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STUDY ON GENETIC DIVERSITY BETWEEN *Malva* L. (Malvaceae): A HIGH VALUE MEDICINAL PLANT USING SCOT MOLECULAR MARKERS

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Li H., H. Yu, X. Zeng, S. Hussein Hamarashid (2021). *Study on genetic diversity between Malva L. (Malvaceae): a high value medicinal plant using SCoT molecular markers.*- Genetika, Vol 53, No.2, 895-910.
The *Malva* L. genus, popularly known as mallow, grows spontaneously in almost all of Europe and the Mediterranean region. The *Malva* genus has 25-40 species and it can be considered as an annual and/or biannual herb. This genus contains species that reveal therapeutic properties and are mostly important in medicine and the functional food industry. Therefore, due to the importance of these plant species, we performed a combination of morphological and molecular data for this species. For this study, we used 97 randomly collected plants from seven species in 7 provinces. According to the SCoT markers analysis, *Malva sylvestris* and *Malva aegyptia* had the lowest similarity and the species of *Malva neglecta* and *Malva vericillata* had the highest similarity. The aims of present study are: 1) can SCoT markers identify *Malva* species, 2) what is the genetic structure of these taxa in Iran, and 3) to investigate the species inter-relationship? The present study revealed that combination of morphological and SCoT data can identify the species.
Keyword: Morphology, *Malva;* Medicinal Plants; Species identification, SCoT (Start Codon Targeted)

INTRODUCTION

Exploration of the genetic resources would be a great interest for further uses in plant improvement programs. As the next step, plant conservation is urgently needed as they are going

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to extinct and many others are threatened and endangered. It is undeniable that a disastrous loss of plant genetic diversity is occurring and many species, genes and alleles are being lost and seems to become even worse in future. Species identification and delimitation are fundamentally important within the fields of biology, biogeography, ecology, and conservation. Therefore, species delimitation is a subject of extensive part of studies in the framework of biology (COLLARD & MACKILL 2009, WU *et al.* 2013).

Malvaceae ('the mallows') is a botanical family with a rich diversity of species for textile, medicinal, and ornamental purposes. It consists of 2300 species and about 200 genera and mallows present a cosmopolitan distribution, but with a high number of species in the tropics. Given its high diversity, its primary genetic center of origin is still discussed, and many species have become generalized over world (RAY, 1995).

The *Malva* L. genus, popularly known as mallow, grows spontaneously in almost all of Europe and the Mediterranean region. The *Malva* genus has 25-40 species and it can be considered as an annual and/or biannual herb. Flowers with an epicalyx and 8-15 reticulated mericarps are the typical one (FRYXELL, 1988). RAY (1995) and ESCOBAR *et al.* (2009) relate their similarity to the *Lavatera* L. genus, where the bractoles of the epicalice are joined at the base, in contrast to in *Malva* where they are totally separated. Although there is inconsistency for some species in relation to the fusion of bracts and other characteristics. Molecular studies have also shown that the separation of these two genera based on this morphology is artificial and unsatisfactory (ESCOBAR *et al*., 2009).

Malva species are indicated with potential therapeutic as cicatrizing and analgesic by the Ministry of Health. However, within the genus, some species (e.g., *M. parviflora, M. pusilla, M. nicaeensis,* and *M. neglecta*) are regularly misidentified based on morphological features (DELLAGRECA, *et al*., 2009). Moreover, the phylogenetic relationships and taxonomic organization of the *Malva* genus are still unclear. Therefore, molecular analysis can be useful in species identification and study of the genetic relationships between *Malva* species/subspecies/varieties (ESCOBAR *et al*., 2009). Several studies have been conducted to clarify the taxonomic affiliation of *Malva* species using different features, such as molecular data (nuclear ribosomal DNA (rDNA), internal transcribed spacer (ITS) region, intron–exon splice junction (ISJ), and inter simple sequence repeat polymerase chain reaction (ISSR) markers) (CELKA, *et al*.,2010), differentiation of seed and seed coat structure (EL NAGGAR, 2001), morphology of pollen grains (EL NAGGAR, 2004), epidermal structures and stem hairs (AKÇIN, and ÖZBUCAK, 2006), and plant morphological traits (MICHAEL *et al.,* 2009).

The variability in mallow species is due, at least in part, to hybridization. Natural crossings between *M. pusilla* Sm. and *M. neglecta*, *M. alcea* L., and *M. moschata* L. as well as *M. sylvestris* and *M. neglecta* were found in Europe. RAY (1995) stated that hybridization or polyploidy is probably a factor in the evolution of these species, but this aspect has not been investigated so far. The taxonomy and systematics of the *Malva* genus are still unclear and very complicated. Taxonomic doubts have appeared because of the high level of homoplasty in morphological traits that are usually used as diagnostic features (ESCOBAR *et al*., 2009). Based on the flower structure, DALBY (1968) divided the *Malva* genus into two sections: *Bismalva* (with *M. alcea, M. excisa Rchb*., and *M. moschata*) and *Malva* (*M. neglecta, M. pusilla, M. sylvestris,* and *M. verticillata*) A different classification based on ITS molecular markers as well as fruit

morphology and seed structure was reported by RAY (1995), and two groups were distinguished: malvoid and lavateroid. A similar division was proposed by ESCOBAR *et al*. (2009) based on five ITS molecular markers (matK plus trnK, ndhF, trnL-trnF, and psbA-trnH). These genetic relationships and the classification of Malva species were also confirmed by CELKA *et al*. (2010) and LO BIANCO *et al*. (2017) based on ITS and ISSR molecular markers along with seed image analysis. Most of the *Malva* species are polyploids with the base chromosome number n = x = 7 (3,43). Numerous species are hexaploids, where the chromosome number is in the range of 40 to 44, and a few species possess higher numbers of chromosomes.

With the progress in plant molecular biology, numerous molecular marker techniques have been developed and used widely in evaluating genetic diversity, population structure and phylogenetic relationships. In recent years, advances in genomic tools provide a wide range of new marker techniques such as, functional and gene targeted markers as well as develop many novel DNA based marker systems (ESFANDANI-BOZCHALOYI *et al.* 2017 a, 2017b, 2017c, 2017d). Start codon targeted (SCoT) polymorphism is one of the novel, simple and reliable gene-targeted marker systems. This molecular marker offers a simple DNA-based marker alternative and reproducible technique which is based on the short conserved region in the plant genes surrounding the ATG (COLLARD & MACKILL 2009) translation start codon (COLLARD & MACKILL 2009). This technique involves a polymerase chain reaction (PCR) based DNA marker with many advantages such as low-cost, high polymorphism and extensive genetic information (COLLARD & MACKILL 2009, WU *et al.* 2013).

The present investigation has been carried out to evaluate the genetic diversity and relationships among different *Malva* species using new gene-targeted molecular markers, i.e. SCoT. This is the first study on the use of SCoT markers in *Malva* genus; Therefore, we performed molecular study of 97 collected specimens of 7 *Malva* species. We try to answer the following questions: 1) Is there infra and interspecific genetic diversity among studied species? 2) Is genetic distance among these species correlated with their geographical distance? 3) What is the genetic structure of populations and taxa? 4) Is there any gene exchange between *Malva* species in Iran?

MATERIALS AND METHODS

Plant materials

A total of 97 individuals were sampled representing 7 geographical populations belong 7 *Malva species* in East Azerbaijan, Mazandaran, Esfahan, Kerman, Hormozgan,and Khuzestan Provinces of Iran during July-Agust 2018-2019 (Table 1). For SCoT analysis we used 97 plant accessions (11 to 18 samples from each populations) belonging to 7 different populations with different eco-geographic characteristics were sampled and stored in -20 till further use. More information about geographical distribution of accessions are in Table 1 and Fig. 1.

Morphological studies

In total 38 morphological (10 qualitative, 28 quantitative) characters were studied. Data obtained were standardized (Mean= 0, variance = 1) and used to estimate Euclidean distance for clustering and ordination analyses (PODANI 2000). Morphological characters studied are: corolla

shape, bract shape, calyx shape, calyx length, calyx width, calyx apex, calyx margins, bract length, corolla length, corolla width, corolla apex, leaf length and leaf width, leaf apex, leaf margins, leaf shape, leaf gland and bract margins.

Table 1. List of the investigated taxa including origin of voucher specimens.

Taxa	Locality	Latitude	Longitude	Altitude(m)
Malva neglecta Wallr.	West Azerbaijan, Kaleybar	37° 07' 48 "	49° 54' 04"	165
Malva parviflora L.	Hormozgan, Bandar Abbas	38 ˚ 52' 93"	47 ˚25' 92	1133
Malva pusilla Sm.	Khuzestan, Behbahan	37° 07' 08"	49°54' 11"	159
Malva sylvestris L.	Esfahan:, Ghameshlou, Sanjab	38 ˚ 52' 93"	47 ˚25' 92	1133
Malva vericillata L.	Kerman, Hamun-e Jaz Murian	38˚52' 93"	47 ˚25' 92'	1139
Malva nicaeensis All.	Mazandaran, Haraz road, Emam Zad-e-Hashem	35 ˚50' 36"	51˚ 24' 28"	2383
Malva aegyptia L.	Golestan, Gorgan	35 ˚42'29"	52 ˚20'51"	2421

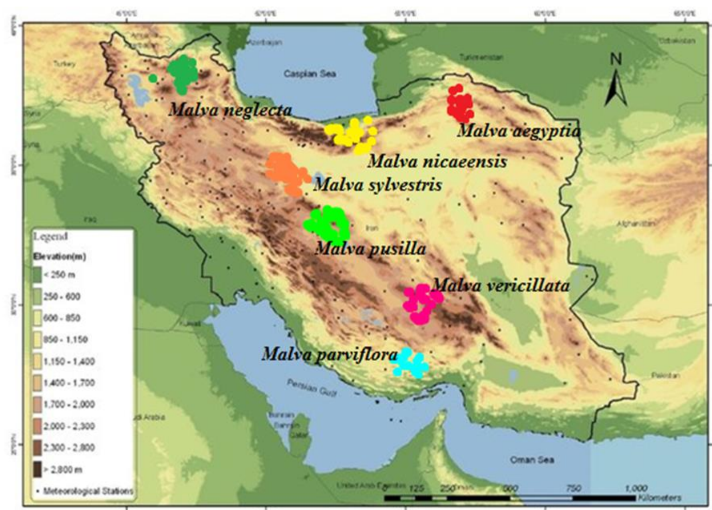

Fig. 1. Map of Iran shows the collection sites and provinces where *Malva* species were obtained for this study

DNA Extraction and SCoT Assay

Fresh leaves were used randomly from one to twelve plants in each of the studied populations. These were dried by silica gel powder. CTAB activated charcoal protocol was used to extract genomic DNA (ESFANDANI-BOZCHALOYI *et al.* 2019). The quality of extracted DNA

was examined by running on 0.8% agarose gel. A total of 22 SCoT primers developed by COLLARD & MACKILL (2009), 10 primers with clear, enlarged, and rich polymorphism bands were chosen (Table 2). PCR reactions were carried in a 25µl volume containing 10 mM Tris-HCl buffer at pH 8; 50 mM KCl; 1.5 mM $MgCl_2$; 0.2 mM of each dNTP (Bioron, Germany); 0.2 µM of a single primer; 20 ng genomic DNA and 3 U of *Taq* DNA polymerase (Bioron, Germany). The amplifications' reactions were performed in Techne thermocycler (Germany) with the following program: 5 min initial denaturation step 94°C, followed by 40 cycles of 1 min at 94°C; 1 min at 52-57°C and 2 min at 72°C. The reaction was completed by final extension step of 7-10 min at 72°C. The amplification products were observed by running on 1% agarose gel, followed by the ethidium bromide staining. The fragment size was estimated by using a 100 bp molecular size ladder (Fermentas, Germany).

Table 2. SCoT primers used for this study and the extent of polymorphism.

Primer name	Primer sequence (5'-3')	TNB	NPB	PPB	PIC	PI	EMR	MI
SCoT-1	CAACAATGGCTACCACCA	11	11	100.00%	0.47	7.32	14.55	5.18
SCoT-3	CAACAATGGCTACCACCG	11	9	85.79%	0.43	6.56	9.34	3.18
SCoT-6	CAACAATGGCTACCACGC	17	17	100.00%	0.62	3.88	8.56	6.44
SCoT-11	AAGCAATGGCTACCACCA	16	16	100.00%	0.39	5.23	10.23	3.65
SCoT-14	ACGACATGGCGACCACGC	22	22	100.00%	0.45	5.66	7.56	4.47
SCoT-15	ACGACATGGCGACCGCGA	14	14	100.00%	0.66	5.34	16.55	6.44
SCoT-16	CCATGGCTACCACCGGCC	24	24	100.00%	0.57	5.88	12.56	5.87
SCoT-17	CATGGCTACCACCGGCCC	20	20	100.00%	0.55	6.23	12.23	6.47
SCoT-18	ACCATGGCTACCACCGCG	8	8	100.00%	0.57	7.32	15.55	4.18
SCoT-19	GCAACAATGGCTACCACC	13	11	80.89%	0.53	6.56	10.34	6.18
Mean		15.6	14.5	98.58%	0.54	5.35	12.55	5.8
Total		156	145					

Note: TNB - the number of total bands, NPB: the number of polymorphic bands, PPB (%): the percentage of polymorphic bands, PI: polymorphism index, EMR, effective multiplex ratio; MI, marker index; PIC, polymorphism information content for each of CBDP primers

Data Analyses

Morphological Studies

Morphological characters were first standardized (Mean = 0, Variance = 1) and used to establish Euclidean distance among pairs of taxa (PODANI 2000). For grouping of the plant specimens, The UPGMA (Unweighted paired group using average) ordination methods were used (PODANI 2000). ANOVA (Analysis of variance) were performed to show morphological difference among the populations while, PCA (Principal components analysis) biplot was used to identify the most variable morphological characters among the studied populations (PODANI 2000). PAST version 2.17 (HAMMER *et al.* 2012) was used for multivariate statistical analyses of morphological data.

Molecular Analyses

SCoT bands obtained were coded as binary characters (presence = 1, absence = 0) and used for genetic diversity analysis. Discriminatory ability of the used primers was evaluated by means of two parameters, polymorphism information content (PIC) and marker index (MI) to characterize the capacity of each primer to detect polymorphic loci among the genotypes (POWELL *et al.* 1996). MI is calculated for each primer as MI = PIC × EMR, where EMR is the product of the number of polymorphic loci per primer (*n*) and the fraction of polymorphic fragments (β) (HEIKRUJAM *et al.* 2015). The number of polymorphic bands (NPB) and the effective multiplex ratio (EMR) were calculated for each primer. Parameter like Nei's gene diversity (H), Shannon information index (I), number of effective alleles, and percentage of polymorphism (P% =number of polymorphic loci/number of total loci) were determined (WEISING *et al*, 2005, FREELAND *et al.* 2011). Shannon's index was calculated by the formula: H' = -Σpiln pi. Rp is defined per primer as: Rp = ∑ Ib, were "Ib" is the band informativeness, that takes the values of 1-(2x [0.5-p]), being "p" the proportion of each genotype containing the band. The percentage of polymorphic loci, the mean loci by accession and by population, UHe, H' and PCA were calculated by GenAlEx 6.4 software (PEAKALL & SMOUSE 2006). Nei's genetic distance among populations was used for Neighbor Joining (NJ) clustering and Neighbor-Net networking (FREELAND *et al.* 2011, HUSON & BRYANT 2006). Mantel test checked the correlation between geographical and genetic distances of the studied populations (PODANI 2000). These analyses were done by PAST ver. 2.17 (HAMMER *et al.* 2012), DARwin ver. 5 (2012) software. AMOVA (Analysis of molecular variance) test (with 1000 permutations) as implemented in GenAlex 6.4 (PEAKALL & SMOUSE 2006) were used to show genetic difference of the populations. Gene flow was determined by (i) Calculating Nm an estimate of gene flow from Gst by PopGene ver. 1.32 (1997) as: Nm = 0.5(1 - Gst)/Gst. This approach considers the equal amount of gene flow among all populations.

RESULTS

Species identification and inter-relationship

Morphometry

ANOVA showed significant differences (P <0.01) in quantitative morphological characters among the species studied. In order to determine the most variable characters among the taxa studied, PCA analysis has been performed. It revealed that the first three factors comprised over 60% of the total variation. In the first PCA axis with 44% of total variation, such characters as corolla shape, calyx shape, calyx length, bract length and leaf shape have shown the highest correlation (>0.7), leaf apex, corolla length, leaf length, leaf width were characters influencing PCA axis 2 and 3 respectively. Different clustering and ordination methods produced similar results therefore, PCoA plot of morphological characters are presented here (Fig 2). In general, plant samples of each species were grouped together and formed separate groups. This result show that both quantitative and qualitative morphological characters separated the studied species into distinct groups. In the studied specimens we did not encounter intermediate forms.

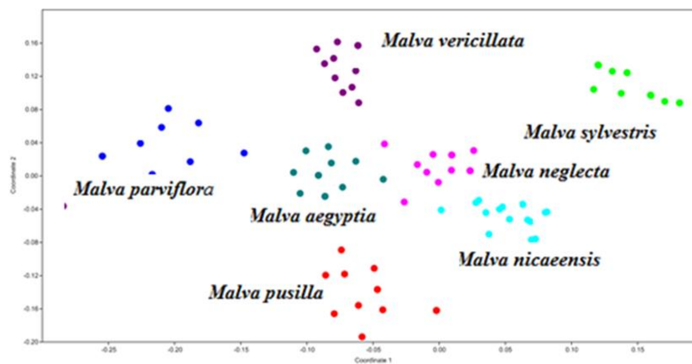

Fig 2**.** PCoA plots of morphological characters revealing species delimitation in the *Malva* species

Species Identification and Genetic Diversity

Ten SCoT primers were screened to study genetic relationships among *Malva* species*;* all the primers produced reproducible polymorphic bands in all 7 *Malva* species. An image of the SCoT amplification generated by SCoT-18,11 primer is shown in Figure 3. A total of 145 amplified polymorphic bands were generated across 7 *Malva* species. The size of the amplified fragments ranged from 100 to 3000 bp. The highest and lowest number of polymorphic bands was 24 for SCoT-16 and 8 for SCoT-18, on an average of 14.5 polymorphic bands per primer. The PIC of the 10 SCoT primers ranged from 0.39 (SCoT-11) to 0.66 (SCoT-15) with an average of 0.54 per primer. MI of the primers ranged from 3.18 (SCoT-3) to 6.47 (SCoT-17) with an average of 5.8 per primer. EMR of the SCoT primers ranged from 7.56 (SCoT-14) to 16.55 (SCoT-15) with an average of 12.55 per primer (Table 2). The primers with the high EMR values were considered to be more informative in distinguishing the genotypes.

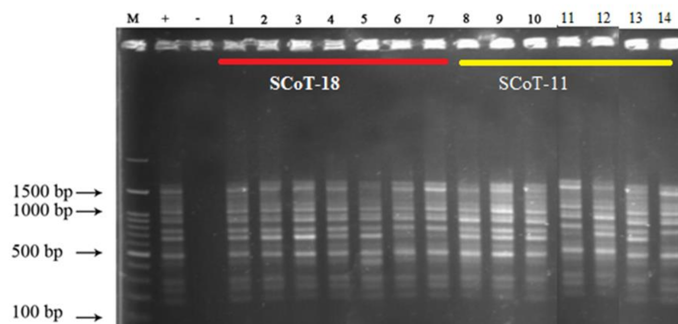

Fig 3. Electrophoresis gel of studied ecotypes from DNA fragments produced by SCoT-18, 1, 8: *Malva neglecta* 2, 9: *Malva parviflora* 3, 10: *Malva pusilla*. 4, 11: *Malva sylvestris* 5, 12: *Malva vericillata* 6, 13: *Malva nicaeensis*7, 14: *Malva aegyptia*

The genetic parameters were calculated for all the 7 *Malva* species amplified with SCoT primers (Table 3). Unbiased expected heterozygosity (*H*) ranged from 0.25 (*Malva vericillata*) to 0.48 (*Malva aegyptia*), with a mean of 0.39. A similar pattern was observed for Shannon's information index (*I)*, with the highest value of 0.31 observed in (*Malva aegyptia*) and the lowest value of 0.10 observed in (*Malva vericillata*) with a mean of 0.18. The observed number of alleles (*N*a) ranged from 0.34 in *Malva sylvestris* to 1.18 in *Malva pusilla.* The effective number of alleles (*N*e) ranged from 0.18 (*Malva pusilla*) to 1.095 (*Malva aegyptia*).

Table 3. Genetic diversity parameters in the studied Malva species.

taxon	N	Na	Ne	I	He	UHe	%P
Malva neglecta Wallr.	14.000	1.021	0.25	0.28	0.22	47.15%	53.75%
Malva parviflora L.	15.000	1.024	0.292	0.23	0.23	43.15%	44.73%
Malva pusilla Sm.	18.000	1.183	0.184	0.116	0.122	44.29%	51.83%
Malva sylvestris L.	15.000	0.344	1.042	0.20	0.33	0.30	57.53%
Malva vericillata L.	13.000	0.369	1.081	0.10	0.11	0.25	35.18%
Malva nicaeensis All.	12.000	0.499	1.067	0.14	0.12	0.34	49.26%
Malva aegyptia L.	11.000	0.462	1.095	0.318	0.48	0.48	62.55%

Abbreviations: (N = number of samples, Na= number of different alleles; Ne = number of effective alleles, I= Shannon's information index, He = gene diversity, UHe = unbiased gene diversity, P%= percentage of polymorphism, populations).

AMOVA test showed significant genetic difference (P = 0.001) among studied species. It revealed that 70% of total variation was among species and 30% was within species (Table 4) Moreover, genetic differentiation of these species was demonstrated by significant Nei's GST (0.22, P = 0.001) and D_est values (0.389, P = 0.001). These results revealed a higher distribution of genetic diversity among *Malva* species compared to within species.

Table 4. Analysis of molecular variance (AMOVA) of the studied species.

Source	df	SS	MS	Est. Var.	%
Among Pops	9	435.979	48.442	9.644	70%
Within Pops	36	152.217	4.228	4.228	30%
Total	45	588.196		13.872	100%

df: degree of freedom; SS: sum of squared observations; MS: mean of squared observations; EV: estimated variance; *Φ*PT: proportion of the total genetic variance among individuals within an accession, (P < 0.001).

Different clustering and ordination methods produced similar results therefore, UPGMA clustering are presented here (Figure 4). In general, plant samples of each species, were grouped together and formed separate cluster. This result show that molecular characters studied can delimit *Malva* species in two different major clusters or groups. In the studied specimens we did not encounter intermediate forms. In general, two major clusters were formed in UPGMA tree

(Figure. 4), populations of *Malva aegyptia* were placed in the first major cluster and were placed with great distance from the other species. The second major cluster included two sub-clusters. Plants of *Malva neglecta* and *Malva sylvestris* comprised the first sub-cluster, while plants of *Malva parviflora, Malva pusilla. Malva vericillata, Malva nicaeensis* formed the second sub-cluster.

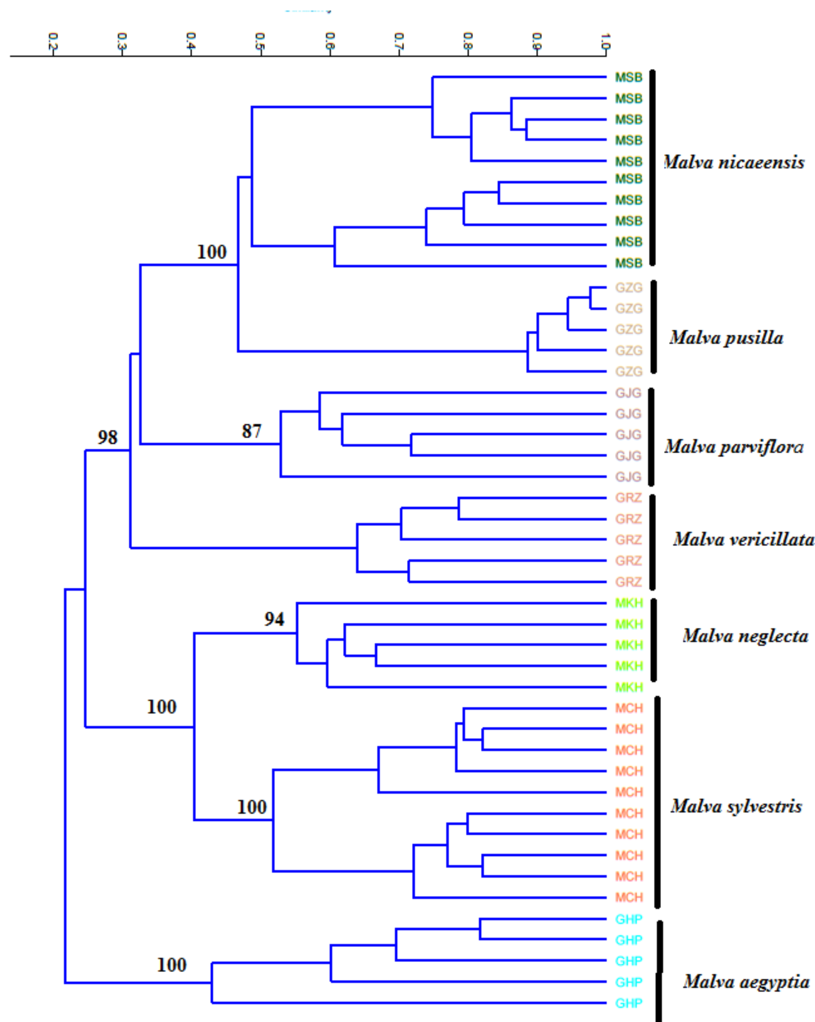

Fig 4. UPGMA tree of SCoT data revealing species delimitation in the *Malva* species.

In general, relationships obtained from SCoT data agrees well with species relationship obtained from morphological. This is in agreement with AMOVA and genetic diversity parameters presented before. The species are genetically well differentiated from each other. These results indicate that SCoT molecular markers can be used in *Malva* species taxonomy. The Nm analysis by Popgene software also produced mean Nm= 0.333, that is considered very low value of gene flow among the studied species.

Mantel test with 5000 permutations showed a significant correlation (r = 0.55, p=0.0002) between genetic distance and geographical distance, so isolation by distance (IBD) occurred among the *Malva* species studied.

Nei's genetic identity and the genetic distance determined among the studied species (Table 5). The results showed that the highest degree of genetic similarity (0.877) occurred between *Malva neglecta* and *Malva vericillata.* The lowest degree of genetic similarity occurred between *Malva sylvestris* and *Malva aegyptia* (0.688). The low Nm value (0.333) indicates limited gene flow or ancestrally shared alleles between the species studied and indicating high genetic differentiation among and within *Malva* species.

Table 5. The matrix of Nei genetic similarity (Gs) estimates using SCoT molecular markers among 7 Malva species. 1: Malva neglecta 2: Malva parviflora 3: Malva pusilla. 4: Malva sylvestris 5: Malva vericillata 6: Malva nicaeensis7: Malva aegyptia

sp1	sp2	sp3	sp4	sp5	sp6	sp7	
1.000							sp1
0.744	1.000						sp2
0.735	0.790	1.000					sp3
0.744	0.754	0.711	1.000				sp4
0.877	0.799	0.757	0.750	1.000			sp5
0.712	0.778	0.759	0.691	0.757	1.000		sp6
0.774	0.843	0.778	0.688	0.754	0.785	1.000	sp7

The Neighbor-Net tree and MDS plot of *Malva* populations based on SCoT data produced similar results therefore only Neighbor-Net tree is presented and discussed (Fig. 5). Neighbor-Net tree revealed that the seven species are well differentiated on the genetic grounds. In both UPGMA and Neighbor-Net, samples of the *Malva aegyptia* were placed far from each other. *Malva neglecta* was placed close to *Malva sylvestris*, and far from *Malva aegyptia.* In both analyses, *Malva nicaeensis* showed closer affinity with *Malva pusilla* and *Malva parviflora.*

We performed STRUCTURE analysis followed by the Evanno test to identify the optimal number of genetic groups. We used the admixture model to illustrate interspecific gene flow or / and ancestrally shared alleles in the species studied.

STRUCTURE analysis followed by Evanno test produced ΔK =7. The STRUCTURE plot (Fig. 6) produced more detailed information about the genetic structure of the species studied as well as shared ancestral alleles and / or gene flow among *Malva* species. This plot revealed that the *Malva* species are genetically differentiated due to different allelic structures. This is in agreement with UPGMA dendrogram presented before.

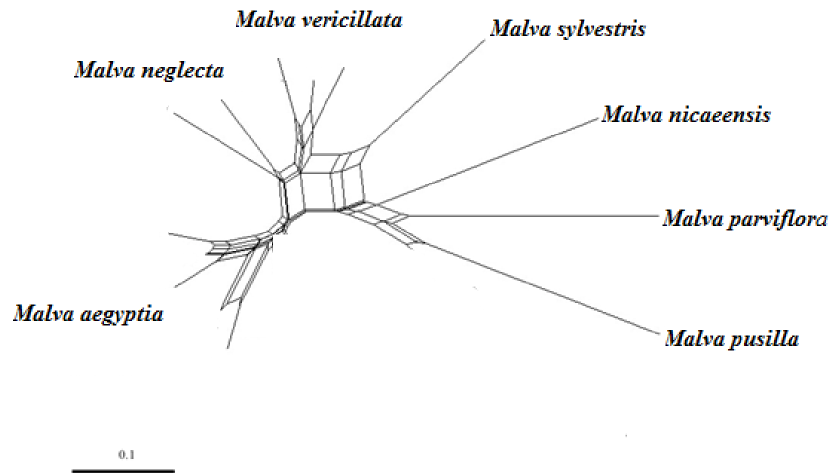

Fig 5. Neighbor-Net in *Malva* species based on RAPD data

The low Nm value (0.333) indicates limited gene flow or ancestrally shared alleles between the species studied and supports genetic stratification as indicated by K-Means and STRUCTURE analyses. Population assignment test also agreed with Nm result and could not identify significant gene flow among members of the studied species.

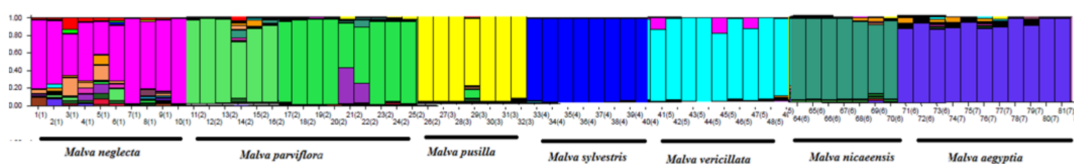

Fig 6. STRUCTURE plot of *Malva* species based on SCoT-14.

DISCUSSION

Medicinal plants represent an important therapeutic resource for the Iranian population and they are part of the country's Public Policies (BRASIL, 2006). The use of medicinal plants can be influenced by the economic condition, the high cost of medicines and the difficult access to public consultations. In addition to that, there is a difficulty of access by residents in rural areas to health care units located in urban areas. Moreover, the increase the trend for considering traditional knowledge that supports using natural resources as an alternative to synthetic drugs (BATTISTI *et al.,* 2013). The indiscriminate use of plants due to the lack of phytochemical, pharmacological and mainly toxicological knowledge is of great concern for public health. The correct identification of medicinal plant species is necessary, especially when they are processed in order to avoid misuse of medicinal plants (ROMITELLI & MARTINS, 2013).

Malvaceous germplasm has been variously investigated by different molecular marker techniques but the earlier studies either focused on the comparison of the Malvaceae with other families in the order Malvales or to explore the genetic relationships and diversity within and among population and limited number of species in the same genus. Very little attention has been given to the analysis at interspecific and intergeneric levels. LA DUKE and DOBLEY (1995) has the only worth mentioning work in this regard. Their results showed that, the genetic relationships and diversity within and between 12 malvaceous species belonging to five genera are investigated by using the Amplified fragment length polymorphism (AFLP).

SHAHEEN *et al*., (2009) with used AFLP (Amplified fragment length polymorphism) marker to explore phenetic relationships and diversity within and between 13 Malvaceae species belonging to 5 different genera. Their primary objective of the study was to evaluate the taxonomic potential, usefulness and applicability of AFLP marker system to reconstruct genetic relationships at interspecific and intergeneric level in Malvaceae. Two primer pairs produced a total of 73 bands, of which 70 were polymorphic.

According to CELKA *et al* (2010) two categories of DNA markers were used to determine genetic relationships among eight *Malva* taxa. A maximum parsimony analysis validated the division of the genus *Malva* into the sections *Bismalva* and *Malva*. The species classified into those sections formed separate clusters. *M. moschata* was a distinctive species in the section *Bismalva*, as confirmed by previous genetic research based on ITS and cpDNA sequence analyses. The applied markers revealed a very high level of genetic identity between *M. alcea* and *M. excisa* and enabled molecular identification of *M. alcea* var. *fastigiata*.

JEDRZEJCZYK and REWERS (2020) applied flow cytometry and inter simple sequence repeat polymerase chain reaction (ISSR-PCR) for fast and accurate species identification. Genome size estimation by flow cytometry was proposed as the first-choice method for quick accession screening. Out of the 12 tested accessions, it was possible to identify six genotypes based on genome size estimation, whereas all species and varieties were identified using ISSR markers. Flow cytometric analyses revealed that *Malva* species possessed very small (1.45–2.77 pg/2C), small (2.81–3.80 pg/2C), and intermediate (11.06 pg/2C) genomes, but the majority of accessions possessed very small genomes. The relationships between the investigated accessions showed the presence of two clusters representing malvoid and lavateroid group of species. Their results showed that Flow cytometry and ISSR molecular markers can be effectively used in the identification and genetic characterization of *Malva* species.

Until now, molecular studies using ISSR markers conducted in the *Malva* genus have only included a few species (CELKA, *et al*., 2012). All primers used in ISSR-PCRs for the *Malva* genus revealed 100% polymorphism between all accessions. Therefore, it was possible to identify all tested species. Moreover, for *M. verticillata* taxon, it was possible to distinguish all studied varieties. The usefulness of most of the used ISSR primers was also confirmed in *Ocimum, Origanum,* and *Mentha* identification (LO BIANCO, *et al.,* 2017). The systematics of the *Malva* genus and closely related genera is complicated. Moreover, the relationships obtained from molecular studies do not confirm traditional classification (ESCOBAR, *et al.,* 2009). So far, only molecular analysis relying on rDNA ITS sequences and ISSR markers have shed light on taxonomical relationships between *Malva* species (ESCOBAR, *et al*., 2009). Phylogenetic analyses of rDNA ITS sequences indicated the presence of two well-supported clusters within the mallow species (malvoid and lavateroid clades), which is consistent with the presented data. There are two hypotheses for the absence of differences between isolated populations. The first hypothesis explained that genetic diversity within and between populations demonstrate gene flow processes, which led to the fragmentation of larger populations (DOSTÁLEK *et al.,* 2010). The second hypothesis presented that geographically proximate populations are more efficiently connected through gene flow than populations separated by greater distance. Between genetic diversity parameters and population size were showing positive correlations that confirmed various studies (LEIMU *et al.* 2006). There are two reasons for the positive correlation between genetic diversity and population size (LEIMU *et al.,* 2006). 1- A positive correlation could imply the presence of an extinction vortex, where the drop-in population size lowers genetic diversity, which leads to inbreeding depression. The second reason is the fact that plant fitness differentiates populations based on variations in habitat quality (VERGEER *et al.,* 2003). This study proved the potential of SCoT marker analyses in the identification and evaluation of genetic relationships between *Malva* species/varieties. Out of the 97 studied accessions, it was possible to identify seven genotypes using SCoT-PCR method. This is the first report about the application of these techniques to estimate the genetic variation and genetic characterization of *Malva* accessions. The results of this study can be useful in mallow breeding programs, planning of conservation strategies, germplasm collection, and taxonomy of the genus.

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ISPITIVANJE GENETIČKOG DIVERZITETA *Malva* L. (Malvaceae): VISOKO VREDNE MEDICINSKE BILJKE PRIMENOM SCOT MOLEKULARNIH MARKERA

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Izvod

Rod *Malva* L. raste spontano skoro svuda u Evropi i Mediteranu. Ima 25-40 vrsta I može se smatrati jednogodišnjom i dvogodišnjom biljkom. Ovaj rod sadrži vrste koje imaju terapeutska svojstva i najvažnije su u medicini i funkcionalnoj prehrambenoj industriji. Zbog toga smo zbog važnosti ovih biljnih vrsta izvršili kombinaciju morfoloških i molekularnih podataka za ovu vrstu. Za ovu studiju koristili smo 97 slučajno sakupljenih biljaka iz sedam vrsta u 7 provincija. Prema SCoT analizi markera, *Malva silvestris* i *Malva aegiptia* su imale najmanju sličnost, a vrste *Malva ignocta* i *Malva vericillata* imale su najveću sličnost. Ciljevi ove studije su: 1) mogu li SCoT markeri identifikovati vrste Malve, 2) kakva je genetska struktura ovih taksona u Iranu i 3) da se istraži međusobni odnos vrsta? Ova studija je otkrila da kombinacija morfoloških i SCoT podataka može identifikovati vrstu.

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