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EVALUATION OF GENETIC DIVERSITY IN SOME SPECIES OF WILD HAWTHORNS (*CRATEAGUS* SPP.) IN VARIOUS REGIONS OF IRAN BY MEANS OF MORPHOLOGICAL MARKERS

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ABSTRACT

The genus *Crataegus* known as hawthorns is the largest genus among the *Maloideae* which comprises 265 species. Iran is one of the genetic centers of *Crataegus* and there are more than 22 species found in Iran. The genus *Crataegus* is well distributed in East and North and West as a wild plant, with numerous, inherently variable species and genotypes. To date, hawthorn has been the subject of only a few genetic studies and, therefore, the present study was aimed to evaluate genetic diversity among some genus of hawthorn (*Crataegus* spp. Pajarkova.) for further use to horticultural research program in Iran. Using morphological characteristics in more than 30 different traits in this experiment related to species was studied and measured. The results of cluster analysis showed that the obtained pattern, in which the species were assigned into three separate major groups. At the first group including four species which belong to the *C. szovitsii*, *C. orientalis*, *C. pentagyna* subsp. *pentagyna* and *C. pentagyna* subsp. *pseudomelanocarpa*. The second groups include four species *C. microphylla* var. *microphylla*, *C. microphylla* var. *dolichocarpa*, *C. sakranensis* and *C. curvisepala*. At the next groups included the *C. monogyna* var. *lasiocarpa*, *C. pseudoheterophylla* subsp. *turcomanica*, *C. pseudoheterophylla* subsp. *turkestanica*, *C. songarica*, *C. atrosanguinea*, *C. meyeri*, *C. ambigua*, *C. assadii*, *C. azarolus* var. *pontica*, *C. azarolus* var. *aronia*, *C. pseudoheterophylla* subsp. *pseudoheterophylla* and *C. kurdistanica*.

Keywords: *Crataegus* spp. Pajarkova, Genetic Diversity, Morphological Characteristics, Clusters Analysis

INTRODUCTION

Hawthorn belongs to the family Rosaceae, and the genus *Crataegus*. Approximately its 250 species have been recorded in the world. The genus *Crataegus* is believed to have originated from northern temperate regions (Phipps *et al.*, 2003). They are found in the northern temperate regions of the Old and New World (Phipps and Muniyamma 1980, Christensen 1992, Mabberley 1997). In the Old World, four sections with ca 60 taxa are known from Europe and Asia (Phipps and Muniyamma 1980, Christensen 1992, Gu and Spongberg 2003). According to Lo *et al.*, (2007), molecular analyses of North American species showed that the genus is monophyletic. The species range from small shrubs to trees and are widely distributed in western Asia, North America, and Europe. Besides its wide horticultural use as an ornamental, it is considered one of the oldest pharmaceutical plants of the western world and is described in various pharmacopoeias (Ferrazzini *et al.*, 2008). It is not easy to separate into species due to individual variations of hawthorn plants (Kadir and Yilmaz, 2010). The long, straight or slightly curved, branches with long, sharp thorns are distinctive characteristics (Ozcan *et al.*, 2005). The leaves can vary in shape, some are slightly or deeply lobed, and others have not any sign of lobes. The ripe fruits are small, and their colors can be yellowish, orange, reddish, black and dark blue. Generally, species of *Crataegus* occur on moist, deep, fine-textured soils, often in hedges (for *Crataegus monogyna* see Sparks and Martin 1999, Jones *et al.*, 2001, Croxton and Sprks, 2002), but also in thickets and old forest edges. As the dominant linear feature in the landscape, hedges have an important function as wildlife habitat in farmland (Croxton and

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Sprks 2002). They usually produce large numbers of fruits during late summer and autumn. Many species and hybrids are used for horticultural purposes and street trees (Phipps *et al.*, 2003). Hawthorn has long been used as an herbal medicine to treat heart failure and cardiovascular disease all around the world (Salehi *et al.*, 2009). Hawthorn can also be used as a rootstock in the practice of grafting. It is graft-compatible with *Mespilus* (medlar), and with pear or quince, and makes a hardier rootstock than quince, but the thorny suckering habit of the hawthorn can be problematic (Phipps *et al.*, 2003). The deeply grown root system and rigid wood made the trees hardy.

On one hand, most of the trees bear weak yields and no pleasant small fruits with low sugar contents (Abdullah, 1988). Hawthorns are native to Iran. Iran is one of the most important diversity centers of wild-grown *Crataegus* species.

It is possible to see wild hawthorn bushes or trees in every regions of Iran. Some hawthorn (*Crataegus* spp.) species are native to mountainous area of Alborz and Zagros of Iran and it is possible to see wild hawthorn species in every regions in semi-arid to the forests of Caspian Sea. Several studies have focused on the genus *Crataegus* including the treatment of the Iranian species (de Candolle 1825, Boissier 1872, Bornm uller and Gauba 1935, 1940, Pojarkova 1939, Parsa 1948, Zohary 1963, 1973, Riedle 1969, Sabeti 1976, Ghahreman 1978, 1987, 1997, 2000, Mobayen 1996, Arjmandi *et al.*, 2009, Donmez 2009, Sharifnia *et al.*, 2011). According to Khatamsaz, 27 taxa representing four sections (Sanguinae Zabel ex Schneider, Pentagynae Schneider, Azaroli Loud and Oxyacanthae Zabel ex Schneider) occur in Iran of which seven taxa are endemic. In the treatment by Christensen (1992), only 13 taxa were accepted from Iran, and thus Khatamsaz revision was overlooked (Christensen and Zielinski 2008).

Many *Crataegus* (Hawthorn) species are polyploids and can reproduce both sexually and apomictically (Lo *et al.*, 2009)). *Crataegus* is a taxonomically difficult genus including several examples of polyploidy, hybridization and clinal variations. Moreover, due to incomplete sampling and low-values, the taxonomy of the hawthorns in Iran is not satisfying and need a more comprehensive evaluation. These, it is important to increase the available knowledge for the species in this genus.

Morphological markers are visible plant traits controlled by Mendelian genes which congregate with genes determining the expression of the trait of interest to allow selection for suitable individuals from a population. Knowledge's regarding hawthorn genetic resources in Iran is limited. This study aims to identify and evaluated of genetic diversity in some species of wild Hawthorn in various regions of Iran by morphological markers for further use to horticultural research programs carried out.

MATERIALS AND METHODS

The present study includes the examination of vast collections of *Crataegus* specimens made aimly by the first author from different regions of Iran over a span of two years. Most localities were visited twice in order to collect material at both flowering and fruiting stages. At first visit, all trees were marked. Habitat, phenology and some important changeable characteristics such as fruit color were recorded in the field Pyrene numbers were counted in about 10 fruits. The species concept of Christensen (1992) was applied for the taxonomic treatment. Moreover, measurement methods for morphometry of morphological characteristics followed Christensen (1992). Plant material of species was collected from different localities shown in table 1.

We reviewed the distribution centers of wild species of hawthorn in the north and east of the Iran, selected some of these centers for our study, and conducted this research for two years (2012-2013). This selection was based on the areas of distribution of the various species in this genus in the provinces of Iran. During spring, summer, and fall, flowering branches, fruit-bearing branches, and fruits were sampled. Using the identification key of the species, more than 30 distinctive morphological characters that had been confirmed by the monographs of the species were measured.

These characters included growth habit and the status of thorns, of hairs on the upper side of leaves, of hairs on the lower side of leaves, and of hairs on the petioles. Some of the other measured characters were leaf blade incisions in flowering branches, number of indentations of the lower edge of leaves in flowering branches, number of flowers in each inflorescence, and open or compact inflorescences.

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Table 1: Localities of 20 species of *Crataegus*

Taxon	Locality	Height
<i>C. pentagyna</i> subsp. <i>pentagyna</i>	Mazandaran, Gyllan and Golestan	645,435,364 m
<i>C. pentagyna</i> subsp. <i>pseudomelanocarpa</i>	Golestan, Bojnord	510,625 m
<i>C. microphylla</i> var. <i>dolichocarpa</i>	Gyllan, Mazandaran and Golestan	631,385,428 m
<i>C. microphylla</i> var. <i>microphylla</i>	Golestan and Gyllan	385,465 m
<i>C. azarolus</i> var. <i>pontica</i>	Bojnord, Mashhad, Kurdistan, Azerbaijan west and east, Kermanshah, Isfahan	1100-1560 m
<i>C. assadii</i>	Kurdistan, Azerbaijan west, Bojnord	1350 m
<i>C. monogyna</i> var. <i>lasiocarpa</i>	Bojnord	1370 m
<i>C. pseudoheterophylla</i> subsp. <i>turcomanica</i>	Bojnord	1280 m
<i>C. pseudoheterophylla</i> subsp. <i>turkestanica</i>	Mashhad and Bojnord, Kurdistan, Azerbaijan west and east	1085-1730 m
<i>C. ambigua</i>	Hamedan, Bojnord, Kurdistan, Azerbaijan west and east	1520 m
<i>C. kurdistanica</i>	Golestan, Kurdistan	785 m
<i>C. songarica</i>	Kerman and Golestan	1105-1980 m
<i>C. meyeri</i>	Bojnord and Mazandaran, Ghazvin	1050-1400 m
<i>C. atrosanguinea</i>	Kerman, Isfahan, fars, Bakhdiyari	2000 m
<i>C. curvisepala</i>	Ghazvin, Kurdistan, Azerbaijan west	1650 m
<i>C. sakranensis</i>	yasuje	1740 m
<i>C. pseudoheterophylla</i> subsp. <i>pseudoheterophylla</i>	Kurdistan, Azerbaijan west, Isfahan	1400 m
<i>C. azarolus</i> var. <i>aronia</i>	Kermanshah, Isfahan, Kurdistan, Azerbaijan west	1200-1350 m
<i>C. szovitsii</i>	Kurdistan and Azerbaijan west	1450 m
<i>C. orientalis</i>	Azerbaijan east	1635 m

So were the status of hairs in inflorescences and at the base of the hypanthium, number of styles, fruit color, color of the upper and the lower surfaces of leaves, leaf blade length, fruit length, and fruit diameter. The ratio of length to diameter of fruit, fruit shape, number of seeds per fruit, stone length, stone diameter, fleshy or dry edible part of fruit, color of the edible part of fruit were also measured and determined. We also measured length to diameter ratio of stones, and the length to diameter ratio of leaf blades and the length of leaf blades in flowering branches. After measuring and determining these morphological characters in the samples, we used the keys in Flora Iranica, Trees, Bushes, and Flora of Iran, volume four, and identified and named monographs of the samples. In all, we measured 30 qualitative and quantitative characters in 60 fresh plant samples with ten replications for each character. The numerical taxonomy method (using the NTSYS software) and the UPGMA method were then employed to perform cluster analysis on the samples.

RESULTS AND DISCUSSION

According to results of cluster analysis of morphological data, the studied species and genotypes were divided into three major groups (Fig. 1). Group (I) includes *C. pentagyna* subsp *pentagyna*, *C. pentagyna* subsp. *pseudomelanocarpa*, *C. szovitsii*, and *C. orientalis*. *C. pentagyna* subsp *pentagyna* and *C. pentagyna* subsp *pseudomelanocarpa* are placed in this group. According to Christensen's classification, all members of the first group belong to the *ser. pentagynae*. Khatamsaz considers the above two species as two independent species. However, Christensen considers them as two subspecies of *C. pentagyna*

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subsp. *pentagyna* and *C. pentagyna* subsp. *pseudomelanocarpa*. Characteristics of these species include: inflorescence with 9-50 flowers without fluff or with cuspidate curved fluffs or distorted wooly fluffs; Bracts length 5-21 times longer than their width and usually entire; black fruit with reddish edible part; 3-5 (-6) seeds. However, *C. szovitsii* and *C.orientalis* belong to ser. *Orientalis*. Group (II) includes *C. sakranensis*, *C.curvisepala*, *C. microphylla* var. *microphylla*, and *C. microphylla* var. *dolichocarpa* that are placed in two series. *C. microphylla* var. *microphylla* and *C. microphylla* var. *dolichocarpa* are placed in ser. *Crataegus* subser. *Crataegus*. In in variety of *C. microphylla* var *microphylla*, fruit is elongated oval and calyx is lax. However, in variety of *C. microphylla* var. *dolichocarpa*, fruit is bottle-like and calyx is a little compressed. Katamsaz and Christensen did not mention the existence of these two varieties in Iran and they consider them equivalent with *C. microphylla*. However, Arjmandi and Sabeti reported two varieties of this species. Results of this study also confirm the existence of two distinct varieties. *C.curvisepala* and *C. sakranensis* belong to ser. *Crataegus* subser. *Erianthae*. All species of this group belong to, ser. *Crataegus*. Important characteristics of *Crataegus* ser. *Crataegus* include: leaf blades of flowering branches with 1-3 (-4) pairs of lips; inflorescence loose, scarcely compressed; bracts diffusive with entire or dentate margin; sepals entire or with 1-6 glandular teeth; fruit red to dark red with yellowish edible part; 1 (-2) seeds with grooved dorsal and lateral-ventral surfaces. Members of group (III) are divided into two major subgroups, each of which is classified into two minor subgroups.

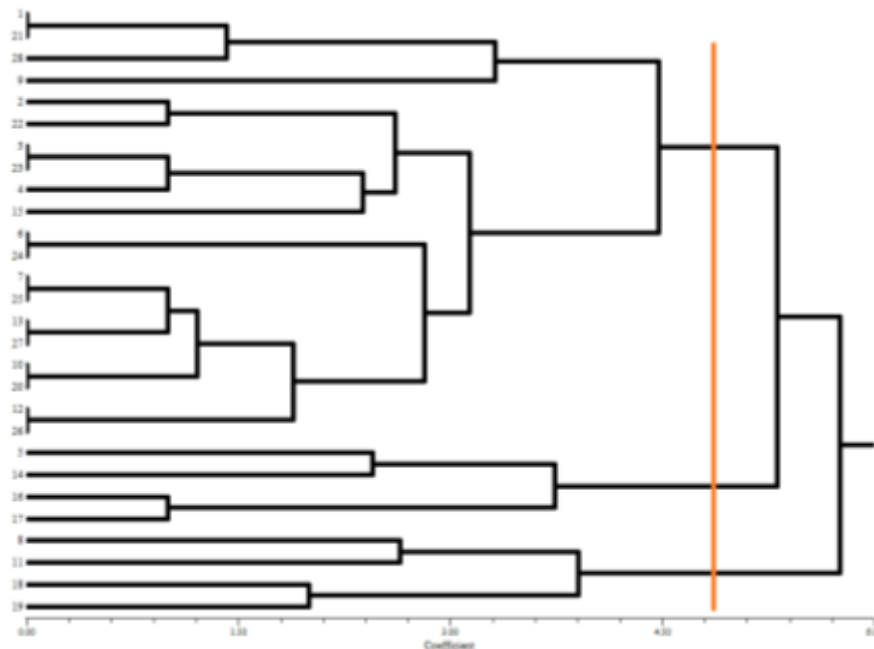


Figure 1: The dendrogram of 28 *Crataegus* spp.(Hawthorn) species based on Morphological Markers analysis using UPGMA



C. azarolus* var. *pontica* *C. pentagyna* subsp. *pentagyna* *C. microphylla* var. *microphylla

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1	<i>C. pseudoheterophylla</i> subsp. <i>turkestanica</i>	15	<i>Crataegus .kurdistanica</i>
2	<i>C. assadii</i>	16	<i>C. microphylla</i> var. <i>dolichocarpa</i>
3	<i>C. azarolus</i> var. <i>pontica</i>	17	<i>C. microphylla</i> var. <i>microphylla</i>
4	<i>C. azarolus</i> var. <i>aronia</i>	18	<i>C. pentagyna</i> subsp. <i>Pentagyna</i>
5	<i>C. curvisepala</i>	19	<i>C. pentagyna</i> subsp. <i>pseudomelanocarpa</i>
6	<i>C. monogyna.</i> var. <i>lasiocarpa</i>	20	<i>C. meyeri</i>
7	<i>C. atrosanguinea</i>	21	<i>C. pseudoheterophylla</i> subsp. <i>turkestanica</i>
8	<i>C. orientalis</i>	22	<i>C. assadii</i>
9	<i>C. pseudoheterophylla</i> subsp. <i>pseudoheterophylla</i>	23	<i>C. azarolus</i> var. <i>pontica</i>
10	<i>C. meyeri</i>	24	<i>C. monogyna.</i> var. <i>lasiocarpa</i>
11	<i>C. szovitsii</i>	25	<i>C. atrosanguinea</i>
12	<i>C. songarica</i>	26	<i>C. songarica</i>
13	<i>C. ambigua</i>	27	<i>C. ambigua</i>
14	<i>C. sakranensis</i>	28	<i>C. pseudoheterophylla</i> subsp. <i>turcomanica</i>

The first minor subgroup is also divided into two smaller subgroups. Four species including *C. Songarica*, *C. atrosanguinea*, *C. meyeri*, and *C. ambigua* are placed in the first minor subgroup. All members of this group belong to *ser. Crataegus subser. Erianthae*. Important characteristics of *ser. Crataegus subser. Erianthae* include: terminal leaf blades of flowering branches with 1-3 (-4) pairs of lips; inflorescence loose and scarcely compressed; bracts diffusive with entire or dentate margin; sepals entire or with 1-2 glandular teeth; fruit with yellowish edible part; 1-2 (-3) or 2-3 (-5) (-1) seeds with grooved dorsal surface and grooved or smooth lateral-ventral surface. *C. monogyna* var. *lasiocarpa* is placed in the second minor subgroup and belongs to *ser. Crataegus subser. Crataegus*. All members of the minor subgroup belong to *ser. Crataegus*. *C. assadii*, *C. azarolus* var. *pontica*, *C. azarolus* var. *aronia* and *C. kurdistanica* are placed in the second minor subgroup. According to Christensen's Classification, *C. azarolus* var. *aronia* and *C. kurdistanica* are placed in *ser. Crataegus subser. Erianthae*. *C. assadii*; and *C. azarolus* var. *pontica*, which compose most of the members of this group, belong to *ser. Orientales*. The important characteristics of this group include: terminal leaf blades of flowering branches with 1-3 (-4) pairs of lips; inflorescence loose and scarcely compressed; bracts diffusive with entire or dentate margin; sepals entire or scarcely with 1-2 glandular teeth; fruit with yellowish edible part; 1 -2 (-3) or 2-3 (-5) (-1) seeds with grooved dorsal surface and grooved or flat lateral-ventral surface. *C. assadii* is not mentioned in this classification. According to *C. assadii* being located beside *C. azarolus* var. *aronia* and the results obtained by Arjmandi(2009), it is recommended that *C. assadii* be placed beside *ser. Orientalis*. Given the considerable distance of *C. assadii* from *C. azarolus* var. *pontica*, this species is considered as an independent species. *C. pseudoheterophylla* subsp. *turkestanica*, *C. pseudoheterophylla* subsp. *turcomanica*, and *C. pseudoheterophylla* subsp. *pseudoheterophylla* are placed in the second sub-group. All of the above species belong to *ser. Crataegus subser. Crataegus*. According to the dendrogram, it can be concluded that the above species are placed in 3 series named *ser. Pentagynae*, *ser. Orientalis*, and *ser. Crataegus* which include two sub-series of *ser. Crataegus* subsp. *Erianthae* and *ser. Crataegus* subsp. *Crataegus*. Separation of series and mostly sub-series are in accordance with Christensen's classification. The Separation of species and taxa are also well-confirmed.

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