

## Investigation of genetic diversity in some Hawthorn (*Crataegus* spp.) species in the North and East of Iran by morphological markers

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### Abstract

Iran is considered as one of the most important centers of genus hawthorn diversity and it is growing wild in the some parts of the country including north to the center and east to west. 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 species of hawthorn (*Crataegus* spp. Pajarkova.) from the north and east Iran carried out. Using morphological characteristics in more than 30 different traits in this experiment related to the genus 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 two species which belong to the ser. *Pentagynae*. The second group included two species *C. microphylla* var. *microphylla* and *C. microphylla* var. *dolichocarpa* At the next groups including species *C. monogyna* var. *lasiocarpa*, *C. pseudoheterophylla* sub sp. *turcomanica*, *C. pseudoheterophylla* subsp. *turkestanica*, *C. songarica*, *C. atrosanguinea*, *C. meyeri*, *C. ambigua*, *C. assadii*, *C. azarolus* var. *pontica* and *C. kurdistanica*.

**Keywords :** *Crataegus* spp., Pojarkova, genetic diversity, morphological characteristics and clusters analysis

### Introduction

Hawthorn (*Crataegus* spp.) ornamentally and medically has a big name in science history. The genus *Crataegus* belongs to the family of Rosaceae and is a mixture of trees and shrubs, which are located in America, Europe and Asia. They grow up to 5 to 15 m tall with small pomes fruit and small sharp tipped branches, typically 1 to 3 cm long. In most species the leaves are either lobed or in serrate margins shape. On long shoots, leaves grow spirally and on spur shoots in clusters. The number of species in the genus varies from hundreds to thousands which is largely dependent on the Taxonomic interpretation (Phipps *et al.*, 2003). Many *Crataegus* (Hawthorn) species are polyploids and can reproduce both sexually and apomictically

(Lo *et al.*, 2009). Usage spectrum of Hawthorn is very wide and is cultivated as edible fruit. The genus is of great importance in nature, and in the vulnerable eco-systems (Ferrazzini *et al.*, 2008).The family Rosaceae is classified into the three subfamilies Spiraeoideae, osoideae and Dryaoideae, and is represented by about 100 genera and 2000– 3000 species in the Northern Hemisphere (Phipps and Muniyamma, 1980; Mabberley 1997; Potter *et al.*, 2007). *Crataegus* L. is a large genus with approximately 200 accepted species in subfamily Spiraeoideae, tribe Pyreae, subtribe Pyrinae (formerly classified in subfamily Maloideae) (Phipps, 1983, Campbell *et al.*, 2007; Donmez, 2007). 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. It is assumed that introgressive hybridization between the different species and successive backcrossing with one of the parental species has resulted in a large number of phenotypically intermediate forms. The general attribution to *Crataegus* of taxonomic complexity has been endlessly repeated (Phipps and Muniyamma, 1980; Phipps *et al.*, 2003) often referred to as the Crataegus-problem. The main center of diversity of *sect. Crataegus* lies in the region between Turkey and Iran, and extends to Crimea, neighboring areas of Russia and Caucasia where a second center of diversity of the section is located (Christensen, 1992). Iran has a rich flora with about 8000 taxa (Ghahreman and Attar, 1999) and *Crataegus*, as might be expected, has many species in this large country. These taxa can be found in meadows, along creeks and rivers, in woodlands as well as in broad-leaved deciduous forests in the phytogeographical regions of the Irano - Turanian and Euro - Siberian elements (Hyrcanian forests) (Zohary 1973, Hamzehee *et al.*, 2008). 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. These may include characters such as flower color, seed shape, and pigmentation. Some hawthorn (*Crataegus* spp.) species are native to the 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 the Caspian Sea. Several studies have focused on the genus *Crataegus* including the treatment of the Iranian taxa (de Candolle, 1825; Boissier, 1872; Bornm uller and Gauba, 1935, 1940; Ghahreman, 2000; Arjmandi *et al.* 2009; Donmez, 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). *Crataegus* is a taxonomically difficult genus including several examples of polyploidy, hybridization and clinical variations. The populations of several species are declining in terms of the number of individuals in the examined areas. More-over, due to incomplete sampling and low-values, the taxonomy of the hawthorns in Iran is not satisfying and need a more comprehensive evaluation. Thus, it is important to increase the available knowledge of the species in this genus. Therefore, Iran is rich in genetic resources of hawthorn. Knowledge's regarding hawthorn genetic resources in Iran is limited. In the country, hawthorn genetic resources have not been previously studied. This study aims to identify and evaluated of genetic diversity in some species of wild Hawthorn from north and east Iran by morphological markers for further use for horticultural purposes carried out.

#### Material and methods

The present study includes the examination of vast collections of *Crataegus* specimens made only by the first author from north and east 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 the first visit, all trees were marked. Habitat, phenology and some important changeable characteristics such as fruit color were recorded in the field. Seed numbers were counted in about 10 fruits. The species concept of Christensen (1992) was applied for the taxonomic treatment. More- over, measurement methods for morphometry of

**Table - 1.** Localities of 14 species of *Crataegus* spp.

Taxon	Locality	height
<i>C. pentagyna</i> subsp. <i>pentagyna</i>	Gyllan and Golestan	645,435 m
<i>C. pentagyna</i> subsp. <i>pseudomelanocarpa</i>	Golestan	510 m
<i>C. microphylla</i> var. <i>dolichocarpa</i>	Mazandaran and Golestan	631,385 m
<i>C. microphylla</i> var. <i>microphylla</i>	Golestan and Gyllan	385,465m
<i>C. azarolus</i> var. <i>pontica</i>	Bojnord, Mashhad and Bojnord	11001560 m
<i>C. assadii</i>	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	1085,1730 m
<i>C. ambigua</i>	Bojnord	1520 m
<i>C. Kurdistanica.</i>	Golestan	785 m
<i>C. songarica</i>	Kerman and Golestan	1105,1980 m
<i>C. meyeri</i>	Bojnord and Mazandaran	1400,1050 m
<i>C. atrosanguinea</i>	Kerman	2000 m

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 Kerman, Khorassan Razavi, North Khorassan, Golestan, Mazandaran, and Gilan. 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 on flowering branches, number of flowers in each inflorescence, and open or compact inflorescences. So were the status of hairs in inflorescences and at the

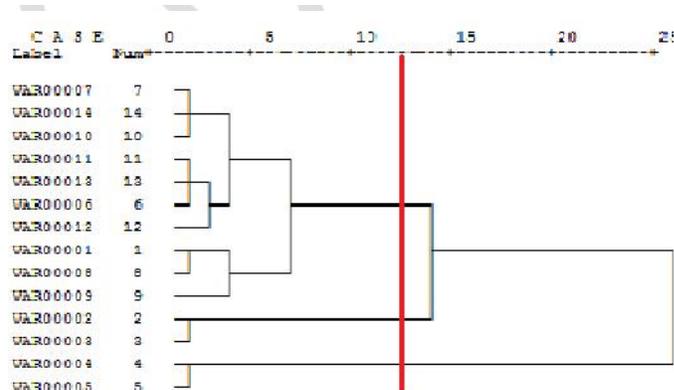


Fig. – 1. Dendrogram obtained from cluster analysis of morphological characters of various species of *Crataegus* spp.

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 blades in flowering branches. After measuring and determining these morphological characters in the samples, we used the keys in Flora Iranica, Trees,



**Fig.- 2a. A Photograph showing leaf and seed length of *C.assadii***



**Fig.- 2b. A Photograph showing leaf and seed length of *C. Pentagyna* subsp. *pentagyna***

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 SPSS software) and the LINKAGE method were then employed to perform cluster analysis on the samples.

### Results and Discussion

Cluster analysis base on morphological traits, results in three groups of genotypes (Fig. - 1). Group (I) included *C. pentagyna* subsp. *pentagyna* and *C. pentagyna* subsp. *Pseudo melanocarpa*. Based on Christensen's classification, all members of the first group belonged to *ser. pentagynae*. Khatamsaz considered *C. pentagyna* subsp. *pentagyna* and *C. pentagyna* subsp. *pseudomelanocarpa* as two independent species, while Christensen considered them as *C. pentagyna* subsp. *pentagyna* and *C. pentagyna* subsp. *Pseudo melanocarpa*. The characteristics of these two species are as follows: inflorescences have with 9-50 flowers, are loose, lack hairs, or have acuminate (or matted wooly) bent hairs; the length of bracts is 5-21 times the width, and they are usually entire; the fruits are black, their edible part is reddish, and they have 3-5 (-6) seeds. Group (II) includes *C. microphylla* var. *microphylla* var. *dolichocarpa*. In the variety microphylla

and *C. microphylla* var. *microphylla*, the fruits are ovate - oblong, the calycle is not compact, but in the variety *C. microphylla* var. *dolichocarpa*, the fruits are bottle-shaped and the calycle is a little compact (Fig.2a & b). Although Khatamsaz and Christensen have not reported these two varieties from Iran, and consider them the same as *C. microphylla*, Riedle and Sabeti have reported two varieties of this species from Iran. The Results of this research also confirm the existence of these two separate varieties. Members of the group (III) are divided into three main subgroups. The first subgroup includes *C. monogyna* var. *lasiocarpa*, *C. pseudoheterophylla* subsp. *turcomanica*, and *C. pseudoheterophylla* subsp. *turkestanica*. Based on Christensen's classification, all members of this group belong to *ser. Crataegus* subser. *Crataegus*. The main characteristics of *Crataegus ser. Crataegus* are leaf blades of flowering branches with 1-3(-4) pairs of lobes, loose inflorescences, rarely compact, non-persistent bracts, the margins entire or indented, sepals entire or with 1-6 glandular indentations. Moreover, the fruits are red to dark-red with the edible parts yellowish, seeds 1(-2), and the dorsal and lateroventral faces are grooved. In the next subgroup, there were four species: *C. songarica*, *C. meyeri*, *C. atrosanguinea*, and *C. ambigua*. Based on Christensen's classification, all the species in this group belong to the *ser. Crataegus*

subser. *Erianthae*. According to the morphological results, members of this subseries have been removed from *ser. Crataegus* subser. *Crataegus* and placed near *ser. Orientalis*. 1-3(-4) pairs of lobes on sub-terminal leaf blades of flowering branches, loose inflorescences, rarely compact, non-persistent bracts, leaf margins entire or indented, entire sepals rarely with 1-2 glandular indentations, yellowish edible part of fruits, (1-) 2-3(-5) seeds, grooved dorsal faces, and latero-ventral faces grooved or smooth are among the main characteristics of *ser. Crataegus* subser. *Erianthae*. In the third subgroup, the species *C. assadii*, *C. azarolus* var. *pontica*, and *C. kurdistanica* were placed in a separate group. Based on Christensen's classification, *C. azarolus* var. *pontica* belongs to *ser. Orientalis*; however, in this classification, *C. assadii* is not mentioned. Since *C. assadii* is placed next to *C. azarolus* var. *pontica*, we suggest that *C. assadii* should be put in *ser. Orientalis*. Moreover, given the considerable distance between *C. assadii* and *C. azarolus* var. *pontica*, this species is an independent one. Branches with more or less matted wooly hairs or slender long hairs, thorns up to 3.5 centimeters long, and the length of petioles in almost terminal leaf blades of flowering branches 0.1-0.4 times the length of the blades are some of the main characteristics of *ser. Orientalis*. So are entire or more or less irregularly serrate stipules 3-15 millimeters long in flowering branches, and 4-25 millimeters long in long branches, and inflorescences with 4-25 flowers, compact, rarely loose, more or less with matted wooly hairs or slender long hairs. Bracts with lengths 2.6-24 times their widths, entire or indented, non-persistent, sepals entire, rarely with 1-2 glandular or non-glandular indentations, and fruits yellow, orange, or red, with the edible part yellowish seeds (1-) 2-5(-6) with dorsal face grooved, and the latero-ventral surface smooth rarely grooved are some of the other main characteristics of *ser. Orientalis*.

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* subser. *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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