Journal of Plant Molecular Breeding (JPMB)
Vol.1/No.2/ October 2013/ 40-53

Assessment of genetic diversity among and within Iranian chamomile populations using semi random intron-exon splice junction (ISJ) markers

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Received: October 2012 Accepted: February 2013

Abstract

Chamomile (*Matricaria chamomilla*), an important medicinal plant belonging to the *Asteraceae* family, has a wide distribution in Iran and other parts of the world. The medicinal and pharmacological effects of chamomile are mainly associated with its essential oil content and it is widely used in food, cosmetics and pharmaceutical industries. Despite its wide geographical distribution in Iran, little is known about its molecular genetic diversity and distribution. In this study, intron-exon splice junction (ISJ) markers, including both intron-targeted (IT) and exon-targeted (ET) primers, were used to assess the genetic diversity of thirty-one chamomile populations, including 28 populations from different parts of Iran, one Hungarian population, and two of unknown origin. Twenty-six out of thirty-five primers used in the study, were reliable, producing a total number of 566 sharp and precise bands, of which 557 bands were polymorphic (98%). The average polymorphic information content (PIC) and the average marker index (MI) were calculated at 0.33 and 7.34, respectively. The average total genetic diversity (H_T), average genetic diversity within population (H_{ST}) and gene differentiation coefficient (G_{st}) were 0.293, 0.219, and 0.251, respectively. The diversity data revealed that the *Matricaria chamomilla* species exhibited the closest relationship with the *Tripleurospermum disciforme* and *Tripleurospermum sevanense* species.

***Key words*:** Iranian Chamomile; *Matricaria chamomilla*; Genetic diversity; ISJ markers; polymorphism

Abbreviations: ISJ: Intron-exon splice junction; IT: Intron-Targeted; ET: Exon-Targeted; PIC: Polymorphic Information Content; MI: Marker Index; H_T: Total Genetic diversity; H_{ST}: Average genetic diversity within population; G_{st}: Gene differentiation coefficient; AMOVA: Analysis of molecular variance; PCO: Principal Coordinates Analysis.

Introduction

Many chemical constituents have so far been identified in chamomile flowers as secondary metabolites as potential pharmacological activity (Singh et al., 2011). Due to many important chemicals, chamomile has anti-diarrheal (De la Motte et al., 1997), anti-inflammatory (Pourohit and Vyas, 2004), antispasmodic (Achterrath-Tuckermann et al., 1980; Singh, 1997), anti-allergic (Pourohit and Vyas, 2004),

and stomachic stimulant (Pourohit and Vyas, 2004) properties. Moreover, researchers have proven that chamomile has a most effective antileishmanial activity (Schnitzler et al., 1995). Recently through selection and breeding efforts, varieties like "Bona," "Kosice-II," and the cultivar "koice-1" have been which normally contain over twice the essential oil content (-)-α-bisabolol and chamazulene) than older varieties (Singh et al., 2011).

In order to improve the line or even develop a hybrid cultivar with highly active ingredients and essential oils, it is always crucial to find/develop lines with higher heterosis potential. To this end, the study of genetic diversity of endogenous germplasms is the first basic step in any national breeding program (Taviani et al., 2002). So far, morphological, molecular, and biochemical markers have been used for evaluation, identification, and determination of genetic diversity throughout the plant kingdom (Chahal and Gosal, 2002). The application of molecular markers in genome whole analysis has been greatly advanced by the development of PCR based markers. So far different molecular marker systems have been evaluated for their efficiency in detecting polymorphism and assessing genetic diversity using various statistical parameters. Intron-exon splice junction (ISJ) markers, unlike other molecular markers such as RAPD, AFLP and RFLP are DNA-based molecular markers that work semi-randomly and whose target location is based on intron-exon splice junction boundaries (Gaweł et al., 2002; Rafalski et al., 2002). These types of DNA markers were developed as an alternative to RAPD or other expensive methods such as AFLP and RFLP molecular markers (Rafalski et al., 2002; Weining and Langridge, 1991). In comparison with random RAPD markers, semi-random markers produce more reproducible banding patterns with lower complexity and higher polymorphism (Rafalski et al., 2002; Przetakiewicz et al., 2002). Semi-random primers are 10-18 nucleotide in length of which 7-9 nucleotides are complementary to the exon-intron junction boundaries and the rest are randomly chosen (Rafalski et al., 2002; Gawel et al., 2002; Nowosielski et al., 2002). Unlike RAPD markers, two different sets of primers, i.e., Exon Targeting (ET) and Intron Targeting (IT) primers can be designed to amplify ISJ markers. ET and IT primers are identifiable due to the fact that ET and IT primers amplify exon and intron parts of the genome (Nowosielski et al., 2002).

So far, few studies have dealt with the genetic diversity of chamomile in Iran and elsewhere (Pirkhezri et al., 2010; Solouki et al., 2008; OkOń and Surmacz-Magdziak, 2011; Wagner et al., 2005). For instance, Wagner et al. (2005) used RAPD and AFLP molecular markers to assess the genetic diversity within and among chamomile populations in Germany (Wagner et al., 2005). The same study was carried out to reveal the genetic similarity and relationship among four wild chamomile genotypes from Poland, nine wild chamomile genotypes from

European gene banks, and seven cultivars from different parts of Europe (OkOń and Surmacz-Magdziak, 2011). Few studies have also dealt with unrevealing genetic diversity among Iranian populations, using different DNA-based molecular markers (Pirkhezri et al., 2010; Solouki et al., 2008). In both cases, however, neither the number of populations used nor the number of primers was reliable enough to draw appropriate conclusions about Iranian chamomile populations. Furthermore, in previous studies, RAPD was used as a molecular marker tool which does possess reproducibility problems, and is therefore unreliable for assessing genetic diversity among gene bank populations. Regarding the medicinal properties of chamomile and the limited number of studies in Iran on its genetic diversity, especially based on molecular markers, the present study aimed at studying the genetic diversity and relationship of chamomile populations revealed by semi-random molecular markers.

Materials and Methods

Plant materials and DNA extraction

A total of thirty-one chamomile populations (with different numbers of genotypes ranging from four to seventeen) from different locations, including twenty-eight populations from Iran, one from Hungary, and two from an unknown origin (collected from Esfahan Research Institute) were used in this study (Table 1).

Table 1. Chamomile populations used in this study along with origin and the allocated codes in cluster analysis. Number were allocated to ease the analysis.

Code	Population	Collection site(province/city)
5	*Anthmis cotula*	Esfahan
14	*Anthmis cotula*	Azerbaijan
15	*Anthmis cotula*	Unknown
12	*Anthmis altissima*	Unknown
9	*Anthmis psedocotula*	Ilam
4	*Tripleurospermum disciforme*	Yazd
6	*Tripleurospermum disciforme*	Gorgan
7	*Tripleurospermum disciforme*	Tehran
16	*Tripleurospermum disciforme*	Fereidunshahr
1	*Tripleurospermum sevanense*	Fars
8	*Tripleurospermum sevanense*	Ardebil
10	*Tripleurospermum sevanense*	Mashhad
11	*Tripleurospermum sevanense*	Shiraz
13	*Tripleurospermum sevanense*	Mashhad
2	*Matricaria chamomilla*	Esfahan
3	*Matricaria chamomilla*	Hungary
17	*Matricaria chamomilla*	Tehran
18	*Matricaria chamomilla*	Zabol
19	*Matricaria chamomilla*	Esfahan
20	*Matricaria chamomilla*	Ardebil
21	*Matricaria chamomilla*	Kerman
22	*Matricaria chamomilla*	Shiraz
23	*Matricaria chamomilla*	Tehran
24	*Matricaria chamomilla*	Kerman
25	*Matricaria chamomilla*	Shiraz
26	*Matricaria chamomilla*	Tehran
27	*Matricaria chamomilla*	Esfahan
28	*Matricaria chamomilla*	Kashan
29	*Matricaria chamomilla*	Arak
30	*Matricaria chamomilla*	Ahvaz
31	*Matricaria chamomilla*	Lorestan

To adopt the most appropriate DNA extraction method, three methods, i.e., Doyle and Doyle also known as CTAB

(Doyle, 1990), Dellaporta (Dellaporta et al., 1983), and Khanuja (Khanuja et al., 1999), were compared based on the quantity and quality of DNA. The quantity of DNA against the standard concentration of lambda DNA was determined using agarose gel electrophoresis.

ISJ analysis

Thirty five primers were initially chosen based on other studies (Table 2). All the primers were screened in small samples to check the amplification patterns and their reproducibility in three separate runs for each individual primer. Therefore, out of thirty-five ISJ primers screened for amplification of DNA of chamomile genotypes, nine primers resulted in either sub-optimal or non-distinct amplification products. The remaining twenty-six primers that generated reproducible ISJ patterns were used for subsequent analyses (Table 2). PCR reaction mixtures contained 45 ng/ l of chamomile genomic DNA, 2 mM $MgCl_2$, 0.5 pm of primers, 0.2 mM dNTPs, 1×PCR buffer(10 mM Tris-HCl(pH 8.0), 10 mM KCl) and 0.15 unit Taq DNA polymerase in a total volume of 15 l. PCR reaction was performed in a two-step procedure according to Przetakiewicz et al. (2002). PCR thermal cycler was programmed as follows: *Step one*: Initial prenaturation at 94°C for 5 min, followed by 7 cycles (94°C for 40 sec, T_m+2 for 1 min and 72°C for 2 min). *Step two*: 33 cycles (94°C for 40 sec, T_m+6 for 1 min, and 72°C for 2 min), followed by an additional final extension at 72°C for 10 min. All PCR amplified products were subjected to horizontal electrophoresis, using 1.5% agarose gel.

Data analysis

The presence and absence of amplicons for each marker system was recorded for all genotypes and then converted into a genetic similarity matrix using Jaccard coefficient (Jaccard, 1908), using NTSYS-PC 2.1 (Rohlf, 1998). The similarity coefficients were used to construct a dendrogram depicting genetic relationships using the unweighted pair group mean average (UPGMA) method (Sneath and Sokal, 1973). The Polymorphism Information Content (PIC) values were calculated using the following equation described by Botstein et al. (1980):

$$PIC = 1 - (\sum_{i=1}^{n} P_{ij}^2)$$

where P_{ij} is the frequency of the jth allele for the ith marker, and summed over n alleles. The marker index (MI) was calculated, using the following equation described by Powell et al. (1996):

$$MI = PIC.N.\beta$$

where N is the total number of bands of a primer, and β is the ratio of polymorphism for each primer. PIC shows the capability of a primer in distinguishing genotypes and MI calculates the potentiality of a primer for producing more bands on the gel. T-test was used to compare the level of significance between the two different sets of primer. Analyse of genetic diversity between and within population

Table 2. Profile and number of bands produced by each semi-random ISJ primer.

Primer code	Sequence ($5' \rightarrow 3'$)	No of bands	Polymorphic bands	MI	PIC	Polymorphism(%)
IT_{10-1}	ACGTCCAGAC	17	17	5.44	0.32	100
IT_{10-2}	ACGTCCAGGT	24	23	8.51	0.37	95
IT_{10-3}	ACGTCCAGCA	24	23	8.74	0.38	95
IT_{10-4}	ACGTCCACCA	15	14	3.78	0.27	93
IT_{10-6}	ACGTCCATCC	20	17	5.95	0.35	85
IT_{15-31}	GAAGCCGCAGGTAAG	27	27	8.91	0.33	100
IT_{15-34}	GCGGCATCAGGTAAG	23	21	6.30	0.30	91
IT_{15-35}	CGAAGCCCAGGTAAG	23	23	8.97	0.39	100
IT_{15-36}	ACCTACCTGGGGCTC	24	24	8.88	0.37	100
IT_{18-1}	CCGGCAGGTCAGGTAAGT	30	30	9.60	0.32	100
IT_{18-2}	GCAGAGGGCCAGGTAAGT	30	30	10.2	0.34	100
ISJ_1	CAGACCTGC	21	21	6.93	0.33	100
ISJ_5	CAGGGTCCCACCTGCA	27	27	11.34	0.42	100
ISJ_9	AGGTGACCGACCTGCA	27	26	10.40	0.40	96
ISJ_{11}	TGCAGGTCAAACGTCG	21	21	7.77	0.37	100
ET_{12-26}	AGCAGGTGGACT	18	18	4.32	0.24	100
ET_{12-27}	AGCAGGTCCTAG	17	17	4.42	0.26	100
ET_{12-28}	AGCAGGTCCAAG	22	22	7.70	0.35	100
ET_{12-29}	AGCAGGTCGTGA	21	21	5.88	0.28	100
ET_{12-30}	AGCAGGTGGTAC	19	19	5.70	0.30	100
ET_{15-32}	ACTTACCTGGGCACG	20	20	6.80	0.34	100
ET_{15-33}	ACTTACCTGGCCGTG	15	15	5.85	0.39	100
ET_{15-35}	ACTTACCTGCCGCAG	13	13	4.55	0.35	100
ET_{15-36}	ACTTACCTGGGGCTC	23	23	8.74	0.38	100
ET_{18-1}	ACTTACCTGAGGCGCGAC	23	23	7.36	0.32	100
ET_{18-2}	ACTTACCTGCTGGCCGGA	22	22	7.84	0.34	100
Total		566	557	7.34	0.33	98

MI: Marker Index, PIC: Polymorphism Information Content

was carried out to describe the structure of populations, using POPGENE software, Ver. 1.31 (Yeh et al., 1997). Since its insensitivity to the bias may be introduced into data by the inability to detect heterozygous individuals (Dawson et al., 1995; Gustafson et al., 1999; Oiki et al., 2001), Shannon's index is suitable for analyzing random marker data. POPGEN was also used to measure genetic variations within the populations in terms of Shannon's indices. Patterns of genetic diversity and genetic variation parameters were also calculated according to Nei (Nei, 1973). All the ISJ data were subjected to a hierarchical analysis of molecular variance (AMOVA) (Excoffier et al., 1992) using three hierarchical levels (individual, population, and their regions). The molecular variance within each population was calculated as an

indicator of intra-population genetic variation. Estimates of the partitioning of the genetic variation among the populations and among individuals within the populations were initially derived from a global analysis without considering differences in region. The analysis was performed using GenALEx software (Peakall and Smouse, 2001). To plot the relationship between distance matrix elements based on their first two principal coordinates, GenALEx was also used to perform a Principal Coordinates Analysis.

Results and Discussion

In order to choose one of the three well known genomic DNA isolation methods in plants, CTAB (Doyle, 1990), Dellaporta (Dellaporta et al., 1983), and Khanuja (Khanuja et al., 1999) methods were compared. Results of this experiment showed that with slight modifications, CTAB was the most appropriate method for genomic DNA isolation from chamomile leaves (Table 3). The DNA yield extracted by the CTAB method was almost three times as high as that of the other two methods (Table 3). It is expected that pure DNA and RNA have $A_{260/A280}$ ratios of 1.8 and 2.0, respectively (Maniatis et al., 1982). Therefore, a ratio of $A_{260/A280}$>1.8 suggests little protein contamination in a DNA/RNA sample. In this study, the $A_{260/A280}$ absorbance ratio for the CTAB method was 1.9, indicating high purity of the isolated DNA. Therefore, genomic DNA used in this study was isolated in this way.

Table 3. Quantitative and qualitative comparison of different DNA extraction methods.

Specifications	DNA extraction methods		
	CTAB	**Dellaporta**	**Khanuja**
Amount of tissue	0.2 g	0.2 g	0.2 g
Washing buffer type	Ammonium acetate	Alcohol 70%	Alcohol 80%
Proteins removal	Chloroform	Chloroform	Chloroform
Centrifuge temperature	4°C	4°C	4°C
Amount/color of DNA sediment	High/white	Low/light brown	Low/milky
Concentration (ng/µl)	670	240	210
$A_{260/280}$	1.9	1.6	1.4
A_{320}	0.003	0.008	0.023

Molecular marker analysis

Out of thirty-five primers used in the study, 26 (15 IT and 11 ET) were reproducible and produced a total number of 566 sharp and precise bands, of which 557 bands were polymorphic (98% polymorphism). The fifteen IT primers produced 344 polymorphic bands (97% polymorphism), whereas the eleven ET primers produced 213 polymorphic bands (100% polymorphism) (Table 2). The average numbers of polymorphic bands per primer were 22.93 and 19.36 for IT and

ET primers, respectively. IT_{18-1} and IT_{18-2} with thirty bands and ET_{15-35} with thirteen bands generated the maximum and minimum number of bands, respectively. IT_{18-1} and IT_{18-2} with thirty polymorphic bands and ET_{15-35} with thirteen polymorphic bands were the most and the least informative locus for DNA profiling and differentiation, respectively. Although it was found that IT primers produced more and sharper bands than ET primers, no significant differences were found in this regard (T-test, $p<0.05$). Rafalski et al. (1997), came to the same results, indicating that both sets of primers revealed the same amount of diversity. Nonetheless, one likely explanation for the superiority of IT primers might be the amplification of non-coding regions of genes (Rafalski et al., 2002; Przetakiewicz et al., 2002; Nowosielski et al., 2002).

Solouki et al. (2008) showed that six IT primers generated a total number of 94 bands, of which 86 bands were polymorphic (91.5% polymorphism) and 8 ET primers resulted to a total number of 101 bands of which 87 were polymorphic (86.1% polymorphism). Furthermore, the numbers of polymorphic bands per primer were 14.3 and 10.9 for IT and ET primers, respectively. Our results showed that the number of polymorphic bands and the level of polymorphism for ET primers were higher in comparison to their study. This can be attributed primarily to the higher number of populations and secondarily to the existence of species other than *chamomilla* in this study.

The average PIC index was 0.33, ranging from 0.24 to 0.42. The ISJ_5 and ET_{12-26} primers showed the highest (0.42) and the lowest (0.24) PIC values, respectively. Since PIC is a parameter to estimate the discriminatory power of molecular marker per primer, it can be concluded that ISJ_5 is the best primer to evaluate the genetic diversity of chamomile populations. The average MI was 7.34, ranging from 3.78 to 11.34. The ISJ_5 and IT_{10-4} primers produced the highest (11.34) and the lowest (3.78) MI values, respectively. The concept of MI was introduced as an overall measure of marker efficiency (Powell et al., 1996). A high MI or diversity index is a reflection of the efficiency of ISJ markers to simultaneously analyze a large number of bands rather than the levels of polymorphism detected.

Cluster analysis based on a simple matching coefficient and the UPGMA method generated a dendrogram for all twenty-six semi-random primers (Figure 1A). Cluster analysis grouped the populations into five separate groups at 0.64, whereas the number of group at this point for IT and ET primers were four and three, respectively (Figure 1B and 1C). The dendrograms displayed that there are many similarities between *Tripleurospermum sevanense* and *Tripleurospermum disciforme* species. Mitsouka et al. (1972) also found these two species very close with regard to their karyotype analysis (Mitsuoka and Ehrendorier, 1972). In the ET-primers group (Figure 1C), the first cluster included all *Tripleurospermum*

populations and one population (Azerbaijan) of *Anthemis cotula*, the second cluster contained all *Anthemis* populations and one population (Gorgan) of *Tripleurospermum disciforme*, and the third cluster contained all *Matricaria chamomilla* populations. One important point observed in all the dendrograms was that no relationship was found between the origin of the populations (geographical location) and genetic distances; i.e. populations collected from the same geographical locations were not necessarily similar and often had large genetic distances. A great similarity was observed between Yazd *Tripleurospermum disciforme,* Tehran *Tripleurospermum disciforme*, Mashhad *Tripleurospermum sevanense,* Shiraz *Tripleurospermum sevanense*, and Tehran *Matricaria chamomilla* , Zabol, Kerman, Esfahan and Ardabil *Matricaria chamomilla.* One of the most significant similarities found in the dendrograms was between the Azerbaijan *Anthemis cotula* and *Tripleurospermum sevanense* populations. These findings are in line with the fact that Tripleurospermum is closely related to Anthemis (Oberprieler et al., 2001). Taxonomy problems in the *Asteraceae* family members, however, cannot be ignored. Part of these discrepancies might be attributed to either sampling errors, misnaming the individual genotypes or the migration of the populations and weakness of the markers applied in the study.

Evaluation of genetic diversity among and within populations

AMOVA for semi-random ISJ primers showed that variance within the populations was higher than variance between the populations (Table 4). Variance within the populations for IT and ET primers were 87%, and 80% of the total variance, respectively. Principle component analysis was calculated to display genetic relationships among thirty-one chamomile genotypes. The first three coordinates derived from this analysis could explained 66.78% of the variation. The two-dimensional plot also confirmed the cluster analysis results (Figure 2). The two-dimensional scatter diagram showed a wide dispersion in the *Matricaria chamomilla* population which to some degree, was observed in the three other populations. As can be seen from Figures 1 and 2, it is not possible to group various populations unambiguously. The results of this type of analysis are in line with the outcome of UPGMA dendrogram (Figure 1A).

Genetic Diversity among the Populations

The total genetic diversity (H_T), genetic diversity within the populations (H_{ST}), and the coefficient of gene differentiation (G_{ST}) for all ISJ primers were 0.293, 0.219, and 0.215, respectively. The H_T, H_{ST}, and G_{ST} for IT were 0.302, 0.229 and 0.239, respectively. The values for ET primers were 0.258, 0.202, and 0.217, respectively. In comparison with the genetic diversity among the populations, the genetic diversity within the populations received a significantly

(P≤0.001) larger share of the total genetic diversity in both two groups of primers. Thus, this finding may indicate that the observed total genetic diversity was more affected by the genetic diversity within than among the populations. Coefficient of gene differentiation had a value between 0.2 to 0.3 in both groups of primers, which indicated that there was little diversity among the populations and species.

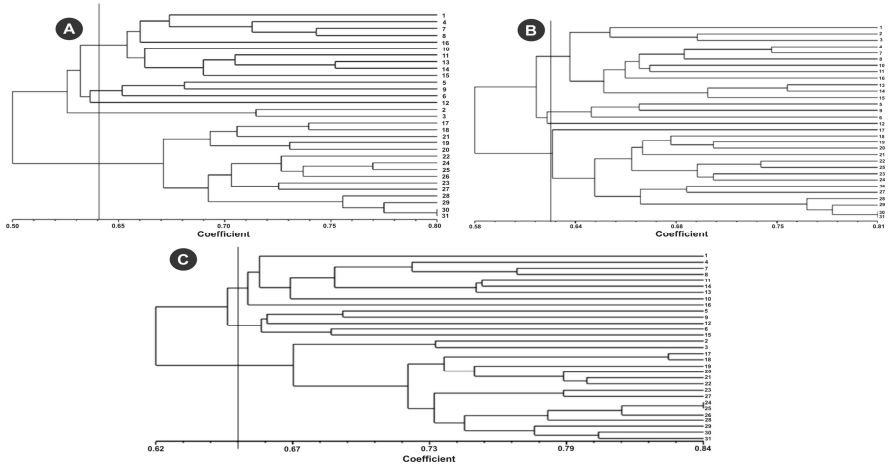

Figure 1. UPGMA dendrogram of 31 chamomile populations by simple matching coefficient based on all primers (A), IT primers (B), and ET primers (C). Numbers were allocated to the member of each population to differentiate the genotypes (Table 1).

Table 4. Analysis of molecular variance (AMOVA) among and within 31 chamomile populations .

Source of variation	df	Mean of squers	Variance components	% of variation
Between populations	3	186.346	16.5	15%
Among populations	25	91.136	91.136	85%
Total	28	-	107.637	100%

df: degree of freedom

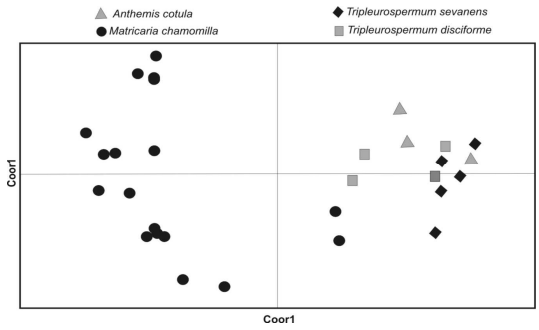

Fig. 2. Two-dimensional scatter diagram of four chamomile populations based on principal coordinate analysis based on semi-random data performed by GenAlex software.

Genetic diversity within the populations

Analysis of the data for genetic diversity within populations revealed that the *Matricaria chamomilla* population had the highest (I=0.377) and the *Anthemis cotula* population showed the lowest (I=0.277) diversity. With respect to the percentage of polymorphic loci, the *Matricaria chamomilla* population had the highest (86.6%) and the *Anthemis cotula* had the lowest (49.4%) polymorphic loci. The *Matricaria chamomilla* population

with I=0.411, showed the highest Shannon information index for IT primers. The highest percentage of polymorphism for IT and ET primers were 90.14% and 82.16%, respectively and both were related to the *Matricaria chamomilla* population. In general, the *Matricaria chamomilla* population had the highest Shannon information index and polymorphism in both groups of primers, which was attributed to the existence of numerous populations and the differences between them.

Cluster analysis results showed that both *Tripleurospermu sevanense* and *Tripleurospermum disciforme* populations are in the same group (Figure 1A). These two populations were also very close to each other in the dendrogram drawn by POPGEN. Furthermore, the *Matricaria chamomilla* population was the closest neighbor to these two populations. The *Anthemis cotula* population had the maximum distance from the other three populations. Nei's genetic distance among the four populations of chamomile is represented in Table 4. According to Table 4, the *Tripleurospermum disciforme* and *Tripleurospermum sevanense* populations had the minimum distance (0.06) and the maximum similarity (0.95). The dendrograms based on IT and ET primers divided the whole populations of chamomile into three groups. In both dendrograms, the *Tripleurospermum sevanense* and *Tripleurospermum disciforme* populations have minimum genetic distances of approximately 0.05 and 0.02 and the highest similarities of 0.95% and 0.97%, respectively (Table 5). This indicated that the ET series of primers could effectively separate these populations. In IT primers, the *Tripleurospermum sevanense* and *Anthemis cotula* populations had the maximum genetic distance (0.1551) and the minimum similarity (0.8563). In ET primers, the *Anthemis cotula* and *Matricaria chamomilla* had the maximum genetic distance (0.1188) and the minimum similarity (0.888). Similar to all ISJ primers, in IT primers, the *Matricaria chamomilla* showed the maximum similarity to *Tripleurospermum species*. Mitsouka et al. (1971) reported that these two populations were approximately similar in terms of karyotype, which in turn might explain the similarity of these two populations.

Table 5. Nei genetic distances (bottom diagonal) and genetic similarity (top diagonal) between chamomile populations based on ISJ analysis.

	T. sevanense	***T. disciforme***	***A. cotula***	***M. chamomilla***
T. sevanense	1.00	0.96	0.85	0.90
T. disciforme	0.06	1.00	0.91	0.94
A.cotula	0.16	0.01	1.00	0.86
M.chamomilla	0.10	0.06	0.15	1.00

In ET primers the *Matricaria chamomilla* and *Tripleurospermum* populations had the maximum genetic distance, indicating differences between them with respect to exon regions.

In conclusion, fundamental genetic information about natural chamomile populations was obtained based on ISJ molecular markers. Results of this study showed that there is no obvious relationship between the genetic diversity of chamomile populations and their geographical origin. Furthermore, the *Matricaria chamomilla* species exhibited the closest relationship with the *Tripleurospermum disciforme* and *Tripleurospermum sevanense* species. We reckon that the findings of this study have important implications for the management of regional genetic variation and for conservation of this very important medicinal resource. A wide genetic diversity of Iranian chamomile populations as revealed in this study is a critical component for future selections and use of this germplasms for future chamomile breeding.

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ارزیابی تنوع ژنتیکی بین و درون جمعیت‌های بابونه‌ی ایرانی (*Matricaria chamomilla*) به کمک نشانگرهای نیمه تصادفی ISJ

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چکیده

بابونه (*Matricaria chamomilla*) یکی از گیاهان داروئی مهم خانواده Asteraceae می‌باشد که پراکندگی زیادی در ایران و سایر نقاط جهان دارد. خواص طبی و داروئی بابونه غالباً از محتوای روغن آن نشأت می‌گیرد و به وفور در صنایع غذائی، آرایشی و داروئی مورد استفاده قرار می‌گیرد. با وجود پراکندگی جغرافیائی زیاد این گیاه در ایران، در مورد تنوع ژنتیکی و پراکنش آن اطلاعات کمی در اختیار است. در این تحقیق، از نشانگرهای ISJ شامل هر دو نوع آعازگر، اینترونی IT و اگزونی ET، جهت بررسی تنوع ژنتیکی ۳۱ جمعیت بابونه شامل ۲۸ جمعیت از نقاط مختلف ایران، یک جمعیت مجارستان و۲ جمعیت با منشأ نامعلوم مورد استفاده شد. از مجموع ۳۵ آغازگر مورد استفاده در این تحقیق، ۲۶ آغازگر قابلیت امتیازدهی داشتند و در مجموع ۵۶۶ باند واضح و قابل شمارش ایجاد کردند. از این تعداد، ۵۵۷ باند چند شکل بودند(۹۸٪). میانگین میزان اطلاعات چندشکلی (PIC) و میانگین شاخص نشانگر (MI) به ترتیب ۳۳ و ۷.۳۴ محاسبه شدند. میانگین تنوع ژنتیکی کل (H_T)، میانگین تنوع ژنتیکی درون جمعیت (H_{ST}) و ضریب تمایز ژنی (GST) به ترتیب ۰.۲۹۳ ، ۰.۲۱۹ و ۰.۲۵۱ بودند. نتایج این تحقیق نشان داد که گونه‌های *Matricaria chamomilla* بیشترین نزدیکی را با Tripleurospermum Disciform و Tripleurospermum Sevanens دارد.

کلمات کلیدی: بابونه ایرانی، *Matricaria chamomill*a، تنوع ژنتیکی، نشانگر نیمه تصادفی ISJ، چندشکلی.