairs of populations, calculated for each marker system is shown in Table 4. RAPD data shows a lower average similarity as compared to AFLP markers. Estimates of genetic similarities of RAPD markers based on the 192 polymorphic bands among 20 populations of *B. persicum* ranged from 0.4 to 0.82 with an average of 0.64. The estimation as revealed by the 228 polymorphic AFLP bands showed an average value of 0.69 and genetic similarities ranging from 0.39 to 0.96. Average, minimum and maximum values of similarity coefficients based on all RAPD +AFLP data were found to be 0.68, 0.50 and 0.85, respectively.

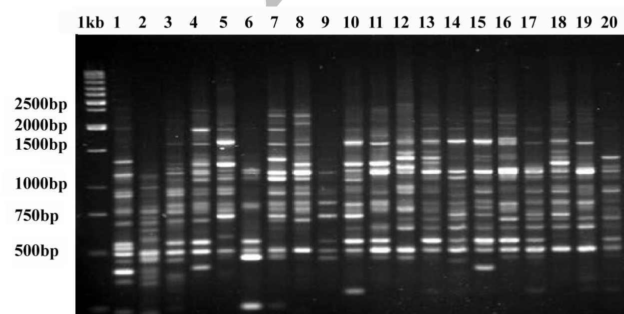

Figure 1. RAPD banding pattern of different populations of *B. persicum* obtained by using primer TIBMBB-17, according to Table 1.

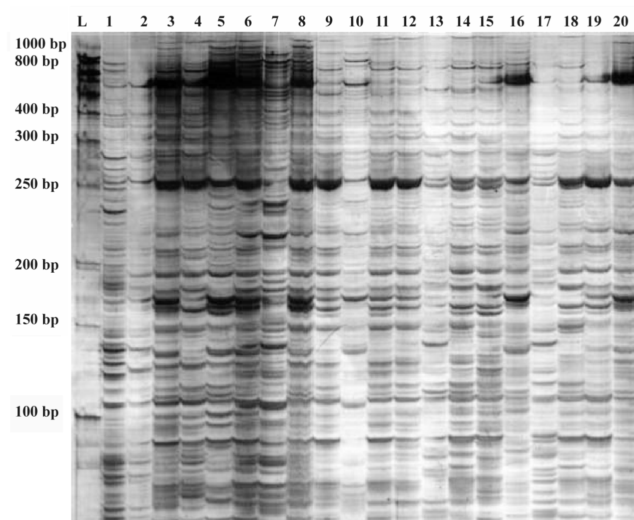

Figure 2. AFLP banding pattern of different populations of *B. persicum* obtained by using the EGTC/MCAG primer, according to Table 1.

Archive of SID
www.SID.ir

Table 5. Correlation between similarity matrices, goodness of fit of cluster analysis and cophenetic values of different markers.

	Marker systems		
	RAPD	AFLP	RAPD+AFLP
RAPD	0.96** b	0.23 c	0.10 c
AFLP	0.24 a	0.96** b	0.21 c
RAPD+AFLP	0.15 a	0.30 a	0.91** b

**Significant at $P < 0.001$. [a]comparison of original similarity matrix obtained from different marker systems. [b]goodness of fit of a cluster analysis to the similarity matrix on which it was based. [c]cophenetic value matrix comparison (after UPGMA clustering procedure).

Correlation between similarity matrices and Cophenetic matrices: The correlation coefficients were used to compare similarity matrices. Cophenetic matrices were statistically significant for two marker systems (Table 5). The cophenetic correlation coefficient between the dendrogram and the original distance matrix was highly significant for RAPD (0.96), AFLP (0.96) and RAPD + AFLP (0.97) markers.

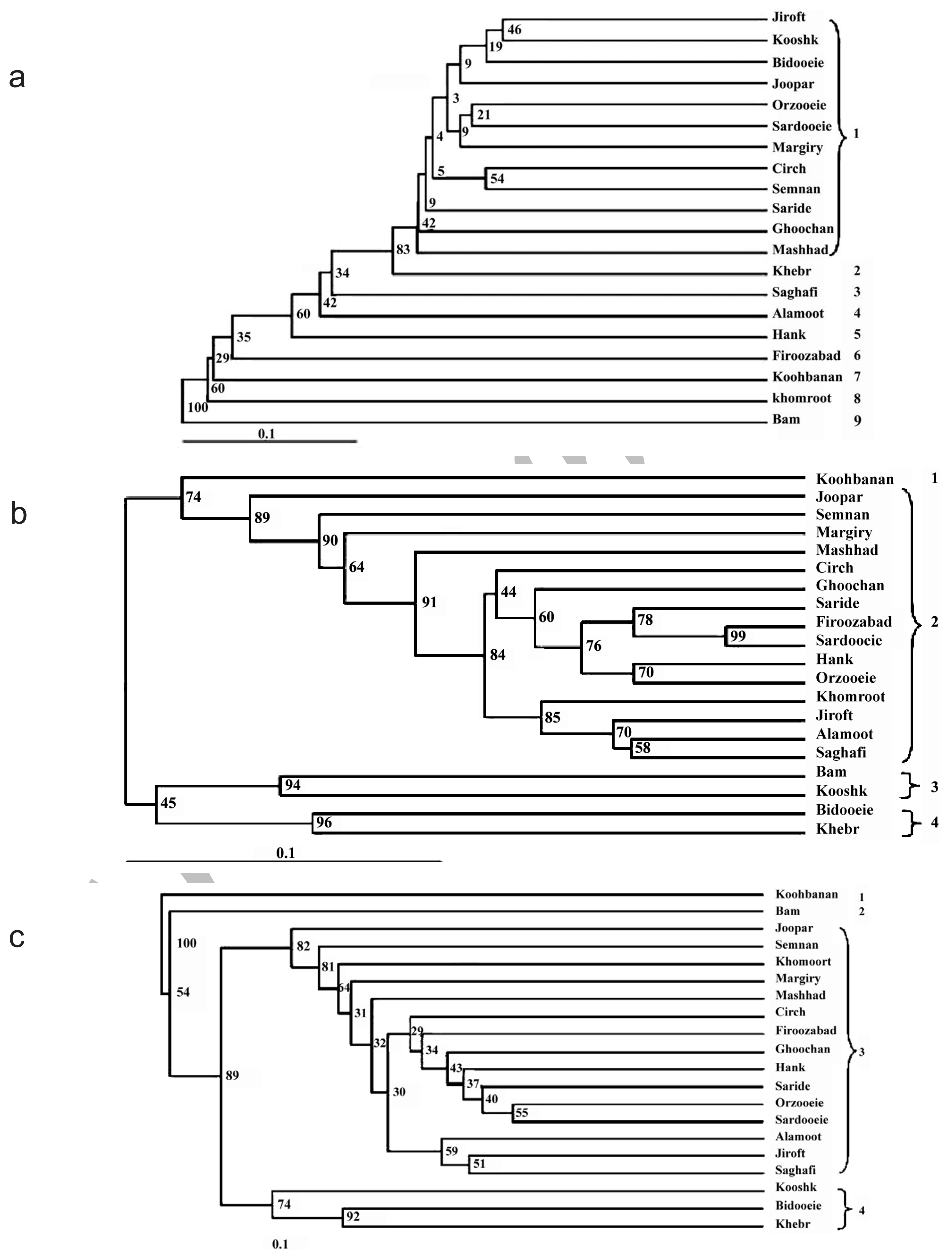

Figure 3. a) Dendrogram of 20 populations of *B. persicum* derived from RAPD analysis. Numbers on the branches are bootstrap values (%) obtained from 1000 replicate analyses. b) Dendrogram of 20 populations of *B. persicum* derived from AFLP analysis. umbers on the branches are bootstrap values (%) obtained from 1000 replicate analyses. c) Dendrograms of 20 populations of *B. persicum* obtained using RAPD + AFLP analyses. Numbers on the branches are bootstrap values (%) obtained from 1000 replicate analyses.

www.SID.ir

Cluster analyses: Three dendrograms were constructed to express the results of cluster analyses based on RAPD, AFLP, and RAPD+AFLP data (Figs. 3a, b, c). The denderograms obtained with AFLP and RAPD+AFLP markers were more similar when compared to the denderogram derived from RAPD markers. The AFLP generated dendrogram was supported with higher bootstrap values. In the RAPD derived cluster, all populations were represented by 9 different groups. According to this analysis, 8 populations from the Kerman province along with 1 population from the Semnan province and 3 populations from the Khorasan province were classified in the same group. Six other populations from the Kerman province and population from Saghafy of the Esfahan province and Alamoot from the Ghazvin province were represented as individual branches in the cluster. In the AFLP cluster, 4 main groups were obtained. Population collected from Koohbanan was represented as an individual group while 9 other populations from the Kerman province together with all other populations from the Semnan, Khorasan, Ghazvin and Esfahan provinces were placed in the same group. Populations of Bam and Kooshk from the Kerman province were represented by the same group in close relationship with another group, which included populations of Bidooeie and Khebr from the same province. Cluster analysis of RAPD+AFLP data classified all 20 populations into 4 different groups. The Koohbanan and Bam populations were clustered as two individual groups. In the third group, 9 populations from the Kerman province demonstrated close relationships with 6 other populations from the Semnan, Khorasan, Ghazvin and Esfahan provinces. Populations of Kooshk, Bidooeie and Khebr from the Kerman province were represented by the fourth group.

DISCUSSION

DNA based markers provide precise information on genetic diversity because of the independence of the confounding effects of environmental factors (Powell *et al*., 1995). RAPD markers are based on random priming, which randomly screen various regions of genomic DNA, while AFLP markers detect polymorphisms based on the restriction enzyme sites on DNA. Hence, each system reveals different levels of polymorphism. In this study both the marker systems revealed high levels of polymorphism among the populations (86% for RAPD and 75% for AFLP), indicating the effectiveness of these DNA markers for evaluation of genetic diversity in Black cummin. The estimates of genetic similarity do not seem to be different and the ranges overlap (for RAPD it goes from 0.4 to 0.82 and for AFLP it goes from 0.39 to 0.96). No association is observed between both the DNA markers for similarity matrices. RAPD and AFLP are providing information, which come from different parts of the genome (Wachira *et al*., 2001). In this study, the genetic distance patterns between *B*. *persicum* populations, expressed by the RAPD and AFLP dendrograms, were relatively different. For example in RAPD analysis, Circh and Semnan were subgrouped together while, AFLP detected higher genetic variation between these two populations, which were divided into two separate branches. The bootstrap support values for the degree of confidence at the nodes of the dendrograms derived from different marker systems were varied. In the RAPD derived dendrogram, bootstrap values were very low (<50%) while these values were relatively high for the AFLP generated cluster with almost all bootstrap values being more than 60%. This shows the reliability and stability of the inferred association and the robustness of AFLP markers. The bootstrap support values of the RAPD+AFLP derived cluster were more than that of RAPD but less than that of the AFLP ones.

The measures of relative genetic distances among populations did not completely correlate with geographical distances of places of their origins. For instance, some populations of Black cummin originating from the Kerman province, such as Koohbanan and Bam are represented as independent groups in contrast to their low geographical distance, thus showing the unique genetic background of these populations. In *Daucus carota* populations as another plant of Apiaceae family, Bradeen *et al*. (2002) also has reported low correlation between geographic distribution and genetic distances measured by AFLP markers. The same results did not show any relation between the geographical distribution and genetic distance of *Phaseolus vulgaris* (Martins *et al*., 2006) and *Matricaria chamomilla* (Solouki *et al*., 2008) landraces.

In this study, RAPD and AFLP revealed a high

Archive of SID

www.SID.ir

level of genetic variation among the black cummin populations. Genetic diversity of black cummin from other countries has been studied previously. Kapila *et al*. (1997), Devi (2004) and Mittal *et al*. (2006) used morphological traits to study genetic diversity of black cummin populations. Majeed (2005) studied genetic diversity of black cummin populations based on morphological and RAPD markers. High levels of genetic diversity among black cummin populations have been reported in all of these investigations. Broad genetic variability of black cummin plays an important role in its adaptation to a wide range of climatic and geographical conditions in Iran. A high level of diversity could be useful in selection of desirable traits in the breeding program and domestication of black cummin.

Acknowledgments

The authors would like to thank Dr. Javad Hadian for his valuable comments and revision of the manuscript.

References

Baser KHC, Oezek T, Abduganiev BE, Abdullaev UA, Aripov KN (1997). Composition of the essential oil of *Bunium persicum* (Boiss.) B. Fedtsch. from Tajikistan. J Essential Oil Res. 9: 597-598.

Bassam BJ, Caetano-Anolles G, Gresshoff PM (1991). Fast and sensitive silver staining of DNA in acrylamide gels. *Anal Biochem*. 196: 80-83.

Boskabady MH, Moghaddas A (2004). Antihistaminic effect of *Bunium persicum* on Guinea Pig tracheal chains. *Iran Biom J*. 8: 149-155.

Bradeen JM, Bach IC, Briard M, Le Clerc V, Grzebelus D, Senalik DA, Simon PW (2002). Molecular diversity analysis of cultivated carrot (*Daucus carota* L.) and wild Daucus populations reveals a genetically nonstructured composition. *J Am Soc Hortic Sci*. 127: 383-391.

Briard M, Le Clerc V, Mausset AE, Veret A (2000). A comparative study on the use of ISSR, microsatellites and RAPD markers for varietal identification of carrot genotypes. *Acta Hortic*. 546: 377-385.

Cabrita LF, Aksoy U, Hepaksoy S, Leitão JM (2001). Suitability of isozyme, RAPD and AFLP markers to assess genetic differences and relatedness among fig (*Ficus carica* L.) clones. *Sci Hortic*. 87: 261-273.

Devi S (2004). Evaluation of *Bunium persicum* (Boiss.) B. Fedtsch. germplasm in Himachal Peradesh. *Ph.D. Thesis*, University of Horticulture and Forestry, Himachal Pradesh, India.

Hampl V, Pavlícek A, Flegr J (2001). Construction and bootstrap analysis of DNA fingerprinting-based phylogenetic trees with a freeware program FreeTree: Application to trichomonad parasites. *Int J Syst Evol Microbiol*. 51: 731-735.

Heywood VH (2002). The conservation of genetic and chemical diversity in medicinal and aromatic plants. In: *Biodiversity*: *biomolecular aspects of biodiversity and innovative utilization*. Þener B. Springer, PP. 13-22.

Kapila RK, Panwar KS, Badiyala D (1997). Variation and association analysis in domesticated population of Black caraway (*Bunium persicum*). *J Med Arom Plant Sci*. 19: 709-711.

Kermani, M., Marashi, H., Nasiri, M., Safar Nezhad, A. and Shahriari, F., 2006. Evaluation of genetic diversity in Iranian cumin (*Cuminum cyminum*) using AFLP markers. The First Agricultural Biotechnology Congress, Kermanshah, Iran.

Khosravi M (2005). Intercropping Black Zira (*Bunium persicum*) with Saffron and annual crops: Agroecological and economic perspectives. *Ph.D. Thesis*, The University of Ferdosi Mashhad, Faculty of Agriculture.

Lee S, Rasmussen SK (2000). Molecular markers in some medicinal plants of the Apiaceae family. *Euphytica* 114: 87-91.

Majeed S (2005). Assessment of genetic divergence and *in vitro* conservation in *Bunium persicum* (Boiss.) Fedtsch. *Ph.D. Thesis*, University of Horticulture and Forestry, Himachal Pradesh, India.

Mantel M (1967). The detection of disease clustering and a generalized regression approach. *Cancer Res*. 27: 209-220.

Martins SR, Vences FJ, Saenz de Miera LE, Barrosa MR, Carnide V (2006). RAPD analysis of genetic diversity among and within Portuguese landraces of common white bean (*Phaseolus vulgaris* L.). *Sci Hort*. 108: 133-142.

Mittal RK, Chahota RK, Gartan SL, Katna G (2006). Genetic variability and component analysis in kalazira (*Bunium persicum*) in dry temperate areas of North-western Himalayas. Crop Improvement. 33: 202-204.

Muminović J, Melchinger AE, Lübberstedt T (2004). Prospects for celeriac (*Apium graveolens* var. *rapaceum*) improvement by using genetic resources of *Apium*, as determined by AFLP markers and morphological characterization. Plant Genetic Resources 2,189-198 Cambridge University Press.

Nei M, Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the Natural Academy of Science of the U.S.A 76, 5269-5273.

Omidbeigi R (1997). *Approaches to production and processing of medicinal plants*. Volume 2, Tarrahan-e-Nashr. Tehran, Iran. PP. 424.

Powell W, Orozco-Castillo C, Chalmers KJ, Provan J, Waugh R (1995). Polymerase chain reaction-based assays for the characterisation of plant genetic resources. *Electrophoresis* 16: 1726-30.

Rohlf FJ (1998). NTSYSpc. Version 2.10p. Applied Biostatistics, Setauket, NY 11733-2870 USA.

Sharp PJ, Kreis M, Shewry PR, Gale MD (1988). Location of b-amylase sequences in wheat and its relatives. *Theor Appl Genet*. 75: 289-290.

Shasany AK, Darokar MP, Dhawan S, Gupta AK, Gupta S, Shukla AK, Patra NK, Khanuja SPS (2005). Use of RAPD and AFLP Markers to Identify Inter- and Intraspecific Hybrids of Mentha. *J Hered*. 96: 542-549.

Sheidai M, Ahmadian P, Poorseyedy S (1996). Cytological studies in Iran zira from three genus: Bunium, Carum and Cuminum. *Cytologia* 61: 19-25.

Solouki M, Mehdikhani H, Zeinali H, Emamjomeh AA (2008). Study of genetic diversity in Chamomile (*Matricaria*

Archive of SID

www.SID.ir

chamomilla) based on morphological traits and molecular markers. *Sci Hort*. 117: 281-287.

Talhinhas P, Neves-Martins J, Leitão J (2003). AFLP, ISSR and RAPD markers reveal high levels of genetic diversity among Lupinus spp. *Plant Breed*. 122: 507-510.

Vos P, Hogers R, Bleeker M, Reijans M, Van der Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res*. 21: 4414-4470.

Wachira F, Tanaka J, Takeda Y (2001). Genetic variation and differentiation in tea (*Camellia sinensis*) germplasm revealed by RAPD and AFLP variation. *J Hortic Sci Biotechno*. 76: 557-563.

Williames JGK, Kubelik AE, Livak KJ, Rafalski JA, Tingey SC (1990). DNA polymorphism amplified by arbitrary primer is useful as genetic markers. *Nucleic Acids Res*. 118: 6531-6535.

Yang X, Quiros C (1993). Identification and classification of celery cultivars with RAPD markers. *Theor Appl Genet*. 86: 205-212.

Yonemoto Y, Chowdhury AK, Kato H, Macha MM, Okuda H (2007). Characterization of white sapote (Casimiroa edulis Llave and Lex.) germplasm using floral morphology, RAPD and AFLP markers. *Sci Hortic*. 112: 366-375.

Archive of SID

www.SID.ir