COMPARATIVE MICROMORPHOLOGICAL CHARACTERISTICS OF LOWER SURFACE OF LEAF EPIDERMIS AND SEED SURFACE IN TWO SERIES OF CRATAEGUS L. (ROSACEAE) AND THEIR TAXONOMICAL ROLE

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Micromorphological features regarding lower surface of leaf epidermis and seed surface in eight *Crataegus* taxa representing the series *Pentagynae* and series *Crataegus* subseries *Erianthae* were studied by scanning electron microscopy. All data were analyzed using Anova and PCA statistical methods. Qualitative traits of lower surface of leaf epidermis include relief of cuticle membrane, striations and kinds of crystalloid waxes allow distinct delimitation at infrageneric, inter- and infraspecific levels, whereas the quantitative characteristics are less informative. The seed traits compared to the leaf features provide less reliable characters in the taxonomy of the genus.

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Key words: *Crataegus*; ser. *Pentagynae*; subser. *Erianthae*; SEM; Taxonomy; Iran

مقایسه ویژگی‌های ریز ریخت‌شناسی اپیدرم سطح زیرین برگ و سطح دانه در دو سری از جنس .Crataegus L از تیره گلسرخیان (Rosaceae) و نقش تاگزونومیکی آن‌ها

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ویژگی‌های ریز ریخت‌شناسی اپیدرم سطح زیرین برگ و سطح دانه در هشت تاگزون از جنس .Crataegus L مربوط به سری series Pentagynae و سری series Crataegus زیر سری subseries erianthae با استفاده از تصاویر الکترونی (SEM) مورد مطالعه قرار گرفت. تجزیه و تحلیل داده‌ها با استفاده از روش‌های آماری تحلیل واریانس یکطرفه (ANOVA) و تحلیل مولفه‌های اصلی (PCA) انجام شد. صفات کیفی اپیدرم سطح زیرین برگ شامل تزیینات غشای کوتیکولی، نواره بندی و انواع واکس‌های کریستالوئیدی در سطح زیر جنس، بین گونه‌ها و زیر گونه ها جدا کننده بودند در حالی‌که صفات کیفی اهمیت کمتری داشتند. صفات مربوط به سطح دانه نیز اهمیت کمتری نسبت به صفات اپیدرم سطح زیرین برگ در تاگزونومی جنس دارند.

INTRODUCTION

Crataegus L. is a well-known genus, classified under the subtribe *Pyrinae*, tribe *Pyreae*, subfamily Spiraeoideae and family Rosaceae. *Crataegus* is a taxonomically highly complicated genus due to biological phenomena such as apomixis, hybridization, polyploidy and morphological variations. This genus with about 200 accepted species in the world is usually found in temperate regions of Northern hemisphere (Phipps 1983; Dickinson & Phipps 1985; Christensen 1992; Potter & al. 2007). Different ideas have been proposed about classification of infrageneric, inter- and infraspecific levels. Four taxonomic sections have been recognized in the old world, two of which i. e. section *Sanguineae* Schneider and section *Crataegus* L. are present in Iran (Pojarkova 1939; Parsa 1948; Riedl 1969; Phipps 1983; Khatamsaz 1988; 1991; 1992; Christensen 1992; Dönmez 2007; 2009; Christensen & Zielinski 2008; Arjmandi & al. 2009; Sharifnia & al. 2011). Iran with three main phytogeographical regions is one of the largest countries in SW Asia (Hamzeh'ee & al. 2008). Iran and Turkey are the main diversity centers of section *Crataegus* (Christensen 1992). Morphological features are the main tools in the study of classical taxonomy and micromorphological characteristics of SEM micrographs, as the complementary tools, have always been used in taxonomical investigations. Unlike several studies that have been done about taxonomical values of morphological features, karyology, apomixis, polyploidy and molecular surveys about evolutionary history, not many studies have been performed about micromorphological characteristics of *Crataegus* genus (Dickinson & Phipps 1985; Campbell & al. 2007; Gladkova 1968; Talent & Dickinson 2007; Dickinson & al. 2007; Albarouki & Peterson 2007; Lo & al. 2007; 2009; 2010; Nieto-Ángel & al. 2009). The most important micromorphological works on *Crataegus* include studies of epidermal pattern of petals (Christensen 1992; Christensen & Hansen 1998), structure of pyrene surface (Dowidar & al. 2003), pollen morphology (Byatt 1976; Dönmez 2008), characteristics of floral nectaries (Weryszko-Chielewska & al. 2003) and leaf epidermis structure (Ganeva & al. 2009).

The goal of this study was to compare by SEM the most important micromorphological characteristics of seed surface and lower surface of leaf epidermis among eight taxa of *Crataegus* section *Crataegus* series *Pentagynae* (Schneider) Russanov and *Crataegus* section *Crataegus* series *Crataegus* subseries *Erianthae* (Pojarkova) Christensen, in order to clarify taxonomic values of these characteristics in relation to infrageneric, inter -and infraspecific levels. Protective potential of seed and inner parts of the fruits is a characteristic of all plants and therefore these organs have low plasticity (Núñez-Colín& al. 2008). This attempt shows whether variations of quantitative and qualitative characters of seed and leaf epidermis are similar to other morphological characters of *Crataegus* and basically, what values does traits of seed surface have in comparison to micromorphological leaf features. Micromorphological features of different organs have been usually described as qualitative characteristics (Barthlott & al. 1998; Christensen & Hansen 1998; Ganeva & al. 2009).

In this study, under SEM observations, quantitative traits such as length and periphery of epidermal cells, length of stomata, thickness of the outer stomatal rim of the guard cells on the lower surface of leaf epidermis and thickness of external periclinal cell wall on seed surface, have been measured for the first time. Moreover, usefulness of quantitaitive and qualitative traits of seed and leaf epidermis are compared in separation of the taxa.

MATERIALS AND METHODS

This study was made from the authors' own collections and herbaria specimens deposited in TARI and TUH according to Holmgren & Holmgren (1990) (table 1). Seeds were extracted from mature fruits and pyrenes. For SEM (Scanning Electron Microscope), an intact seed and a piece of subterminal leaf of the flowering shoot cut parallel to the main midrib were directly mounted on aluminium stubs with double sided cellotype without any treatment. Coating gold was done by sputtering, Hummer 11, Technics. The micrographs were taken with Scanning Electron Microscope Hitachi, Japan, FESEM 15 KV. Measurements of length and periphery of cells and thickness of periclinal cell wall were randomly obtained from 20 cells and from 13 - 20 cells for length of stomata and the outer stomatal rim of the guard cells seen in SEM x250 scales micrographs by MicroMeasure software ver. 3. 3. Terminology used in sculpturing of seed surface and the type of waxes on the leaf followed orderly Stearn, (1978), Barthlott & al. (1998) and Ganeva & al. (2009). Quantitative data were processed by the statistical variation method (one - way Anova). The mean values of quantitative traits were used for multivariate analyses (PCA), while qualitative characteristics were coded as binary characters i. e. 0 and 1 representative of absence or presence (table 2).

Table 1: Voucher specimens and abbreviations.

Taxa & abbreviations	Locality
Crataegus pentagyna Waldstein & Kitabel ex Willdenow subsp. *pentagyna*1= C. penta1	Gorgan: ca. 5 km. S of Aliabad, 100 m, Assadi & Abouhamzeh 43449 (TARI).
Crataegus pentagyna subsp. *pentagyna* 2 = C. penta 2	Semnan: Fulad Mahaleh, Rudbarak village, N: 36 03 29 E: 53 34 25, 2031 m, Panahi 95162 (TARI).
Crataegus pentagyna Waldstein & Kitabel ex Willdenow subsp. *pseudomelanocarpa* (Pojarkova) Christensen1= C. pesud1	Golestan: Galikosh, 7 km. after Sadeghabad to Gloestan forest, 389m, N: 37 23 E: 55 43, Hamzeh'ee, Mohebi & Rahmanpour 95371 (TARI).
Crataegus pentagyna subsp. *pseudomelanocarpa*2 = C. pesud2	Gorgan: Kurdkuy, between Radkan and Jahan-nama, 1450 m, V. Mozaffarian 77838 (TARI).
Crataegus ambigua Meyer ex Becker subsp. *ambigua*1= C. ambig1	W Azerbaijan: 2 km from Baneh to Sanandaj, Nekerouz routh, Hamzeh'ee, Attar, Alavi 95293 (TARI).
Crataegus ambigua subsp. *ambigua*2 = C. ambig2	Yazd: Taft, Aliabad, Markaz-e Behdasht, Hamzeh'ee, Arabzadeh 95455 (TARI).
Crataegus babakhanloui Khatamsaz1 = C. babak1	Markazi, Karaj-Chalus pass, Aderan, Arangeh, 1700 m, Khatamsaz 47505 (holotype: TARI).
*Crataegus babakhanloui*2 = C. babak2	Tehran: Karaj to Gachsar, Arangeh, 1810 m, Attar, Hamzeh'ee, Zamani, Raei, 40347 (TUH).
Crataegus caucasica Koch = C. cauca	Azerbaijan: Kalaybar, Arasbaran Protected Area, Aynelou forest, Attar, Zamani, Raei, Maleki 40450 (TUH).
Crataegus kurdistanica Hadac& Chrtek1 = C. kurdi1	W Azerbaijan: Sardasht, Mirabad, Molla Allah Cemetery, N: 36 24 44 E: 45 21 55, 1425 m, Panahi 95170 (TARI).
*Crataegus kurdistanica*2 = C. kurdi2	W Azerbaijan: Sardasht to Pyranshahr, 2 km of Shiveh Mardan village, N: 36 52 526 E 45 21 735, 1200 m, Hamzeh'ee, Attar, Alavi 95306 (TARI).
Crataegus meyeri Pojarkova1 = C. meyer1	Ardabil: 3 Km after Lanbar to Givi, 1650 m, Attar, Zamani, Raei, Maleki 40435 (TUH).
*Crataegus meyeri*2 = C. meyer2	Qazvin: Rdubar-e Alamut, before Juladak village, 1648 m, Rashvand 95156 (TARI).
Crataegus songarica Koch1 = C. songa1	Semnan: Turan protected area, Nahar valley at N. foot of Kuh-e Peyghambar, gardens above the village, 1250-1300 m, H. Freitag 13792 (TARI).
*Crataegus songarica*2 = C. songa2	Mazandaran: 12 km. to Baladeh, Valasht, 2000 m, Khatamsaz & Abouhamzeh 47524 (TARI).

Table 2: Comparison of the qualitative characters of seed surface and lower surface of leaf epidermis in studied *Crataegus* species.

Taxa	Level	Seed					Leaf	
		Seed surface	Shape of epidermal cell	Relief of anticlinal cell wall	Cell surface	Sculpturing of cell surface	Cuticle membrane	Crystalloid wax
Crataegus pentagyna subsp. *pentagyna*	series Pentagynae	Reticulate	Elongate / Isodiametric	Raised	Unevenly Convex	Wrinkled	Smooth with sparse striae around stomata	Granules and dense Entire platelets
C. pentagyna subsp. *pseudomelanocarpa*	series Pentagynae	Reticulate	Elongate / Isodiametric	Raised	Unevenly Convex	Wrinkled	Smooth with sparse striae around stomata	Granules and sparse Entire platelets
C. ambigua subsp. *ambigua*	subseries Erianthae	Reticulate	Elongate / Isodiametric	Raised	Unevenly Convex	Wrinkled	Smooth-Rugose with dense striae around stomata	Granules and Rosettes
C. babakhanloui	subseri. Erianthae	Reticulate	Isodiametric	Raised / Depressed	Convex	Coiled	Striate throughout	Granules
C. caucasica	subseries Erianthae	Reticulate	Elongate / Isodiametric	Depressed	Convex	Wrinkled	Striate throughout	Granules and Rosettes
C. kurdistanica	subseries Erianthae	Reticulate	Elongate	Raised	Unevenly Convex	Wrinkled	Smooth-Rugose with sparse striae around stomata	Granules and sparse Entire and Non-entire platelets
C. meyeri	subseries Erianthae	Reticulate	Elongate / Isodiametric	Raised	Unevenly Convex	Wrinkled to Smooth	Striate throughout	Granules
C. songarica	subseries Erianthae	Reticulate	Elongate / Isodiametric	Raised	Unevenly Convex	Wrinkled	Smooth-Rugose with sparse striae around stomata	Granules and sparse Entire and Non-entire platelets

The Single Linkage and Euclidean Distance methods were used as similarity level in cluster analysis of data. All data were analyzed by Minitab software ver. 14.

RESULTS

Based on SEM micrographs, the studied taxa of the genus *Crataegus* have generally similar traits in both leaf and seed epidermis, but have also indicated significant differences among infrageneric, inter -and infraspecific levels.

Quantitative traits

The first two PCA axes, account for ca. 84% of total variance. The most important quantitative traits in PCA axis 1 are related to the seed surface. These characteristics are thickness of periclinal cell wall, length and periphery of cells respectively (table 3). Based on increasing values of above characters, from the left to right of the axis, two taxa of series *Pentagynae* (*C. pentagyna* subsp. *pentagyna* and *C. pentagyna* subsp. *pseudomelanocarpa*) with the lowest values are placed at the beginning of the axis close to *C. babakhanloui* and *C. kurdistanica*1 of subseries *Erianthae*. All these are separated from the other taxa of subseries *Erianthae.* At the end and lower end of PCA axis 1, *C. meyeri* and *C. ambigua* subsp. *ambigua* with the highest eigenvector scores are completely separated from *C. caucasica*, *C. ambigua*2 and *C. songarica*, with medium values, at the center and near the center of the axis (fig. 1). The main traits determining PCA axis 2 with ca. 25% of total variance are the length of stomata and the thickness of the outer stomatal rim of the guard cells (table 3). At the negative part of PCA axis 2, the two subsp. of *C. pentagyna* (Plate 1a-b and d-e) with the lowest values of quantitative traits leaf are separated from the taxa of subseries *Erianthae* with the highest scores. At the end of PCA axis 2, *C. caucasica* (Plate 1m-n) and *C. songarica* (Plate 2d-e) have the highest scores of stomatal length

which gradually decreases (fig. 1). Comparing the average thickness of the external periclinal cell walls (4. 29 ± 1. 526), periphery of cells (133. 30 ± 26. 06), length of cells (45. 30 ± 8. 12), length of stomata (30. 07 ± 3. 41) and thickness of the stomatal rim (2. 73 ± 0. 60) in series *Pentagynae* with these of subser. *Erianthae* (orderly 5. 47 ± 1. 72, 158. 25 ± 34. 32, 57. 01 ± 15. 73, 36. 69 ± 5. 13 and 3. 64 ± 0. 75) have shown significant differences at level 95% (fig. 2).

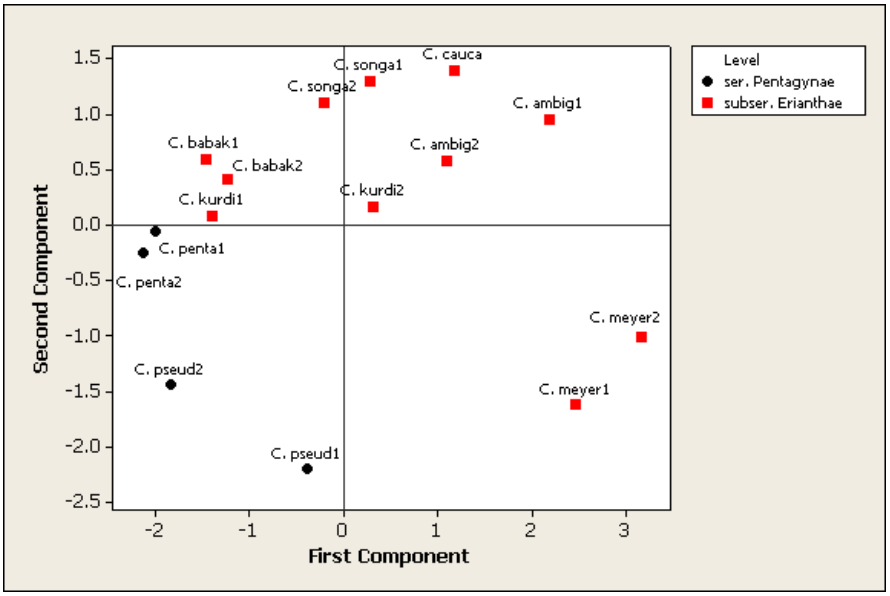

Fig. 1. PCA ordination of eight taxa belonging to two taxonomical levels based on quantitative traits of seed surface and lower epidermis of leaf (see table 1 for full names of taxa).

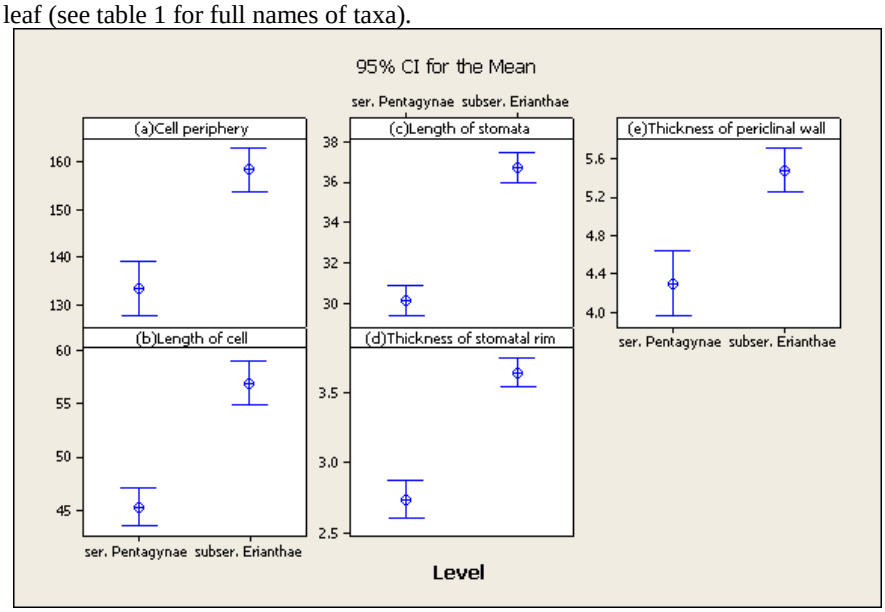

Fig. 2. Comparative mean values of quantitative traits among *Crataegus* of series *Pentagynae* and subseries *Erianthae*. a-b and e = traits of seed surface, c-d = traits of lower leaf epidermis. Meaurments = µm.

Table 3. Eigenvector scores of quantitative traits of seed surface and lower epidermis of leaf on three main PCA axes.

Trait	PCA1 (59%)	PCA2 (24%)	PCA3 (11%)
Thickness of periclinal wall	0. 462	-0. 340	0. 518
Length of cell	0. 541	-0. 178	-0. 284
Periphery of cell	0. 523	-0. 287	-0. 335
Length of stomata	0. 297	0. 688	-0. 429
Tickness of stomatal rim	0. 364	0. 545	0. 596

Full name of traits:
Thickness of periclinal wall = Thickness of external,periclinal cell walls of seed surface.
Length of cell = Length of seed surface cells.
Periphery of cell = Periphery of seed surface cells.
Length of stomata = Length of stomata on lower surface of leaf epidermis.
Thickness of stomatal rim = Thickness of the outer stomatal rim of the guard cells on the lower surface of leaf epidermis.

Qualitative traits

All taxa have cyclocytic stomatal type with round to broadly elliptic and elliptic shape on the surface of lower leaf epidermis (Plate 1a-b, d-e, g-h, j-k, m-n, p-q, Plate 2a-b, d-e). Also, reticulated seed surface with polygonal cells were found in all the representatives (Plates 2 & 3).

The first two PCA axes represented ca. 71% of the total variation in the dataset (table 4). The highest eigenvector scores on PCA axis 1 as well as PCA axis 2 are related to the characteristics of the lower surface of leaf epidermis i. e. striation, kind of wax and sculpturing of cuticle membrane that followed by sculpturing of cell surface of seed (table 4 and fig. 3). PCA axis 1 differentiated between three main groups. First, *C. pentagyna* subsp. *pentagyna* (Plate 1a-b), *C. pentagyna* subsp. *pseudomelanocarpa* (Plate 1d-e), *C. songarica* (Plate 2d-e) and *C. kurdistanica* (Plate 1p-q), at the negative part of the axes, are chracterized with sparse striae around stomata. Second, *C. babakhanloui* (Plate 1j-k), *C. caucasica* (Plate 1m-n) and *C. meyeri* (Plate 2a-b), at the end and lower end of positive part of the axis, are striated throughout the cuticle membrane. Third, *C. ambigua* subsp. *ambigua* with dense striae around stomata (Plate 1g-h), an intermediate state, is placed between two last groups (fig. 4 and table 2). Granular waxes are found on the surface of lower epidermis leaves of all studied taxa (table 2). Smooth cuticle membrane and crystalloid wax – entire platelets separated the taxa of series *Pentagynae* (Plate1 a-f) from the members of subseries *Erianthae* (Plate1 g-r and Plate2 a-f) with smooth-rugose cuticle membrane and kinds of crystalloids waxes. Sparse and dense entire platelets are found on leaf epidermis of subsp. *pentagyna* and subsp. *pseudomelanocarpa* respectively (Plate 1c, f).

Two types of waxes –entire and non entire platelets are found on leaf epidermis of *C. kurdistanica* (Plate 1r) and *C. songarica* (Plate 2. f) that cause to separation them from *C. ambigua* subsp. *ambigua* (Plate 1i) and *C. caucasica* (Plate 1o) with wax rosette (fig. 4). On the cuticle membrane of *C. meyeri* and *C. babakhanloui*, one kind of crystalloid wax are observed –granules (Plate 2c and Plate 1l respectively).

The second and third PCA axes have ca. the same proportions of total variation (table 4). The PCA axis 2 with ca. 14% of the total variation as well as PCA 1 is mainly related to traits of leaf epidermis which followed by seed surface features. Values of eigenvector scores of PCA axis 3 indicated that PCA 3 was mainly driven by traits of seed surface (table 4).

Seed surface of the most taxa have similar traits. However a few diffenrences have been observed in some species of subseries *Erianthae*. *C. babakhanloui* is characterized by isodiametric cell, raised/depressed anticlinal cell wall as well as convex and coiled cell surface (Plate 2m-n). Also, convex cell surface has been found on seed epidermis of *C. caucasica* (Plate 2o-p) which differs from *C. babakhanloui* to having isodiametric/elongate cells, depressed anticlinal cell wall and wrinkled cell surface. Isodiametric/elongate cells, raised anticlinal cell wall, unevenly convex and wrinkled cell surface have been observed on seed surface of two membres of series *Pentagynae* (Plate 2g-j), *C. ambigua* subsp. *ambigua* (Plate 2k-l) and *C. songarica* (Plate 3c). Wrinkled to smooth cell surface belongs to *C. meyeri* (Plate 3b) and elongate cells have only been found in *C. kurdistanica* (Plate 2q-r) (to compare seed surface traits of studied taxa see table 2).

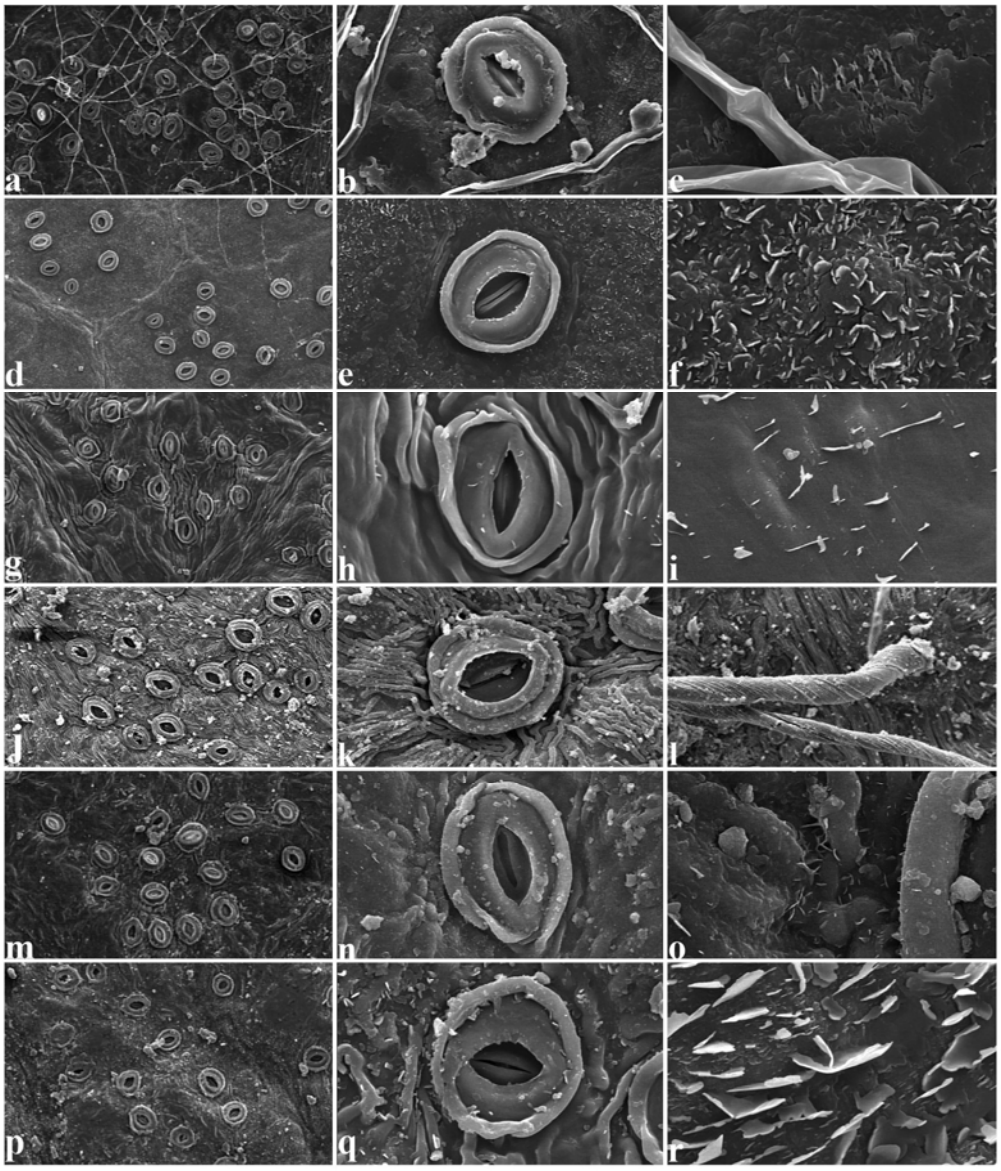

Plate 1. SEM micrographs of lower surface of leaf epidermis in *Crataegus* taxa: a-c, *C. pentagyna* subsp. *pentagyna*; d-f, *C. pentagyna* subsp. *pseudomelanocarpa*; g-i, *C. ambigua* subsp. *ambigua*; j-l, *C. babakhanloui*; m-o, *C. caucasica*; p-r, *C. kurdistanica*. a, d, g, j, m, p: (x 250); b, c, h, k, n, q: (x 1500); e, f, i, l, o, r: (x 5000).

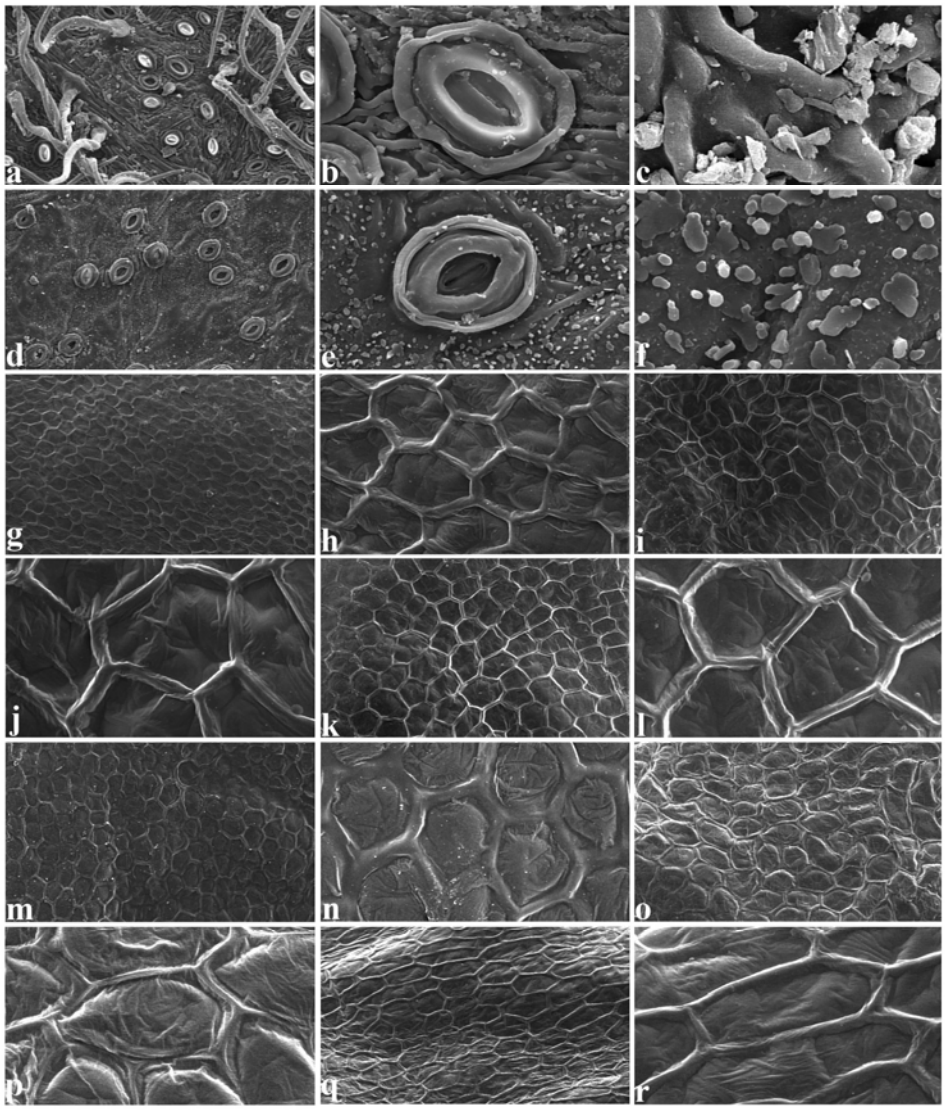

Plate 2. SEM micrographs of lower surface of leaf epidermis and seed surface in *Crataegus* taxa: a-c, *C. meyeri*; d-f, *C. songarica*; g-h, *C. pentagyna* subsp. *pentagyna;* i-j, *C. pentagyna* subsp. *pseudomelanocarpa*; k-l, *C. ambigua* subsp. *ambigua*; m-n, *C. babakhanloui*; o-p, *C. caucasica*; q-r, *C. kurdistanica*. Leaf: a, d: (x 250); b, c: (x 1500); c, f: (x 5000); seed: g, i, k, m, o, q: (x 250); h, j, l, n, p, r: (x 1000).

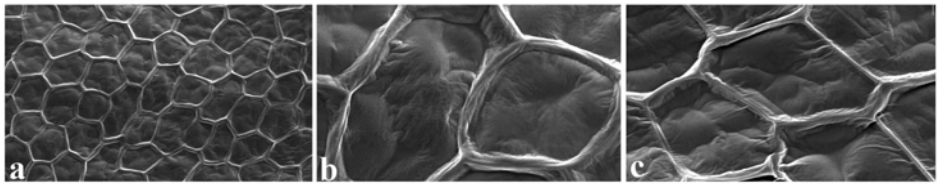

Plate 3. SEM micrographs of seed surface in *Crataegus* species: a-b, *C. meyeri*; c, *C. songarica* a: (x 250); b, c: (x 1000).

Table 4. Eigenvector scores of qualitative traits of leaf and seed epidermis in three main PCA axes (see table 2 for full names of traits).

Trait	PCA1 (57%)	PCA2 (14%)	PCA3 (13%)
Smooth-Rugose	-0. 006	0. 575	0. 265
Smooth	-0. 206	-0. 317	-0. 114
Striation	0. 763	0. 131	-0. 119
Granule	-0. 088	-0. 001	0. 363
Entire platelet	-0. 407	-0. 189	0. 102
Non-entire platelet	-0. 860	0. 306	0. 261
Rosette	0. 080	0. 268	0. 003
Elongate/Isodiametric	-0. 083	0. 097	-0. 418
Isodiametric	0. 125	-0. 256	0. 213
Elongate	-0. 042	0. 160	0. 205
Raised	-0. 051	-0. 061	-0. 063
Depressed	0. 176	-0. 195	0. 275
Unevenly Convex	-0. 176	0. 195	-0. 275
Convex	0. 176	-0. 195	0. 275
Wrinkled	-0. 213	0. 258	0. 150
Smooth- Wrinkled	0. 088	-0. 001	-0. 363
Coiled	0. 125	-0. 256	0. 213

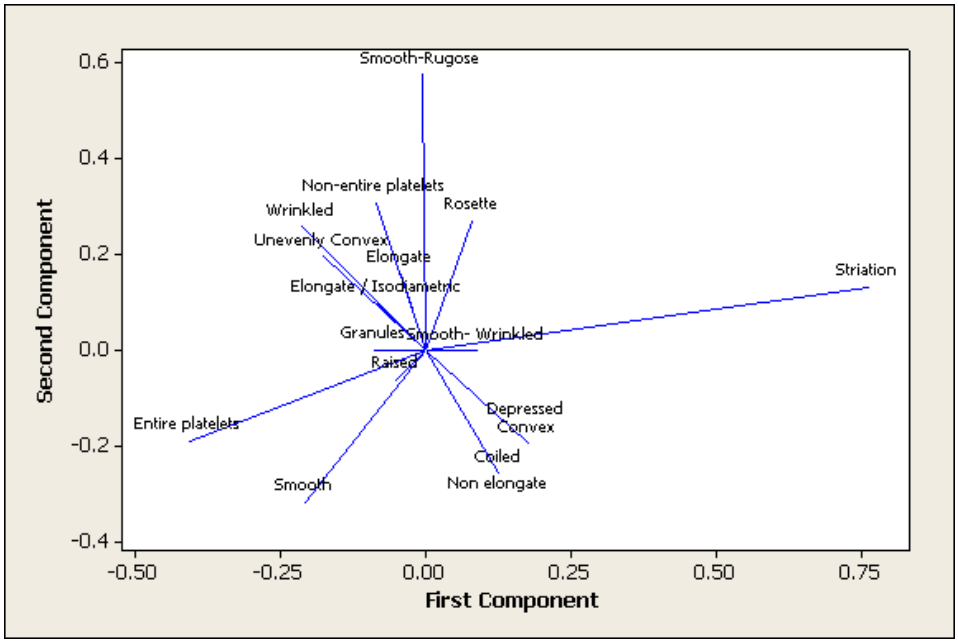

Fig. 3. PCA ordination of qualitative traits of seed surface and lower epidermis of leaf in eight *Crataegus* taxa (see table 2 for full names of traits).

Clustering of all samples based on similarities of qualitative traits of leaf and seed, leads to creation of two major clusters A and B. The cluster A consists of seven subgroups that the two subspecies of *C. pentagyna* have

the highest similarities among other subgroups. Three taxa include *C. ambigua* subsp. *ambigua*, *C. kurdistanica* and *C. songarica*, with ca. the same similarities, are placed adjacent together. Considering the rate of similarities, *C. meyeri* and *C. caucasica* are located between groups A & B. Group B, includes two samples of *C. babakhanloui* completely isolated from all other taxa (fig. 5).

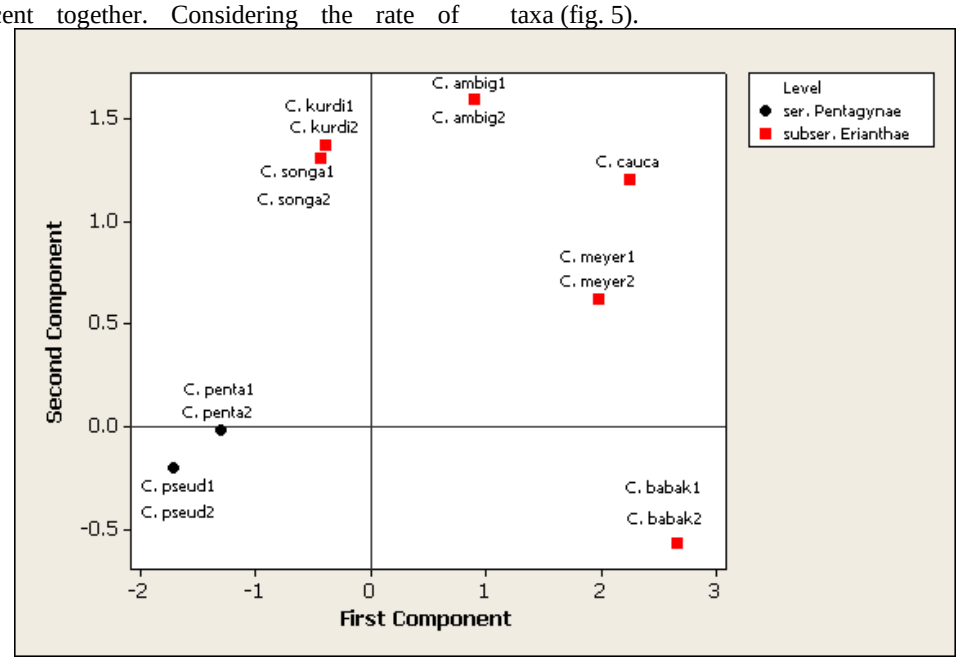

Fig. 4. PCA ordination of eight *Crataegus* taxa based on qualitative traits of seed surface and lower leaf epidermis (see table 1 for full names of taxa).

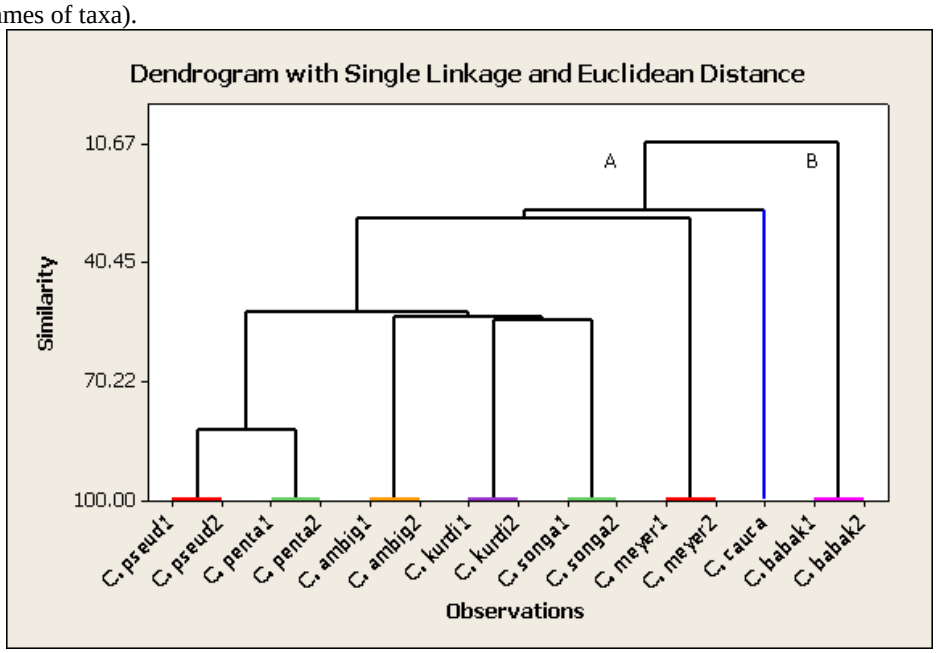

Fig. 5. Dendrogram obtained from the cluster analysis of eight *Crataegus* taxa based on qualitative traits of seed and leaf epidermis (see table 1 for full names of taxa).

DISCUSSION

Our results showed the usefulness of micromorphological characteristics of lower surface of leaf epiderims and seed surface. These characters allowed determination and separation of studied taxa on infrageneric, inter -and infraspecific levels. According to morphological characters and controversial nomenclature as well as species concept used by different authors, we are encountered with three taxonomical groups. These taxonomical groups include section or ser. *Pentagynae* with two species or subspecies, subseries *Erianthae* and section *Sanguineae* Zabel ex Schneider. Two species *C. pentagyna* and *C. pseudomelanocarpa* of section *Pentagynae* (Pojarkova 1939) were reduced to subspecies and series *Pentagynae* by Christensen (1992). All quantitative traits of leaf and seed have shown significant differences, at level 95%, between the *C. pentagyna* group with small sizes and species of subseries. *Erianthae* with larger sizes. All the measured traits of the two subspecies of *C. pentagyna*, except thickness of the outer stomatal rim of the guard cells, also were statistically significant. These differences may be related to different ecological conditions. The two subspecies *pentagyna* and *pseudomelanocarpa* occurred in Hyrcanian forest, from ca. 100 to 2000 meters above sea level, with humid climate, but species of subseries *Erianthae* exist at different altitudes of Irano-Turanian phytogeographical region with semi-arid to dry climate. Qualitative characters of leaf epidermis have taxonomical role in identification of *Crataegus* species (Ganeva & al. 2009). Many kinds of epicuticular wax crystalloids are of great systematic significance in angiosperms (Barthlott & al. 1998). Comparative qualitative characteristics between European and Iranian *C. pentagyna* indicated that lower epidermis of leaves are smooth and covered with wax granules in European taxa (Ganeva & al. 2009). But small entire platelets with different density are also found on leaf epidermis of Iranian taxa.

Clustering of data showed that similarities of the members of series *Pentagynae* are close to some members of subseries *Erianthae*. On the other hand, limited differences between qualitative micromorphological traits of seed and leaf of series *Pentagynae* sound that the use of a series and subspecies levels is more appropriate than sectional and species rank. Among infrageneric levels in *Crataegus* genus, members of series *Crataegus* subseries *Erianthae* have many morphological variations (Christensen 1992). These variations have been observed in micromorphological traits of the studied species. Replicates of different populations of the same species are separated from each other on PCA axes of quantitative characteristics. Moreover, despite existence of similarity 100% between qualitative traits of different replicates, two distinct subgroups include *C. songarica*, *C. kurdistanica* and *C. ambigua* subsp. *ambigua*, with the same similarities, are separated from *C. meyeri* and *C. caucasica*. Khatamsaz (1992) has described *C. babakhanloui* as a new species of section *Sanguineae* Zabel ex Schneider. According to Dönmez (2007), morphological features of *C. babakhanloui* do not fit to section *Sanguineae* and it is close to series *Peshmeniae* Dönmez, also based on Sharifnia & al. (2011) it is related to *C. songarica* of subseries *Erianthae*. Our results showed, unlike, the qualitative characteristics that have been isolated *C. babakhanloui* into a separated cluster, quantitative traits put it near *C. kurdistanica* and *C. pentagyna* on PCA axes. It seems that molecular studies are needed to exact determination the section of *C. babakhanloui*.

The results showed that the quantitative traits of leaf and seed are partially effective in separation of the taxa in infragenenric level. These traits also showed some variations in samples of different ecological conditions. In contrast, the qualitative characteristics are more stable and of great taxonomical values. Also comparison of qualitative traits of leaf and seed epidermis indicated that features of leaf are more valuable in determination of infrageneric, inter and infraspecific levels.

This attempt has clarified some taxonomical ambiguities of two parts of *Crataegus*, section *Crataegus*, series *Pentagynae*, subseries *Erianthae* which will be completed by micromorphological studies of two remaining parts of the genus, i. e. taxa of section *Sanguineae* and *Crataegus* series *Cretageus* subseries *Crataegus* in Iran as the one of the main diversity centers of *Crataegus*.

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REFERENCES

Albarouki, E. & Peterson, A. 2007: Molecular and morphological characterization of *Crataegus* L. species (Rosaceae) in southern Syria. *Botanical Journal of the Linnean Society* 53: 255-263.

Arjmandi, A. A., Nazeri, V., Ejtehadi, H. & Joharchi, M. R. 2009: Revision of the genus *Crataegus* L. in the East and Northeast of Iran. *Rostaniha* 10: 1-36.

Barthlott, W., Neinhuis, C., Cutler, D., Ditsch, F., Meusel, I., Theisen, I. & Wilhelmi, H. 1998: Classification and terminology of plant epicuticular waxes. *Botanical Journal of the Linnean Society* 126: 237-260.

Byatt, J. I. 1976: Pollen morphology of some European species of *Crataegus* L. and *Mespilusgermanica* L. (Rosaceae). *Pollen Spores* 18: 335-349.

Campbell, C. S., Evans, R. C., Morgan, D. R. Dickinson, T. A. & Arsenault, M. P. 2007: Phylogeny of subtribe Pyrinae (formerly the Maloideae, Rosaceae): Limited resolution of a complex evolutionary history. *Plant Systematics and Evolution* 266: 119-145.

Christensen, K. I. 1992: Revision of *Crataegus* sect. *Crataegus* and nothosect. *Crataeguineae* (Rosaceae-Maloideae) in the Old World. *Systematic Botany Monographs* 35: 1-199.

Christensen, K. I. & Hansen, H. V. 1998: SEM studies of epidermal patterns of petals in the angiosperms. *Opera Botanica* 135: 1-91.

Christensen, K. I. & Zielinski, J. 2008: Notes on the genus *Crataegus* (Rosaceae-Pyreae) in southern Europe, the Crimea and western Asia. *Nordic Journal of Botany* 26: 344-360.

Dickinson, T., E. Lo & N. Talent 2007: Polyploidy, Reproductive Biology, and Rosaceae: understanding evolution and making classifications. *Plant Systematics and Evolution* 266: 59-78.

Dickinson, T. A. & Phipps, J. B. 1985: Degree and pattern of variation in *Crataegus* section *Crus-galli* in Ontario. *Systematic Botany* 10: 322-337.

Dönmez, A. A. 2007: Taxonomical notes on the genus *Crataegus* (Rosaceae) in Turkey. *Botanical Journal of the Linnean Society* 155:231-240.

Dönmez, E. O. 2008: Pollen morphology in Turkish *Crataegus* (Rosaceae). *Botanica Helvetica* 118: 59-70.

Dönmez, A. A. 2009: *Crataegus zarrei* (Rosaceae), a new species from Iran. *Annales Botanici Fennici*. 46: 439-442.

Dowidar, A. E., Loutfy, M. H. A., Kamel, E. A., Ahmad, A. H. M. & Hafez, H. H. 2003: Studies on the Rosaceae I-Seed and/or Achene Macro and Micromorphology. *Pakistan Journal of Biological Sciences* 6: 1778-1791.

Gladkova, V. N. 1968: Karyological studies of the genera *Crataegus* L. and *Cotoneaster* Medic. (Maloideae) as related to their taxonomy. *Botanicheskii Zhurnal* 53: 1203-1273.

Ganeva, T. S., Uzunova, K. R. & Koleva, D. 2009: Comparative leaf epidermis investigation in species of genus *Crataegus* L. (Rosaceae) from Bulgaria. *Feddes Repertorium* 120: 169-184.

Hamzeh´ee, B., Naqinezhad, A., Attar, F., Ghahreman, A., Assadi, M. & Prieditis, N. 2008: Phytosociological survey of remnant *Alnus glutinosa* ssp. *barbata* communities in the lowland Caspian forests of northern Iran. *Phytocoenologia* 38: 1-17.

Holmgren, P. K., Holmgren, N. H., & Barnett, L. C. 1990: Index Herbariorum I: The Herbaria of the world, 8th ed. Regnum Veg. 20.

Khatamsaz, M. 1988: Studies on the Rosaceae family in Iran, new taxa and new records. *Iranian Journal of Botany* 4: 111-125.

Khatamsaz, M. 1991: The genus *Crataegus* L. (Rosaceae) in Iran. *Iranian Journal of Botany* 5: 47-56.

Khatamsaz, M. 1992: Rosaceae in Assadi M. & al. (eds.), *Flora of Iran* 6: 241-267. Research Institute of Forests and Rangelands, Tehran (in Persian).

Lo, E., Stefanovic, S. S. & Dickinson, T. A. 2007: Molecular reappraisal of relationships between *Crataegus* and *Mespilus* (Rosaceae, Pyreae) - two genera or one?. *Systematic Botany* 32: 596-616.

Lo, E., Stefanovic, S. S. & Dickinson, T. A. 2010: Reconstructing reticulation history in a phylogenetic framework and the potential of allopatric speciation driven by polyploidy in an agamic complex in *Crataegus* (Rosaceae). *Evolution* 64: 3593-3608.

Lo., E., Stefanovic, S. S., Christensen, K. I. & Dickinson, A. 2009: Evidence for genetic association

between East Asian and western North American *Crataegus* L. (Rosaceae) and rapid divergence of the eastern North American lineages based on multiple DNA sequences. *Molecular Phylogenetics and Evolution Journal* 51: 157-168.

Nieto-Ángel, R., Pérez-Ortega, S., Núñez-Colín, CA., Martinez-Solís, J., Gonzáles-Andrés, F. 2009: Seed and endocarp traits as markers of the biodiversity of regional sources of germplasm of tejocote (*Crataegus* spp.) from Central and Southern Mexico. *Scientia Horticulturae* 121: 166–170.

Núñez-Colín, CA.,Nieto-Ángel, R., Barrientos-Priego, AF., Shahagún-Castellanos, J, Segura, S., Gonzáles-Andrés, F. 2008: Variability of three regional sources of germplasm of Tejocote (*Crataegus* spp.) from central and southern Mexico. *Genetic Resources and Crop Evolution* 55:1159–116.

Parsa, A. 1948: *Crataegus* L. *Flore de l'Iran*, 2: 550-555. Tehran university publication.

Phipps, J. B. 1983: Biogeographic, taxonomic, and cladistic relationships between East Asiatic and North American *Crataegus*. *Annals of the Missouri Botanical Garden* 70: 667-700.

Pojarkova, A. I. 1939: Genus *Crataegus* L. (Rosaceae). In: Komarov, V. l., Yuzepchuk, S. V. (eds.): Flora of the USSR, vol. 9: 416-468. Moscow-Leningrad: (translated from Russian by Israel Program for Scientific Translations, Jerusalem 1971), 317-356.

Potter, D., Erikson, T., Evans, R., Smedmark, J., Morgan, D. K. M., Robertson, K., Arsenault, M. Dickinson, T. & Campbell, C. 2007: Phylogeny and classification of Rosaceae. *Plant Systematics and Evolution* 266: 5-43.

Riedl, H. 1969: *Crataegus* L. in: Rechinger, K. H. (ed.). *Flora Iranica*, Rosaceae 1, 66: 49-65.

Sharifnia, F., Christensen, K. I., Seyedipur, N., Shirdelpur, F. & Mehregan, I. 2011: *Crataegus grossidentata*, sp. nov. (Rosaceae, Pyreae), a new hawthorn from northern Iran. *Nordic Journal of Botany* 29: 534-437.

Stearn, W. T. 1978: Botanical Latin, ed. 2. Newton Abbot. U. K.

Talent, N. & Dickinson, T. A. 2007: Ploidy level increase and decrease in seeds from crosses between sexual diploids and asexual triploids and tetraploids in *Crataegus*L. (Rosaceae, Spiraeoideae, Pyreae). *Canadian Journal of Botany* 85: 570-584.

Weryszko Chmielewska, E., Masierowska, M. L. & Konarska, A. 2003: Characteristics of floral nectaries and nectar in two species of *Crataegus* (Rosaceae). *Plant Systematics and Evolution* 238: 33-41.