

ACHENE ULTRASTRUCTURE IN SOME SPECIES OF *SENECIO* (ASTERACEAE)

*Hatice Nurhan Büyükkartal¹, Hatice Çölgeçen² and Ümit Budak³

¹Department of Biology, Faculty of Science, Ankara University, 06100, Tandoğan, Ankara,

²Department of Biology, Faculty of Science and Arts, Bülent Ecevit University, Zonguldak

³Department of Biology, Faculty of Science and Arts, Bozok University, Yozgat

*Author for Correspondence

ABSTRACT

The genus *Senecio* L. belongs to the tribe Senecioneae Cass. of the family *Asteraceae* (*Compositae*) which is the richest plant family of Turkey in terms of endemic species. The study was conducted on a total of 6 taxa belonging to *Senecio* section (*Senecio eriospermus* DC. subsp. *eriospermus*, *Senecio eriospermus* DC. subsp. *lorentii* (Hochst.) Hamzaoglu & Budak, *Senecio cilicius* Boiss., *Senecio hypochionaeus* Boiss. subsp. *lazicus* (Boiss. & Balansa) Hamzaoglu & Budak, *Senecio hypochionaeus* Boiss. subsp. *hypochionaeus*, *Senecio cariensis* Boiss.). Achene structure was examined by light microscopy and transmission electron microscopy (TEM) in order to identify anatomical and histological modifications and resolve taxonomic problems of the genus. A pericarp differentiated into four main region; the exocarp (outer epidermis), mesocarp, sclerenchyma region and endocarp (inner epidermis). In all the taxa it was observed that there was a thick and darkly stained cuticle layer on the epidermal cells. *Senecio cilicius* Boiss. and *Senecio hypochionaeus* Boiss. subsp. *hypochionaeus*, papillary cuticle was found on the epidermis layer. But in other taxa, papil was not observed on cuticle. *Senecio eriospermus* DC. subsp. *eriospermus* was found in the secretory ducts along the achene wall. In the other 5 taxa, no secretory ducts was observed. In the *Senecio eriospermus* DC. subsp. *eriospermus*, *Senecio eriospermus* DC. subsp. *lorentii* (Hochst.) Hamzaoglu & Budak and *Senecio hypochionaeus* Boiss. subsp. *hypochionaeus* taxa, prismatic shaped crystals were found in mesocarp cells. In other species, crystals were not found in the middle layer or mesocarp cells.

Keywords: *Asteraceae*, *Anatomy*, *Achene*, *Senecio*, *Ultrastructure*

INTRODUCTION

The members of the *Asteraceae* family occupy a wide range of habitat types and are found in almost every area except Antarctica (Heywood, 1978). *Asteraceae* (*Compositae*) is the richest plant family of Turkey in terms of endemic species. The genus *Senecio* L. belongs to the tribe Senecioneae Cass. of the family *Asteraceae* (*Compositae*). Senecioneae is the largest tribe of the *Asteraceae* comprising 150 genera and 3,500 species distributed worldwide (Nordenstam, 2007). The *Senecio*, the largest genus of the tribe, is represented in Turkish flora by 52 taxa (43 species, 3 subspecies and 6 varieties). 21 of these taxa are endemic for Turkey and the rate of endemism is 40%. As a result of taxonomic studies, Turkish *Senecio* species were suggested to be transferred to the genera *Senecio* and *Tephrosieris* (Rchb.) Rchb. (Hamzaoglu et al., 2009).

Many morphological, anatomical (Aguado et al., 2011; Bona, 2014; Özbek et al., 2014; Sosa et al., 2014; Selvi et al., 2014; Karaismailoğlu, 2015; Özcan et al., 2015; Özcan, 2017; Talukdar, 2015; Sukhorukov and Nilova, 2015; Batista et al., 2015; Sourav and Mukherjee, 2017; Candan et al., 2016; Zareh et al., 2016), palynological (Ghahreman et al., 2007; Qureshi et al., 2009; Özmen et al., 2009; Akyalcın et al., 2011), taxonomic and genetical (Frangiote-Pallone and Antonio de Souza, 2014; Karanovic et al., 2016; Ruvimbo and Glynis, 2016) studies have been conducted to identify relationships among *Asteraceae* taxa. Fruit and seed morphological and anatomical features provide much taxonomically useful information, and have been used in *Asteraceae* classification at various levels (Anderberg, 1991; Cron et al., 1993; Pak et al., 2001; Zhu et al., 2006; Inceer et al., 2012; Talukdar, 2013; Özcan, 2017). Similarly, Karaismailoğlu (2015), who investigates the anatomical structure of the fruit in seven taxa of *Crepis* by

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scanning electron microscopy, stated that fruit morphology and anatomy are important in taxonomically in Asteraceae. The fruit of Asteraceae is technically called as cypsela or achene which is developed from bicarpellary, unilocular ovary (Marzinek *et al.*, 2008; Biswas *et al.*, 2014; Özcan, 2017)

This study was conducted on a total of 6 taxa belonging to *Senecio* section seed coat (achene+testa) was examined by light microscopy and transmission electron microscopy (TEM) in order to identify anatomical and histological modifications and contribute to fruit terminology and taxonomic assessment of the genus.

MATERIALS AND METHODS

All plant materials used in the present study were collected from different regions of Turkey (Figure 1A-F). The localities and collection data are listed in table 1. Achene samples per each taxon were first fixed with 3% glutaraldehyde and then with 1% osmium tetroxide. Following dehydration and saturation, samples were embedded in Epon 812 (Luft, 1961). Ultrathin sections were sectioned on a Reichert-OMU ultramicrotome, these ultrathin sections were stained with uranyl acetate (Stempak and Ward, 1964) and lead citrate (Sato, 1968) then examined with Jeol CX-100 Transmission Electron Microscope (TEM) at 80 kV. Semi-thin sections were cut at 1.5 or 2 μ m thickness, stained with methylene blue and toluidin blue and mounted. These preparations were photographed using Leica DMLS2 binocular light microscope with a Leica DFC 320 camera.

RESULTS AND DISCUSSION

Results

A pericarp differentiated into four main region; the exocarp (outer epidermis), mesocarp, sclerenchyma region and endocarp (inner epidermis). Achene of *Senecio Eriospermus* var. *Eriospermus* is circular (Figure 2A). Transverse sections taken from the achene fruit is wavy-walled and oval in shape (Figure 2B). The exocarp is composed of cuticle, thick walled, rectangular of epidermal cells. The cuticle is dark stained. Below the exocarp, there is the mesocarp composed of 3-4 layers of thick-walled sclerenchyma cells (Figure 2C-D). Throughout the achene wall, the secretory ducts are found in the sclerenchyma cells (Figure 2D). Below sclerenchyma cells are mid-layer cells (Figure 2E), some having prismatic crystals. In electron microscopy images, a thick cuticle was detected above epidermal cells (Figure 2F). The schizogenous space is filled by the secretion of phytomelanin which were electron dense. Sclerenchyma cells have thick and electron-opaque walls (Figure 2G). Prismatic crystals are seen in mid-layer cells (Figure 2H). Endosperm was persisted in mature achene and parenchymatous.

In *Senecio Eriospermus* Subsp. *Lorentii* achene fruit (Figure 2I) is wavy-walled and oval in shape. In wavy parts of the exocarp, made up of uniseriately arranged thick walled, parenchymatous cells which develop indents from the outside inward (Figure 2J). Mesocarp made up of continuous four to five layers of sclerenchymatous cells; cells were thick-walled, compactly arranged, variously shaped, with large and round lumen (Figure 2K). The endocarp composed of parenchyma more or less collapsed which contains an elongated calcium oxalate crystal (Figures 2L, O). The schizogenous space is filled by the secretion of phytomelanin. Testa attach with achene wall, made up of crusted layer of uniseriately arranged parenchyma cells. The seed coat or testa is located under the pericarp. Histologically, testal zone has lost its cellular structure and it was represented by a narrow layer of collapsed cells which was located above the endosperm. Ultrathin sections show that sclerenchyma cells have thick walls and lumens are full in some of the cells (Figure 2M). Mid-layer cells have thick walls. Phytomelanin accumulation was detected in some cells (Figure 2N). Prismatic crystals are seen in mid-layer cells (Figure 2O).

In *Senecio Cilicius* achene fruit (Figure 3A) is wavy-walled and oval in shape (Figure 3B). The cuticula layer above epidermal cells in exocarp is dark-stained. Sclerenchyma bundles are observed in undulating sides of the fruit which are conspicuous. Short hairs are seen partly in epidermal cells (exocarp) (Figure 3C). There are mid-layer cells below sclerenchyma bundles (Figure 3D). The schizogenous space is filled by the secretion of phytomelanin. The mesocarp composed of parenchyma more or less collapsed. In electron microscopy images, a thick cuticle was detected above epidermal cells (Figure 3E).

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Sclerenchyma cells are polygonal shaped and thick-walled (Figure 3F). Mid-layer cells have thick walls. Phytomelanin accumulation was detected in some cells (Figure 3G).

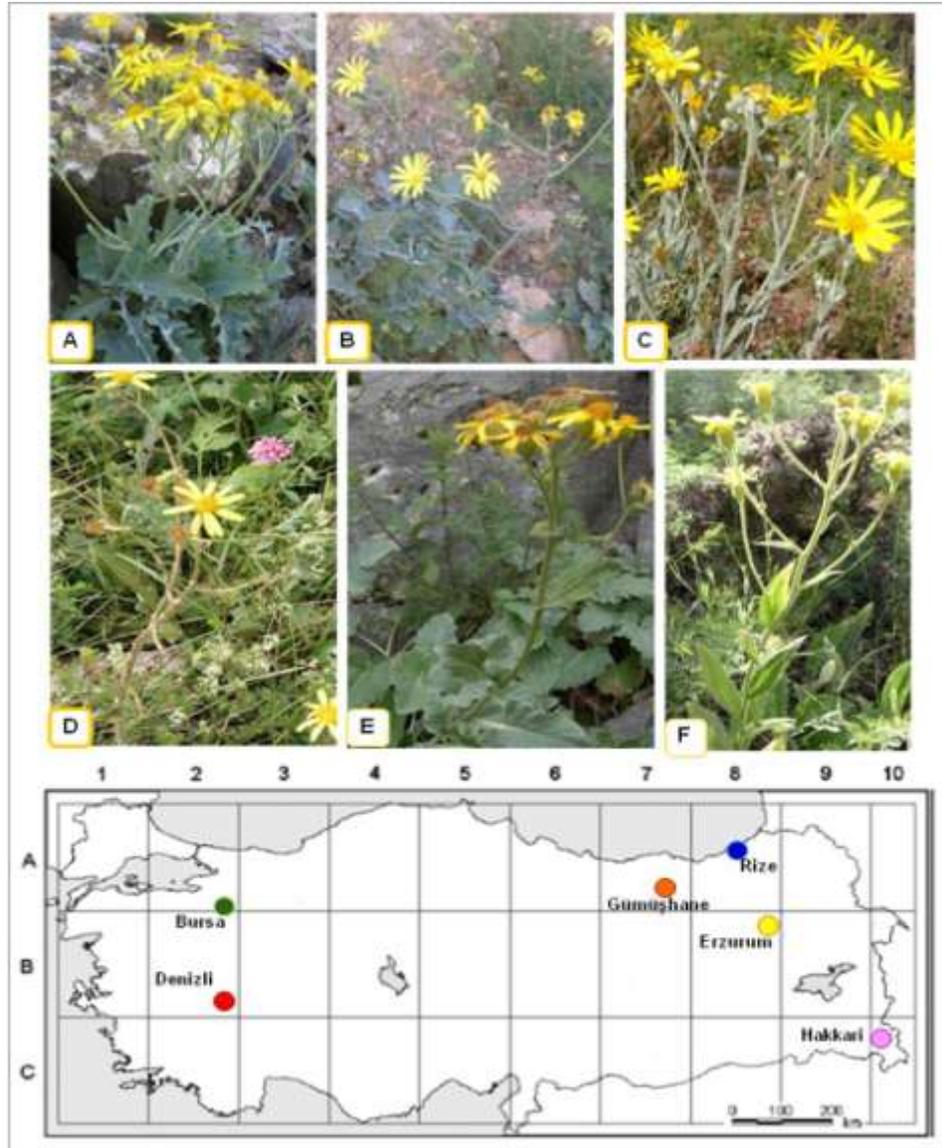


Figure 1: Habitat Views and Collection Localities of Examined Senecio taxa in Turkey. (A) *Senecio Eriospermus* Subsp. *Eriospermus* (●); (B) *Senecio Eriospermus* Subsp. *Lorentii* (●); (C) *Senecio Cilicius* (●); (D) *Senecio Hypochionaeus* Subsp. *Lazicus* (●); (E) *Senecio Hypochionaeus* Subsp. *Hypochionaeus* (●); (F) *Senecio Cariensis* (●)

Achene fruit (Figure 3H) of *Senecio Hypochionaeus* subsp. *Lazicus* made up of uniseriately arranged thick walled, parenchymatous cells and has a dark-stained cuticle above epidermal cells (Figure 3I-J). Mesocarp made up of continuous four to five layers of sclerenchymatous cells which were thick-walled, compactly arranged, variously shaped, with large and round lumen (Figure 3J-K). Mid-layer cells have wavy and thick walls and phytomelanin is detected in some of the cells (Figure 3L).

Achene fruit (Figure 4A) of *S. Hypochionaeus* subsp. *Hypochionaeus* is oval-shaped wavy parts of the fruit (Figure 4B). The cuticle above epidermal cells is thick and dark-stained (Figure 4C). Below exsocarp, mesocarp made up of continuous four to five layers of sclerenchymatous cells; cells were

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thick-walled, compactly arranged, variously shaped, with large and round lumen. Below sclerenchyma cells are mid-layer cells. Testa attach with achene wall, made up of crusted layer of uniseriately arranged parenchyma cells. The inner testa, endosperm cells, surrounding the cotyledons and the embryo, can be observed (Figure 4D).

Endosperm was persisted in mature achene and parenchymatous. Ultrathin sections show that epidermal cells are oval-shaped and the cuticle is dark-stained (Figure 4E). Sclerenchyma cells have narrow lumens (Figure 4F).

Wavy-walled, dark-stained mid-layer cells are detected below sclerenchyma cells (Figure 4G). Phytomelanin accumulation was detected in some cells. Exocarp contains an elongated calcium oxalate crystal (Figure 4H).

Table 1: Examined Taxa of *Senecio* and Collection Localities

No.	Taxon Name	Location	Collector
1	<i>Senecio eriospermus</i> DC. subsp. <i>eriospermus</i>	Hakkari: Between Yüksekova-Dağlıca, 1830 m, 30.06.2006, Budak 2077, Hamzaoğlu & Aksoy (Bozok Hb.).	Budak 2077
2	<i>Senecio eriospermus</i> DC. subsp. <i>lorentii</i> (Hochst.) Hamzaoğlu & Budak	Erzurum: Between Erzurum-İspir, 2270 m, 31.07.2005, Budak 1923, Hamzaoğlu & Aksoy (Bozok Hb.);	Budak 1923
3	<i>Senecio cilicius</i> Boiss.	Gümüşhane: Kelkit, Akdağ village, Kuzuçimeni plateau, 1930 m, 31.07.2005, Budak 1909, Hamzaoğlu & Aksoy (Bozok Hb.);	Budak 1909
4	<i>Senecio hypochionaeus</i> Boiss. subsp. <i>lazicus</i> (Boiss. & Balansa) Hamzaoglu&Budak	Rize: İkizdere, the hills on the southern plateau of Gölyayla village, 2665 m, 02.08.2005, Budak 1945, Hamzaoğlu & Aksoy (Bozok Hb.);	Budak 1945
5	<i>Senecio hypochionaeus</i> Boiss. subsp. <i>hypochionaeus</i>	Bursa: Upper parts of Uludağ Kırkpınar valley are close relatives of akışkan rocks, 2100-2200 m, 29.07.2006, Hamzaoğlu 4391, Aksoy & Budak (Bozok Hb.).	Hamzaoğlu 4391
6	<i>Senecio cariensis</i> Boiss.	Denizli: Honaz Mountain, Honaz exit, Arpacik location, Kayapınarı, 17.07.2005, 1720 m, 23.06.2006, Budak 2070, Hamzaoğlu & Aksoy (Bozok Hb.).	Budak 2070

In *Senecio Cariensis* achene (Figure 4I) fruit is wavy-walled and oval in shape (Figure 4J). Sclerenchyma bundles are observed in undulating sides of the fruit (Figure 4K). The cuticula layer above epidermal cells in exsocarp is very dark-stained.

There are mid-layer cells below sclerenchyma bundles. Sclerenchyma cells are thick-walled and electron-opaque (Figure 4L). Mid-layer cells have wavy and thick walls and phytomelanin is detected in some of the cells (Figure 4M).

Discussion

Our work is the first important study that explains in detail the anatomy and the ultrastructural formation of mature achenes for some *Senecio* taxa by using light microscopy and transmission electron microscopy (TEM). The pericarp is composed of an outer epidermis (exocarp) and an inner epidermis (endocarp). The cells between this two layers form the mesocarp. The mesocarp is mainly composed of parenchyma and sclerenchyma.

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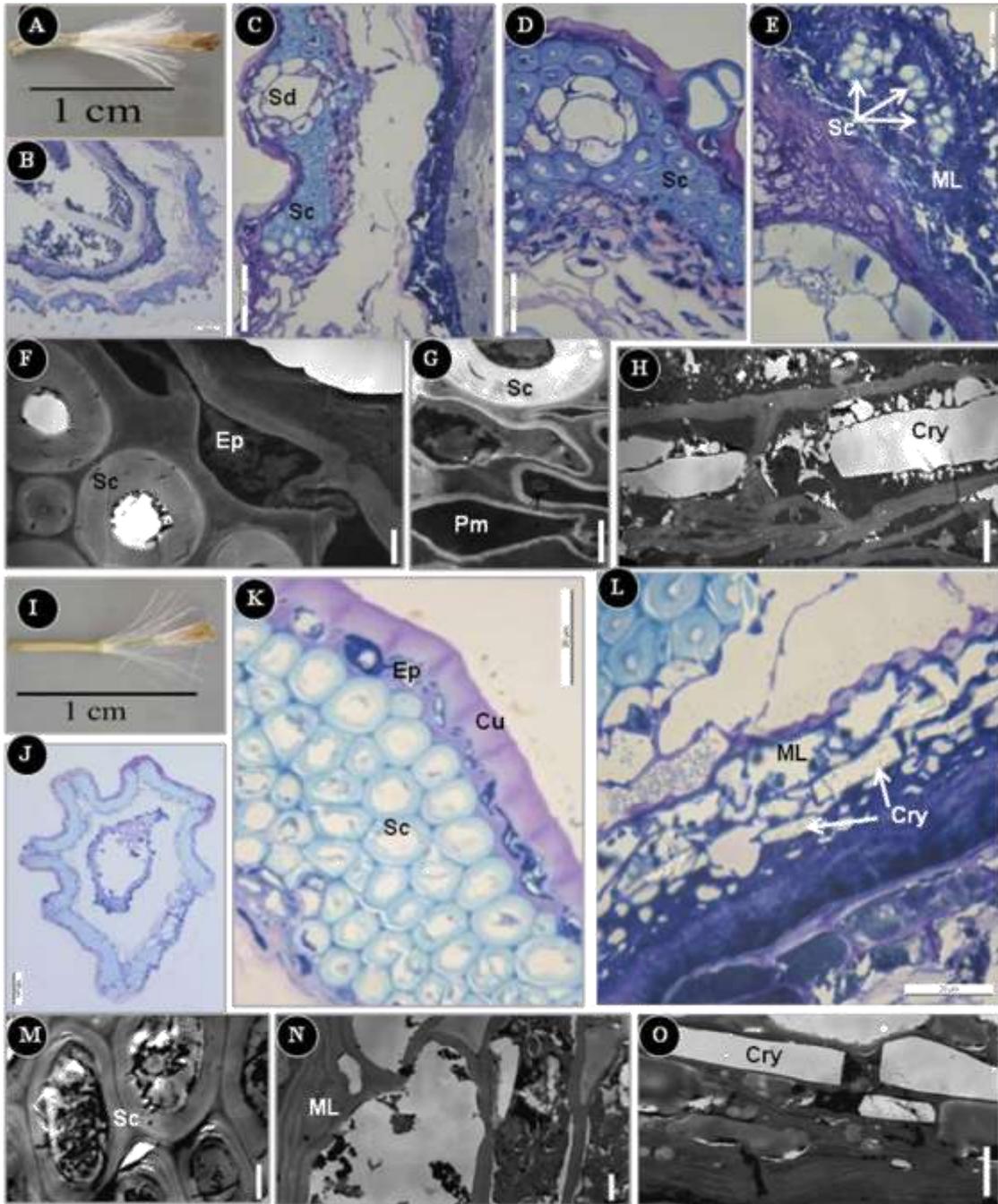


Figure: 2(A) General View of Achene of *Senecio Eriospermus* Subsp. *Eriospermus*; (B) General Appearance of Achene Fruit Structure; (C) Secretory Duct in Sclerenchyma Bundle; (D) Sclerenchyma Cells; (E) Mid-Layer Cells in Achene Fruit; (F) Electron Micrograph of Epidermal Cells (Scale Bar = 5 μ m); (G) Electron Micrograph of Mid-Layer Cells; Note the Schizogenous Space is Filled by the Secretion of Phytomelanin (Scale Bar = 1 μ m); (H) Electron Micrograph of Mid-Layer Cells Including Crystal (Scale Bar = 3 μ m); (I) General View of Achene of *Senecio Eriospermus* Subsp. *Lorentii*; (J) General Appearance of Achene Fruit Structure; (K) Sclerenchyma Cells; (L) Mid-Layer Cells; (M) Electron Micrograph of Sclerenchyma Cells (Scale Bar = 5 μ m); (N) Electron Micrograph of Mid-Layer Cells (Scale Bar = 1 μ m); (O) Electron Micrograph of Mid-Layer Cells Including Crystal (Scale Bar = 1 μ m)

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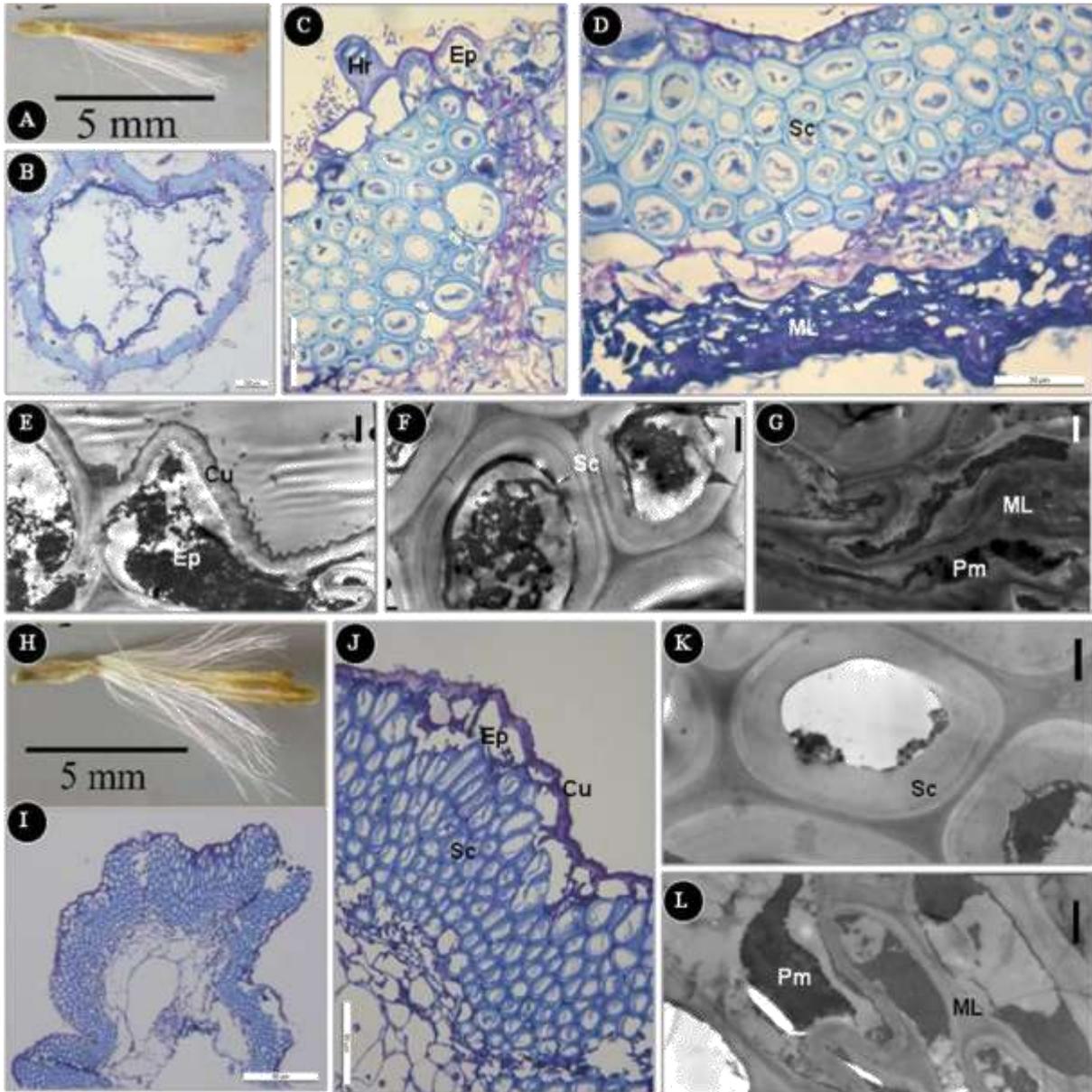


Figure: 3(A) General View of Achene of *Senecio Cilicius*; (B) General Appearance of Achene Fruit Structure; (C) Hair in Achene Fruit; (D) Sclerenchyma and Mid-Layer Cells; (E) Electron Micrography of Epidermal Cells (Scale Bar = 1 μ m); (F) Electron Micrography of Sclerenchyma Cells (Scale Bar = 5 μ m); (G) Electron Micrography of Mid-Layer Cells Including Phytomelanin (Scale Bar = 1 μ m); (H) General View of Achene of *Senecio Hypochionaeus* Subsp. *Lazicus*; (I) General Appearance of Achene Fruit Structure; (J) Sclerenchyma Cells; (K) Electron Micrography of Sclerenchyma Cells (Scale Bar = 3 μ m); (L) Electron Micrography of Mid-Layer Cells Including Phytomelanin (Scale Bar = 1 μ m)

In all the taxa it was observed that there was a thick and darkly stained cuticle layer on the epidermal cells. *Senecio cilicius* and *Senecio hypochionaeus* subsp. *hypochionaeus*, papillary cuticle was found on the epidermis layer. But in other taxa, papil was not observed on cuticle. *Senecio eriospermus* subsp. *eriospermus* was found in the secretory ducts along the achene wall. There are single row of gland cells around the secretory ducts.

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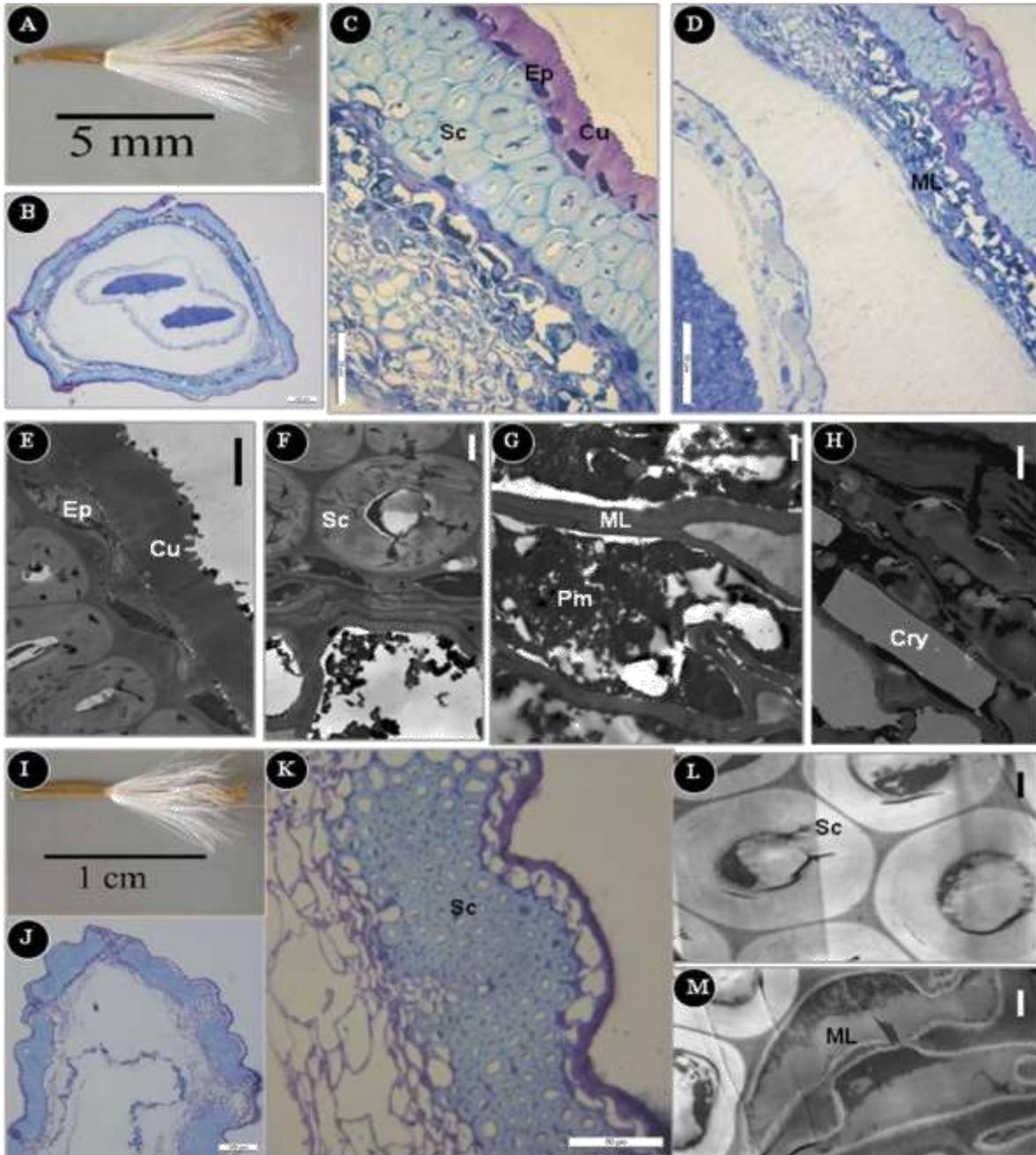


Figure: 4(A) General View of Achene of *Senecio Hypochionaeus* Subsp. *Hypochionaeus*; (B) General Appearance of Achene Fruit Structure; (C-D) Sclerenchyma and Mid-Layer Cells; (E) Electron Micrography of Epidermal Cells (Scale Bar = 1 μ m); (F) Electron Micrography of Sclerenchyma Cells (Scale Bar = 1 μ m); (G) Electron Micrography of Mid-Layer Cells Including Phytomelanin (Scale Bar = 1 μ m); (H) Electron Micrography of Mid-Layer Cells Including Crystal (Scale Bar = 5 μ m); (I) General View of Achene of *Senecio Cariensis*; (J) General Appearance of Achene Fruit Structure; (K) Sclerenchyma Cells; (L) Electron Micrography of Sclerenchyma Cells (Scale Bar = 1 μ m); (M) Electron Micrography of Mid-Layer Cells Including Phytomelanin (Scale Bar = 1 μ m)

Abbreviations: Cu: Cuticula, Ep: Epidermis, Cry: Crystal, ML: Middle layer, Pm: Phytomelanin, Sc: Sclerenchyma cell, Sd: Secretory duct.

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In the other 5 taxa, no secretory ducts was observed. In all of the taxa, thick-walled sclerenchymatous cells were observed in achene. In the *Senecio eriospermus* subsp. *eriospermus*, *Senecio eriospermus* subsp. *lorentii* and *Senecio hypochionaeus* subsp. *hypochionaeus* taxa, prismatic shaped crystals were found in mesocarp cells. In other species, crystals were not found in the middle layer or mesocarp cells. In addition, several small bundles of sclerenchymatous cells were observed in the middle layer of *Senecio eriospermus* subsp. *eriospermus*. Similarly, Zarembo and Boyko (2008) studied the morphology and anatomy of Cardueae (Asteraceae) fruits; the structure of the pericarp epidermal cells has determined that the formation and localization of calcium oxalate crystals and the formation and location of secretory ducts in the mesocarp are important between the taxa. Mukherjee and Nordenstam (2008), Jana and Mukherjee (2014), have extensively studied the crystal structure in the ovaries of certain Asteraceae and mentioned the presence of crystal in *Centaurea* and others. Singh and Pandey (1984) have contributed the presence of druses in *Centaurea cyanus*. Karaismailoğlu (2015), who investigates the anatomical structure of the fruit in seven taxa of *Crepis* by scanning electron microscopy, stated that fruit morphology and anatomy are important in taxonomically in Asteraceae. According to Karanović *et al.*, (2016), the morphological and anatomical properties of fruit and seed provide taxonomically useful information and are used at various levels in the Asteraceae classification.

In all the taxa it was observed that the schizogenous space was filled by the secretion of phytomelanin. Also, phytomelanin accumulation was detected in some cells. According to Roth (1977), the phytomelan layer varies within the outer pericarp: it may be in the outer epidermis, in the subepidermal layer beneath the outer epidermis, or on the outer or inner surface of the sclerenchymatous tissue. Phytomelan layer is characteristic of many achenes (cypselas), which may serve as a protective screen against excessive insolation or as a protection of the pericarp (Roth, 1977; Pandey and Dhakal, 2001). Marzinek and Oliveira (2010) report that phytomelanin covers the entire area between the outer and inner mesocarp, but does not react positively to any histochemical tests applied. Biswas *et al.*, (2014) suggests that crystal formation in Asteraceae has an important taxonomic parameter and crystal formation occurs; the phytomelanin layer was not found.

In this study, 6 taxa belonging to genus *Senecio* in Turkey, achene structure was examined by light microscopy and transmission Electron Microscopy (TEM), and then histological and cytological differences were revealed to contribute to the solution of taxonomic problems of the genus. All these data will be the main source for the persons and units dealing with systematic botanical and related sciences.

Conclusion

A pericarp differentiated into four main region; the exocarp (outer epidermis), mesocarp, sclerenchyma region and endocarp (inner epidermis). In all the taxa it was observed that there was a thick and darkly stained cuticle layer on the epidermal cells. *Senecio cilicius* Boiss. and *Senecio hypochionaeus* subsp. *hypochionaeus*, papillary cuticle was found on the epidermis layer. But in other taxa, papil was not observed on cuticle. *Senecio eriospermus* subsp. *eriospermus* was found in the secretory ducts along the achene wall. In the other 5 taxa, no secretory ducts was observed. In the *Senecio eriospermus* subsp. *eriospermus*, *Senecio eriospermus* subsp. *lorentii* and *Senecio hypochionaeus* subsp. *hypochionaeus* taxa, prismatic shaped crystals were found in mesocarp cells. In other species, crystals were not found in the middle layer or mesocarp cells.

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