Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

A comparative analysis of RAPD and ISSR markers for assessing genetic diversity in Iranian populations of *Nigella sativa* L.

Samira Hosseini KorehKhosravi, Asad Masoumiasl*, Masoud Dehdari

Department of Agronomy and Plant Breeding, Yasouj University, Iran

Correspondence to: Masoumiasl@yu.ac.ir, Masumiasl@yahoo.com

Received September 5, 2017; **Accepted** January 25, 2018; **Published** January 31, 2018

Doi: http://dx.doi.org/10.14715/cmb/2018.64.1.10

Abstract: The genetic diversity of 18 Iranian populations of *Nigella sativa* L. was tested using RAPD (10 primers) and ISSR (12 primers) molecular markers. RAPD primers produced 103 acceptable bands and out of them, 42 bands were polymorphic and the total percentage of polymorphism was estimated 40.5%. Out of the 12 ISSR primers, only 5 of them produced acceptable bands. Five ISSR primers generated 39 amplified products and out of them, 19 bands were polymorphic and the total percentage of polymorphism was estimated 57.4%. As a result, the level of polymorphism obtained from the ISSR markers was higher than of the RAPD markers. Mean of marker index (MI) (0.822 for RAPD and 0.74 for ISSR) and Polymorphic information content (PIC) (0.402 for RAPD and 0.35 for ISSR) for each of the marker systems indicated that both markers were effective for evaluating genetic diversity. Cluster analysis classified the populations into 5 groups for both DNA markers. By integration and analyzing of the two marker systems, the populations were classified into 4 groups. The results of this research showed that RAPD and ISSR molecular markers can be useful for identifying and classifying the Iranian *Nigella sativa* L. populations.

Key words: Black cumin; Genetic distance; Polymorphic information content; Similarity.

Introduction

Black cumin (*Nigella sativa* L.) belongs to Ranunculaceae family. *Nigella sativa* L. is used for treating cancer, peptic ulcer, liver detox, regulating the digestive system and also for increasing the secretion of breast milk. Thymoquinone, dithymoquinone and thymohydroquinone and thymol are the main active ingredients of the aqueous extract of Black cumin seed (1). Thymoquinone has an anticonvulsant effect. A substance called nigilline is extracted from the essence of Black cumin which can kill worms, be laxative and increase the secretion of breast milk (2). The seeds can be found in some places in Iran, such as Kermanshah and Arak, it is naturally growing plant in some areas in Iran, like Isfahan and Khorasan (3). Throughout the study of genetic diversity, differences and similarities between species, populations or individuals were studied using statistical models and methods and the pedigree information or molecular characteristics were evaluated based on morphological traits (4).

A suitable marker is a marker that is different for two individuals and also has heritability (5). Morphological traits are controlled by one or multiple genes and are affected by the environmental conditions and stage of growth and development. Totally, morphological markers have not enough polymorphism; they are mainly dominant, show epistasis and are affected by the environment (5). Salamati and Zeinali (3) evaluated the genetic diversity of some genotypes of *Nigella sativa* L. using morphological traits. Their results showed high phenotypic and genotypic coefficients of variations; this indicated a high diversity of the investigated genotypes. Molecular markers such as RFLP, RAPD and ISSR are more powerful tool to evaluate genetic diversity while morphological markers and their relevant field experiments and comparisons are time-consuming and labor-intensive. Among molecular markers, RAPD and ISSR have been widely used for analyzing genetic diversity. The RAPD marker is based on arbitrary primers and does not need exclusive restriction enzymes, transferring, labeling, hybridization and the basic information about the sequence of genome. In addition to being a high-speed method, it needs a few DNA; it is based on PCR and easily automated (5). ISSRs have a larger length of primer and a high intensity and resolution of the obtained bands in the annealing temperature, and that is it has high reproducibility (6). Roose and Fang (7) have reported a reproducibility of higher than 99 percent for the ISSR markers in the DNA samples extracted from leaves of different ages in citrus cultivars. Domblides et al. (8) evaluated the genetic diversity of 32 parsley samples (belonging to the Apiaceae family) using 6 ISSR primers and on average, they observed 10.2 polymorphic bands. They showed that this marker is highly efficient for evaluating the genetic diversity of parsley. Gajera et al. (9) evaluated the genetic diversity of 20 genotypes of plant species at the risk of *Mangifera indica* with 21 ISSR primers and observed 94.4 percent of polymorphism. Domblides et al. (8) also evaluated the genetic diversity of 32 parsley samples using 5 RAPD primers and on average, they observed 11 polymorphic bands. Morales et al. (10) reported the grouping proposed by the ISSR markers was more coherent with the origin and the genealogy of the cultivars than that proposed by the RAPD markers, and it can be conside-

Table1. Name and location of studied Iranian populations of Black cumin.

Name	site of collection
Hamedan	Hamedan Province (1)
Botanical garden of BuAli	Hamedan Province (1)
Qazvin 1	Qazvin Province (2)
Qazvin 2	Qazvin Province (2)
Takestan	Qazvin Province (2)
Ardebil 1	Ardebil Province (3)
Ardebil 2	Ardebil Province (3)
Arak	Markazi Province (4)
Lordegan	Chaharmahal & Bakhtiari Province (5)
Kermanshah	Kermanshah Province (6)
Khorroamabad	Lorestan Province (7)
Sarbisheh	South Khorasan Province (8)
Shahreza	Isfahan Province (9)
Semirom	Isfahan Province (9)
Ardestan	Isfahan Province (9)
Meybod	Yazd Province (10)
Shahediieh	Yazd Province (10)
Share Rey	Tehran Province (11)

red the most efficient method for the study of genetic divergence in strawberry. Moradi and Chogamirza (11) evaluated the genetic diversity of various genotypes of Durum wheat and showed that ISSR markers (86.44 percent) show more polymorphism than RAPD markers (64.91 percent). They concluded that each of these two markers is a quick and inexpensive method for evaluating the genetic diversity of a large number of samples.

Despite the fact that Black cumin is considered to be an important medicinal plant that is widely used in the global market, but in Iran, there is not sufficient information about the local populations that exist and its cultivation has not been common yet. Thus, it seems essential to us to accurately evaluate the local populations which exist in Iran in order to prepare identification for them to be used for planning in association with breeding studies. For this purpose, the present study has aimed to evaluate the genetic diversity of various populations of Black cumin collected from several places in Iran and to determine their relationships using molecular markers.

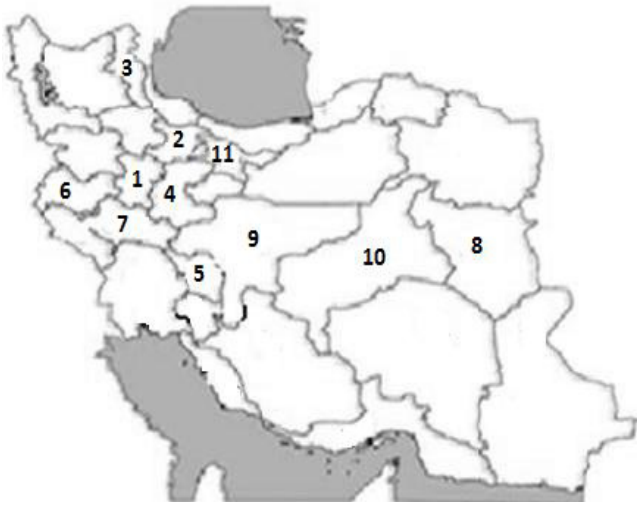

Figure 1. Display the collected evaluated populations of Black cumin on Iran map.

Materials and Methods

In this study, 18 populations of Black cumin collected from various places in Iran (Table 1, Figure 1) was evaluated. Out of these 18 populations, 13 populations from the gene bank of Research Institute of Forests and Rangelands of Iran (Tehran, Iran), 3 populations (Semirom, Shahreza and Ardestan) from Pakan-Bazr Company (Isfahan, Iran) and 2 populations from the Research Centers of the Kermanshah and Shahre Rey were prepared. The collected seeds were cultivated in a mixture of sand, soil, and vermicompost (1:1:2) after break dormancy by Gibberellic acid (1250ppm). The experiment was organized in Complete Random Design in greenhouse.

In order to DNA extraction, young leaves were prepared and DNA extraction was done through the method of Murray and Thompson (12). The quality and quantity of extracted DNA were investigated using spectrophotometry and electrophoresis techniques. 10 RAPD primers (13) and 12 ISSR primers (14) were selected and synthesized by the CinnaGene Company (Tehran, Iran) (Table 2). In order to do PCR, the instruction presented by Williams et al. (15) was used with little changes. The PCR reaction for the both markers contained 2 microliter of template DNA (50ng), 1.6 microliter of $MgCl_2$

Table 2. ISSR and RAPD primer sequences and annealing temperatures (Ta).

ISSR			RAPD		
Primer	**Sequence (5'-3')**	**Ta (°C)**	**Primer**	**Sequence (5'-3')**	**Ta (°C)**
ISSR5H	AGAGAGAGAGAGAGAGCA	43.3	H1	GAG GAT CCC T	32
ISSR6H	AGAGAGAGAGAGAGAGCC	44.6	H2	ACG GTA CCA G	34
ISSR8H	GTCACACACACACACAC	48.7	H3	GGA CCC TTA C	33
ISSR9H	CAGCAGCAGCAGCAG	51.5	H4	CGA CAG TCC C	36
ISSR12H	AGCCTCTCTCTCTCTCT	41.9	H5	GCC CGC GAG T	38
ISSR1H	CACACACACACAGT	31.6	H6	GGT GGC GGG A	35
ISSR2H	CACACACACACAGG	37.1	H7	TGT AGC TGG G	34
ISSR3H	CACACACACACAAC	32.5	H8	CCA GTT CGC C	33
ISSR4H	CTCTCTCTCTCTCTCTTG	40.1	H9	GCA CCG AGA G	38
ISSR7H	AGAGAGAGAGAGAGAGCT	41.2	H10	CCT GGG CCT A	40
ISSR10H	TCTCTCTCTCTCTCTCRG	48			
ISSR11H	TGTAGAGAGAGAGAGAG	34.6			

Table 3. Thermal program for PCR in ISSR and RAPD primers.

Reaction step	T (°C)		Time (s)		Cycle	
	ISSR	RAPD	ISSR	RAPD	ISSR	RAPD
Primary denaturation	95	94	120	240	1	1
Denaturation	93	94	30	60	-	-
Annealing	34-45	32-52	55	55	-	-
Extension	72	72	90	120	-	-
Repeat 2-4 steps	-	-	-	-	35	40
Final extension	72	72	120	420	1	1

(50mM), 0.5 microliter of dNTP mix (10mM), 0.15 microliter of *Taq* DNA polymerase (5 unit/µl), 0.8 microliter of the primer (10 ppm), 2 microliter of Buffer PCR (10X) and 13 microliter of sterile double distilled water which was put in the thermocycler device (BIO-RAD, Germany) with a separate thermal program for each marker (Table 3). PCR products were separated on 2% agarose gel and the observed bands were scored as zero (lack of a band) and as one (presence of a band). The data were analyzed by the NTSYS-pc (Ver.2.02) and GenAlex 6.3 software (16). The following indicator were calculated for the two markers: the percentage of polymorphism (4), Polymorphic information content (PIC) (4), marker index (MI) (17), Effective multiplex ratio (EMR) (17) and Resolution power (RP) (18). In order to draw the dendrogram, the STATGRAPHICS software and in order to Principle component analysis SAS (9.1) software was used.

Results

RAPD analysis

Each 10 RAPD primers produced acceptable bands. Totally, 103 bands were produced and out of them, 42 bands were polymorphic and the total percentage of polymorphism 40.5 percent. The maximum percentage of polymorphism was associated with the H5 primer and the minimum was associated with the H3 primer. The maximum PIC was obtained for H7 and the minimum for H4 primer. The maximum and maximum MI belonged to the H5 and H4 primers, respectively; which means that the H5 primer has a higher level of RP in comparison to the other primers. The highest EMR and RP were associated with the H3 primer (Table 4, Figure 2).

The populations were classified into 5 groups using cluster analysis based on Jaccard's similarity matrix (Figure 4) (the cut-off point was specified based on the highest Cophenetic coefficient). In group 1, there were two populations from Isfahan province and a population from Hamedan province. Group 2 was divided into two subgroups and both of them had populations from Qazvin Province, an interesting point here is that the first subgroup included a population from Yazd and a population from Qazvin. Group 3 had two subgroups which included one population from Arak and one from Qazvin. There were many subgroups in group 4 and included most of Iranian populations. The first sub-

Table 4. The total number of loci, polymorphic loci, polymorphic percentage, PIC, MI, EMR and RP revealed by ISSR, RAPD and RAPD+ISSR primers

	Primer code	No. locus	Polymorph locus	Polymorphism percentage	PIC	MI	EMR	RP
ISSR	ISSR5H	10	2	20	0.49	0.196	0.4	2.33
	ISSR6H	7	5	71	0.47	1.668	3.55	6.33
	ISSR8H	3	3	100	0.11	0.33	3	5.66
	ISSR9H	9	5	56	0.38	1.064	2.8	7.44
	ISSR12H	10	4	40	0.30	0.48	1.6	6.55
	Total	39	19	-	-	-	-	-
	Mean	**7.8**	**3.8**	**57.4**	**0.35**	**0.74**	**2.27**	**5.66**
RAPD	H1	13	4	31	0.49	0.603	1.24	4.55
	H2	13	4	31	0.36	0.449	1.24	6.11
	H3	8	2	25	0.43	0.214	0.5	2.77
	H4	5	2	40	0.24	0.193	0.8	3.44
	H5	16	10	63	0.493	3.08	6.3	11.22
	H6	14	4	29	0.44	0.497	1.16	5.44
	H7	10	5	50	0.494	1.232	2.5	5.55
	H8	7	2	29	0.34	0.196	0.58	3.11
	H9	10	5	50	0.44	1.088	2.5	6.77
	H10	7	4	57	0.30	0.675	2.28	6.55
	Total	103	42	-	-	-	-	-
	Mean	**10.3**	**4.2**	**40.5**	**0.402**	**0.822**	**1.91**	**5.55**
RAPD+ISSR	**-**	142	61	43	-	-	-	-

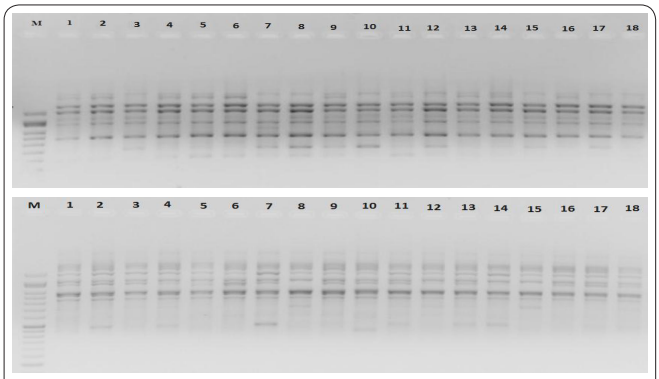

Figure 2. RAPD marker profiles of H7 (above) and H5 (below) primers. M= Ladder 50-1500 bp, CinnaGen Company, Iran. 1= Hamedan, 2= Qazvin 1, 3= Takestan, 4= Ardebi 1, 5= Botanical garden of bouAli, 6= Lordegan, 7= Ardebil 2, 8= Kermanshah, 9=Khorramabad, 10=Sarbisheh, 11=Shahreza, 12=Meybod, 13=Ardestan, 14=Arak, 15=Share Rey, 16=Shahediieh, 17=Semirom, 18=Qazvin 2.

group had 6 populations and the second subgroup had 2 populations. In the first subgroup, there were populations from Hamedan, Chaharmahal & Bakhtiari, Kermanshah, Tehran, Yazd and Ardebil provinces and there were populations from Lorestan and southern Khorasan provinces in the second subgroup. What is interesting here is that the populations from Sarbisheh (belonging to the southern Khorasan province) and Khoramabad (belonging to Lorestan province) were in one subgroup. Group 5 only had a population from Semirom. The maximum genetic distance was seen between the populations from Semirom and Hamedan. The three-dimensional distribution of the populations has been presented in Figure 5 using main components analysis based on Jaccard's similarity coefficient matrix. The Eigen values of the similarity matrix of the first 10 components regarding the level of variations explained by each of these components and the accumulative percentage of the variations explained by the independent samples which have been presented in Table 5.

ISSR analysis

Out of the 12 ISSR primers, only 5 of them produced acceptable bands. The total number of the produced bands was 39 bands. 19 of these bands were polymorphic and thus, the percentage of total polymorphism was estimated to be equal to 57.4 percent.

Table 5. Characteristics of ten first components based on RAPD data.

Component	Eigen value	Component variance	Accumulative variance
1	0.97	13.55	13.55
2	0.90	12.56	26.12
3	0.83	11.59	37.71
4	0.75	10. 45	48.17
5	0.53	7.44	55.61
6	0.47	6.68	62.23
7	0.46	6.45	68.68
8	0.42	5.94	74.62
9	0.32	4.51	79.13
10	0.29	4.14	83.28

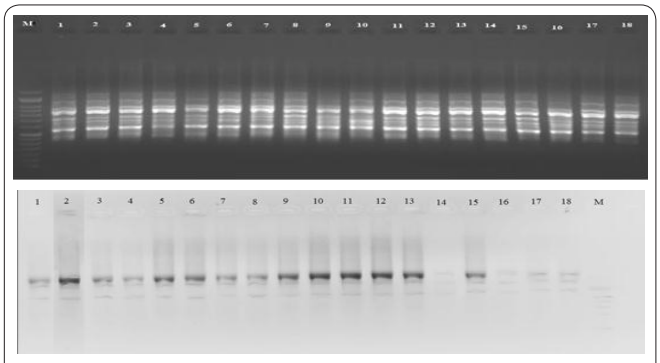

Figure 3. ISSR marker profiles of ISSR12H (above) and ISSR8H (below) primers. M= Ladder 50-1500 bp, CinnaGen Company, Iran. 1=Hamedan, 2=Qazvin 1, 3=Takestan, 4=Ardebi 1, 5=Botanical garden of bouAli, 6=Lordegan, 7=Ardebil 2, 8=Kermanshah, 9=Khorramabad, 10=Sarbisheh, 11=Shahreza, 12=Meybod, 13=Ardestan, 14=Arak, 15=Share Rey, 16=Shahediieh, 17=Semirom, 18=Qazvin 2.

The size of the bands varied between 350-1700 base pairs. The maximum percentage of polymorphism was for ISSR8H primer (100 percent) and the minimum for ISSR5H primer (20 percent). The maximum PIC was obtained for ISSR5H primer (0.49) and the minimum for ISSR8H primer (0.11). The maximum value of MI was for ISSR6H primer (1.668) which means that the RP of this primer is more significant than other primers. The marker index is calculated using the number of the polymorphic gene loci obtained from the primers in the respect of estimating the efficiency and RP of the marker. The maximum EMR was seen for the ISSR6H primer (3.55) and the minimum value of this index was observed for the ISSR5H primer (0.4). The maximum RP index was calculated for ISSR9H primer (7.44) (Table 4, Figure 3).

The cluster analysis classified the populations into 5 groups based on Jaccard's similarity matrix (Figure 4). Group 1 was divided into two subgroups. The Hamedan, Ardebil1 and Ardebil2 populations were placed in the first subgroup and the Qazvin1 population alone was placed in the second subgroup. Group 2 was also divided into two subgroups with 7 populations in the subgroup 1 and the Sarbisheh population alone was placed in the second subgroup. Subgroup 1 of this group itself was divided into two secondary subgroups and the second subgroup was also divided into two secondary subclasses of its own. It can be argued that most of the populations were situated in the second group (i.e. 8 populations). Group 3 only had Lordegan population and group 4 only had Kermanshah population. In group 5, the populations were divided into two subgroups: one of these subgroups included the Shahediieh population alone and the other included three other subgroups. This classification makes it possible for the researcher to geographically separate the different populations from one another. For instance, in group 1, there was no population collected from the south and southwest of the country and all of them were collected from Ardebil, Qazvin and Hamedan. In contrast, samples collected from other places in the country were placed in other clusters. Nonetheless, there were some contradictions in the classifications with geographical distribution that might be because the presence of a high genetic diversity among the populations under investigation in

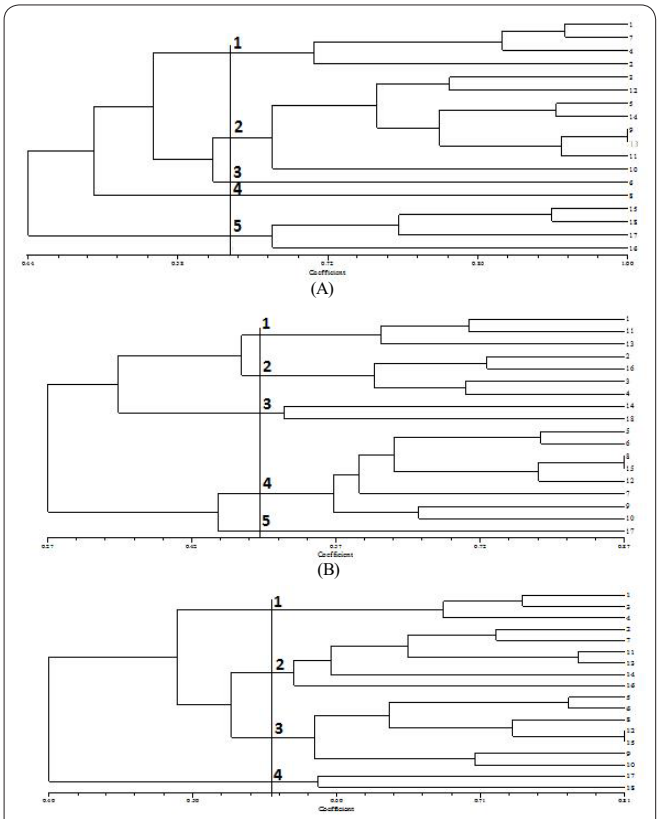

Figure 4. Dendrogram generated using complete analysis, showing relationships among Black cumin accessions using ISSR, RAPD and combining RAPD and ISSR data. (A) ISSR, (B) RAPD, (C) RAPD and ISSR. 1= Hamedan, 2= Qazvin 1, 3= Takestan, 4= Ardebi 1, 5= Botanical garden of bouAli, 6= Lordegan, 7= Ardebil 2, 8= Kermanshah, 9=Khorramabad, 10=Sarbisheh, 11=Shahreza, 12=Meybod, 13=Ardestan, 14=Arak, 15=Share Rey, 16=Shahediieh, 17=Semirom, 18=Qazvin 2.

each region. The maximum genetic distance was seen between Hamedan and Shahediieh populations. The three-dimensional distribution of populations has been presented in Figure 5 using principle component analysis based on Jaccard's similarity coefficient matrix. The results also showed that most of the populations which placed in a group in the cluster analysis were also stayed next to each other in the distribution graph. The results of principle components analysis (Table 6) showed that the first five components were able to explain 71.09 percent of the total variance, respectively.

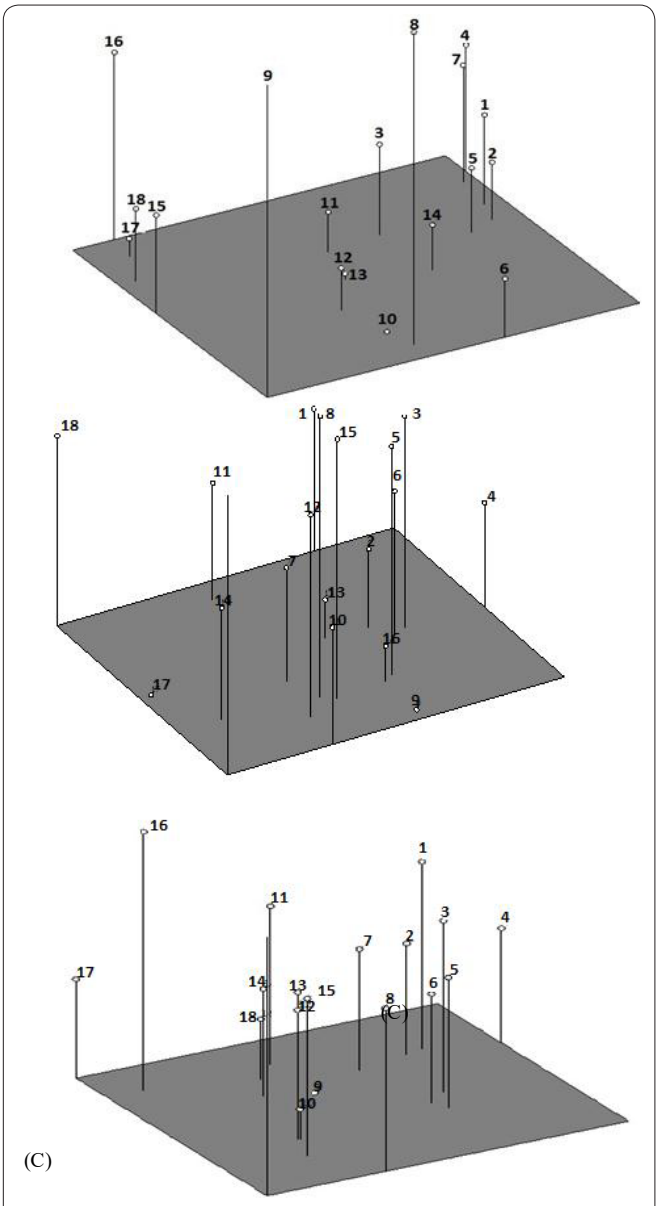

Figure 5. Three-dimensional plot of principle component analysis of 18 black cumin accessions using (A) ISSR, (B) RAPD, (C) ISSR+RAPD data. 1= Hamedan, 2= Qazvin1, 3= Takestan, 4= Ardebi1, 5= Botanical garden of BuAli, 6=Lordegan, 7=Ardebil2, 8=Kermanshah, 9=Khorramabad, 10=Sarbisheh, 11=Shahreza, 12=Meybod, 13=Ardestan, 14=Arak, 15=Share Rey, 16=Shahediieh, 17=Semirom, 18=Qazvin2.

Analysis of a combination of RAPD and ISSR

In order to have a more comprehensive understanding of the results obtained from our research, the data of the two marker systems were combined. Therefore, a total of 142 bands were created. Out of these 142 bands, 61 bands were polymorphic, thus, the percentage of polymorphism was expected about 43 percent (Table 4). The matrix of the genetic similarity between the samples was drawn based on Jaccard's similarity coefficient. The range of variations of the Jaccard's genetic similarity coefficients between the genotypes was between 0.4 and 0.81. The maximum genetic similarity, based on the calculated similarity coefficient, was seen between Meybod and Shahre Rey populations (0.81) and the minimum similarity was seen between Ardebil1 and Semirom populations (0.4). According to the coefficient of Cophenetic correlation between Jaccard's similarity matrix and dendrogram output matrix, the cluster was prepared (Figure 4). Populations were classified into 4 groups (the cut-off point was specified based on

Table 6. Characteristics of principle components based on ISSR data.

Component	Eigen value	Component variance	Accumulative variance
1	1.24	25.56	25.56
2	0.74	15.29	40.85
3	0.62	12.70	53.56
4	0.46	9.51	63.07
5	0.39	8.01	71.09
6	0.35	7.23	78.32
7	0.32	6.58	84.90
8	0.22	4.48	19.39
9	0.14	2.99	92.39
10	0.12	2.53	94.93

Table 7. Characteristics of ten first components based on ISSR+RAPD data.

Component	Eigen value	Component variance	Accumulative variance
1	0.91	14.18	14.18
2	0.74	11.54	25.72
3	0.68	10.55	36.28
4	0.56	8.78	45.06
5	0.49	7.69	52.76
6	0.43	6.67	59.44
7	0.41	6.51	65.95
8	0.36	5.70	71.65
9	0.29	4.51	76.17
10	0.27	4.22	80.39

the highest Cophenetic coefficient). In the dendrogram, distribution of the populations in four groups was balanced and most of the populations were in groups 2 and 3. There was no group containing just one population. In group 1, there were three populations from three neighbor provinces (Ardebil, Hamedan and Qazvin) which have comparable climates. In group 2, there were 6 populations from 5 different provinces. In this group, Shahediieh populations (belonging to Yazd province) which are from a tropical zone was placed in a separate subgroup; while other populations were placed in the second subgroup. The habitat of these populations had relatively similar climatic conditions. There were 7 populations from Hamedan, Chaharmahal & Bakhtiari, Kermanshah, Yazd, Tehran, Lorestan and southern Khorasan provinces in group 3. The interesting point here is that in the three subgroups of this group, the populations from Lordegan and Botanical garden of BuAli were quite similar and the populations from Khoramabad and Sarbisheh were similar as well. The populations from Kermanshah, Meybod and Shahre Rey were placed in a subgroup and the populations from Meybod and Shahre Rey were quite similar. Group 4 contained populations from Semirom and Qazvin. The maximum genetic distance was seen between populations from Hamedan and Qazvin. Distribution of the populations was evaluated based on a combination of ISSR and RAPD markers through the main component analysis using Jaccard's similarity matrix. The three-dimensional graph of the distribution of populations has been presented in Figure 5. Most of the populations that were next to each other in the distribution graph were also stayed in one group in dendrogram (Figure 4). The first five components were able to explain 52.76 percent of the total variance and in total; the first 10 components were able to explain 80.35 percent of the total variance (Table 7).

Discussion

RAPD analysis results indicate that there is a high genetic diversity among the populations under investigation in comparison with the results of Huang et al. (19) evaluated 22 *Astragalus adsurgens* populations, the results of Chengxin et al. (20) evaluated one of the plants belonging to the Apiaceae family as well as the results obtained by Pezhmanmehr et al. (21) who evaluated 20 Iranian populations of *Bunium persicum*. The first five components have been able to explain a total of 55.61 percentage of total variance. Given that each of these components had a small share of the explanation of the total variance, it can be concluded that each of the markers has assessed different parts of the sample genomes. ISSR analysis results are indicative of a significant genetic diversity among the populations and they comply with the results of Fabriki Orang et al. (22) who studied the genetic diversity of local populations of Iranian melon but they did not comply with the results of Feyzian et al. (23) who studied the genetic diversity of Iranian melons. The results of principle components analysis showed that the first five components were able to explain 71.09 percentage of the total variance, respectively; which means that the used markers had a relatively suitable distribution at the genome and have been efficient in evaluating the genetic diversity of the Black cumin populations. Ismaili et al. (28) evaluated genetic diversity in populations of *Thymus kotschyanus* using RAPD markers and showed that these markers are useful to study the genetic diversity of this plant and can also reveal geographical differences and *T. kotschyanus* showed a high percentage of polymorphism (77.57%). Therefore, RAPD markers were useful tool for evaluation of germplasm. Obtained polymorphism percentage in this study was more than the polymorphism percentage obtained for *T. kotschyanus* by Ismaili et al. (28), which may be due to the high diversity between different populations of Black cumin. Also, Ismaili et al. (29) assessed genetic diversity in two subspecies of *Thymus daenensis* using intron-exonic markers. Grouping based on cluster analysis also could separate two subspecies of *Thymus daenensis*. Results obtained from this study showed that intron-exonic markers had an effective potential in assessment of genetic relationships between the two sub-species of daenensis. The DNA markers not only have been used for plants (30) but also for other organism such as insects (31-33).

RAPD and ISSR combination results comply with the results obtained by Moradi and Chogamirza (11) that evaluated the genetic diversity of various genotypes of Durum wheat using RAPD and ISSR markers and showed that the ISSR markers show a higher diversity (86.44 percent) than the RAPD markers (64.91 percent). Moreover, Morales et al. (10) reported that the grouping proposed by the ISSR markers was more coherent with the origin and the genealogy of the cultivars than that proposed by the RAPD markers in strawberry. Researchers who have compared RAPD and ISSR markers have reported that ISSR exhibited higher level of polymorphism compared with RAPD markers (24, 25, 26). The result of the present study also showed that ISSR markers were more informative than RAPD markers. Comparison of MI values for the two marker systems indicated that the range of MI values for RAPD primers was from 0.196 (H8) to 3.08 (H5). Also the comparison of the MI values of ISSR primers showed that the lowest value was 0.33 (ISSR8H) and the highest value was 1.66 (ISSR6H). RAPD and ISSR markers provided five groups, but RAPD plus ISSR analysis provided four groups.

A possible explanation for these results is that the two marker systems targeted different regions of the genome. It has been reported that ISSR marker to be

more reproducible and produce more complex marker patterns than the RAPD system, and is valuable when differentiating closely related cultivars (27).

The obtained marker indices showed that the RAPD and ISSR were suitable markers for investigation of the genetic diversity of Black cumin populations. Based on obtained results from cluster and principal components analyses, the two markers had a relatively suitable distribution at the genome and have been relatively efficient in evaluating the genetic diversity of our Black cumin populations. The level of polymorphism of RAPD and ISSR markers was equal to 40.5 and 57.4 percent, respectively. This indicates that the ISSR markers were more polymorphic than RAPD markers. By combining the two marker systems, the level of polymorphism was equal to 43 percent. Ultimately, these results indicated that RAPD and ISSR molecular markers can be useful for identifying and classifying various Black cumin populations.

Acknowledgments

This work was supported by Yasouj University. We are thankful to Mrs. Hajizadeh and Mrs. Karami for technical services in the Genetics and Central laboratory of Yasouj University. Thanks to Zagros Bioidea Co. for all supports.

References

1. Morikawa T, Xu F, Kashima Y, Matsuda H, Ninomiya K., Oshikawa M. Novel dolabellane-type diterpene alkaloids with lipid metabolism promoting activities from the seeds of Nigella sativa. Org Lett 2004; 6: 869-872.

2. Riyaz M, Syed M, Chaudhary FM. Chemistry of the medicinal plants of the genus Nigella sativa L. Hamdard Med 1996; 39(2): 40-45.

3. Salamati MA, Zeinali H. Assessment genetic variation of some accessions of Nigella sativa L. using morphological and agronomic traits. Iranian J Med Aroma Plants 2013; 29(1): 201-214.

4. Mohammadi SA, Prasanna BM. Analysis of genetic diversity in crop plants–salient statistical tools and considerations. Crop Sci 2003; 43:1235-1248.

5. Dominique VD. Molecular markers in plant genetics and biotechnology. Taylor & Francis Inc. USA 2003; 248 pp.

6. Han YC, Teng CZ, Zhong Sh, Zhou MQ. Genetic variation and clonal diversity in populations of Nelumbo nucifera (nelumbonaceae) in central china detected by ISSR markers. Aquat Bot 2007; 86: 69- 75.

7. Roose ML, Fang DQ. Identification of closely related citrus cultivars with inter-simple sequence repeats markers. Theor Appl Genet 1997; 95(3):408-417.

8. Domblides AS, Domblides EA, Kharchenko VA, Potekhin GA. Study of genetic variation among Parsley (Petroselinum Crispum (Mill.) Nym.) samples using RAPD and ISSR markers. Moscow Univ Biol Sci Bullet 2010; 4: 25-28.

9. Gajera HP, Tomar RS, Patel SV, Viradia RR, Golakiya BA. Comparison of RAPD and ISSR markers for genetic diversity analysis among different endangered Mangifera indica genotypes of Indian Gir forest region. J Plant Biochem Biotechnol 2011; 10:1000-1007.

10. Morales RGF, Resende JTV, Faria MV, Andrade MC, Resende LV, Carla Andrea Delatorre CA, da Silva PR. Genetic similarity among strawberry cultivars assessed by RAPD and ISSR markers. Sci Agr 2011; 68(6): 665-670.

11. Moradi A, Chogamirza K. Evaluation of genetic diversity in different genotypes of durum wheat using RAPD and ISSR markers. Proceeding of the 5th National Biotechnol Cong Islamic Republic of Iran in 2007; Tehran.

12. Murray MG, Thompson WF. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 1980; 8: 4321–4326.

13. Garcia E, Jamilena M, Alvarez JI, Arnedo T, Oliver JL, Lozano R. Genetic relationships among melon breeding lines revealed by RAPD markers and agronomic traits. Theor Appl Genet 1998; 96: 878-885.

14. Al-Huqail A, Al-Saad F. DNA Fingerprinting and genotyping of four Black seed (Nigella sativa L.) taxa. Environ Arid Land Agr Sci 2010; 21(1): 93-108.

15. Williames JGK, Kubelik AE, Livak KJ, Rafalski JA, Tingey SC. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 1990; 18: 6531-6535.

16. Peakall R, Smouse PE. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 2006; 6: 288-295.

17. Kumar M, Mishra GP, Singh R, Kumar J, Naik PK, Singh ShB. Correspondence of ISSR and RAPD markers for comparative analysis of genetic diversity among different apricot genotypes from cold arid deserts of trans-Himalayas. Physiol Molecul Biol Plants 2009; 15(3): 225-236.

18. Altintas S, Toklu F, Kafkas S, Kilian B, Brandolini A, zkan HO. Estimating genetic diversity in durum and bread wheat cultivars from Turkey using AFLP and SAMPL markers. Plant Breed 2008; 127(1): 9-14.

19. Huang L, Chen Z, Zhang X, Wang Z, Liu C. A comparative analysis of molecular diversity of Erect Milkvetch (Astragalus adsurgens) germplasm from north China using RAPD and ISSR markers. Biochem Genet 2009; 47: 92–99.

20. Chengxin FU, Yingxiong Q, Hanghui K. RAPD analysis for genetic diversity in Changium smyrnioides (Apiaceae), an endangered plant. Botan Bullet Academia Sinica 2003; 44: 13-18.

21. Pezhmanmehr M, Hassani ME, Jahansooz F, Najafi AA, Sefidkon F, Mardi M, Pirseiedi M. Assessment of genetic diversity in some Iranian populations of Bunium persicum using RAPD and AFLP markers. Iranian J Biotechnol 2009; 7(2): 92-100.

22. Fabriki Orang S, Shams-Bakhsh M, Jalali-Javaran M, Ahmadi J. Assessment genetic variation of endemic accessions of Iranian melon (Cucumis melo L.) using molecular inter sequence repeat (ISSR) markers. Iranian Biol J 2009; 22(2): 271-281.

23. Feyzian A, Jalali-Javaran M, Dehgani H, Zamiad H. Assessment genetic variation of some endemic melons in Iran using morphological and molecular RAPD markers. J Sci Technol Agri Nat Resour 2007; 41(11): 151-162.

24. Martins-Lopes P, Lima-Brito J, Gomes S, Meirinhos J, Santos L, Guedes-Pinto H. RAPD and ISSR molecular markers in Olea europaea L.: Genetic variability and molecular cultivar identification. Genet Resour Crop Evol 2007; 54:117–128.

25. Sadigova S, Sadigov H, Eshghi R, Salayeva S, Ojaghi J. Application of RAPD and ISSR markers to analyses molecular relationship in Azerbaijan wheat accessions (Triticum aestivum L.). Bulgarian J Agri Sci 2014; 20 (1): 87-95.

26. Elmeer Kh, Alghanem M, Al-Latifi L, Alhemairi H. Efficiency of RAPD and ISSR Markers for the Detection of Polymorphisms and Genetic Relationships in Date Palm. Biotechnol 2017; 16(1): 19-26.

27. Goulao L, Cabrita L, Oliveira CM, Leitao JM. Comparing RAPD and AFLP analysis in discrimination and estimation of genetic similarities among apple (Malus domestica Borkh) cultivars. Euphytica 2001; 119: 259–270.

28. Ismaili A, Zabeti SM, Hosseini SZ. Genetic diversity of different accessions of Thymus kotschyanus using RAPD marker.

Taxon Biosyst J 2014; 6(19): 55-66.
29. Ismaili A, Mojiri F, Hosseini SZ. Use of intron-exonic marker in assessment of genetic diversity of two subspecies of Thymus daenensis. Taxon Biosyst J 2013; 5(16): 41-45.
30. Rostami-Ahmadvandi H, Cheghamirza K, Kahrizi D, Bahraminejad S. Comparison of morpho-agronomic traits versus RAPD and ISSR markers in order to evaluate genetic diversity among Cuminum cyminum L. accessions. Aust J Crop Sci 2013; 7(3):361-367.
31. Yari K, Mirmoayedi A, Marami M, Kazemi E, Kahrizi D. Genetic diversity analysis of chrysopidae family (Insecta neuroptera) via molecular markers. Mol Biol Rep 2013; 41:6241-6245.
32. Asadi N, Rahimi A, Ghaheri M, Kahrizi D, Bagheri Dehbaghi M, Khederzadeh S, Esmaeilkhanian S, Veisi B, Geravandi M, Karim H, Vaziri S, Daneshgar F, Banabazi MH, Zargooshi J. Genetic diversity of the Dwarf honeybee (Apis florea Fabricius 1787) populations based on microsatellite markers. Cell Mol Biol 2016; 62(12): 51-55.
33. Rahimi A, Mirmoayedi A, Kahrizi D, Zarei L, Jamali S. Genetic diversity of Iranian honey bee (Apis mellifera meda Skorikow 1829) populations based on ISSR markers. Cell Mol Biol 2016; 62(4): 55-60.