

Using micro-morphological fruit characters in resolving some of ambiguities in Iranian *Acer* L. (Sapindaceae) species

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Abstract. Asadi F, Sharifnia F, Salimpour F, Majd A. 2019. Using micro-morphological fruit characters in resolving some of ambiguities in Iranian *Acer* L. (Sapindaceae) species. *Biodiversitas* 20: 297-304. *Acer* L. belongs to the Sapindaceae family consists of 126 species which are distributed in the temperate regions of the northern hemisphere. There are 12 taxa (including *Acer monspessulanum* with four subspecies and *Acer velutinum* with two varieties) in Iran. In this study 21 fruit characteristics are partially investigated using Scanning Electron Microscope (SEM) and Stereomicroscope. According to the obtained results, four types of faveolate, folded, wrinkled and pavement wing surface ornamentations as well as four types of faveolate, folded, verrucous, and wrinkled achene surface ornamentations were observed. Hair of wing margin, achene hair, wing ornamentation, inside angle of wing, and outside angle of wing have taxonomic value. According to fruit morphology, separation of 4 subspecies of *A. monspessulanum* can be verified. Also varieties of *A. velutinum* var. *velutinum*, and *A. velutinum* var. *glabrescens* were totally separated due to differences in characteristics such as wing ornamentation, achene ornamentation, hair density of achene, wing length, wing width, the ratio of length to width of wing, total fruit length, total fruit width, the ratio of length to width of fruit, inside angle of wing, outside angle of wing. The results showed that micro-morphological fruit characters of *Acer* genus are valuable in terms of taxonomy and classification of subspecies level.

Keywords: *Acer*, Iran, micromorphology, subspecies

INTRODUCTION

Sapindaceae including. Aceraceae-Hypocastanaceae with 131-135 genus (Xu et al. 2008; Simpson 2010) is distributed mainly in moderate regions. *Acer* is one of the largest genera of this family and it is distributed in Asia, Europe, North America and North of Africa. At present, approximately 200 maple species are known, half of which occurs in China. Within this genus, polyploid species occur and the number of cultivated species derived from various genotypes of *Acer* in terms of ploidy amounts to over 600 (Van Gelderen et al. 1994; Hasebe et al. 1998). The genus is easily recognized by opposite leaves and samaras fruit. However, other morphological characters are highly diversified. Due to morphological diversity, many varieties and forms were generated that makes the genus taxonomically very difficult (Delendick 1990; Park et al. 1993; Judd et al. 2002). The arrangement of the two and seldom three winged pericarps (samaras) are seen in this genus and they have ranged from subparallel to diverging at about a right angle. A number of characters that are beneficial for identification of species in the field may have evolved independently (lobe shape leaves and margin of leaves lobes). These traits may be highly variable within species like pubescence of lower leaf surface (Grimm et al. 2007). Several different florescence types, including racemes, panicles, corymbs and spikes, occur in this genus. These variations make infrageneric divisions very difficult. Species delimitation and phylogenetic relationships within the genus *Acer* are also very controversial (Kholie 1967;

Judd et al. 2002).

Classification and phylogenetic relations of *Acer* species have always been questionable and different categorizations on sections or series have been proposed (Pax 1902; Pojarkova 1933; Momotani 1962; Fang 1966; Ogata 1967; Murray 1970; De Jong 1976; Tanai 1978; Van Gelderen et al. 2010; Weakley 2011). Fang (1966) proposed a different system in which the genus was divided into two subgenera, mainly on the basis of simple versus compound leaves. In Ogata's system (Ogata 1967), the genus was classified into 26 sections. In 1970, Murray published his monograph of the Aceraceae with 7 subgenera, 24 sections and 35 series within *Acer* (Murray 1970). Ogata's system was essentially followed by Xu (1966), with some additions and amendments. More recently De Jong (1994) recognized only 19 series in 16 sections, providing a quite different arrangement from those of other authors (Pax 1902; Xu 1966, 1998; Ogata 1967; Xu et al. 2008). In recent classifications, *Acer* consists of 16 sections and 126 species (Acevedo-Rodriguez et al. 2011). There are so high hybridization and diversity between *Acer* species that taxonomic identification is difficult (Grimm et al. 2007, 2014). Inter- and intraspecific variability of the group is the main cause of its taxonomic complexity. In addition, with hybridization as a potentially effective phenomenon in the formation of species complexes, it is difficult to identify species of maples (Grimm et al. 2007). Characterizing the new sequence types, Grimm and Denk (2014) showed that cryptic speciation can occur in isolated populations of

maples and reported cryptic species in maples from Georgia. Cytogenetic studies of *Acer*, which has a basic chromosome number of 13 ($x = 13$) and various levels of ploidy (Grimm and Denk 2014), confirms inter- and intraspecific complications in the genus. According to different sources, eight species of *Acer*, i.e., *A. hyrcanum*, *A. cappadocicum*, *A. campestre*, *A. monspessulanum*, *A. platanoides*, *A. velutinum*, *A. mazandaranicum*, and *A. tataricum* are distributed in Iran (Murray 1969; Maroofi and Sharifi 2006; Amini et al. 2008). According to Buerki et al. (2009), these taxa are classified in three sections: Sec. *Acer* (*A. mazandaranicum*, *A. hyrcanum*, *A. velutinum*, *A. monspessulanum*), Sec. *Platanoides* (*A. campestre*, *A. cappadocicum*, *A. platanoides*), and Sec. *Ginalla* (*A. tataricum*). Alborz and Zagros mountains are main centers of this generic distribution in Iran.

A. monspessulanum L. is the unique species that appears a medium-sized deciduous tree or densely branched shrub that grows to a height of 10-15 m (rarely to 20 m) (Fontaine 2011). The trunk is up to 75 cm diameter, with smooth, dark grey bark on young trees, becoming finely fissured on old trees. Among similar maple is easily distinguished by its small three-lobed leaves, 3-6 cm long and 3-7 cm wide, glossy dark green, sometimes a bit leathery, and with a smooth margin, with a 2-5 cm petiole. The leaves fall very late in autumn, typically in November. The flowers are produced in spring, in pendulous, yellow to white corymbs 2-3 cm long. The samaras are 2-3 cm long with rounded nutlets (Rushforth 1999, Van Gelderen 1999). *Acer monspessulanum* from *Acer* section (Van Gelderen et al. 1994) has distinct small, 3-lobed leaves, while its close relatives *A. hyrcanum* and *A. opalus* normally have 5-lobed leaves.

Acer monspessulanum fossils are fairly common in Late Miocene and Pliocene floras from southern Europe and south-western Asia (Kvacek et al. 2002; Sachse 2004). *A. monspessulanum* L. is the unique species that appears a small tree and it is very complicated (incl. *A. assyriacum* Pojark., *A. cinerascens* Boiss., *A. persicum* Pojark. and *A. microphyllum* (Boiss.) Dinsm.). Now, these species are treated as subspecies. Meanwhile, they are very similar to each other. This species has a large number of subspecies and varieties, most of them are not accepted because they are variations affected by environmental conditions (Amini et al. 2008). On the other hand, there are similarities between *A. mazandaranicum* and *A. hyrcanum*, especially in leaves characters. And so, there are some doubts about *A. velutinum* and *A. cappadocicum* varieties. According to Russian flora *Acer velutinum* or velvet maples are endemic of Caucasus (Iran has two varieties) and tall trees with even trunk and broad crown, up to 40 m high, 1.2 m in diameter; bark gray, juvenile shoots reddish-brown, glabrous; leaves up to 30 cm long and wide, averagely 13-15 cm, dark green above, always glabrous, paler beneath, glabrous (var. *glabrescens* Boiss. et Buhse, var. *van volxemii* (Mast.) Pax) or completely velutinous-hairy beneath (var. *velutinum* Boiss. et Buhse), usually cordate, rarely more or less rounded or truncate at base, always 5 lobed, the three upper lobes ovate, acute. The flower panicles are long-pedunculated, erect, dense, up to 5 cm long, with hairy axes

and bracts. The sepals are oblong-ovate; petals narrow, slightly longer. The sepals and petals are hairy inside. The flowers are small, greenish-yellow. The fruiting panicle has almost 60 samaras, wings tapering at the base, then rounded above, diverting at a right angle. Flowering from April to May. Fruiting in August. The trees are found in damp tertiary relict forests, usually as sparse trees, rarely in small groups, growing together up to 1700 m (Pojarkova 1949).

It seems that the micro-morphological characters could be useful for taxonomic separation of close taxa. (Gran and Sharifnia 2008; Sharifnia et al. 2009; Sharifnia and Behzadi Shakib 2012; Bani et al. 2016). Some researchers discussed the infrageneric phylogenetic relationships in the genus by analyzing gross morphology, seed proteins, fossils and geographic distributions, but the conclusions were not in consensus (Momotani 1962; Rechinger 1969; Pax 1985, 1986; Wolfe and Yanai 1987; Thorne 1992).

Variation in fruits characters of *Acer* is crucial for separating the similar taxa. The aim of this study was to investigate micro-morphological data of fruits for evaluation and taxonomic implications of Iranian *Acer* species.

MATERIALS AND METHODS

Samara fruits of 12 samples of *Acer* taxa were investigated here (Table 1). Samples are kept in the herbarium of Islamic Azad University, Tehran North Branch (IAUNT). 21 quantitative and qualitative (bi-state as 0 and 1 or sometimes three state 0, 1 and 2) characteristics of the fruits such as shape of wing ornamentation (SWO), wing ornamentation (WO), shape of achene ornamentation (SAO), presence or absence of openings on achene surface (AS), hair density of achene (HAD), wing angle (WA), outside angle of the wing (OAW), inside angle of the wing (IAW), and ratio of inside angle of the wing to outside angle of the wing (IAW/OAW) (investigated traits in table 2). The characteristics were investigated with BEL stereomicroscope model STMRO-B and as for micro-morphological study, the wing of fruits was stabled on the aluminum Stub and was covered by golden coat after freezing. Samples were observed via Scanning Electron Microscope (SEM) (EM 3200). Clustering analysis was performed according to Unweighted Pair Group Method with Arithmetic (UPGMA) method; the UPGMA algorithm constructs a rooted tree (dendrogram) that reflects the structure present in a pairwise similarity matrix (or a dissimilarity matrix). At each step, the nearest two clusters are combined into a higher-level cluster. The data of characters were standardized before analyzing, finally, factor analysis and principal component analysis (PCA) have been done (Podani 2000).

RESULTS AND DISCUSSION

According to the obtained results, four types of faveolate, folded, wrinkled and pavement wing surface

ornamentations as well as four types of faveolate, folded, verrucous, and wrinkled achene surface ornamentations were observed (Figure 2-3). Based on the results obtained from analyzing 12 samples, the ratio of inside angle of the wing to outside angle of the wing varies from 1 (*A. campestre*) to 4 (*A. monspessulanum* subsp. *Persicum*); fruit length varies from 1.5 cm (*A. monspessulanum* subsp. *microphyllum*, *A. monspessulanum* subsp. *persicum*) to 4.5 cm (*A. velutinum* var. *glabrescens*); the longest wing belonged to *A. velutinum* var. *glabrescens* while the shortest wing belonged to *A. platanoides*; the widest wing was observed in *A. mazandaranicum*. In order to study the fruit, phenotypic similarities cluster analysis showed two major clusters: Cluster A consisted of section *Platanoidae* (*A. platanoides*, *A. campestre*, *A. cappadocicum* var. *stenocarpum*, *A. cappadocicum* var. *cappadocicum*) and cluster B included the species of section *Acer* (*A. mazandaranicum*, *A. hyrcanum*, *A. velutinum* var. *velutinum*, *A. velutinum* var. *glabrescens*, *A. monspessulanum* subsp. *microphyllum*, *A. monspessulanum* subsp. *cinerascens*, *A. monspessulanum* subsp. *assyriacum*, *A. monspessulanum* subsp. *persicum*) (Figure 1)

According to Dendrogram cluster A consist of. *A. platanoides* (*A1*) and *A. campestre*, *A. cappadocicum* var. *cappadocicum*, *A. cappadocicum* var. *stenocarpum* (*A2*). Wing ornamentation arrangement, achene surface arrangement, the ratio of length to width of wing, total fruit length, inside angle of wing, and the ration of inside angle of wing to outside angle of wing are the main characters in them. Also, varieties of *A. cappadocicum* were separated from *A. campestre* in *A2* cluster. At taxonomic distance of 2, which shows that some characteristics such as wing ornamentation, type of wing and achenes ornamentation are important separating of *A. cappadocicum* varieties. Cluster B is divided into two sub-clusters. Sub-cluster *B1* includes *A. mazandaranicum*, *A. hyrcanum*, *A. velutinum* var. *velutinum*, *A. velutinum* var. *glabrescens* and sub-cluster *B2* consists of *A. monspessulanum* subsp. *microphyllum*, *A. monspessulanum* subsp. *cinerascens*, *A. monspessulanum* subsp. *assyriacum*, *A. monspessulanum* subsp. *persicum*.

two groups are showed in *B1*. Some characters such as wing margin hair, wing parallelism, wing angle, achene hair, can be distinguished their taxa. Also, subspecies of *A. monspessulanum* are showed in sub-cluster *B2*. Some characters such as wing surface hair, wing margin hair, achene hair density, wing angle, outside angle of wing, and inside angle of wing can be distinguished their taxa. Factor analysis and Principal Component Analysis (PCA) showed that wing surface hair, wing margin hair, achene hair, and achene hair density are the most variable and characters to separate the taxa (Figure 4).

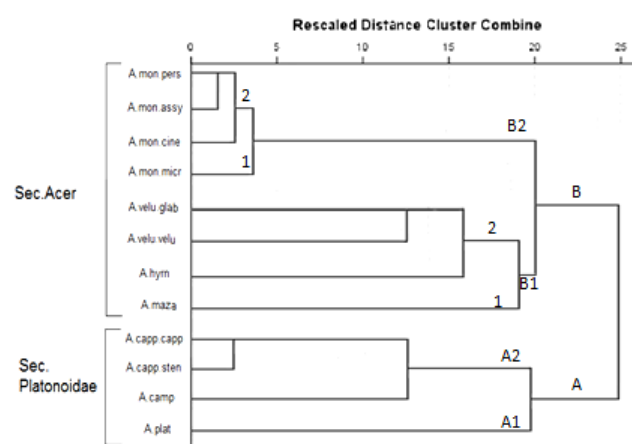


Figure 1. Cluster dendrogram of according to average linkage method based on qualitative and quantitative characteristics. Note: A mon pers= *A. monspessulanum* ssp. *persicum*, A mon assy= *A. monspessulanum* ssp. *assyriacum*, A mon cine= *A. monspessulanum* ssp. *cinerascens*, A mon micr= *A. monspessulanum* ssp. *microphyllum*, A velu glab= *A. velutinum* var. *glabrescens*, A hydr= *A. hyrcanum*, A maza= *A. mazandaranicum*, A capa capp= *A. cappadocicum* var. *cappadocicum*, A capp sten= *A. cappadocicum* var. *stenocarpum*, A camp= *A. campestre*, A plat= *A. platanoides*

Table 1. List of studied taxa of *Acer* with localities and voucher numbers

Specimen	Locality	Alt	N°	E°	Voucher number
<i>A. campestre</i> L.	Gilan, Talesh	1250	48.81	37.82	IAUNT-16110
<i>A. hyrcanum</i> Fisch. & C.A.Mey.	Mazandaran, bisheband	1740	52.40	36.13	IAUNT-16113
<i>A. mazandaranicum</i> Amini, H.Zare & Assadi	Gilan, Asalem to Khalkhal road	900	48.65	37.58	IAUNT-16112
<i>A. cappadocicum</i> var. <i>cappadocicum</i>	Ardabil, Gardane Heyran	420	48.30	38.24	IAUNT-16104
<i>A. cappadocicum</i> var. <i>stenocarpum</i> Yalt.	Gilan, lavandevill	190	48.86	38.30	IAUNT-16109
<i>A. platanoides</i> L.	Gorgan, Paband	2000	53.95	36.48	IAUNT-16126
<i>A. velutinum</i> var. <i>glabrescens</i> (Boiss. & Buhse) A. E. Murray	Ardabil, Hyran pass to Astara road	850	38.27	48.39	IAUNT-16101
<i>A. velutinum</i> var. <i>velutinum</i>	Tehran, Jamshidieh park	1530	51.46	35.82	IAUNT-16103
<i>A. monspessulanum</i> subsp. <i>persicum</i> (Pojark.) Rech.f.	Kerman, Kushk	2400	54.96	29.54	IAUNT-16115
<i>A. monspessulanum</i> subsp. <i>microphyllum</i> (Boiss.) Bornm.	Lorestan, Doroud	2050	49.06	33.49	IAUNT-16119
<i>A. monspessulanum</i> subsp. <i>cinerascens</i> (Boiss.) Yalt.	Lorestan, Doroud	1850	49.07	33.50	IAUNT-16117
<i>A. monspessulanum</i> subsp. <i>assyriacum</i> (Pojark.) Rech.f.	Lorestan, Khorramabad	670	48.18	32.98	IAUNT-16116

Note: SWO: Shape of wing ornamentation, WS: Wing surface, WO: Wing ornamentation, HWS: Hair of wing surface, HWM: Hair of wing margin, WP: Wing parallelism, WT: Wing type, WA: Wing angle, SAO: Shape of achene ornamentation, AS: Achene surface, AH: Achene hair, HDA: Hair density of achene, WL: Wing Length, WW: Wing Width, WL/WW: Ratio of wing length to wing width, FL: Fruit length, FW: Fruit width, FL/FW: Ratio of fruit length to fruit width, IAW: Inside angle of the wing, OAW: Outside angle of the wing, IAW/OAW: Ratio of Inside angle of the wing to outside angle of the wing.

Table 2. Fruit characters of studied taxa

Specimen	S	W	O	WS	WO	HWS	HWM	WP	WT	WA	SAO	AS	AH	HDA	WL _{cm}	WW _{cm}	WL/WW	FL _{cm}	FW _{cm}	FL/FW	IAW gradian	OAW gradian	IAW/ OAW
<i>A. campestre</i>	0	1	1	1	1	1	1	1	0	2	1	1	1	2	1.5	0.5	3	2	1.3	1.5	180	180	1
<i>A. hyrcanum</i>	0	1	1	1	1	0	1	1	1	1	1	1	0	0	2.6	0.5	5.2	3.1	1.2	2.6	102	67	1.5
<i>A. mazandaranicum</i>	0	1	1	1	1	1	0	1	1	2	1	1	1	2	1.5	1	1.5	2	1.5	1.3	20	10	2
<i>A.cappadocicum</i> var. <i>cappadocicum</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	2	1.6	0.6	2.6	2.2	1.2	1.8	103	86	1.4
<i>A. cappadocicum</i> var. <i>stenocarpum</i>	0	1	1	1	1	1	1	1	1	1	0	1	1	2	2.2	0.9	2.4	3.1	2.2	1.4	123	86	1.4
<i>A. platanoides</i>	1	1	0	1	1	1	1	1	1	1	1	0	1	2	1	0.5	2	1.8	1	1.8	100	50	2
<i>A. velutinum</i> var. <i>glabrescens</i>	1	0	1	0	0	1	1	1	1	1	1	1	0	0	4	0.8	5	4.5	1.6	2.8	108	63	1.7
<i>A.velutinum</i> var. <i>velutinum</i>	1	1	0	0	0	1	1	1	1	1	1	1	0	1	2	0.5	4	2.4	1	2.4	112	68	1.6
<i>A. monspessulanum</i> subsp. <i>persicum</i>	0	1	1	1	1	1	0	1	1	2	1	1	1	2	1	0.5	2	1.5	1	1.5	40	10	4
<i>A. monspessulanum</i> subsp. <i>microphyllum</i>	0	1	1	0	0	1	1	1	0	0	1	0	0	0	1.2	0.3	4	1.5	0.5	3	60	25	2.4
<i>A. monspessulanum</i> ssp. <i>cinerascens</i>	0	1	1	1	1	1	1	1	1	0	1	0	1	2	1.7	0.4	4.2	2.2	1	2.2	67.5	25	2.7
<i>A. monspessulanum</i> ssp. <i>assyriacum</i>	0	1	1	1	1	1	1	1	1	0	1	1	1	2	2	0.5	4	3	1.5	2	80	50	1.6

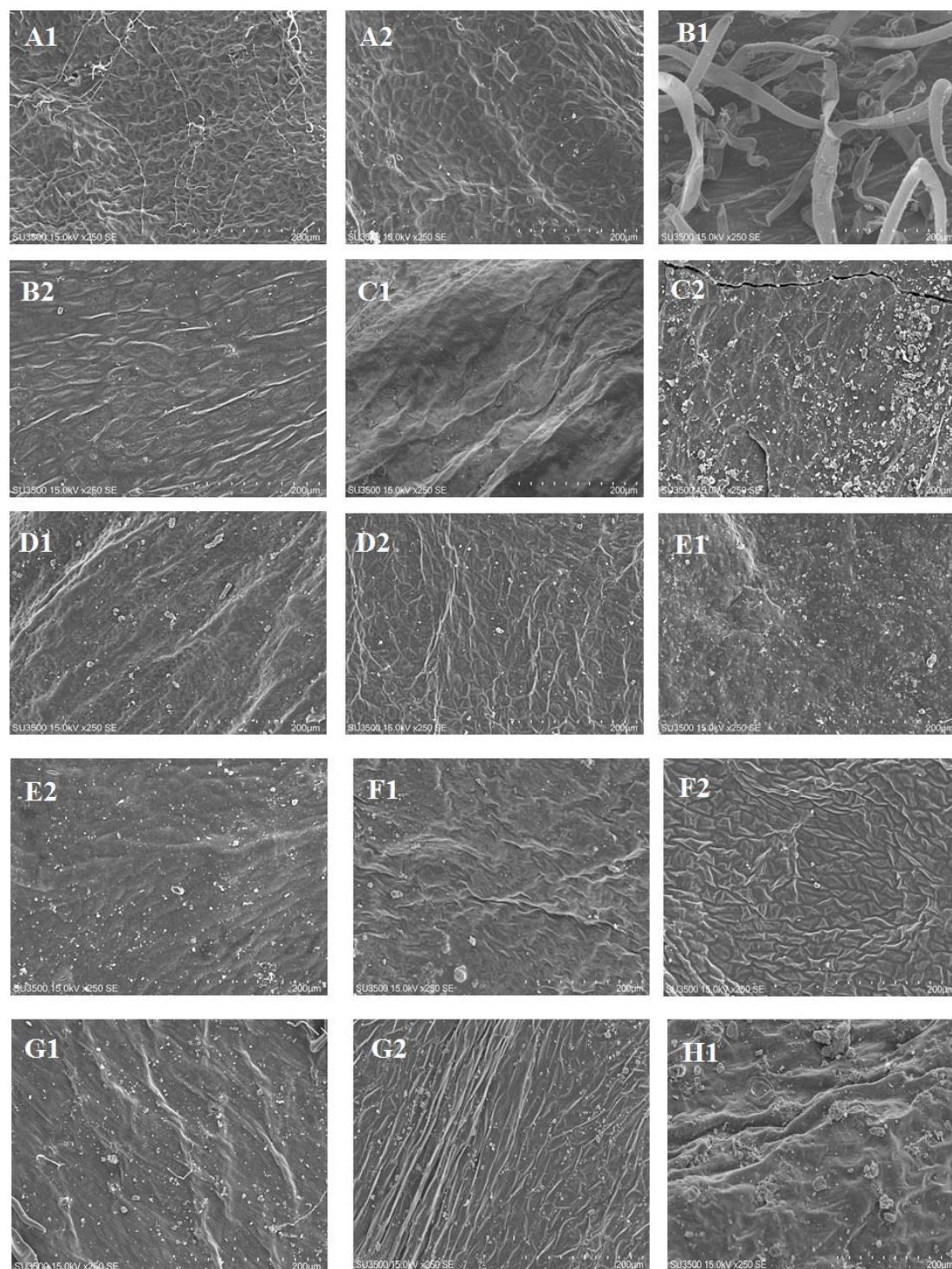


Figure 2. SEM photographs show fruit morphology of the studied species of genus *Acer* L. The first photographs represent the surface ornamentation of the achene and the second ones show the details of the surface ornamentation of the wing. A1-A2: *A. campestre*; B1-B2: *A. hyrcanum*; C1-C2: *A. mazandaranicum*; D1-D2: *A. cappadocicum* var. *cappadocicum*; E1-E2: *A. cappadocicum* var. *stenocarpum*; F1-F2: *A. platanoides*; G1-G2: *A. velutinum* var. *glabrescens*; H1: *A. velutinum* var. *velutinum*

The science of micromorphology is important sometimes for the definition and classification of taxa and their relation to certain groups studies on plants enable micro-level analysis of pollen, leaves, fruit, epidermal patterns, tissues, seeds, etc. of plants via scanning electron microscope. *Acer* is a genus which is taxonomically

diverse due to differences in leaf shape, inflorescence structure, and other morphological characteristics (Delendick 1990). Determination of the limits of *Acer* species is difficult due to differences are fine and continuous. According to the results from pollen morphology (Nikzat Siahkolahi et al. 2017), the varieties

of *A. velutinum* overlapped and the result practically pollen morphology could not be applied in the separation of these two varieties. In this work, the morphology of fruits, showed that these two taxa are separated at higher taxonomic level than variety. Varieties of *A. velutinum* var. *velutinum*, and *A. velutinum* var. *glabrescens* were totally separated due to differences in characteristics such as achene ornamentation (SAO), hair density of achene (HAD), wing length (WL), wing width (WW), the ratio of length to width of wing (WL/WW), inside angle of wing (IAW), outside angle of wing (OAW) (investigated traits in table 3) and this result complied with molecular results obtained for the two varieties of the mentioned species (Asadi et al. not published yet).

Due to similarities between *A. hyrcanum* and *A. mazandaranicum*, there is a necessity to revising their taxonomic positions and considering the possibility that they belong to one taxon. Micro-morphological study of leaf by Mohtashamian et al. in 2017 showed that the structure and shape of epidermis cannot separate *A. mazandaranicum* and *A. hyrcanum* as two species. Therefore, it can be concluded that these two species are very close and *A. mazandaranicum* may be similar to *A. hyrcanum*. But, in this work, the two species were separated due to differences in fruit morphology characters such as hair of margin, wing parallelism, wing angle, achene hair, etc (Table 4).

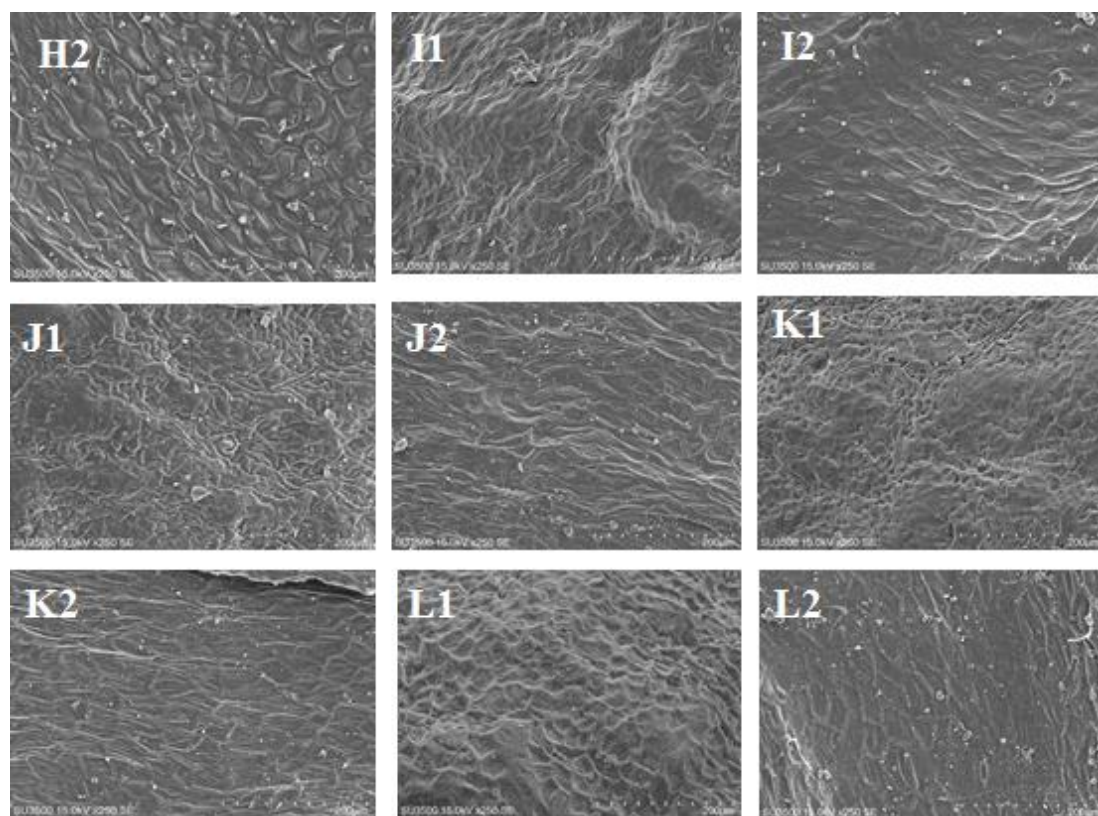


Figure 3. SEM photographs show fruit morphology of the studied species of genus *Acer* L. The first photographs represent the surface ornamentation of the achene and the second ones show the details of the surface ornamentation of the wing. H2: *A. velutinum* var. *velutinum*; I1-I2: *A. monspessulanum* subsp. *persicum*; J1-J2: *A. monspessulanum* subsp. *microphyllum*; K1-K2: *A. monspessulanum* subsp. *cinerascens*; L1-L2: *A. monspessulanum* subsp. *assyriacum*

Table 3. Separating characteristics of *A. velutinum* var. *velutinum* and *A. velutinum* var. *glabrescens*

Specimen	WS	WO	HDA	WL _{cm}	WW _{cm}	WL/WW	FL _{cm}	FW _{cm}	FL/FW	IAW gradian	OAW gradian
<i>A. velutinum</i> var. <i>glabrescens</i>	0	1	0	4	0.8	5	4.5	1.6	2.8	108	63
<i>A. velutinum</i> var. <i>velutinum</i>	1	0	1	2	0.5	4	2.4	1	2.4	112	68

WS: Wing surface, WO: Wing ornamentation, HDA: Hair density of achene, WL: Wing Length, WW: Wing Width, WW/WL: Ratio of wing width to wing length, FL: Fruit length, FW: Fruit width, FW/FL: Ratio of fruit width to fruit length, IAW: Inside angle of the wing, OAW: Outside angle of the wing

Table 4. separating characteristics of *A. mazandaranicum* and *A. hyrcanum*

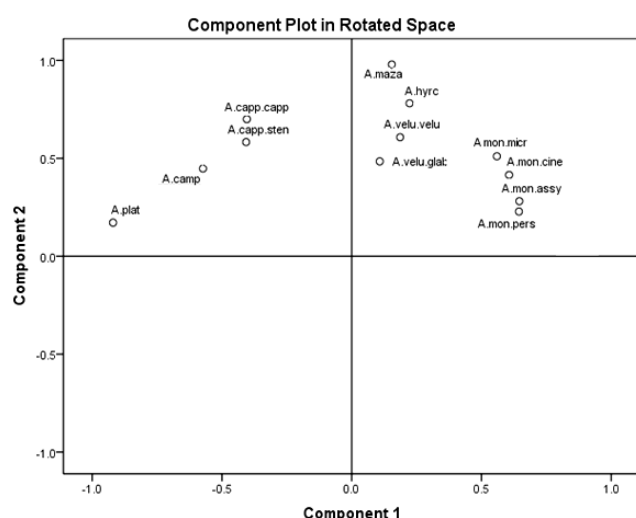
Specimens	HWM	WP	WA	AH	HDA	WL	WW	WL/WW	FL	FW	FL/FW	IAW	OAW	IAW/OAW
						cm	cm		cm	cm		gradian	gradian	OAW
<i>A. hyrcanum</i>	0	1	1	0	0	2.6	0.5	5.2	3.1	1.2	2.6	102	67	1.5
<i>A. mazandaranicum</i>	1	0	2	1	2	1.5	1	1.5	2	1.5	1.3	20	0	2

Note: HWM: Hair of wing margin, WP: Wing parallelism, WA: Wing angle, AH: Achene hair, HDA: Hair density of achene, WL: Wing Length, WW: Wing Width, WL/WW: Ratio of wing length to wing width, FL: Fruit length, FW: Fruit width, FL/FW: Ratio of fruit length to fruit width, IAW: Inside angle of the wing, OAW: Outside angle of the wing, IAW/OAW: Ratio of Inside angle of the wing to outside angle of the wing.

Table 5. Separating characteristics of subspecies of *A. monspessulanum*

Specimen	HWS	HWM	WP	WA	AS	AH	HDA	WL	WL/WW	FL	IAW	OAW	IAW/OAW
								cm		cm	gradian	gradian	OAW
<i>A. monspessulanum</i> subsp. <i>persicum</i>	1	1	0	2	1	1	2	1	2	1.5	40	10	4
<i>A. monspessulanum</i> subsp. <i>microphyllum</i>	0	0	1	0	0	0	0	1.2	4	1.5	60	25	2.4
<i>A. monspessulanum</i> subsp. <i>cinerascens</i>	1	1	1	0	0	1	2	1.7	4.2	2.2	67.5	25	2.7
<i>A. monspessulanum</i> subsp. <i>assyriacum</i>	1	1	1	0	1	1	2	2	4	3	80	50	1.6

Note: HWS: Hair of wing surface, HWM: Hair of wing margin, WP: Wing parallelism, surface, AH: Achene hair, HDA: Hair density of achene, WL: Wing Length, WL/WW: Ratio of wing length to wing width, FL: Fruit length, IAW: Inside angle of the wing, OAW: Outside angle of the wing, IAW/OAW: Ratio of Inside angle of the wing to outside angle of the wing.

**Figure 4.** PCA plot of fruit characters

Four subspecies of *Acer monspessulanum* are very similar together. Khademi et al., 2016 was reported presence or lack of hair on the surface or inside achene are diagnostic features for subspecies level. In this work, micromorphological characters are used for separation of *A. monspessulanum* (*A. subsp. microphyllum*, *A. subsp. assyriacum*, *A. subsp. cinerascens*, and *A. subsp. persicum*) which have always been hard to distinguish. Except for subsp. *microphyllum*, other subspecies are native to Iran and among them subsp. *persicum* is endemic (Amini et al 2008) Micromorphological characteristics of fruit showed that the presence or lack of hair on wing margin (HWM), presence or lack of hair on wing surface (HWS), wing angle (WA), achene hair (AH), inside angle of wing

(IAW), outside angle of wing (OAW), and the ratio of inside angle to outside angle of wing (IAW/OAW) (investigated traits in Table 5) which can separate four subspecies of this species.

In this work, micro-macromorphological characteristics of fruit especially wing ornamentation, hair of wing, hair of achene was found to be very useful for the separation of taxa in *Acer* genus even at subspecies and varieties level.

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