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Study of genetic diversity in lemon balm (*Melissa officinalis* l.) populations based on morphological traits and essential oils content

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ABSTRACT

Lemon balm (Melissa officinalis L.) is one of the most important medicinal plants in the world trade that has many applications in drug and flavoring industrials. In order to evaluate the genetic diversity of different lemon balm populations based on morphological, agronomic and essential oils characters, nine populations from different areas of Iran were collected. In addition, two populations from Germany and Japan were examined. The experiment was carried out based randomized complete block design with three replications. Analysis of variance showed that these populations were significantly different for majority of characters such as plant height, number of tiller, chlorophyll index, leaf width, leaf length, leaf area index, fresh weight, dry weight and dry weight to fresh weight ratio except for number of node and stem diameter. Populations were classified in four groups using cluster analysis based on Ward's algorithm. High levels of phenotypic variation using Shannon-Weaver diversity index (DI) were found among the countries of origin (DI= 0.95-1.00). Qazvin2 population was more diverse than others (DI = 0.99) whereas populations from Germany and Kurdistan displayed lower diversity indices (DI = 0.96). No clear association was detected between phenotypic diversity and origin of Iranian populations. The results of the character distribution and phenotypic diversity analysis permitted some broad generalization about collection and conservation of lemon balm landraces. This also indicated the presence of important genes for future lemon balm breeding purposes. Despite the limitation in estimating the total genetic variation, the present study indicated that morphological traits were useful for preliminary evaluation and can be used as a general approach for assessing genetic diversity among morphologically distinguishable lemon balm populations.

Keywords: *Melissa officinalis* L, Genetic diversity, Morphological traits, Essential oils

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INTRODUCTION

Due to continental and geographical conditions, Iran is a suitable location for growth of many medicinal plants that are compatible with different area and are genetically valuable resource in fundamental and application research in plant breeding.

Lemon balm is a medicinal plant and native to southern Europe, northern Africa, east as far as the Caucasus and northern of Iran [1]. Due to the several applications in pharmacy, nutritional and sanitary industrials, lemon balm was one of the most important commercial plants during the recent decades. This plant occurs naturally in sandy and scrubby area [2, 3] but has also been reported to grow on damp wasteland, at elevations ranging from sea level to the mountains [4].

Lemon balm has medicinal attribute such as tonic, antispasmodic, carminative, surgical dressing for wounds, diaphoretic effect [5], antidepressant [6] and treatment of fever, flatulence, headaches, influenza and toothaches [7, 8]. Although over 100 chemicals have been identified in *Melissa officinalis* L. [9], Major components are citral (neral+geranial) representing 48% of the essential oil, followed by citronellal with 39.47% and β-caryophyllene with 2.37% [10].

Genetic diversity is a key to plant breeding programs. Knowledge of the genetic relationships between different populations supplying this diversity can greatly aid the development of efficient germplasm-management and -utilization strategies. Various methods, including morphological traits, molecular and biochemical markers have been employed to estimate genetic diversity [11]. For breeders, distinguish of the genetic relationship among genotypes are related to phenotypic information [12].

Morphological trait measurements are commonly used parameters since they provide a simple technique of quantifying genetic variation while simultaneously assessing genotype performance under relevant growing environments [13]. Despite the fact, that morphological traits have a number of limitations including low polymorphism, low heritability, late expression and vulnerability to environmental influences. However, morphological characterization is the first step in the classification and evaluation of the germplasm [14, 15]. Several morphological characters are the major determining factors of essential oils yield of lemon balm. The recognition of genetic variability in any character involved in the yield synthesis provides scope to the possibility of improvement and breeding of lemon balm's populations. The genetic differentiation among the populations is also useful for breeding of other populations possessing desire traits.

In our knowledge, no enough systematic study has been carried out to survey the extent of genetic variation and the relationships between various agro-ecotypes of lemon balm's populations. It is necessary to assess the patterns of character variation and distribution of lemon balm in different agro-ecological areas. So the main objective of this study was to characterize and classify the diversity for quantitative traits in the 11 populations of *Melissa officinalis* L. collected from Different sources of Iran and two genotypes from Germany and Japan.

MATERIALS AND METHODS

Plant material

Plant material consisted of 11 lemon balm (*Melissa officinalis* L.) populations including nine Iranian wild accessions, namely Karaj, Ardabil, Esfahan, Hamadan, Fars, Qazvin2, Qazvin3, Kurdistan, and two improved cultivars from Germany and Japan (Fig. 2). Seeds of Iranian landraces were provided by Iranian Forests and Rangelands Research Institute, and seeds of improved cultivars were obtained from Iranian Institute of Medicinal Plants.

Methods

12 morphological traits were scored on the 11 lemon balm landraces that were cultivated in Tabriz Agriculture Research Station (Karkaj), Tabriz, Iran (1360 m altitude). After disinfection with 5% sodium hypochlorite for two minutes, sterilized seeds were evenly placed on two layers of filter paper in sterile Petri dishes. Then the normal seedlings were planted in randomized complete block design (RCBD) with three replications. Lemon balm seedlings were transplanted with 50 cm row space and 30 cm apart in the row. Five plants on the rows in each plot were cut in the early stages of flowering [16, 17] and morphological and agronomic traits consist of plant height (cm), number of tillage, stem diameter (mm), length of leaf (cm), width of leaf (cm), number of node per plant, chlorophyll index, LAI (cm^2) and fresh weight (g) were evaluated. The harvested plants were dried for 72 hours at 35°C and dry weight measured (g), then dry weight/fresh weight ratio was calculated.

To determine the essential oils content in samples, a sample of 20 gram of dried herb was powdered and mixed with 1000 ml of distilled water in flask, and the water was distilled for 175 min using a Clevenger-type apparatus. The

oils were dried over sodium sulphate and its rate was measured based on ml oil per 20 g dry matter of herb and the essential oils percentage were calculated.

Statistical analysis

The proportions of the different populations for each trait and origins were calculated. Origin of populations was used as classifying variables. The phenotypic frequency data of the 12 traits calculated by the Shannon-Weaver diversity index (HI) that defined as [18]:

$$DI = -\sum_{i=1}^{s}\left[\left(\frac{K_i}{K}\right) \times \ln\left(\frac{K_i}{K}\right)\right]$$

Where K_i is the number of plant in i^{th} population, K is total number and S is the total number of populations. H was estimated for each trait and population. In order to keep the values of DI in the range of 0-1, each value of HI was divided by its maximum value $\ln(K_i)$.

Cluster analysis was conducted with Ward's method based on squared euclidean distance using SPSS (SPSS Inc. v. 16.0) statistical package.

RESULTS AND DISCUSSION

Most considered traits such as essential oils percentage, plant height, number of tiller, chlorophyll index, leaf width, leaf length, LAI, fresh weight, dry weight and dry weight to fresh weight ratio showed significant differences in their distribution and amount of variations within studied populations. Except that stem diameter and number of nodes was found to be invariant in all populations. The polymorphism was observed and evident in varying degrees at the population level (table 1). The populations of Esfahan with the average values of 0.14 ml per 20 g dry matter and Fars with 0.01 ml had the highest and lowest essential oils percentage, respectively. There were significant differences between studied populations based on the essential oil percentage. In a study by patora *et al.* [17] percentage of essential oils of 16 populations collected from several regions of Poland was evaluated. In this research, a high variation between populations based on essential oils percentage (0.06 - 0.167 %) was observed.

The dendrogram based on morphological traits and essential oils content classified studied populations into four groups (Fig. 1). According to the cluster analysis, two German and Japanese populations were located in two different groups. The first cluster consisted of two populations Karaj and Tehran. These were characterized by very high amount of height, leaf length, leaf width and LAI. The second cluster included Fars and Qazvin2. These landraces displayed moderate height, fresh and dry weight, and low rate of leaf length, leaf width and LAI. Cluster 3 comprised of Hamadan, Kurdistan, Qazvin3 and Japan. They had medium height and high tiller. The fourth cluster consisted of Ardebil, Esfahan and German. This landraces was distinct from high amount of essential oils and dry weight, medium LAI and low rate in height, tiller and fresh weight.

Table1. Analysis of Variance in lemon balm (*Melissa officinalis* L.) populations based on morphological traits and essential oils.

Source of variation	df	Mean of square (MS)											
		Essential oils (%)	Plant height	No. of tiller	Chlorophyll index	No. of nodes	Leaf width	Stem diameter	LAI	Leaf length	Fresh weight	Dry weight	Dry weight to fresh weight ratio
Block	2	0.001^{ns}	68.22^{*}	1107^{ns}	0.95^{ns}	8.21^{ns}	0.26^{ns}	1.009^{**}	1.16^{ns}	0.23^{ns}	4720.7^{ns}	493.7^{ns}	0.003^{**}
Populations	10	0.004^{**}	44.77^{*}	2210.36^{**}	14.46^{*}	5.65^{ns}	0.32^{*}	0.09^{ns}	1.61^{*}	0.65^{**}	5141.75^{**}	583.8^{**}	0.001^{**}
Error	20	0.001	19.19	378.42	5.1	3.34	0.04	0.05	0.51	0.13	1521.31	168.26	2.02×10^{-4}

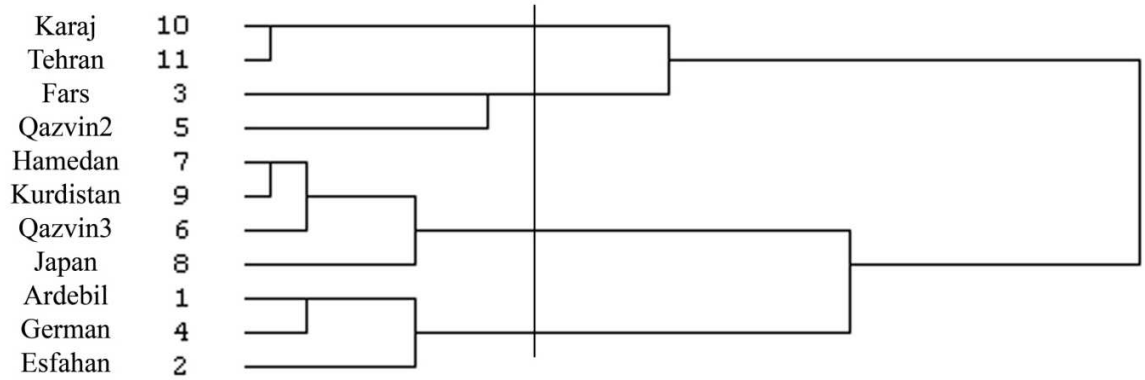
** And ** are statistically significant at 0.05 and 0.01 level, respectively, ns: not significant.*
LAI= Leaf Area Index

Although the cluster analysis of populations together with greater morphological similarity, the clusters did not necessarily include all the populations from the same origin. It was reported that no association between

morphological characters and geographical origin [19, 20]. Sari and Ceylan [21] also observed a large variability of essential oils and morphological characters in 11 different populations of lemon balm from different regions of Turkey and European countries. This may be due to open pollination of lemon balm.

Estimates of phenotypic diversity for individual traits and populations are shown in table 3. The diversity indices (DI) among populations varied in based on studied traits. Polymorphism was common in varying degrees for all characters, thus implying the existence of a wide range of variation in the materials.

For individual traits, the DI values ranged from 0.85 for essential oils (%) to 1.00 (highly polymorphic) for most traits such as plant height, number of tiller, chlorophyll index, stem diameter, number of nodes, width and length of leaf. On the population basis, the highest mean diversity (0.99) was obtained from Qazvin2 and the lowest one (0.96) is from German and Kurdistan populations. Agro-ecological zone identified as Qazvin2 displayed the highest diversity over all characters (DI = 0.96−1.00). All populations revealed the high total diversity index DI pooled over traits so that this index was between 0.96 to 1.00.



Dendrogram established from Euclidean squared for standard variables using Ward's method based on morphological traits and essential oil content between 11 populations of lemon balm (*Melissa officinalis* L.)

The diversity might indicate the presence of important genes for breeding purposes of aromatic rice in future. This finding reflected that morphological traits and essential oils percentage are considerably important characteristics for conservation and utilization in *Melissa officinalis* L. breeding programs.

Table 2. Diversity index (DI) of each phenotypic class for populations and overall collection

Trait	Population											total
	Kurdistan	Hamadan	Esfahan	Karaj	Ardabil	Qazvin3	Tehran	Japan	German	Fars	Qazvin2	
Essential oils (%)	0.85	0.98	0.95	0.98	0.96	0.95	0.96	0.94	0.96	0.94	0.96	0.96
Plant height	0.99	0.99	1.00	0.99	1.00	0.99	1.00	0.99	0.99	1.00	0.99	0.99
No. of tiller	0.91	1.00	0.92	0.93	0.95	0.93	0.92	0.97	0.93	0.95	0.97	0.95
Chlorophyll index	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
No. of nodes	0.99	1.00	1.00	1.00	0.99	0.99	1.00	1.00	1.00	1.00	1.00	1.00
Leaf width	0.99	1.00	1.00	0.99	0.99	0.99	0.98	0.99	0.98	1.00	0.99	0.99
Stem diameter	0.99	0.99	0.99	0.99	1.00	1.00	0.99	0.99	0.99	0.99	1.00	0.99
Leaf Area Index (LAI)	0.97	0.96	0.98	0.98	0.95	0.95	0.97	0.94	0.95	0.98	0.97	0.98
Leaf length	0.99	0.99	1.00	0.99	0.98	0.99	0.99	0.99	0.99	1.00	1.00	0.99
Fresh weight	0.94	0.94	0.91	0.93	0.99	0.93	0.94	0.97	0.91	0.95	0.98	0.96
Dry weight	0.94	0.95	0.92	0.94	0.98	0.93	0.95	0.97	0.91	0.94	0.98	0.96
Dry weight to fresh weight ratio	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total	0.96	0.98	0.97	0.98	0.98	0.97	0.97	0.98	0.96	0.98	0.99	

CONCLUSION

Up to now, phenotypic diversity in Iranian lemon balm populations has not been studied widely. The present study indicated that the amounts of variability were distributed in agro-ecological zones and highlighted the strong

difference among populations. Cultivar collection of this study as indicative source of diversity should take account of distribution of polymorphism. Priorities of germplasm collecting should focus on populations with relatively large variation, i.e. Japan, German, Karaj, with due consideration to the cause of genetic erosion and environmental degradations.

Morphological variation does not always reflect real genetic variation because of interaction between genotype and environment, and the large unknown genetic control of polygenic morphological and agronomic traits [22]. Beyene *et al*. [23] also identified that the morphological traits were relatively less efficient for precise discrimination of closely related accessions and analysis of their genetic relationships. Despite this limitation, morphological traits are useful for preliminary evaluation because it is fast, simple, and can be used as a general approach for assessing genetic diversity among morphologically distinguishable populations.

Because morphological variation alone does not reflect the total variation which is necessary for breeding new lemon balm genotypes from demands of production and customers, further comprehensive investigation including markers such as isozyme, protein, molecular markers and quantitative characters will probably provide a complete view about the genetic variation of lemon balm populations. In addition, amount of populations with larger agro-ecological distribution must be increased in order to estimate the association between different geographic distance and genetic distance to morphologic variables and others.

Iranian lemon balm landraces have a long history of use as tea and several applications in the traditional and industrial pharmacy. These plants maintain an important genetic conservation that can be used in plant breeding. As the widely distribution of landraces in area of 1,648,000 km^2 (about 60% of area is barren land) it would be logical to use molecular methods for the recognition of the lemon balm characters with a high number of samples and molecular methods. Furthermore, biochemical test from the genetic material would causes desirable selection for the economical and extensive culture.

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