Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 8, No. 5, p. 88-96, 2016
http://www.innspub.net

INNSPUB
RESEARCH PAPER
OPEN ACCESS

# Genetic diversity in *Melissa officinalis* accessions by leaf protein patterns

**Zahra Danaeipour[1], Mohammad Hossein Fotokian[1*], Daryush Talei[2]**

[1]*Department of Biotechnology, Faculty of Agriculture, Shahed University, Tehran, Iran*

[2]*Medicinal Plants Research Center, Shahed University, Tehran, Iran*

Article published on May 12, 2016

**Key words:** Genetic diversity, Medicinal plants, *Melissa officinalis*, Protein pattern, SDS-PAGE.

**Abstract**

Lemon balm is a medicinal plant belongs to the family Lamiaceae. Proteins are important biochemical parameters in genetic diversity in plants. The objective of the present study was to evaluate 20 accessions of *M. officinalis* in terms of leaf protein banding patterns. For this reason, the leaf protein was extracted using HEPES/KOH buffer and separated by SDS-PAGE technique. The results indicated a total of 22 bands ranged from 10 to 150 kDa which 59.09 percentages of them were polymorphic. The maximum number of the bands (20) was observed in Germany and Malard accessions, while N. Khorasan accession had the minimum number of bands (13). The cluster analysis of 20 accessions based on the protein data produced three clusters. The first cluster comprised of 15 accessions, the second cluster consisted of four accessions and the third cluster contains only Italy accession. Overall, the outcomes of the present study were indicated the presence of high genetic diversity among the *M. officinalis* accessions. Our findings suggest that the plants belong to different clusters can be used for improvement of *M. officinalis* through hybridization to generate useful recombinants in the segregating generations.

***Corresponding Author:** Mohammad Hossein Fotokian ✉ fotokian@shahed.ac.ir

## Introduction

The lemon balm (*Melissa officinalis* L.) is one of the most important medicinal herbs in the family Lamiaceae. This species includes three subspecies: *M. officinalis* subsp. *Altissima, M. officinalis* subsp, *Inodora* and *M. officinalis* subsp, *officinalis*, however, only subsp *officinalis* has commercial and medicinal value (Marongiu *et al.,* 2004). This plant mainly grown in the Mediterranean region such as Turkey, Southern Europe and Northern Africa, and Iran (Bağdat and Coşge, 2012). In Iran, the plant has a broad distribution in Northern, Northeast (Golestan forest, Mazandaran, Haraz Valley), West region of Kermanshah and Rijab, Tehran (Tochal altitudes) and between Qazvin and Karaj (Zargari, 1991).

The essential oil of *M. officinalis* L. contains three main compounds namely protocatechuic acid, caffeic acid and rosmarinic acid (Kim *et al.*, 2010). Among these phytochemicals, rosmarinic acid possesses the highest bioactivity in treating the hardly curable diseases. The herb exhibited a wide scope of pharmaceutical properties such as anti-oxidant, anti-allergic, sedative, carminative, anti-spasmodic, anti-microbial, anti-fungus, anti-inflammatory, hypolipidemic, and anti-oxidant (Kim *et al.*, 2010; Stefanovic and Comic, 2012). The total content of oil in *M. officinalis* is about 0.02-0.3% per dry weight, which is relatively low as compared with other members of the Lamiaceae (Sanchez-Medina *et al.*, 2007). For this reason, the production cost of essential oil is too high in the market (Moradkhani *et al.*, 2010).

The success of any breeding or genetic conservation program is dependent on an understanding of the amount and distribution of the genetic diversity available in the gene pool. Traditionally, a combination of morphological and agronomic traits has been used to measure genetic diversity. Genetic diversity is estimated using different methods such as morphological traits, protein and DNA markers (Chahal and Gosal, 2002). Morphological traits have a number of limitations, including low polymorphism, low heritability, late expression, and may be controlled by epistasis and pleiotropic gene effects (Nakamura, 2001), While molecular markers such as protein markers reflect the genotype more directly, independent of environmental influences (Brown and Weir, 1983). Protein markers are useful tools to identify cultivar, registration of new varieties and classification of crop species to study genetic diversity, thereby improving the efficiency of plant breeding programs (Gianibelli *et al.*, 2001). Among biochemical techniques, Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is commonly used in the studies of plants genetic diversity due to its simplicity, minimum cost in time and labor, and effectiveness. (Sadia *et al.*, 2009; Masoumi *et al.*, 2012; Radwan *et al.*, 2013; Abou-Ellali *et al.*, 2014).

With respect to genetic diversity, several polymorphic proteins have been reported in the genus of *Mentha* (Hassan *et al.*, 2003), *Ocimum* species (Mustafa *et al.*, 2006), legume (Boulter *et al.*, 1967), wheat (El-Bakatoushi, 2010) and brassicaceae (Khurshid and Rabbani, 2012). An enormous lack of information related to the genetic diversity of *M. officinalis* is tangible. Therefore, the present study emphasized on genetic diversity of *M. officinalis* accessions mostly from different parts of Iran using protein analysis, which might increase efficiency of conservation of germplasm in order to utilize in genetics and breeding programs.

## Material and methods

*Plant material*

A total of 20 accessions of *M. officinalis* were collected from different country (England, Germany, Iran, Italy and Japan) (Table 1). The seeds were germinated into the growth chamber at 28°C and after 48 h, the germinated seeds were then transferred into the soil media in the greenhouse conditions with temperature of 25°C and irrigation every 48 h at Agricultural college, Shahed University, Tehran. The leaves were harvested from the seedling to extract protein.

*Leaf protein extraction*

One gram of bulk fresh leaf from each accession was ground in liquid nitrogen using pre-chilled mortar and pestle to obtain a fine powder. The powder was homogenized with 2.5 mL HEPES/KOH buffers according to the method of Talei *et al.* (2013).

Each sample was vortexed for 5 min, then was centrifuged at 20000 rpm for 20 min at 4°C using centrifuge (Model Sigma 3-30K) for 2-3 times. Next, chloroform (¼ volume supernatants) was added to each sample and kept at room temperature for 5 min. Finally, the sample was centrifuged for 15 min at 16,000 rpm at 4°C. The supernatants were collected and stored at -20°C. The total protein concentration was determined by the Bradford method (1976) at 595 nm, using a spectrophotometer (Lambda 25, UV/VS) (Talei *et al.*, 2013).

*Protein separation*

One dimensional SDS-PAGE was performed according to the method of Laemmli (1970). Twenty µg of protein from each sample were solubilised with 4x SDS/sample buffer [0.125 M Tris base (pH 6.8), 80% (w/v) glycerol, 10% (w/v) SDS, 4% (v/v) 2-mercaptoethanol and 0.05% (w/v) bromophenol blue] and heated at 100°C for 3 min. The protein samples were run on 12% separating gel at 90 V for 90 min using a Bio-Rad, Mini Protein Tetra Cell electrophoresis system. The electrophoresis gels were stained with Coomassie Blue [0.25% Coomassie Brilliant Blue G-250, 40% (v/v) methanol and 7% (v/v) acetic acid] for 1 h and distained with 40% (v/v) methanol and 7% (v/v) acetic acid. Scanning the gels was done using a Gel Documentation (Bio-Rad, XR-S Plus).

*Statistical analysis*

The SPSS software version 22 was used for analysis of variance and mean comparison test. The NTSYS-PC version 2.1 software was used to calculate the Jaccard coefficient and cophenetic correlation coefficient (Rohlf, 1998). The cluster analysis was performed based on protein bands using weighted pair group method arithmetic averages (WPGMA) (or complete linkage method) of DARwin 5 software and PCoA (Perrier X, 2006).

**Results**

The analysis of variance indicated that there were statistically significant differences among the accessions in terms of protein content ($P \leq 0.01$) (Table 2). The highest protein content (0.59 $mg.g^{-1}$) and the lowest protein content (0.24 $mg.g^{-1}$) were observed in Karaj-1 and England accessions, respectively (Table 3).

The SDS-PAGE results showed a total of 22 bands with 59.09 percentage polymorphism in the 20 *M. officinalis* accessions.

**Table 1.** Geographical origins and code number of lemon balm.

| Code | Collection region | Code | Collection region | Code | Collection region | Code | Collection region |
|---|---|---|---|---|---|---|---|
| 1 | Alborz -Karaj | 6 | Gilan -Damash | 11 | Gilan- Roodbar | 16 | Kordestan |
| 2 | Esfahan- Pakan Bazr | 7 | Alborz- Karaj- Shahrak | 12 | Germany | 17 | North Khorasan |
| 3 | Qazvin-Hir | 8 | Qazvin-Herif | 13 | Japan | 18 | Esfahan- Najafabad |
| 4 | Alborz -Malard | 9 | Hamedan A | 14 | England | 19 | Hamedan B |
| 5 | Shiraz | 10 | Esfahan- Esfahan | 15 | Italy | 20 | Ardabil |

The maximum number of the bands (20) was observed in Germany and Malard accessions and the minimum number of bands (13) observed in N. Khorasan (Fig. 1). The protein profiles had different molecular weight ranged from 10 to 150 kDa with 0.16 averages Polymorphic Information Content (PIC). The most of PIC was distinguished in three area included 15 kDa (PIC=0.33), 32-40 kDa (PIC=0.34) and 50-100 kDa (PIC=0.33).

Jaccard's similarity coefficients were computed for all possible pairs of electrophoregrams among the 20

accessions (Table 4). The highest genetic similarity was observed between Germany and Malard accessions with a value of 1.0 and the lowest one's obtained between Italy and N. Khorasan accessions with a value of 0.5.

The WPGMA cluster analysis of 20 *M. officinalis* accessions based on the protein profiles with the highest cophenetic correlation coefficient (r= 0.78) produced three clusters (Fig. 2). The first cluster (red cluster) involved 14 accessions. Interestingly, Germany, Japan and some Iranian accessions with high geographical distance were happened in the first cluster. The second cluster (green cluster) contained 5 accessions (Shiraz, Damash, Hamedan-1, Esfahan and England), and Italy accession alone was assigned in the third cluster (blue cluster) (Fig. 2). The lowest distance (0.00) was happened between Malard and Germany accessions, while the highest distance (6.49) was happened between Karaj-1 and Shiraz accessions. A principle coordinate's analysis (PCoA) of 20 *M. officinalis* accessions were done for establishing the relationship among the accessions (Fig. 3). The distribution pattern of accessions in PCoA was principally similar to the result of cluster analysis.

**Table 2.** Analysis of variance of Leaf protein content among lemon balm accessions.

| Source of Variation | df | Mean of square |
|---|---|---|
| Between accessions | 19 | 0.028** |
| Within accessions | 40 | 0.008 |
| Total | 59 | |

** Significant at p < 0.01. df, degree of freedom.

**Table 3.** Leaf protein content and number of bands in *M. officinalis* accessions (Means ± SEM).

| Accession No. | protein content (mg.g$^{-1}$) | No. of Bands | Accession No. | protein content (mg.g$^{-1}$) | No. of Bands |
|---|---|---|---|---|---|
| 1 | 0.45±0.002$^{abcd}$ | 19 | 11 | 0.37±0.019$^{cdef}$ | 17 |
| 2 | 0.33±0.009$^{def}$ | 19 | 12 | 0.52±0.020$^{abc}$ | 20 |
| 3 | 0.54±0.004$^{ab}$ | 19 | 13 | 0.26±0.016$^{f}$ | 18 |
| 4 | 0.47±0.111$^{abcd}$ | 20 | 14 | 0.24±0.004$^{f}$ | 15 |
| 5 | 0.47±0.049$^{abcd}$ | 16 | 15 | 0.46±0.057$^{abcd}$ | 14 |
| 6 | 0.37±0.116$^{cdef}$ | 17 | 16 | 0.42±0.018$^{abcde}$ | 16 |
| 7 | 0.59±0.004$^{a}$ | 19 | 17 | 0.50±0.074$^{abcd}$ | 13 |
| 8 | 0.51±0.015$^{abc}$ | 17 | 18 | 0.49±0.005$^{abcd}$ | 16 |
| 9 | 0.47±0.085$^{abcd}$ | 17 | 19 | 0.27±0.018$^{ef}$ | 19 |
| 10 | 0.39±0.030$^{bcdef}$ | 17 | 20 | 0.47±0.066$^{abcd}$ | 17 |

The eigenvectors and eigenvalues of the PCoA obtained from data of the total proteins revealed that the accessions were separated on the five principal factors and influencing 53.7 percentages of the variation accumulated up to the first two factors.

## Discussion

*M. officinalis* is an important medicinal plant and characterization of genetic diversity in plants is susceptible to ontogeny and environmental condition. In the present study, protein markers were used to distinguish genetic diversity among 20 *M. officinalis* accessions.

The gel analysis resulted in detection of 22 protein bands with 59.09 percentage polymorphism. The results indicated that the main bands (common proteins) were same in all accessions and the fundamental differences were for minor bands.

On the basis of Jaccard's similarity coefficients results, the highest genetic distance was between Italy

and N. Khorasan accessions. The results matched up well with the findings of Radwan *et al.* (2013) that show crosses between accessions with high polymorphism could create more genetic diversity. Genetic diversity among the accessions was independent in terms of the collecting region. Some accessions with near geographically relationships were really unlike in their leaf protein bands, while some distant geographically accessions were too similar in their leaf proteins.

**Table 4.** Jaccard similarity coefficients between twenty accessions of *M. officinalis*.

| Accession No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.00 | | | | | | | | | | | | | | | | | | | |
| 2 | 0.81 | 1.00 | | | | | | | | | | | | | | | | | | |
| 3 | 0.90 | 0.90 | 1.00 | | | | | | | | | | | | | | | | | |
| 4 | 0.95 | 0.86 | 0.95 | 1.00 | | | | | | | | | | | | | | | | |
| 5 | 0.84 | 0.67 | 0.75 | 0.80 | 1.00 | | | | | | | | | | | | | | | |
| 6 | 0.89 | 0.71 | 0.80 | 0.85 | 0.94 | 1.00 | | | | | | | | | | | | | | |
| 7 | 0.81 | 0.90 | 0.81 | 0.86 | 0.67 | 0.71 | 1.00 | | | | | | | | | | | | | |
| 8 | 0.89 | 0.80 | 0.89 | 0.85 | 0.83 | 0.79 | 0.71 | 1.00 | | | | | | | | | | | | |
| 9 | 0.80 | 0.80 | 0.89 | 0.85 | 0.74 | 0.79 | 0.71 | 0.79 | 1.00 | | | | | | | | | | | |
| 10 | 0.80 | 0.80 | 0.80 | 0.85 | 0.74 | 0.79 | 0.80 | 0.70 | 0.89 | 1.00 | | | | | | | | | | |
| 11 | 0.89 | 0.80 | 0.89 | 0.85 | 0.74 | 0.79 | 0.71 | 0.89 | 0.89 | 0.79 | 1.00 | | | | | | | | | |
| 12 | 0.95 | 0.86 | 0.95 | 1.00 | 0.80 | 0.85 | 0.86 | 0.85 | 0.85 | 0.85 | 0.85 | 1.00 | | | | | | | | |
| 13 | 0.85 | 0.85 | 0.85 | 0.90 | 0.79 | 0.84 | 0.85 | 0.75 | 0.75 | 0.84 | 0.75 | 0.90 | 1.00 | | | | | | | |
| 14 | 0.70 | 0.70 | 0.70 | 0.75 | 0.82 | 0.78 | 0.70 | 0.68 | 0.78 | 0.88 | 0.68 | 0.75 | 0.83 | 1.00 | | | | | | |
| 15 | 0.65 | 0.65 | 0.65 | 0.62 | 0.76 | 0.72 | 0.57 | 0.72 | 0.63 | 0.55 | 0.63 | 0.62 | 0.60 | 0.61 | 1.00 | | | | | |
| 16 | 0.68 | 0.68 | 0.68 | 0.65 | 0.53 | 0.58 | 0.68 | 0.67 | 0.67 | 0.67 | 0.76 | 0.65 | 0.63 | 0.56 | 0.50 | 1.00 | | | | |
| 17 | 0.84 | 0.75 | 0.84 | 0.80 | 0.68 | 0.74 | 0.75 | 0.83 | 0.74 | 0.65 | 0.83 | 0.80 | 0.70 | 0.55 | 0.67 | 0.81 | 1.00 | | | |
| 18 | 0.90 | 0.81 | 0.90 | 0.86 | 0.75 | 0.80 | 0.73 | 0.89 | 0.80 | 0.71 | 0.89 | 0.86 | 0.76 | 0.62 | 0.65 | 0.68 | 0.84 | 1.00 | | |
| 19 | 0.71 | 0.80 | 0.71 | 0.68 | 0.57 | 0.62 | 0.80 | 0.70 | 0.62 | 0.62 | 0.70 | 0.68 | 0.67 | 0.52 | 0.63 | 0.76 | 0.83 | 0.80 | 1.00 | |
| 20 | 0.84 | 0.84 | 0.84 | 0.80 | 0.68 | 0.74 | 0.84 | 0.83 | 0.74 | 0.74 | 0.83 | 0.80 | 0.79 | 0.63 | 0.58 | 0.81 | 0.88 | 0.84 | 0.83 | 1.00 |

The WPGMA cluster analysis of 20 *M. officinalis* accessions based on the protein profiles generated three clusters. The results showed that some Iranian accessions belonged to the first cluster were geographically close intervals, while Germany and Japan accessions with high geographical distance were geographically more distant from each other. The sources of the Pakan Bazr Company may be being collected on the close or the same original population of accessions segregation in this group. Accessions of the second groups were collected from different region far apart from each other, which may be diverging from the same original population. These accessions could be mixed from different genotypes, or random mating populations with the same alleles but differing allele frequencies and probability due to open pollination of *M. officinalis*. Our result up well with the finding of Aharizad *et al.* (2012), who reported that the morphological and essential oil content data of different *M. officinalis* accessions with high geographical distances were placed in the same group (Aharizad *et al.*, 2012). In addition, Ghaffariyan *et al.* (2011) in a study on genetic diversity of twelve ecotypes of lemon balm using IRAP markers, based on long terminal repeats (LTRs) of barley retrotransposons showed that the average PIC and average marker index between ecotypes were 0.27 and 14.36, respectively. Molecular analysis of variance indicated that the variance of within population is greater than the variance between the

populations, and finally, the populations were grouped into three clusters. Mustafa *et al.* (2006) reported that the cluster analysis based on morphological trait, isozyme and seed protein data demonstrated genetic diversity among and within population of two species of *Ocimum* that may be due to environmental modification and natural hybridization. SDS-PAGE analysis of eleven samples of leaf protein in *Ocimum* showed that the method was adequate to determine genetic diversity (Bompalli and Nallabilli, 2013).

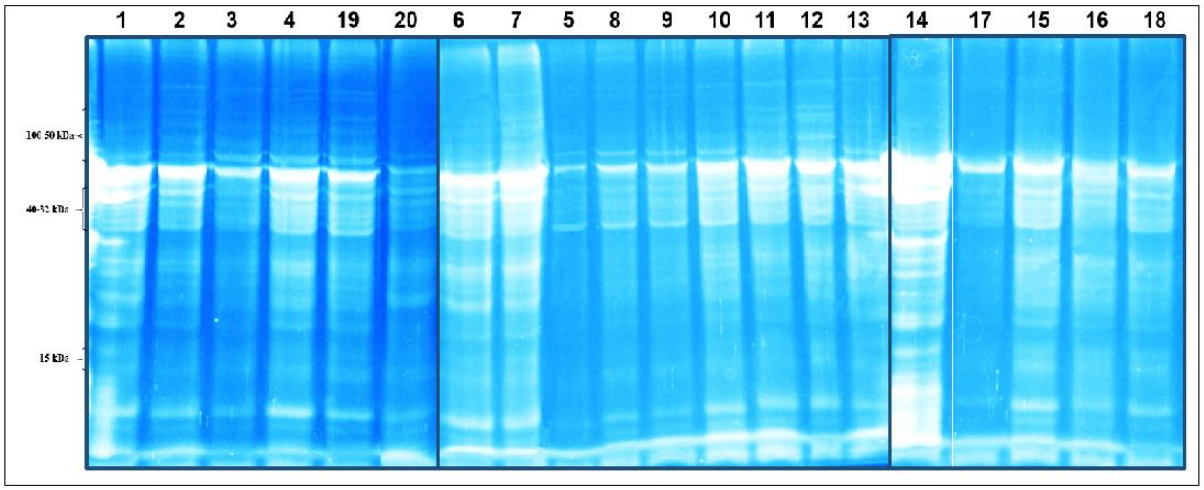


**Fig. 1.** Protein profile of 21 accessions of *M. officinalis*. The accessions were; 7: Karaj-shahrak, 5: Shiraz, 4: Malard, 3: Hir, 2: Pakanbazr, 11: Roodbar and 12: Germany.

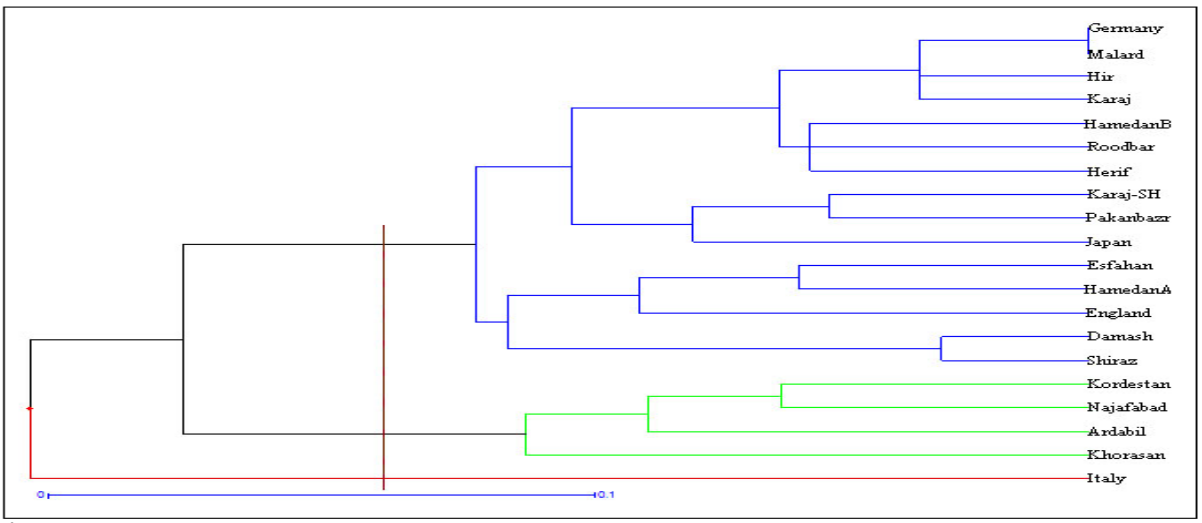


**Fig. 2.** Dendrogram generated by WPGMA clustering method based on the genetic relationships among 20 accessions of *M. officinalis*.

Protein diversity is not random, since they associated with the genome expression and supply additional opportunities for polymorphism that noticed the presence of important genes for breeding purposes. Improvement in plant breeding program through selection is possible, especially if we extend the genetic basis from various habitats to comprise most of the genetic determinants of a desirable trait, such as; yield, a biotic stress tolerance, and quality (Ghafoor and Arshad, 2008). Overall, the results of the present study indicated a reasonable genetic variation in the leaf protein pattern of *M. officinalis* accessions.

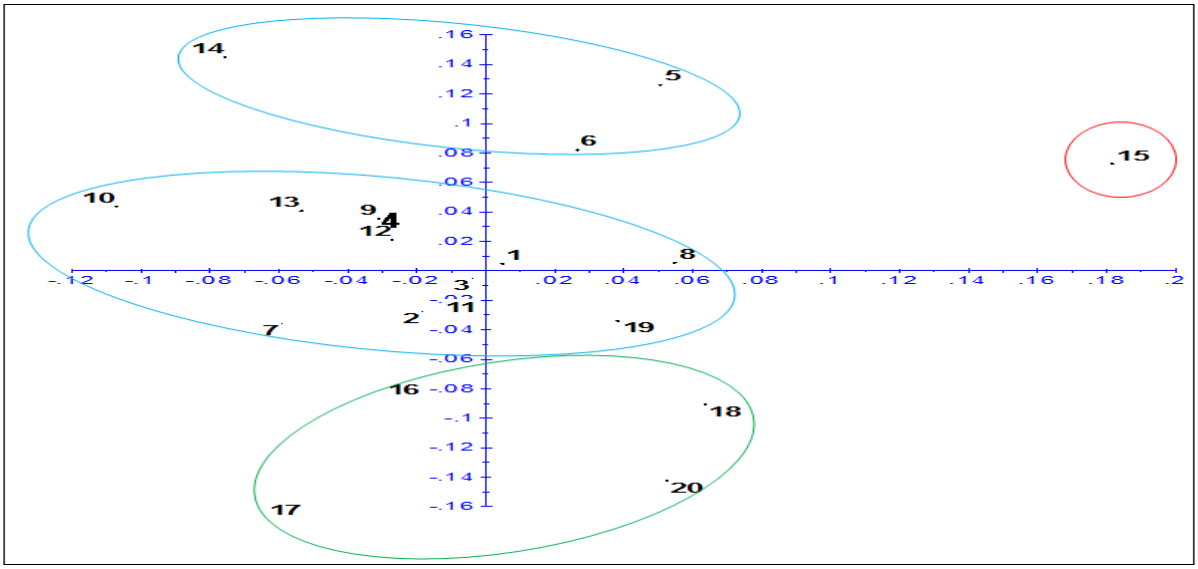


**Fig. 3.** Plot of *M. officinalis* accessions by principal coordinate analysis based on factors 1/2. The accessions were; 1: Karaj, 2: Pakan bazaar, 3: Hir, 4: Malard, 5: Shiraz, 6: Damash, 7: Karaj-shahrak, 8: Herif, 9: Hamedan A, 10: Esfahan, 11: Roodbar, 12: Germany, 13: Japan, 14: England, 15: Italy, 16: Kordestan, 17: Khorasan, 18: Najafabad, 19: HamedanB, and 20: Ardabil.

This variation might be was depended to environmental conditions such as geographically regions, season of culturing, altitude, annual rainfall, temperature, land fertility and genotype variation (Vollmann *et al.*, 2000). Consequently, it is evident that the protein variation among the *M. officinalis* populations can be seriously taken into account in molecular studies of this herb, henceforth. Furthermore, the electrophoresis of leaf proteins can be utilized as an effective strategy in the programs involved with *M. officinalis* conservation.

The differences observed among the accessions would be of immediate importance for development of the *M. officinalis* gene bank and may be used in hybridization and breeding programs to identify diverse parental combinations and creating segregating progeny with high genetic diversity.

**Reference**

**Abou-Ellali M, Hattem MES, Mohamad AM, Usama LA, Hasan AG, Essa EA.** 2014. Appraisal of biochemical and genetic diversity of *Mango* cultivars using molecular makers. African journal of biotechnology **13,** 2796-2806.

**Aharizad S, Rahimi MH, Moghadam M, Mohebalipour N.** 2012. Study of genetic diversity in lemon balm (*Melissa officinalis* L.) populations based on morphological traits and essential oils content. Annals of Biological Research **3,** 5748-5753.

**Bagdat RB, Cosge B.** 2012. The essential oil of lemon balm (*Melissa officinalis* L.), its components and using fields. Anadolu Journal of Agricultural Sciences **21,** 116-121.

**Bompalli L, Nallabilli.** 2013. Genetic diversity of *Ocimum* species through biochemical technique and UPGMA cluster analysis. International jornal of pharmaceutical sciences. Sci **5,** 155-159.

**Boulter D, Thurman D, Derbyshire E.** 1967. A disc electrophoretic study of globulin proteins of legume seeds with reference to their systematics. New Phytologist **66,** 27-36.

**Bradford MM.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry **72,** 248-254.

**Brown AH, Weir BS.** 1983. Measuring genetic variability in plant populations. Isozymes in plant genetics and breeding, part A., 219-239.

**Chahal G, Gosal S.** 2002. Principles and procedures of plant breeding: Biotechnological and conventional approaches. Alpha Science Int'l Ltd.

**El-Bakatoushi R.** 2010. Genetic diversity of winter wheat (*Triticum aestivum* L.) growing near a high voltage transmission line. Romanian Journal of Biology - Plant Biology **55,** 71-87.

**Ghaffariyan S, Mohammadi S, AhariZad S.** 2011. Patterns of population diversity in Lemon balm (*Melissa officinalis* L.) as revealed by IRAP markers. Journal of Plant Physiology and Breeding **2,** 39-51.

**Ghafoor A, Arshad M.** 2008. Multivariate analyses for quantitative traits to determine genetic diversity of blackgram *Vigna mungo* (L.) Hepper germplasm. Pakistan Journal of Botany **40,** 2307-2313.

**Gianibelli M, Gupta R, Lafiandra D, Margiotta B, MacRitchie F.** 2001. Polymorphism of high Mr glutenin subunits in *Triticum tauschii*: Characterisation by chromatography and electrophoretic methods. Journal of Cereal Science **33,** 39-52.

**Hassan M, Badr A, Mustafa A-Z, El-Galaly M, Mobarak A.** 2003. Genetic diversity among Mentha population in Egypt as reflected by morphological and protein electrophoretic variation. Journal of Agricultural and Food Chemistry **1,** 269-286.

**Khurshid H, Rabbani MA.** 2012. Comparison of electrophoretic protein profiles from seed of different oilseed *Brassica* cultivars. Practice **4,** 6.

**Kim S, Yun EJ, Bak JS, Lee H, Lee SJ, Kim CT, Lee J-H, Kim KH.** 2010. Response surface optimised extraction and chromatographic purification of rosmarinic acid from (*Melissa officinalis*) leaves. Food chemistry **121,** 521-526.

**Laemmli UK.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227,** 680-685.

**Marongiu B, Porcedda S, Piras A, Rosa A, Deiana M, Dessì MA.** 2004. Antioxidant activity of supercritical extract of *Melissa officinalis* subsp. *officinalis* and *Melissa officinalis* subsp. *inodora*. Phytotherapy Research **18,** 789-792.

**Masoumi SM, Kahrizi D, Rostami-Ahmadvandi H, Soorni J, Kiani S, Mostafaie A, Yari K.** 2012. Genetic diversity study of some medicinal plant accessions belong to Apiaceae family based on seed storage proteins patterns. Molecular biology reports **39,** 10361-10365.

**Moradkhani H, Sargsyan E, Bibak H, Naseri B, Sadat-Hosseini M, Fayazi-Barjin A, Meftahizade H.** 2010. *Melissa officinalis* L., a valuable medicine plant: A review. Journal of Medicinal Plants Research **4,** 2753-2759.

**Mustafa A, Badr A, El-Galaly MA, Mobarak AA, Hassan MG.** 2006. Genetic diversity among *Ocimum* population in Egypt as reflected by morphological, seed proteins and isozyme polymorphism. International Journal of Botany **2,** 261-269.

**Nakamura H.** 2001. Genetic diversity of high-molecular-weight glutenin subunit compositions in landraces of hexaploid wheat from Japan. Euphytica **120,** 227-234.

**Perrier X, Jacquemoud-Collet JP.** 2006. DARwin software: Dissimilarity Analysis and Representation for Windows. http://darwin.cirad.fr/darwin.

**Radwan SA, El-Koly AS, Sammour RH.** 2013. Genetic variation among accessions of *Lathyrus*

*inconspicuous* (L.) as revealed by SDS Polyacrylamide Gel Electrophoresis. Acta agriculturae Slovenica **101,** 21-30.

**Rohlf F.** 1998. NTSYS-pc version 2.0. Numerical taxonomy and multivariate analysis system. Exeter software, Setauket, New York.

**Sadia M, Malik SA, Rabbani MA, Pearce S.** 2009. Electrophoretic characterization and the relationship between some *Brassica* species. electronic Journal of Biology **5,** 14.

**Sanchez-Medina A, Etheridge CJ, Hawkes GE, Hylands PJ, Pendry BA, Hughes MJ, Corcoran O.** 2007. Comparison of rosmarinic acid content in commercial tinctures produced from fresh and dried lemon balm (*Melissa officinalis*). Journal of Pharmacy & Pharmaceutical Sciences **10,** 455-463.

**Stefanovic O, Comic L.** 2012. Synergistic antibacterial interaction between *Melissa officinalis* extracts and antibiotics. Journal of Applied Pharmaceutical Science **2,** 1-5.

**Talei D, Valdiani A, Puad MA.** 2013. An effective protein extraction method for two-dimensional electrophoresis in the anticancer herb *Andrographis paniculata* Nees. Biotechnology and applied biochemistry **60,** 521-526.

**Vollmann J, Fritz CN, Wagentristl H, Ruckenbauer P.** 2000. Environmental and genetic variation of soybean seed protein content under Central European growing conditions. Journal of the Science of Food and Agriculture **80**, 1300-1306.

**Zargari A.** 1991. Medicinal plants. Tehran University Publications, Tehran.