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ACHENE MICROMORPHOLOGY OF SEVEN TAXA OF *ACHILLEA* L. (ASTERACEAE) FROM TURKEY

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Abstract

Micromorphological characters of achenes in seven taxa of Turkish *Achillea* L. (Asteraceae) were investigated using stereomicroscope and scanning electron microscope (SEM). Some morphological descriptions of achenes were given for each species. *A.biserrata* Bieb. has the biggest (0.69±0.092 x 2.01±0.252 mm) and *A. grandiflora* Friv. has the smallest (0.30±0.018 x 1.12±0.058 mm) achenes. The achenes are oblong-lanceolate in *A.biserrata* and *A. teretifolia* Willd. and they are oblong in the remaining taxa. In surface sculpturing, the ornamentation and slime cell distribution varied among the taxa. However, *A. biebersteinii* Afan. has distinct slime cells forming groups scattered over the achene surface. Mature achenes are ribbed and glabrous in all studied taxa. *A. biserrata* has distinct carpopodium structure.

Introduction

The genus *Achillea* L. (Asteraceae) includes about 140 species distributed in South-west Asia and South-eastern Europe (Akyalcın *et al*., 2011). According to recent studies, the genus *Achillea* is represented in Turkey by 48 species (54 taxa), 24 of which are endemic for Anatolia (Akyalcın *et al*., 2011). *Achillea* L. is classified into five sections, namely sect. *Othantus* (Hoffmanns. & Link) Ehrend. & Y. P. Guo (one species), sect. *Babounya* (DC.) O. Hoffm. (30 species), sect. *Ptarmica* (Mill.) W.D.J. Koch (2 species), sect. *Anthemoideae* (DC.) Heimerl (2 species) and sect. *Achillea* (13 species) (Huber-Morath, 1975; Duman, 2000; Arabacı and Yıldız, 2006; Arabacı and Budak, 2009).

Achene micromorphological characters have been found useful in systematics of the family Asteraceae (Abid and Qaiser, 2007a, b; Shekhar *et al.*, 2011). Cypselar external morphology and anatomy in members of different tribes of Asteraceae are found important for delimitation of genera (Garg and Sharma, 2007; Pandey and Kumari, 2007). Abid and Qaiser (2002) studied cypselar morphology of *Dittrichia* Greuter, *Duhaldea* DC., *Inula* L., *Iphiona* Cass. and *Pentanema* Cass. (Asteraceae) from Pakistan and Kashmir and concluded that two distinct groups of taxa can be recognized. It was confirmed that most of the species of *Anaphalis* DC. (Asteraceae) were delimited due to their distinct micromorphological characters of cypsela (Abid and Qaiser, 2007a). Zhu *et al.* (2006) concluded that achene wall anatomy and surface sculpturing of *Lactuca* L. (Asteraceae) and related genera displayed variation within genera.

Slime cells are widespread in higher plants, especially in fruits and/or seeds in different families, *viz.,* Brassicaceae, Euphorbiaceae, Plantaginaceae, Linaceae, Malvaceae and Lamiaceae (Huang *et al*., 2000; Western *et al.,* 2000). Slime cells are usually rectangular and they form ladder-like columns that are elongated in a parallel form to the long axis of the achene. Within the Asteraceae, slime cells have been reported in *Achillea* (Kreitschitz and Valles, 2007; Akcin and

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Akcin, 2010), *Anthemis* L., *Chrysanthemum* L. (Grubert, 1974) and *Artemisia* L. (Huang *et al.*, 2000; Yakovleva *et al*., 2002). It is also suggested that slime plays an important role in controlling of germination, mostly in plants that grow in the condition of water deficiency in arid and semiarid environments (Kreitschitz and Valles, 2007). It can also help fruit or seed dispersal and defence against pathogens (Huang and Gutterman,1999; Huang *et al.*, 2000). Despite several studies on achene micromorphology of *Achillea* were carried out recently (Abid and Qaiser, 2009; Akcin and Akcin, 2010), however, there is very little information on the Turkish taxa. The main aim of the present work is to study the detailed achene morphology of some Turkish taxa belonging to the genus *Achillea* and to find out how useful these characters are in the systematics of the genus *Achillea*.

Materials and Methods

Achenes of *Achillea* L. taxa were studied by stereomicroscope (Leica T1A) and Scanning Electron Microscope (SEM) (JEOL-Neoscope JCM-5000). The studied plant materials were collected from different populations in Turkey. A list of specimens examined is given in Table 1. The specimens have been deposited at the Herbarium of the Department of Biology, University of Ondokuz Mayıs, Turkey (OMUB).

Table 1. List of *Achillea* L. taxa examined along with their vouchers.

Taxon	Vouchers
Sect. ***Achillea*** *s. lat.*	
1. *Achillea biebersteinii* Afan.	A5 Amasya: Vicinity of Amasya Education Faculty, road side, 550 m, 11.6.2010, T. Akcin, OMUB 6446
2. *A. coarctata* Poir.	B5 Kayseri: Kayseri to Avanos, road side, 1100 m, 10.6. 2008, T. Akcin, OMUB 6447
3. *A. grandifolia* Friv.	A6 Samsun: Kocadağ, 1200 m, 20.7.2008, A. Akcin, OMUB 6448
4. *A. millefolium* L. subsp. *millefolium*	A5 Kırşehir: Mucur, 900 m, 21.6.2009, A. Akcin, OMUB 6444
5. *A. millefolium* L. subsp. *pannonica* (Scheele) Hayek	A5 Amasya: Yemişen village, 800 m, 28.7.2010, A. Akcin, OMUB 6445
Sect. ***Babounya*** (DC.) O. Hoffm.	
6. *A. teretifolia* Willd. (endemic)	A5 Amasya: Yemişen village, road side, 750 m, 28.7.2010, T. Akçin, OMUB 6442
Sect. ***Ptarmica*** (Mill.) W.D.J. Koch	
7. *A. biserrata* Bieb.	A6 Samsun: Kocadağ, 1200 m, 14.7.2009, T. Akcin, OMUB 6443

The achenes were examined using a stereomicroscope to ensure size, shape, colour and maturity. In order to determine the average achene sizes, 25 mature achenes were measured. For SEM observations, the mature achenes were placed on stubs using double-sided adhesive tape. Following that, they were observed and photographed with a JEOL-Neoscope JCM-5000 scanning electron microscope. The terminology of achene surface patterns adopted is mainly from Barthlott (1981, 1984), Barthlott *et al.* (1998) and Johnson *et al.* (2004).

Results and Discussion

In the present study, achene morphology of seven taxa belonging to the genus *Achillea* were reported for the first time. Within the examined taxa, *A. teretifolia* is endemic to Turkey (Ekim *et al.*, 2000). Achene shapes were oblong-lanceolate in *A. biserrata* and *A. teretifolia*, oblong in *A. millefolium* subsp. *millefolium*, *A. millefolium* subsp. *pannonica*, *A. coarctata*, *A. biebersteinii* and *A. grandifolia* (Table 2). When achene width and length were considered, different groups were obtained ($P < 0.001$, Table 2). The widest (0.69±0.092 mm) and longest (2.01±0.252 mm) achenes were seen in *A. biserrata* (Table 2). Mature achenes ribbed and glabrous in all studied taxa. Achene colours vary from brown to light brown and yellowish-brown.

The position of carpopodium is almost similar (basal) in all taxa studied. However, carpopodium is with narrow circular ring in *A. teretifolia* (Fig. 1a), *A. millefolium* subsp. *millefolium* (Fig. 1e), *A. millefolium* subsp. *pannonica* (Fig.1h), *A. coarctata* (Fig. 2m), *A. biebersteinii* (Fig. 2j) and *A. grandifolia* (Fig. 2o). On the other hand, *A. biserrata* could be separated from other taxa due to its carpopodium shape (Fig. 1c). Abid and Qaiser (2009) reported that cypselar features may also be utilized for specific delimitation as the species of

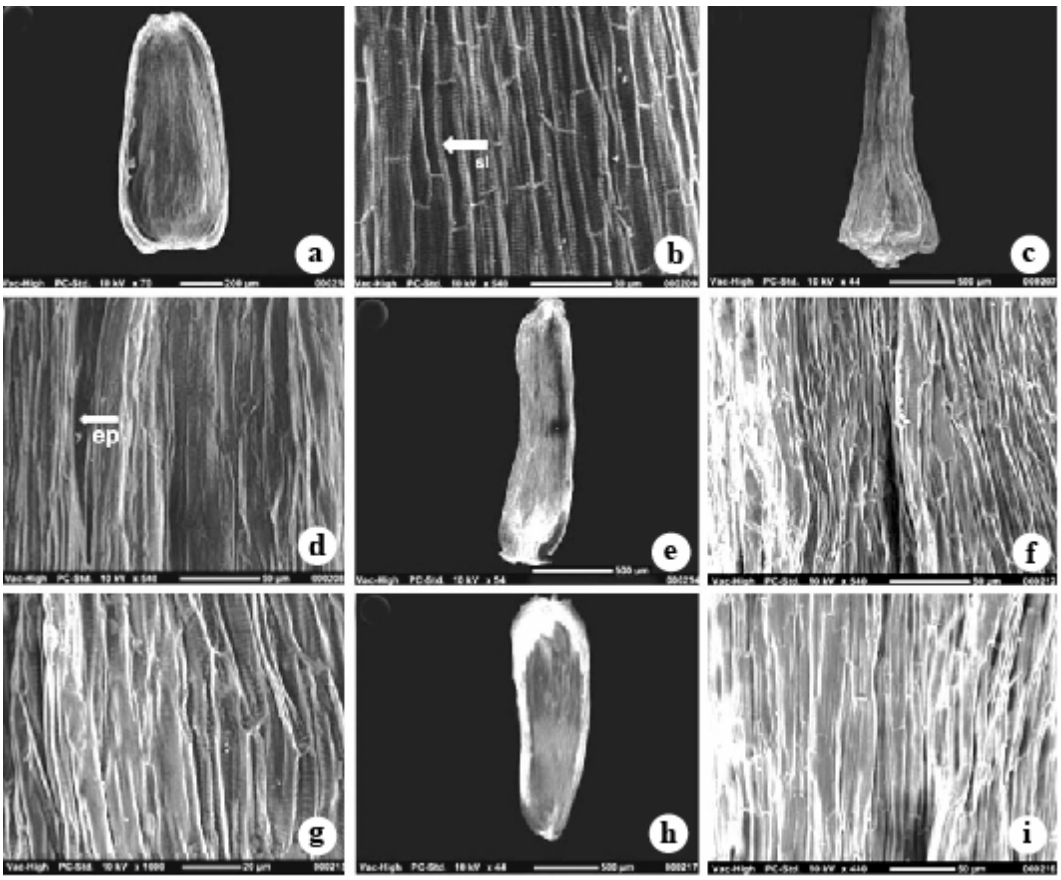

Fig. 1. SEM micrographs of achenes in *Achillea* L. a-b) *A. teretifolia*; c-d) *A. biserrata*; e-g) *A. millefolium* subsp. *millefolium*; h-i) *A. millefolium* subsp. *pannonica*, ep = epidermal cells; sl = slime cells.

Table 2. Achene features of *Achillea* taxa [means in the column the same letter are not significantly different using Tukey's test (P < 0.001), data are given as mean ± sd].

Name of taxa	Achene					Carpopodium		
	Shape	Surface	Colour	Width (mm)	Lenght (mm)	Diameter of Carpopodium (µm)	Diameter of Foramen (µm)	Shape
1. *Achillea biebersteinii*	Oblong	Ribbed, glabrous, slime cells forming groups	Brown	0.54 ± 0.096b	1.24 ± 0.092cd	120.68 ± 4.088c	99.04 ± 7.778	With narrow circular ring
2. *A.biserrata*	Oblong	Ribbed, glabrous, slime cells cover the entire surface	Yellowish-brown	0.69 ± 0.092a	2.01 ± 0.252a	215.93 ± 41.751a	108.66 ± 17.605	Ring lobed
3. *A.coarctata*	Oblong	Ribbed, glabrous, slime cells cover the entire surface	Yellowish-brown	0.54 ± 0.048b	1.30 ± 0.038c	139.07 ± 7.742c	133.81 ± 27.195	With narrow circular ring
4. *A.grandifolia*	Oblong-lanceolate	Indefinite ribbed, glabrous, slime cells alternate with the epidermal cells	Light brown	0.30 ±0 .018c	1.12 ± 0.058d	215.91 ± 9.703a	96.40 ± 9.713	With narrow circular ring
5. *A. millefolium* subsp. *millefolium*	Oblong	Ribbed, glabrous, slime cells alternate with the epidermal cells	Light brown	0.33 ± 0.045c	1.63 ± 0.077b	170.13 ± 11.783b	119.54 ± 4.590	With narrow circular ring
6. *A. millefolium* subsp. *pannonica*	Oblong	Ribbed, glabrous, slime cells alternate with the epidermal cells	Light brown	0.53 ± 0.048b	1.74 ± 0.089b	196.35 ±1 8.799a	122.49 ± 24.227	With narrow circular ring
7. *A. teretifolia*	Oblong-lanceolate	Ribbed, glabrous, slime cells alternate with the epidermal cells	Light brown	0.54 ± 0.024b	1.37 ± 0.063c	134.57 ± 12.769b	86.27 ± 11.000	With narrow circular ring

Achillea are grouped on the basis of ribbed and non-ribbed cypsela. *A. millefolium* was observed to have 10-12 ribbed yellowish-brown cypsela, while non-ribbed cypselas are characteristics for *A. wilhemsii* (Abid and Qaiser, 2009). Similarly, *A. millefolium* as indicated by Abid and Qaiser (2009) seperated from *A. wilhemsii* by the presence of circular ring shaped carpopodium, while *A. wilhemsii* has carpopodium with 4-6 lobed ring. The present investigation also support the observation of Abid and Qaiser (2009). The achenes of *A. millefolium* subsp. *millefolium* and *A. millefolium* subsp. *pannonica* have a circular ring shaped carpopodium and the ribbed surface of achenes (Figs 1 e-i). However, carpopodium diameters of the achenes differ in the taxa ($P< 0.001$, Table 2). The widest carpopodium diameter was present in *A. biserrata* (215.93 ± 41.751 μm) followed by *A. grandifolia* (215.91 ± 9.703 μm), while it was narrow in *A. biebersteinii* (120.68 ± 4.088 μm). The diameters of foramen of carpopodium among the studied taxa were not significant ($P < 0.001$, Table 2).

Slime envelope formation is known in several plant families, including the Asteraceae (Kreitschitz and Valles, 2007). Within the *Achillea* genus, slime has been reported previously (Grubert, 1974; Akçin and Akçin, 2010). Our results confirmed the presence of slime in the investigated *Achillea* taxa. Slime cells are usually rectangular and they form ladder-like columns that are elongated in a parallel form to the long axis of the achene (Figs. 1b, 2k,l,n). Slime cells can either cover almost entire surface of the achene as in *A. biserrata* (Fig. 1d) and *A. coarctata* (Fig. 2n), or alternate with the epidermal cells in *A. millefolium* subsp. *millefolium* (Figs 1f-g), and *A. biebersteinii* (Figs 2k-l). A distinct pattern occurs in *A. biebersteinii* achenes (Figs 2k-l), where slime cells form groups scattered over the achene surface. Slime formation on the fruit and/or seed surface is known to be an ecological adaptation to limited availability of water (Huang and Gutterman, 1999; Huang *et al*., 2000). Several authors showed that the presence of the slime envelope facilitates the adherence of achenes to the soil surface and makes germination easier (Huang *et al*., 2000; Kreitschitz and Valles, 2007).

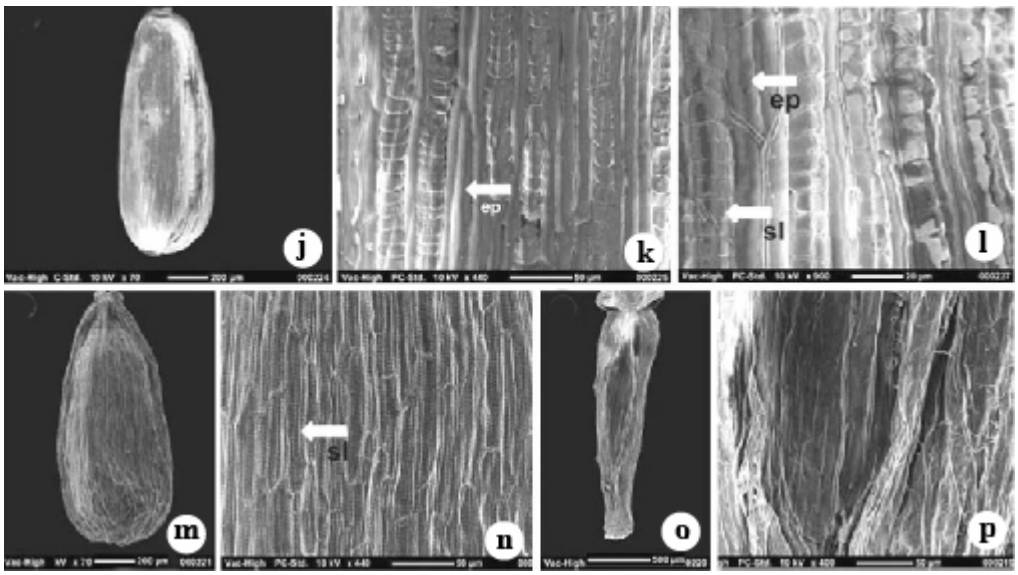

Fig. 2. SEM micrographs of achenes in *Achillea* L. j-l) *A. biebersteinii*; m-n) *A.coarctata*, o-p) *A. grandifolia*, ep = epidermal cells; sl = slime cells.

Taxa such as *A. millefolium* subsp. *millefolium, A. millefolium* subsp. *pannonica*, *A. teretifolia*, *A. coarctata*, *A. biebersteinii* occurring in less fertile and often arid location (Huber-Morath, 1975) are characterized by the presence of numerous strands of slime cells. Slime formation also plays an important role in the control of germination, mostly in plants that grow in the condition of water deficiency in arid and semiarid environments, thus faciliating imbibing and maintenance of the water (Kreitschitz and Valles, 2007).

It was also reported that slime cells in species growing in wet environment such as stream sides and meadows may not mature or function properly (İnceer *et al*., 2012). However, in *A. biserrata* and *A. grandifolia*, often growing around wet environment such as coniferous forest (Huber-Morath, 1975), the presence of a slime envelope may play an important role in dispersal of this species. Huang *et al.* (2000) reported that the slime can reduce the specific weight of diaspores thus facilitating their transportation with water current (hydrochory). In the present study, there are differences in distribution of the slime cells in the examined taxa. Slime cells in *A. teretifolia* similar to *A. coarctata* are usually ladder-like coloumns elongated parallelly to long axis of the achene (Figs 1b, 2n). However, *A. biebersteinii* has a distinct slime cells forming groups scattered over the achene surface (Figs 2k-l). For this reason, these species could be further seperated by the sculpture of achene surface. *A. grandifolia* is separated from the remaining species by having indefinite individual ribs (Fig. 2p). It was also determined that slime cells alternate with epidermal cells in *A. millefolium* (Figs 1f,g,i), while it covers almost the entire surface of achene in *A. biserrata* (Fig. 1d) and *A. coarctata* (Fig. 2n). These results confirmed that slime cells on the fruit surface is associated with ecological adaptation. Akçin and Akçin (2010) reported that achenes of *A. phrygia* have myxogenic cells (slime-producing) almost throughout the entire surface but slime cells are more restricted in *A. gypsicola*. Such a relationship was reported for Lamiaceae (Mosquero *et al.*, 2004), *Artemisia* (Asteraceae) (Kreitschitz and Valles, 2007) and *Tripleurospermum* (İnceer *et al*., 2012).

We can conclude that this work helps to charracterize the taxa of *Achillea* L. Studies employing additional taxa of the genus are necessary which might further contribute to utilization of achene micromorphological characters as significant attributes in classification of the species.

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