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Original Article

A Study of Genetic and Chemical Diversities of some Chamomile Ecotypes Based on RAPD Markers and Essential oil Compositions

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Abstract

Chamomile is a medicinal plant with high economic value. In this research, 20 chamomile ecotypes collected from different regions of Iran were evaluated for genetic and chemical diversity. DNA was extracted by CTAB and polymerase chain reaction was performed using 13 RAPD markers. Essential oils extraction was performed by water distillation using Clevenger system. Components of the essential oils were detected by Gas Chromatography at 62 retention times. The results showed matching relation between chemical composition and geographical diversity. It is important to note that in this study, Pseudo chamomile ecotypes were placed next to German chamomile ecotypes in both molecular and chemical analyses. In order to identify essential oil compositions, the essential oils of 5 ecotypes were analysed using GC/MS method. The major components in the oil were, α-bisabolon oxide A, α-bisabolol oxide A , and (Z)- β-Farnesene. Regarding percentage of three important medicinal compositions of chamomile, Esphandegheh ecotype was chosen as the best one.

Key words: *Matricaria chamomilla,* Essential oil composition**,** Genetic diversity, GC/MS

Introduction

Chamomile is an important medicinal plant belonging to family Asteraceae (compositae) and has high medicinal and economic value and it dated back to the ancient age [1]. Chamomile extract is sedative and antidistention and it is used to remove stress and stomach disorders. It is used widely in making cosmetics due its anti-inflammatory effect on the skin [2-5]. There are different known species from Asteraceae family as Chamomile, such as: German chamomile (*Matricaria chamomilla; M .recutita*), Roman chamomile (*Anthemiss nobilis*), May Weed chamomile (Dag Chamomile; Maruta foetida; *Anthemis cotula*) and Pseudo chamomile (*Tripleurospermum*) [1]. German and Roman species have more medicinal uses than other species. Fresh essential oil of chamomile is blue due to presence of Azolen compounds and changes gradually into brown. Yellow colour of the essential oil of Pseudo Chamomile indicateslack of camazolen and other azolen derivatives. The largest group of medically important compounds forming the chamomile essential oilsare primarilychamazulene, (-)- α-bisabolol, bisabololoxides, bisabolonoxide A, trans-β-farnesene, α-farnesene, spathulenol and the cis/trans-en-in-dicycloethers [3]. The RAPD is one of the molecular markers based on PCR that will be useful to study genetic species of medicinal herbs due to having the advantages of simplicity without the need to know DNA sequence of the genome [6]. Different studies have been done to investigate genetic diversity of chamomile ecotypes based on morphological trait [7,8], molecular markers [9] and chemical compositions of the essential oil [1,10] that each of them has their own advantages. Using these factors simultaneously will be useful to identify valuable ecotypes for breeding projects. Solouki *et al.*

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studied genetic diversity of some commercial and Iranian chamomile ecotypes using morphological trait and molecular markers [11]. In 2010, Pirkhezri.et.al studied genetic ecotypes of the southwest of Iran using morphologic trait and molecular markers [12]. Nazaralipour and sefidkon studied chamomile in 2003. In this study, the aerial parts of *Tripleurospermum disiforme* were collected in complete flowering stage from Taleghan in July 2001. After drying in shade, the plant material was subjected to steam distillation. The pale yellow oil was obtained at 0.45% (w/w) yield. The oil was analyzed by a combination of GC and GC/MS. Results of this investigation that was done the first time in Iran were identification of 14 compounds (representing 94.93% of the oil) by percentage more than 0.07% in the oil. The main components of *Tripleurospermum disiforme* oil were trans, trans matricaria ester (39/93%), cis calamenene (22.99%), (Z) –β- farnesene (12.54%), β- maaliene (7.98%) and β- sesquiphellandrene (2.22%). This study aimed to investigate genetic and chemical diversities of chamomile ecotypes in different areas of Iran and to understand the relation between genetic, chemical and geographical diversity of these ecotypes [13].

Material and Methods

20 chamomile ecotypes including 16 German ecotypes (Dehdasht, Jiroft, Behbahan, Kazeron, Shirvan, Bafgh, Shiraz, Esfandegheh, Mashhad, Gonabad, Ravar, Baft, Isfahan, Noorabad-Mamasani, Kermanshah and Golbaft) and 4 ecotypes of pseudo chamomile (Kerman, Torbat-Heydarieh, Sharekord and Marand) were gathered from different regions in Iran (Fig. 1).

DNA extraction was done by CTAB method. The quality and quantity of DNA samples were investigated by electrophoresis and spectrophotometer. 13 RAPD markers were used to do PCR and replicate DNA fragments. After electrophoresis, 1575 bands were detected that were placed between 200 and 2300 bp. 99.2 percent of bands (1563) were polymorph and were used as a criterion to study genetic diversity among ecotypes. The accurate position of bands on electrophoresis gels were determined by AlphaEaseFC and a 0-1 matrix was made. Then, in order to cluster ecotypes and plot Dandrogram, data from the 0-1 matrix were analyzed by NTSYS. Dandrogram was plotted based on Dice similarity coefficient and UPGMA method.

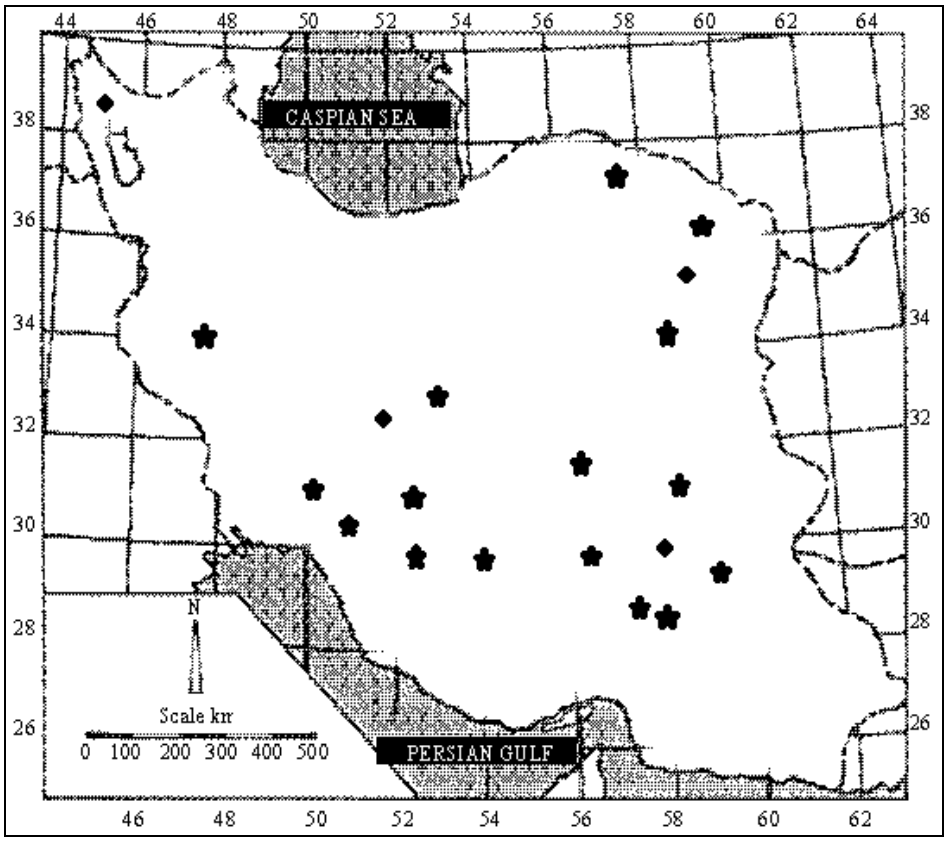

Fig. 1 distribution map of chamomile ecotypes in Iran, German Chamomile (*), Pseudo Chamomile (♦).

Essential oil extraction was done by distillation with water using Clevenger system. Pentane solvent was used to gather the essence. Components of the essence were studied by Gas Chromatograph belonging to Agilent. Co, Model 6890. GC was considered to take 40 minutes. 62 retention times were used as a variable in order to study biochemical diversity of ecotypes. Cluster analysis of data of Gas Chromatograph was done by SPSS and in between group linking.

in order to recognize the essential oil chemical compositions, 5 ecotypes of chamomile including 3 ecotypes related to the German Chamomile (ecotypes of Dehdasht, Esfandagheh and Golbaft) and 2 ecotypes related to the PseudoChamomile (ecotypes of Torbat-Heydarieh and Shahrekord) were chosen. The consisting components of the essential oils were studied by the Shimadzu Q50 gas-chromatograph device connected to a Shimadzu Q50 mass-chromatograph. The condition of analysis is as follows: Capillary columnMS-DB5:with layer thickness of .18 Um,DB5-MS(40M, 18%MM)-temprature progrom:5 minute in 60 ºC, and then 60-275-60 degree Centigrade with 5 ºC/min ,speed-fission ratio: 1 to 43-amoun of injection.1 Microliter–The source temperature of ionization 230 ºC - Mod of ionization: EI-ionization energy 70ev, the carrying gas: Helium-the speed of gas movement:9% Mililiter / Minute–the temperature of injection:1000MS - ionization flow1000 Micro Amper–scan scope :40-300 ºC. And finaly essence elements identified as follow: by comparing standard Mass spectrometry in 2000 willey Electric library in lab solution of GC-MS device and calculating preventive index based on Alkan series C8-C19 injected with even positions and their comparison with available standard numbers in sources. In order to increase the detection accuracy of separated components, the approximate retention index (Quartz Index) was used for approving work of the chromatograph.

Results and Discussion

RAPD analysis

The ecotypes were classified in four groups based on the denderogram resulted from the molecular data in the Euclidean distance of 0.55. Any of Torbat-Heydarieh and Kerman ecotypes, which belong to the pseudospecies,were placed in a different group. The third group included the ecotypes of Dehdasht, Jiroft, Behbahan, Kazeroun, Shirvan and Bafgh, and the last group included the ecotypes of Shahrekord, Marand, Mashhad, Shiraz, Esfandagheh, Gonabad, Ravar, Baft, Esfahan, Nourabad-Mamasani, Kermanshah and Golbaft (Fig.2).Classification of the ecotypes of Shahrekord and Marand related to the pseudospecies, in the same group with the ecotypes of German species is a considerable point in this classification. This that indicated the genetic similarity of these Pseudo Chamomile ecotypes with the German Chamomile species and this result conforms to the findings of Jaymand and Rezaee [10].

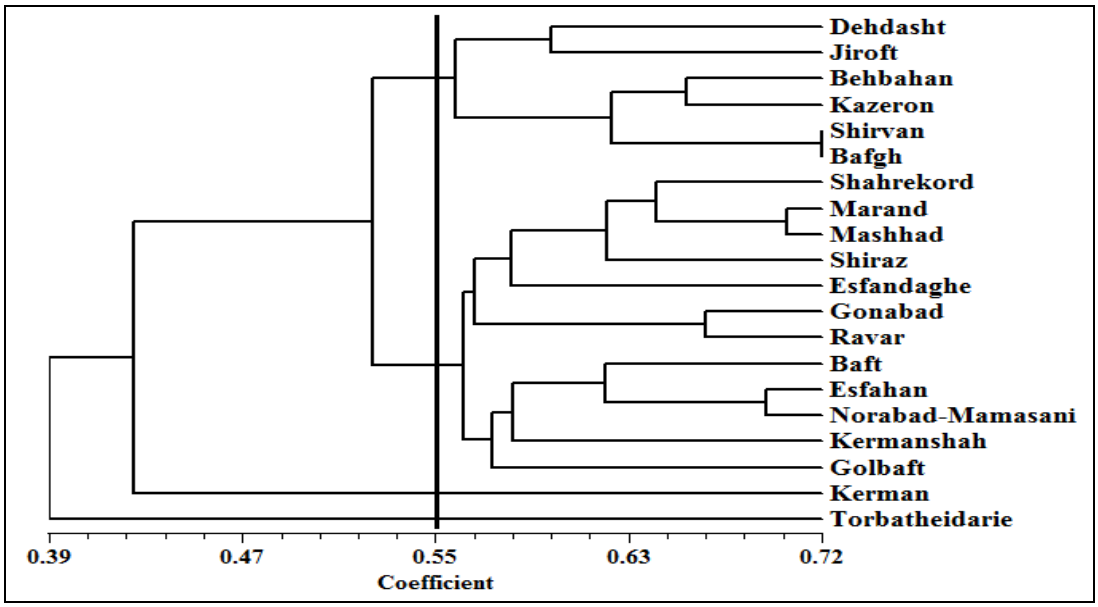

Fig. 2 Dendrogram of chamomile20 populations based on RAPD analysis

According to the findings resulted from this research, no conformity was observed between the molecular and geographical diversities. For instance, the ecotypes collected from Kerman Province were placed in quite different groups, and, on the other hand, the ecotypes which were far from each other from geographical point of view, such as the ecotypes of Jiroft, Kerman Province, and the ecotypes of Behbahan, Khuzistan Province, were placed in one group. Such relations were also observed in the works of other researchers, including [11] in their research the ecotypes of Tehran Province were placed in different groups that the reason can be distribution of seeds by people.

Essential oil analysis

The ecotypes were classified in five groups based on the dendrogram resulted from the cluster analysis of GC data using SPSS software in the Euclidean distance of 12 (Fig. 3).The observed chemical diversity conformed with the geographical distribution of the ecotypes to somehow; one example is the classification of five ecotypes of Kerman Province in one group. Any of the ecotypes of Esfandagheh, Gonabad and Esfahanwere placed in different groups although these ecotypes were placed in one group in the molecular analysis. This difference is probably due to the effect of different environmental conditions on the essential oil compositions. Placing of the ecotypes of the Pseudo Chamomile along with the ecotypes of the German Chamomile indicates the similarity among the essential oil chemical compositions of such ecotypes despite of their genetic difference. Classification of the ecotypes of Kerman and Shiraz in one group, based on the essential oil chemical compositions, has been also observed in the research of Ghenavati *et al*. [14].

Result Comparison of molecular and chemical studies

Comparing the results showed that the samples of Marand, Baft, Ravar, Shiraz and Golbaft were placed in the same group in both molecular and chemical studies. Also, the ecotypes of Dehdasht and Bafgh were placed in one group in both studies. The ecotypes of Gonabad, Esfandagheh and Esfahan which were placed at a same group in the molecular study were classified to three different groups in the chemical study. The ecotypes of Kerman and Torbat-Heydarieh related to the Pseudo chamomile which were classified to different groups in the genetic study were placed at a same group in the chemical study.

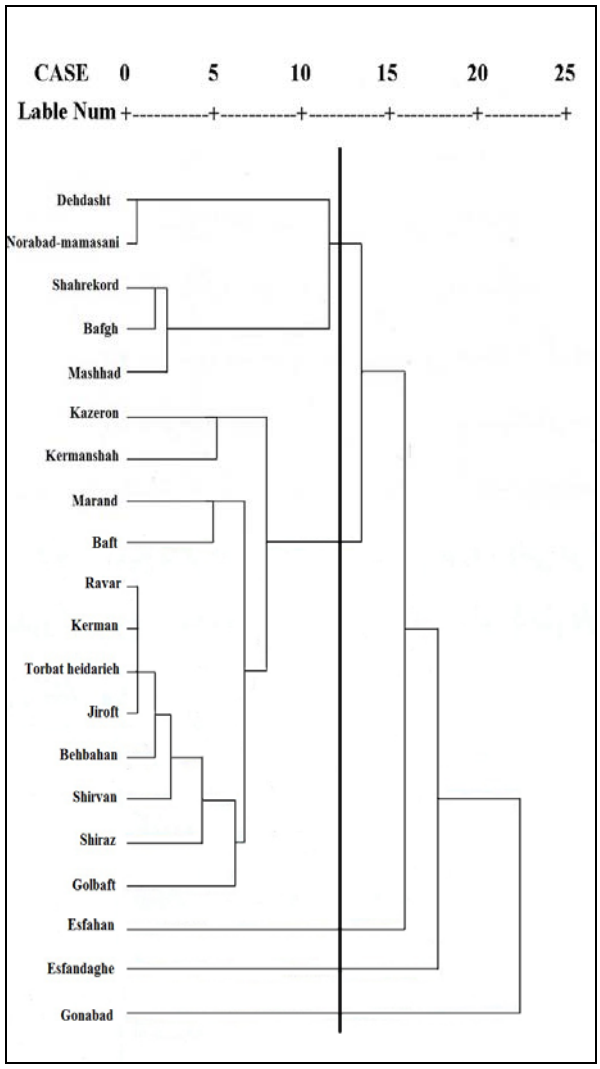

Fig. 3 Dendrogram of 20 chamomile populations based on GC analysis

Recognizing the essential oil chemical compositions

Table 1 shows the consisting compositions of the essential oil, retention index and the quantity percentage of any of the compositions in the 5 genotypes under evaluation. 96.94% essential oil compositions in the ecotype of Dehdasht, 99.49 percent essential oil compositions in the ecotype of Torbat-Heydarieh, and 100 percent essential oil compositions in the ecotypes of Golbaft, Esfandagheh and Shahrekord were identified. As it was expected, chamazolen compound was not observed in the ecotypes of Pseudo chamomile (Shahrekord and Torbat-Heydarieh). Fig.4 compares 3 important medicinal compounds of chamomile (α-bisabolon oxide A, α-bisabolol oxide A , and (Z)- β-Farnesene).

Table 1 Essential oil composions of 5 chamomile ecotypes

	Compounds	RI	Percentage of compound in essential oil				
			Esfandaghe	Dehdasht	Golbaft	Shahrekord	Torbatheydarie
1	α-Pinene	939	-	17.1	-	-	-
2	n-Amyl isobatyrat	1015	-	0.57	-	-	-
3	p-cymene	1025	-	0.63	0.78	-	-
4	Linalool	1097	-	15.6	-	-	-
5	Phenyl ethyl alcohol	1107	-	0.82	-	12.93	-
6	Camphor	1147	-	7.56	-	-	-
7	Borneol	1169	-	0.68	-	-	-
8	Citronelol	1226	-	3.97	-	2.98	-
9	Carvon	1243	-	-	-	3	-
10	Geraniol	1253	0.21	1.81	-	-	-
11	Nonanoic acid	1271	1.8	-	-	-	-
12	Thymol	1290	-	1.24	-	-	-
13	Modheph-2-ene	1384	-	-	0.53	-	4.7
14	(Z)- β-Farnesene	1443	3.42	3.01	3.13	1.74	1.72
15	n- pentadecane	1500	-	-	-	3.67	-
16	Unknown	1523	-	1.41	-	-	-
17	Unknown	1529	-	1.65	-	-	-
18	6-methyl-coumarin	1555	-	-	-	-	-
19	Caryophyllene oxide	1583	-	3.8	-	-	-
20	2E-dodececnyl acetate	1608	-	-	-	-	-
21	α-bisabolol oxide B	1658	2.4	-	1.18	1.03	0.89
22	Ar-turmerone	1669	-	-	6.75	-	1.51
23	Z-nerolidolol acetate	1678	0.33	-	-	-	1.07
24	Deodarone	1699	0.34	-	-	-	-
25	n-heptadecane	1700	-	0.97	-	-	-
26	α-bisabolon oxide A	1711	66.24	24.31	38.4	-	-
27	Chamazulene	1732	1.09	1	0.53	-	-
28	α-bisabolol oxide A	1749	18.8	7.15	12.13	11.37	0.02
29	En-en-dicycloether	1777	0.99	-	1.31	1.31	-
30	n-octadecane	1800	-	4.8	0.96	2.25	-
	Total		95.53	98.08	65.7	40.28	9.91

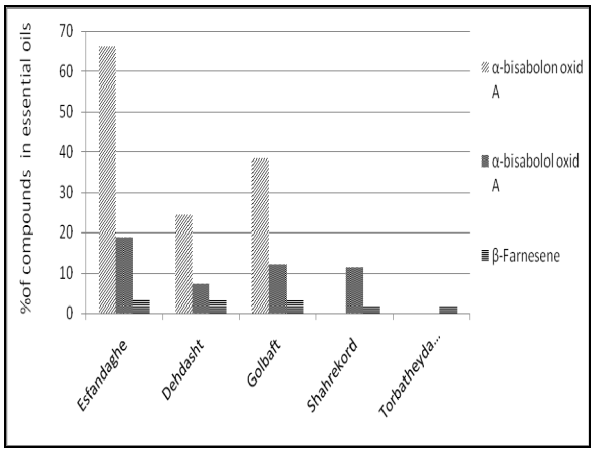

Fig. 4 compares 3 important medicinal compounds of chamomile (Alpha BizablonOxid A, Alpha BizablolOxid A, and Farnezn)

Conclusion

Having knowledge about genetic diversity of available germplasm is one of the most prerequisites for designing a successful breeding project [15]. In addition to genetic differences, different environmental conditions can make chemical compositions of medicinal plants different [16,17]. Therefore, it is better to study simultaneously genetic and chemical diversity of available ecotypes in different geographical areas in order to find ecotypes that are more desirable and improve breeding projects. The effect of different environmental conditions on production of different chemical compositions can be identified by comparing chemical and genetic diversity and understanding the relation between these two factors.

Existence of similarity between chemical and geographical diversities despites lack of similarity between genetic and geographical diversities can be due to two reasons: first, lack of complete coverage of the chamomile genome with the few number of RAPD primers, and second, high influence of the

environmental conditions in the compositions of the essential oil, that this issue has been approved in several studies. Classification of the ecotypes of Pseudo chamomile along with the German chamomile in both molecular and chemical studies indicates very high genetic and chemical similarity of these species that has been also observed in the studies of Jaimand and Rezaei[10]. According to these results, it can be concluded that existence of morphological differences between these two species and also lack of Azolen compounds in the pseudo chamomile are probably related to difference in one or more especial chromosomal areas. Based on comparison of the ecotypes from the point of view of three main medicinal compositions it can be concluded that the ecotypes of Esfandagheh is one of best medicinal ecotypes of Iran and it is recommended to be used in the breeding activities. Also, it can be deduced that the environmental conditions of this region is proper for commercial culture of chamomile.

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