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GENETIC DIVERSITY OF *Melica* POPULATIONS AND THEIR STABILITY IN ECOLOGICAL LANDSCAPE

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ABSTRACT

Melica **grass has effective role in ecological landscape and its stability is affected by population genetics. Main purpose of this study was to investigate the relation and distance of** ***Melica*** **populations genetically that are grown in unlike topographic and climatic conditions. Karyotyping and leaf storage protein SDS PAGE were employed to investigate the genetic diversity of 10 populations of 2 species of** ***Melica viz.*****,** ***M. persica*** **and** ***M. jacquemontii*** **as major plants in ecological landscape of Esfahan, Alborz, Ghom, Golestan, Kerman and Yazd habitats in Iran. The result of karyotyping showed that all the populations have similar chromosome number (2n = 2x = 18), but cytogenetic parameters exhibited significant differences in all the populations. Ward similarity analysis revealed that all the populations can be clustered into 4 groups. SDS PAGE of leaf storage protein indicated that the population of** ***M. persica*** **'21856' had highest protein fragment while the lowest fragments belonged to** ***M. jacquemontii*** **[7683 and 10506]. Jaccard similarity coefficient revealed genetic variation in all the studied populations of** ***M. persica,*** **but** ***M. jacquemontii*** **showed no variation.** ***M. persica*** **had high diversity and more stability in ecological landscape and these results suggest that the species of** ***M. jacquemontii*** **is a subspecies of** ***M. persica*****.**

Keywords: Climate, cytogenetic, diversity, natural habitat, population

INTRODUCTION

Melica L., a genus of Order Poales, family Poaceae and subfamily Pooideae, are found in temperate regions as perennial grasses (Mejia-Saules and Bisby, 2003; Cântar and Dincă, 2017). About 274 species of *Melica* with varying grown habits (clumping, erect stems and corms grass form) are widespread in the world (Clayton and Renvoize. 1986; Mejia-Saules and Bisby, 2003; Cântar and Dincă, 2017). *Melica* grass is a bisexual plant with bisexual spikelet and hermaphrodite florets that expose cleistogamous or chasmogamous (Sheidai and Moghadam, 2009). The genus *Melica* includes phylogenetically old forms found in East Asia and continuous evolution forms observed in arid and semi-arid regions of West Eurasia, North and South America (Niklfeld and Schratt-Ehrendorfer, 1999; Eliáš Jun *et al*., 2017). The base number chromosomes of this genus is x = 9 with different diploid and tetraploid levels, while x = 7 is rarely reported for somatic chromosome in this genus (Dallwitz and Watson, 2005; Sheidai and Moghadam, 2009).

Melica species grow as popular plants in ecological landscape because of their massive root system and play effective role in minimizing soil erosion, especially on slopes (Tomaškin and Tomaškinová, 2012), so are recognized as capable grass for ecosystem reclamation. *M. californica* and *M. imperfecta* are the two important melic grasses found in ecological landscape in valley grassland and foothill woodland (SSQR, 2003). *M. altissima* is a popular species in ecological landscape in Slovakia (Eliáš Jun *et al.*, 2017). The species of genus *Melica* have significance in durability of ecological landscape owing to their effective role in erosion control, slope stabilization, tolerance to serpentine soil, drought tolerance, fast growth rate, fire resistance, robust to invasion, as a forage plant for wildlife and livestock, well performance under oaks, etc. (Long and Anderson, 2012; Porensky *et al*., 2012). The native grass plants serve as an important element in reducing the degradation of rural agricultural landscape (Tomaškin and Tomaškinová, 2012). *Melica*, known as melic grass or melic, is a perennial grass grown in the plains and hills of Iran (Sheidai and Moghadam, 2009; Zarif Ketabi, 2010). This genus has several species in Iran with *M. persica* Kunth and *M. jacquemontii* Decne the widely found species. *M. persica* is widely distributed and well established as a range plant in most areas of rocky foothills in Iran (Sheidai *et al*., 2006; Zarif Ketabi, 2010).

Genetic diversity assessment is a careful and rapid approach used in the cognition and classification of native accessions of turfgrass (Tehrani *et al*., 2009; Hand *et al*., 2012; Cuyeu *et al*., 2013). The genetic diversity assessment of turfgrass species helps in the selection and collection of elite genotypes for future breeding programs (Raggi *et al*., 2015; Meyer *et al*., 2017). On the other hand, the perennial grasses growing in natural habitats show higher genetic variations in comparison to the interspecific hybrids (Honig *et al*., 2010; Averello *et al*., 2015). Meyer *et al*. (2017) have suggested the need to exploit genetic diversity of turfgrass species for their stability under climate change. Among and within the species diversity, the plants regulate their productivity and lastingness in native ecosystems and resist disruptions (Prieto *et al*., 2015). The complementary effects of taxonomic and genetic diversity have been confirmed for yield and stability of forage species, and such genetic diversity has major impact on the control of perdurability of biomass (Prieto *et al*., 2015).

Chromosome number is an important stable feature in plants that helps in elucidating the relationship and interpretation of ecological changes and their effect on ploidy levels of species and populations (Stebbins, 1971). Chromosomes have genes that carry genetic information about the plant phenotype and are important in cellular studies (Stace and Aquier, 1978). Cytogenetic studies have been conducted on a large number of species (Hesamzadeh and Rasuli, 2006; Sheidai and Moghadam, 2009; Honig *et al*., 2010; Bagheri Abyaneh *et al*., 2017). Protein electrophoresis technique is used to assess the genetic diversity and classification of plants. The sodium dodecyl sulfate-polyacrylamide electrophoresis (SDS-PAGE) technique is one of the practical methods used to assess the evolutionary relationship between plants (Sharma and Maloo, 2009). Perusal of literature reveals no report on the studies related to the biochemical markers of *Melica* leaf storage proteins. In present study the genetic variations between different populations of two *Melica* species (*M. persica* and *M. jacquemontii*) based on leaf storage proteins electrophoresis and cytogenetic studies were conducted. *Melica* is identified as the only diploid genus of Poaceae (Sheidai, 2008). The present study was aimed to assess the chromosome number and basic cytogenetic information of *M. persica* and *M. jacquemontii* and to find out the relationship between these species.

MATERIALS AND METHODS

Plant materials

Ten natural habitats and ecological landscape with *Melica* grass were selected in Alborz, Esfahan, Ghom, Golestan, Kerman, Semnan and Yazd provinces in Iran (Fig. 1). Five populations were

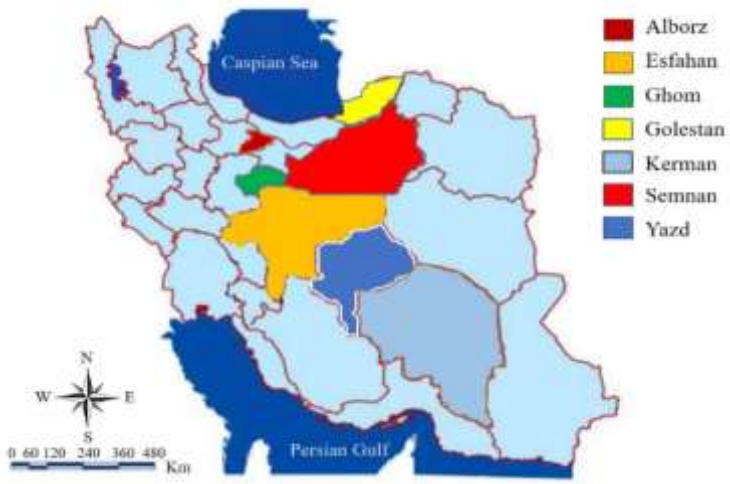

Fig. 1: Origin of investigated populations of *Melica*

recognized for each species of *M. persica* and *M. jacquemontii* in the selected ecological landscapes and their labeled seeds obtained from the Gene Bank of Research Institute of Forest & Rangelands, Iran. The information about the studied populations is given in Table 1. Seeds were germinated in Petri-dishes using wet filter paper at 20-22°C under 16 h light and 8 h darkness. After germination, some seedlings with 1.0-1.5 cm root length were selected for cytological studies and rest seedlings transplanted into pots of 2 L size, maintained in a green-house with controlled conditions for proper growth. The leaves were used for assessment of leaf storage proteins.

Table 1: Climate information of the natural habitat of *Melica* population

Melica species	Gene bank code	Origin province/city	Elevation (m masl)	Annual mean rainfall (mm)	Annual mean temperature (°C)
M. persica	22699	Ghom/Ghom	950	150	18.17
	18477	Esfahan/Golpayegan	1870	327	15.60
	18586	Golestan/Gorgan	175	583	17.75
	25558	Alborz/Taleghan	1300	500	11.40
	21856	Semnan/Semnan	1150	140	17.10
M. jacquemontii	17599	Ghom/Ghom	950	150	18.17
	7683	Kerman/Jiroft	750	220	26.00
	24299	Yazd/Taft	1800	145	16.05
	10506	Yazd/Khezrabad	1200	100	18.60
	13116	Yazd/Tafresht	1600	145	16.05

†Climate data presented according to Meteorological Bureau of the respective provinces

Cytological preparations

To decipher the metaphases of chromosomes, the selected seedlings were immersed in ice-cold water for 24 h at 4°C, then the root tip meristems were immersed in 0.5% saturated aqueous solution of α-bromonaphthalene for 3-4 h at 4°C. The pretreated root tips were washed with distilled water for 5 min (Falistocco *et al*., 2013; Bagheri Abyaneh *et al*., 2017). The root tips were then fixed at room temperature in a fixative solution of formaldehyde (10%) and chromic acid (1%) [1:1 ratio] for 16 h and rinsed for 3 h with distilled water. The samples were then dried with filter paper and kept in 70% ethyl alcohol at 4°C till use. The root tip meristems were hydrolyzed by NaOH (1 M) at 60°C for 7 min and then dyed with hematoxylin-iron for 1.5-2.0 h at room temperature. The root tip meristems were then placed on lam and gently squashed in a droplet of 45% acetic acid and lactic acid [10:1] (Falistocco *et al*., 2013; Bagheri Abyaneh *et al*., 2017).

Cytological investigations

Cytological observations were made using an optical microscope (BH_2 Olympus supplemented digital colour video camera) at a magnification of 1908X, and five best meta-physical plates were selected for measuring the chromosome arms by using micro-measure software (Reeves and Tear,

2000). The parameters estimated in each metaphase plate to characterize the karyotypes numerically were: long arm (LA), short arm (SA), total length (TL) [LA + SA], relative length percentage (RL %), arm ratio (AR) [LA/SA], centromeric index (CI) [SA/ (LA + SA)] and the value of relative chromatin (VRC) [ΣTL/n]. Karyotype asymmetry was estimated by using three methods *viz.*, total form percentage (TF %) [(ΣSA/ΣTL) ×100] (Huziwara, 1962); the difference in relative length (DRL) [Max RL% - Min RL%]; intrachromosomal asymmetry index (A1) [1-Σ (SA—/LA—)/n] and inter-chromosomal asymmetry index (A2) [σ/X] (Romero Zarco, 1986). Both indices (A1 and A2) are independent of chromosome number and size. Also, karyotypic evolution was determined using the symmetry classes of Stebbins (1971). Karyotype formula were determined by chromosome morphology based on centromere position as per the classification of Levan (1964). For each population, karyograms were drawn based on chromosome length.

Protein extraction and electrophoresis

One leaf from each plant sample was separated for protein extraction. The leaves were individually ground to a fine powder using a mortar and pestle. Leaf powder (0.5 g) of each population was taken in an Eppendorf tube and 900 µL protein extraction buffer was added. The extraction buffer contained 50 mM tris-HCl, pH 8.0, 2 mM EDTA, 1 mM PMSF, 2% β-mercaptoethanol, 1% triton X-100 and 20 mM $MgCl_2$ (Laemmli, 1970). The Eppendorf tubes were vortexed and the homogenates purified by centrifugation at 13000 rpm for 10 min at room temperature. The clear supernatants were collected, transferred to new 2 mL Eppendorf tubes and stored at 4°C for electrophoresis. Electrophoresis was done using discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) system with 12.5% (w/v) separating gel and 5% stacking gel (Scheibe*et al.*, 2001). The supernatant (5 µL) was combined with 1.25 µL β-mercaptoethanol and 3.75 µL sample buffer (0.002% blue bromophenol, 0.0625 M tris-HCl, pH 6.8, 0.2% SDS and 15% glycerol) and loaded in gel wells with the help of micropipette and then run at a constant current of 90 V at room temperature (25°C) until the dye migrated to the gel bottom. In next step, the gels were stained in 0.1% Coomassie brilliant blue R-250 in methanol, acetic acid and distilled water (40:10:50 v/v) for 8-10 h and then destained in solution without Coomassie brilliant blue R-250 under shaking till the gels became clear. Finally, the gels were photographed to visualize the protein band patterns.

Statistical analysis

One-way unbalanced ANOVA was performed on normal data to determine the variation between populations, and parameter means were compared by Duncan's test. Principal component analysis (PCA) was performed to evaluate the contribution of each karyotypic parameter to the ordination of species. Clustering was performed using the unweighted pair group method with arithmetic (UPGMA) after the calculation of cophenetic correlation coefficient (r) to examine the karyotype similarity among populations. Numerical analyses were performed using SAS, JMP and StatistiXL software.

RESULTS AND DISCUSSION

Karyotyping is a useful approach for detecting the development of *Melica* species. The ordered karyotype images and karyotypic characteristics of populations for two species of *M. persica* and *M. jacquemontii* were x = 9 and 2n = 2x = 18 for all accessions studied. The information on population karyotype and metaphase for two *Melica* species is given in Fig. 2. The investigated populations of both the species had most metacentric (m) or sub-metacentric (sm) chromosomes (Table 2). Karyotyping showed logical variation in chromosome of the investigated populations of two endemic

species of *Melica* in Iran. Our results are in line with the karyotype studies in other endemic plant species such as Brassicaceae (Sun *et al*., 2020), Asteraceae (Sun *et al*., 2017), *Silene schimperiana* (El-Ghamery *et al*., 2016), *Fritillaria montana* (Samaropoulou *et al*., 2016), and *Astragalus sericeocanus* (Konichenko *et al*., 2014). Karyotyping has previously been used to investigate many populations, genus or species of different plants such as *Allium cepa* and *Lactuca sativa* (Lima *et al*., 2019), *Salvia* population (Kursat *et al*., 2018), genus *Melica* in Bulgaria (Angelov, 2015), *Hibiscus mutabilis* f. *mutabilis* (Li *et al*., 2015), and *Allium* sect. Melanocrommyum (Genç *et al*., 2013).

All the studied populations were classified into four groups (A, 2A, B and 2B) as per Stebbins (1971) symmetry type and Romero Zarco (1986) asymmetry index (Table 2). The asymmetry indices

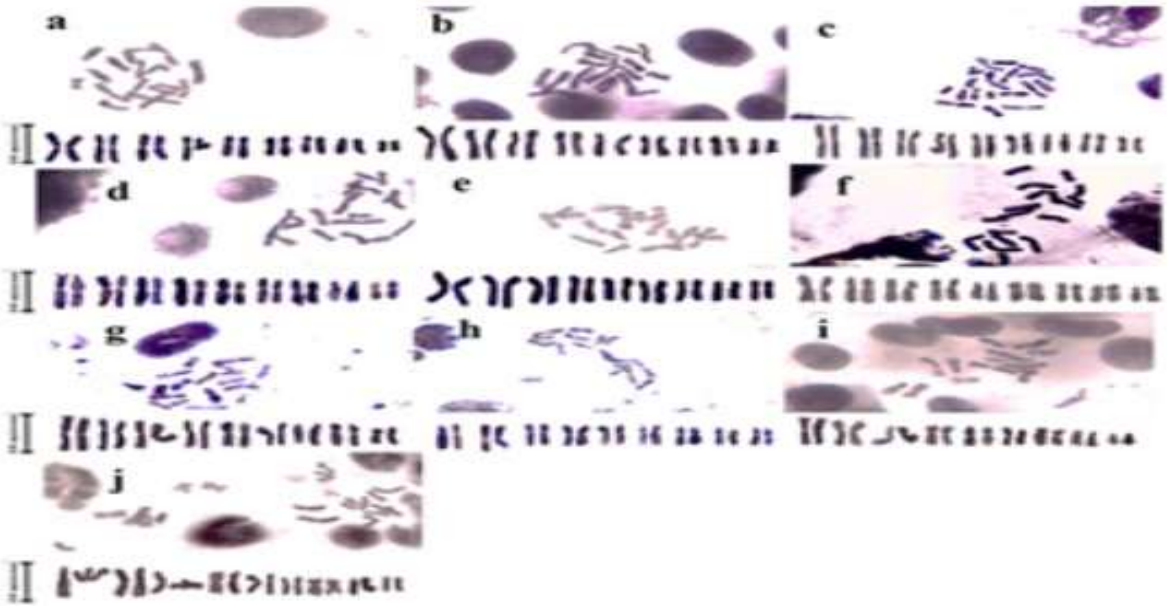

Fig. 2: Microphotograph of somatic metaphases. a) *M. jacquemontii* '7683'; b) *M. jacquemontii* '13116'; c) *M. jacquemontii* '10506'; d) *M. jacquemontii* '24299'; e) *M. jacquemontii* '17599'; f) *M. persica* '25558'; g) *M. persica* '18477'; h) *M. persica* '18586'; i) *M. persica* '21856'; j) *M. persica* '22699'; magnification mentioned [1908x]

Table 2: Karyotype characteristics of 10 populations of two species of *Melica*

Populations	Gene bank codes (RIFR)	2n	X	VRC	% TF	DRL	SC	A2	A1	KF
M. jacquemontii	7683	18	9	5.700	40.631	7.631	A	0.220	0.312	7 m + 2 sm
	13116	18	9	6.431	37.472	7.033	2B	0.220	0.400	5 m + 4 sm
	10506	18	9	5.281	41.193	8.545	B	0.247	0.292	8 m + 1sm
	24299	18	9	5.454	38.569	8.138	2B	0.240	0.378	6 m + 3 sm
	17599	18	9	4.725	38.803	8.207	2B	0.220	0.369	7 m + 2 sm
M. persica	25558	18	9	5.854	38.906	7.003	2A	0.222	0.376	5 m + 4 sm
	18477	18	9	6.042	39.640	8.598	2B	0.241	0.331	8 m + 1 sm
	18586	18	9	5.126	40.170	8.273	B	0.239	0.393	8 m + 1 sm
	21856	18	9	5.258	37.804	8.273	2B	0.239	0.393	6 m + 3 sm
	22699	18	9	4.631	38.985	8.219	B	0.252	0.364	6 m + 3 sm

2*n* = Somatic chromosome number, *x* = Basic chromosome number, DRL = Difference in relative length, VRC = Value of relative chromatin, %TF = Total form percentage, A1= Intra-chromosome asymmetry index, A2 = Inter-chromosome asymmetry index, SC = Symmetry Classes of Stebbins, K.F. = Karyotype formula, m = Metacentric, sm = Submetacentric, st = Subtelocentric

of intra-chromosomal asymmetry index (A1) and inter-chromosomal asymmetry index (A 2) were identified as asymmetric karyotypes among a population that had similar symmetry in Stebbins classes (Table 2). The class 2B population of *M. jacquemontii* '13116', collected from Tafresht of Yazd, had highest A1 value (0.4), and this population had more asymmetric karyotypes than the other populations in class 2B. The population of *M. persica* '18477', collected from Golpayean of Esfahan, had lowest A1 value (0.331) and this population had more symmetric karyotype than the other populations in class 2B. Consequently, based on the percentage of TF and A1 values, the population of *M. jacquemontii* '13116' had the most asymmetrical and evolutionary karyotype, and the population of *M. jacquemontii* '10506' had the most symmetrical karyotype. Moreover, due to A2 values, *M. persica* '22699' had the most asymmetrical karyotype of all population. Further, the differences in relative length (DRL) and value of relative chromatin (VRC) were used to determine the karyotype asymmetry. In present study the highest and lowest relative length percentage (DRL) of chromosomes were detected from 8.598 µm in *M. persica* '18477' from Esfahan-Golpayegan to 7.003 µm in *M. persica* '25558' from Alborz-Taleghan, and VRC values in studied populations ranged from 4.631 and 6.431 (Table 2). All the populations of two endemic species of *Melica* had moderate inter- and intra-asymmetry that caused high asymmetrical and evolutionary of their karyotype. Our results indicated the role of chromosomal asymmetry in improving the evolution of species (Liang and Chen, 2015; Medeiros-Neto *et al*., 2017; Riasat and Sadeghian, 2019).

The studied populations of *M. jacquemontii* were categorized into 4 groups based on karyotype formula (KF). The 1st group had two populations of Kerman-Jiroft and Ghom (17599) with KF; n = 9 = 7 m + 1 sm. The other three populations grouped separately included the population of Yazd-Tafresht with KF; n = 9 = 5 m + 4 sm, population of Yazd-Khezrabad with KF; n = 9 = 8 m + 1 sm, and population of Yazd-Taft with KF, n = 9 = 6 m + 3 sm (Table 2). The populations of studied *M. persica* were grouped into three. Populations of Esfahan-Golpayegan and Golestan-Gorgan with KF; n = 9 = 8 m + 1 sm were placed in the 1st group, the other two populations of Semnan and Ghom (22699) with KF; n = 9 = 6 m + 3 sm was located in 2nd group, and finally the population of Taleghan (25558) with KF; n = 9 = 5 m + 4 sm was located in a group lonely. Significant differences between measured indices for different populations were observed. The results revealed that the two endemic species of *Melica*, growing in different areas of Iran are diploid and their basic chromosome number is x = 9. This is in conformity with the previous studies of some grass genera of subfamily Pooideae in Damavand and Absard in Iran (Sheidai, 2008; Sheidai and Moghadam, 2009). Tutin (1980) reported that *M. uniflora* and *M. nutans* are two diploid species with 2n = 18. The results revealed significant difference between KF for populations of the wo investigated *Melica* species and more chromosomes has metacentric KF. This result can serve to clarify the taxonomic position of *M. jacquemontii* and *M. persica* in BOP clades (Bambusoideae, Oryzoideae, and Pooideae).

The population of *M. jacquemontii* '13116' had highest length arm (LA) while *M. persica* '22699' had lowest LA. The results showed highest short arm (SA) for two populations of *M. jacquemontii* '13116' and *M. persica* '18477' and lowest SA for two populations of *M. jacquemontii* '17599' and *M. persica* '22699' (Table 2). The highest and lowest total length of chromosomes was found in *M. jacquemontii* '13116' and *M. persica* '22699', respectively (Table 3; Fig. 3). A significant difference in arm ratio (AR) and centromic index (CI) was noticed in the test populations. Further, differences were noticed with respect to the presence and absence of satellites in the studied populations (Table 3). Principal component analysis (PCA) for karyotypic indices revealed that the first two independent components included A1 and A2 had 59.04 and 40.91 variance, respectively. Total variance of A_1 and A_2 components were 99.96%. The coefficients of specific vectors in 1st component showed that LA, TF percentage, CI index and AR had the most role in 1st component (Table 4A). Significant variability was observed in the cytological indices of the studied populations of *Melica*. These findings are in line with Kumari and Saggoo (2016) who reported high cytological and morphological variability in *M. persica* populations grown in Himachal Pradesh in India.

Table 3: Comparisons of chromosome analysis for *Melica* populations

Populations	LA	SA	TL	AR	CI	Sat
M. jacquemontii '13116'	4.021^{a}	2.410^{a}	6.431^{a}	1.672^{a}	0.376^{c}	+
M. jacquemontii '7683'	3.386^{abc}	2.282^{ab}	5.668^{abcd}	1.479^{bc}	0.404^{ab}	-
M. jacquemontii '24299'	3.350^{abc}	2.103^{abc}	5.45^{abcd}	1.587^{abc}	0.387^{abc}	-
M. jacquemontii '10506'	3.163^{bc}	2.175^{abc}	5.281^{bdc}	1.429^{c}	0.412^{a}	-
M. jacquemontii '17599'	2.892^{bc}	1.834^{c}	4.725^{dc}	1.577^{abc}	0.388^{abc}	+
M. persica '18477'	3.647^{ab}	2.395^{a}	6.042^{ab}	1.522^{abc}	0.397^{abc}	+
M. persica '25558'	3.576^{abc}	2.277^{ab}	5.854^{abc}	1.571^{abc}	0.389^{abc}	-
M. persica '21856'	3.270^{abc}	1.988^{bc}	5.258^{bdc}	1.651^{a}	0.378^{bc}	-
M. persica '18586'	3.067^{bc}	2.059^{abc}	5.126^{bdc}	1.486^{abc}	0.403^{abc}	+
M. persica '22699'	2.826^{c}	1.805^{c}	4.631^{d}	1.563^{abc}	0.391^{abc}	-

LA = Long arm, SA = Short arm, TL = Total length, AR = Arm ratio, CI = Centromic index and Sat = Presence (+) or absence (–) of satellites; The mean values within a column superscripted by same letter do not differ from each other at 5% level of significance as per Duncan's test

In addition, in 2nd component two traits of TL and SA showed highest effect on the studied population variation (Table 4A). The population dispersion revealed that the populations were located in four groups due to karyotypic difference presented by two first components (Fig. 4A). The population of *M. jacquemontii* '13116' had highest values in 1st component, and in 2nd component, three populations of *M. persica* '18477', *M. jacquemontii* '7683' and *M. jacquemontii* '10506' depicted highest coefficients. Also, the other diagrams that performed due to electrophoresis of leaf protein fragments revealed that all the studied populations dispersed into four groups. All the populations of *M. jacquemontii* had low genetic distance and their PCA analysis suggested that they are located in a group. The population of *M. persica* '18586' had highest variation in protein fragments with another population so depicted higher importance on 1st component. But, in 2nd component *M. persica* '21856' showed highest variation with other population due to the presence of protein fragments on this component (Fig. 4B). The PCA for protein fragments revealed that the first three main components had highest variation (A1 = 39.66; A2 = 32.72; A3 = 14.22, respectively). The results showed four fragments in 1st component (10, 11, 12, 13), and three fragments in 2nd component (2, 5, 7) had highest importance on population variation (Fig. 4B; Table 4B). Our results are in agreement with the previous results on genus *Melica* (Angelov, 2015) and some populations of *Melica* and *Milium* species (Sheidai and Moghadam, 2009).

Simple matching similarity coefficients and genetic distance between populations are presented in Tables 5 & 6. In populations of *M. jacquemontii*, the highest similarity coefficient was found in *M.*

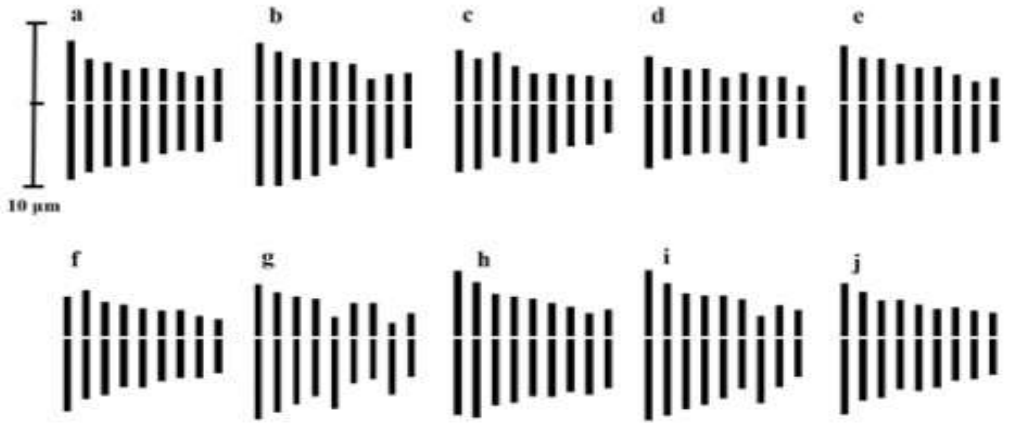

Fig. 3: Ideograms of populations a) *M. jacquemontii* '7683'; b) *M. jacquemontii* '13116'; c) *M. Jacquemontii* '10506'; d) *M. jacquemontii* '17599'; e) *M. jacquemontii* '24299'; f) *M. persica* '22699'; g) *M. persica* '21856'; h) *M. persica* '25558'; i) *M. persica* '18477' & j) *M. persica* '18586'

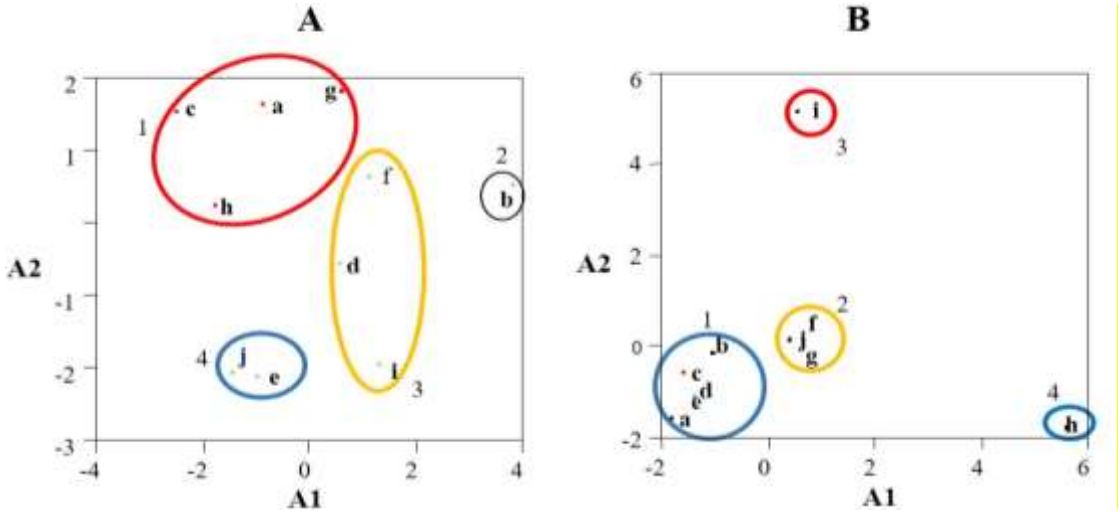

Fig. 4: Scatter plot of the studied population for the first two principals of A) Caryological indices B) Electrophoresis of leaf proteins. a) *M. jacquemontii* '7683'; b) *M. jacquemontii* '13116'; c) *M. jacquemontii* '10506'; d) *M. jacquemontii* '24299'; e) *M. jacquemontii* '17599'; f) *M. persica* '25558'; g) *M. persica* '18477'; h) *M. persica* '18586'; i) *M. persica* '21856'; and j) *M. persica* '22699'

Table 4: Eigen vectors of two first principal components for 6 karyotype indices (A) and 12 fragments of protein to classify 10 population of *Melica*

A			B		
Karyotype indices	A1	A2	Protein fragments†	A1	A2
TL	0.388	0.435	1	0.279	0.227
LA	0.444	0.350	2	0.039	0.469
SA	0.267	0.552	4	0.321	0.226
AR	0.440	-0.36	5	0.039	0.469
CI	-0.439	0.358	6	0.321	0.226
TF	-0.439	0.358	7	0.039	0.469
			10	-0.413	0.153
			11	-0.413	0.153
			12	0.413	-0.153
			13	-0.413	0.153
			19	0.134	0.134
			20	0.103	-0.274
Eigen value	3.542	2.455		4.760	3.927
PV (%)	59.04	40.91		39.68	32.72
CPV (%)	59.04	99.96		39.68	72.40

†These fragments of protein showed variation between different populations.
TL = Total length, LA = Long arm, SA = Short arm, AR = Arm ratio, CI = Centromic index, TF = Total form, PV = Percentage of variance, CPV = Cumulative percentage of variance.

jacquemontii '17599' and *M. jacquemontii* '24299', and the lowest similarity existed among *M. jacquemontii* '13116' and *M. jacquemontii* '7683' populations. In case of *M. persica* populations the highest similarity was observed in *M. persica* '18477' and *M. persica* '22699' and *M. persica* '25558', while in these populations the lowest similarity was noticed for *M. persica* '18586' with *M. persica* '21856' (Table 5). The protein fragment analysis revealed that *M. jacquemontii* '17599' had lowest genetic distance with *M. jacquemontii* '24299', while *M. jacquemontii* '13116' had highest genetic distance with *M. jacquemontii* '7683' among the 5 populations of *M. jacquemontii* studied (Table 6).

Table 5: Simple matching similarity coefficient between different populations for protein fragments

Populations	A	B	C	D	E	F	G	H	I	J
A	1.000									
B	0.955	1.000								
C	0.955	0.909	1.000							
D	0.955	0.909	1.000	1.000						
E	0.909	0.864	0.955	0.955	1.000					
F	0.818	0.864	0.864	0.864	0.818	1.000				
G	0.636	0.682	0.682	0.682	0.636	0.818	1.000			
H	0.727	0.773	0.682	0.682	0.636	0.818	0.636	1.000		
I	0.818	0.864	0.864	0.864	0.818	1.000	0.818	0.818	1.000	
J	0.818	0.864	0.864	0.864	0.818	1.000	0.818	0.818	1.000	1.000

A) *M. jacquemontii* '10506', B) *M. jacquemontii* '13116', C) *M. jacquemontii* '17599', D) *M. jacquemontii* '24299', E) *M. jacquemontii* '7683', F) *M. persica* '18477', G) *M. persica* '18586', H) *M. persica* '21856', I) *M. persica* '22699', and J) *M. persica* '25558'

Table 6: Population genetic distance in *Melica* populations studied as per the variation of their globulin proteins

Populations	A	B	C	D	E	F	G	H	I	J
A	1.000									
B	0.037	1.000								
C	0.037	0.071	1.000							
D	0.037	0.072	0.000	1.000						
E	0.077	0.111	0.037	0.037	1.000					
F	0.133	0.097	0.097	0.097	0.133	1.000				
G	0.286	0.242	0.241	0.241	0.286	0.125	1.000			
H	0.188	0.152	0.212	0.212	0.250	0.111	0.636	1.000		
I	0.133	0.097	0.097	0.097	0.133	0.000	0.818	0.235	1.000	
J	0.133	0.097	0.097	0.097	0.133	0.000	0.818	0.125	0.000	1.000

A) *M. jacquemontii* '10506', B) *M. jacquemontii* '13116', C) *M. jacquemontii* '17599', D) *M. jacquemontii* '24299', E) *M. jacquemontii* '7683', F) *M. persica* '18477', G) *M. persica* '18586', H) *M. persica* '21856', I) *M. persica* '22699', and J) *M. persica* '25558'

Among the *M. persica* population studied, the lowest genetic distance was found between *M. persica* '18477' with *M. persica* '22699' and *M. persica* '25558', and between *M. persica* '22699' with *M. persica* '25558' (Table 6). The highest genetic distances were found among *M. persica* '18586' and *M. persica* '21856'. The results revealed significant genetic diversity between different populations of *Melica* perhaps due to their origin and native habitat. The present results are in agreement with the previous results of DNA marker variation of *M. ciliata* and *M. transsilvanica* (Szczepaniak and Cieślak, 2011) and result variation due to isoenzyme investigation of *M. uniflora* (Angelov, 2015) and *M. nutans* [Tyler, 2002a,b). Contrary to our results, Kumari and Saggoo (2016) reported two morphotypes of *M. persica.* In case of *M. persica*, the populations growing in the hills and plains of Gorgan with more rainfall had highest genetic diversity especially the population growing in Semnan deserts. The populations that grow in Esfahan, Ghom and Taleghan with almost similar climate had high genetic similarity. Also, *M. jacquemontii*, population of Ghom had lowest distance genetically with the population that grows in Taft region. These two regions (Ghom and Taft) have the same climate of cold-dry winter and warm-dry summer. The previous studies on different populations of *M. persica* showed that these were growing in cold desert region of Kinnaur in India (Kumari and Saggoo, 2016), North India (Gohil and Koal, 1986; Gupta *et al*., 2014). There was logical genetic distance between two species of *Melica* studied and *M. jacquemontii* can be considered subspecies of *M. persica*.

Conclusion: All population of two species of *Melica* that grow in Iran are not genetically separate and have significant diversity. The study presents a clear report through karyology of ten populations of *Melica* that are highly widespread in arid and semi-arid areas of Iran. All the populations of *Melica* had 2n = 18 with basic chromosome numbers x = 9. Also, all the studied populations of *Melica* had A, 2A, B, and 2B of symmetry classes of Stebbins. In addition, the values of karyotype were asymmetrically different in *Melica* population. Further, some *Melica* populations had satellites. Karyological and leaf storage protein studies in the population of *M. jacquemontii* showed lowest genetic distance as compared to *M. persica* populations. Based on present study, the ten populations of *Melica* are the approved species of *M. jacquemontii* as subspecies of *M. persica.*

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