Gazi University Journal of Science
GU J Sci
29(2):279-283 (2016)

Seed Coat Ultrastructure of the Genus *Astragalus* L. Section *Uliginosi* Gray (Fabaceae)

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Received:26/02/2016 Revised:03/05/2016 Accepted: 06/05/2016

ABSTRACT

The seed coat ultrastructure of the genus *Astragalus* L. section *Uliginosi* Gray in Turkey was examined by Light Microscopy and Transmission Electron Microscopy in order to determine cytological features of two species and to contribute to their taxonomy. The macrosclereid, osteosclereid and parenchymatic cell layers are found to be in the seed coats.Testa is composed of a quite long, 2-3 layered, with tannin in vacuoles and thick walled macrosclereid cells, underneath there is a single osteosclereid layer with large intercellular space cells. The bottom layer consists of parenchymatic cells stored with starch grains, protein and lipid. The seed coat of these species is similar in structure; but the thickness of the layers is different. In all seed coat layers, *A. odoratus* is thicker than the *A. falcatus*.

Keywords: *Astragalus, Uliginosi, seed coat, ultrastructure, TEM*

1. INTRODUCTION

Astragalus L. is one of the largest genus of vascular plants with about 2500 species which are classified in 10 subgenera and nearly 136 sections. In Turkey, it is represented by 478 taxa in 63 sections and 202 of them are endemic [1-5]. The genetic center of the genus seems to be Eurasia. The Central Asian steppes, Iran and Turkey in particular are areas where the number of this genus is quite high. Turkey with 41 % endemism rate is one of the major distribution center of this genus. The members of the genus *Astragalus* generally grow in a steppeic formation (in dry climates) and they are much found in the Irano-Turanian phytogeographic region of Holarctic world.

The structure of the seed coat has been carried out in some species of Fabaceae [6-18]. There are a few reports about *Astragalus* seed coat but these studies are based on the properties of seed in different stages of developing [19-22].

The section *Uliginosi* is represented by two species in Turkey, namely *A. falcatus* Lam. and *A. odoratus* Lam. The testa ultrastructure of this section has not been studied before. The aims of this study are to describe the seed coat ultrastructure of this section and to contribute to systematics of the section.

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2. EXPERIMENTAL

The research materials were collected from their different localities of Turkey, between May and August, in both flowering and fruiting periods as detailed below:

A. falcatus: A9 Kars: Kağızman-Cumaçay 26. km, roadsides, 1800 m, 14.07.2002, *M.Ekici* 3148 (GAZI), ibid., 11.06.2010, *F.Özbek* 1007 (GAZI); Sarıkamış-Selim 5. km, roadsides, 2000 m, 09.07.2011, *U.Özbek* 2839 (GAZI).

A. odoratus: B5 Kayseri: Erciyes University Campus, 1100 m, 31.05.2011, *F.Özbek* 1023 (GAZI); B6 Kayseri: Bünyan-Pınarbaşı 20. km, roadsides, 1491 m, 09.07.2002, *M.Ekici* 3024 (GAZI); B9 Van: Y.Y.U., Faculty of education garden, 1640 m, 10.06.2010, *F.Özbek* 1001 (GAZI).

Seeds were fixed first 3 % glutaraldehyde buffered with 0.1 M phosphate for 3 h at room temperature. The tissue was washed 3 times in 0.1 M phosphate buffer and postfixed 1 % osmium tetraoxide in 0.1 M phosphate buffer for 3 h at room temperature. The materials were dehydrated through a graduated ethanol series and then embedded in Epon 812 [23]. Tissue for TEM investigations was sectioned on a Reichert ultramicrotome, these ultrathin sections were stained with uranyl acetate and lead citrate [24], and photographed under JEOL CX-100 Transmission Electron Microscope at 80 kV. Semi-thin sections were cut at 1.5 or 2 µm thickness, stained with methylene blue and toluidin blue and mounted. These preparations were measured and photographed using Leica DM1000 Binocular Light Microscope with a Leica DSCF3200 camera.

3. RESULTS AND DISCUSSION

In this report, a detailed structural characterizations of seed coats of two species in section *Uliginosi* are presented. The significance features and parameters of testas are given in Table 1.

The results of LM and TEM investigations, we observed that the seed coat differentiated into three regions: the macrosclereid layer, the osteosclereid layer and the parenchymatic cells region. The outer layer of the seed coat is composed of macrosclereid (Malpighian or palisade cells). The mean thickness of the these cells is 50.42 ± 1.62 µm in *A. falcatus* and 54.26 ± 2.72 µm in *A. odoratus*. This region is 2-3 layered, prolonged cells and covered by a thick cuticle. The thickness of cuticle is variable in two species; 4.59 ± 0.53 µm in *A. falcatus* and 7.12 ± 0.75 µm in *A. odoratus*. It has been observed that the small lumen of the malpighian cells filled with tannin and darkly stained. The light-line is distinct in the tangential walls of the macrosclereid cells.

Below the macrosclereid, the osteosclereid layer is composed of quite thick-walled, vacuolated and large intercellular space cells. Some of cell vacuoles contains tannin. The average thickness of the osteosclereid layer is 12.89 ± 1.78 µm in *A. falcatus* and 15.93 ± 4.3 µm in *A. odoratus*.

The parenchymatic region consists of multilayered, large vacuolated and wavy, irregular thickened walled cells. In *A. falcatus*, the thickness of this layer is 49.16 ± 13.13 µm and also in *A. odoratus* it is 64.76 ± 31.94 µm. A layer of endosperm (aleurone layer) remained attached to the parenchyma layer. Aleurone cells are quite-thickened walled and large cells. Numerous starch grains, lipid, protein bodies and amyloplasts are observed in these cells (Figs. 1, 2).

Table 1. Seed coat layers of the studied *Astragalus* species

Species	*A. falcatus*	*A. odoratus*
Cuticle Thickness (µm)	4.59 ± 0.53	7.12 ± 0.75
Macrosclereid Layer		
Shape of cells	prolonged	prolonged
Line of cells	2-3	2-3
Thickness (µm)	50.42 ± 1.62	54.26 ± 2.72
Osteosclereid Layer		
Shape of cells	spool	spool
Line of cells	single	single
Thickness (µm)	12.89 ± 1.78	15.93 ± 4.3
Parenchymatic Layer		
Shape of cells	isodiametric	isodiametric
Line of cells	multilayer	multilayer
Thickness (µm)	49.16 ± 13.13	64.76 ± 31.94

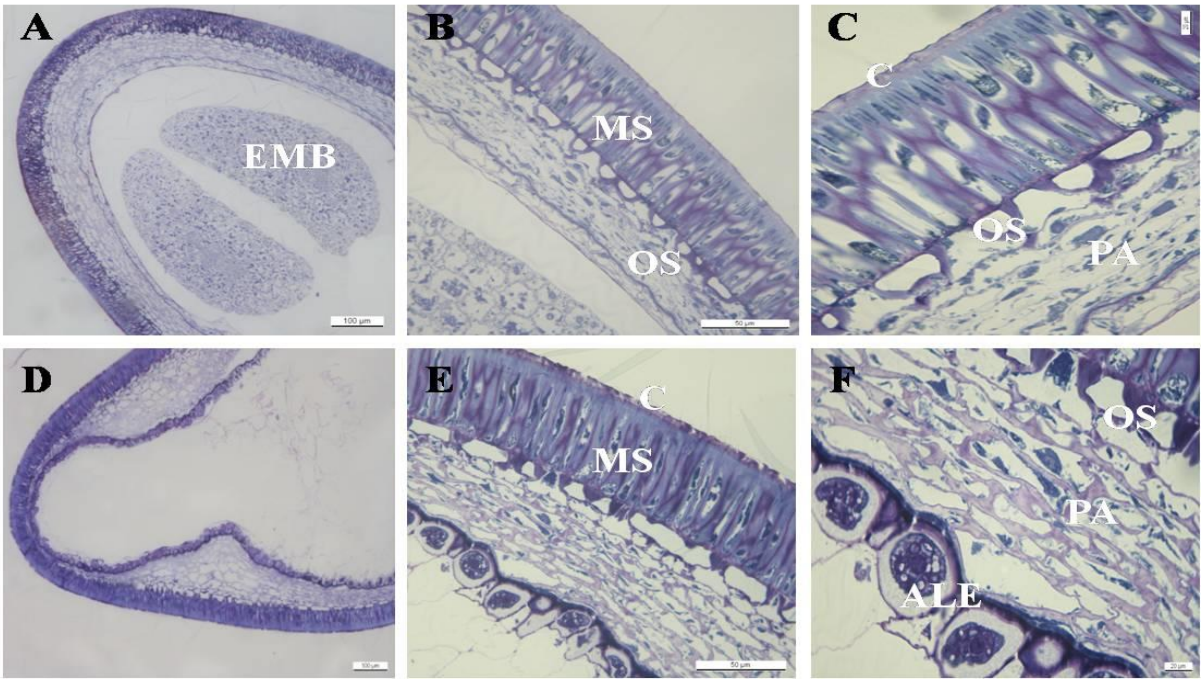

Fig. 1. LM micrographs of longitudinal semithin sections of *A. falcatus* (A-C) and *A. odoratus* (D-E).(ALE- aleurone cells, C- cuticle, EMB- Embriyo, MS- macrosclereid layer, OS- osteosclereid layer, PA- parenchyma cells)

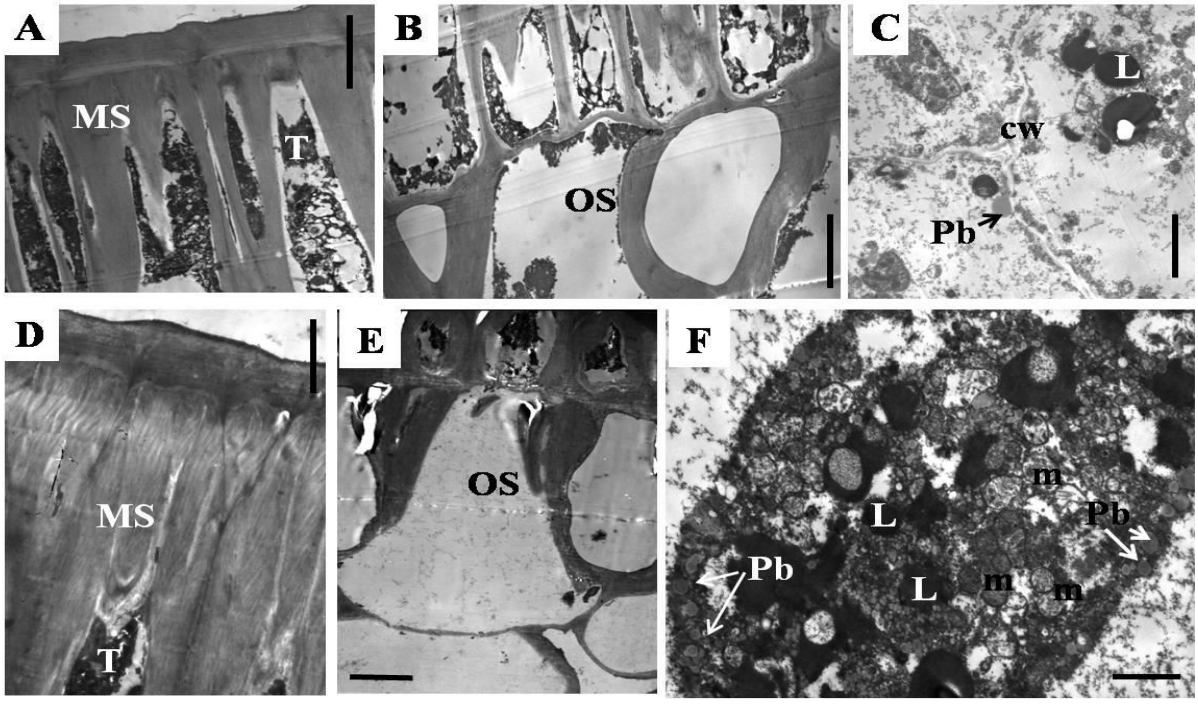

Fig. 2. TEM micrographs of *A. falcatus* (A-C) and *A. odoratus* (D-E). A, D- Epidermal outer walls of the macrosclereid cells, A and D = 5 µm; B, E- Electron micrographs showing the osteosclereid cells, B = 5 µm, E = 1 µm;C, F- Electron micrographs of endosperm cells, C = 3 µm, F= 2 µm.(MS- macrosclereid layer, OS- osteosclereid layer, T- tannin, cw- cell wall, Pb- protein body, m- mitochondria, L- lipid).

The seed coats of the taxa in section *Uliginosi* are similar in structure to the other leguminous plants [8, 10, 11, 25, 27]. The seeds of *A. falcatus* and *A. odoratus* have similar major seed coat characteristics; but some differences are observed in their structure. The thickness of the seed coat layer varies in the investigated species. Generally, the seed coat regions in *A. odoratus* are thicker than the *A. falcatus*.

Pitot [28, 29] stated that the testa of legumes contains external palisade and hourglass cells. Williams [30] and Corner [7] mentioned that three parts of the testa; the epidermal layer of palisade cells (macrosclereids), subepidermal layer (osteosclereids with parenchyma) and aleurone layer. Our findings are partially consistent with those of Williams [30] and Corner [7]. Some authors suppose the aleurone layer in soybean to be derived from the endosperm of the ovular integument [31-33].

Malpighian (called macrosclereid or palisade) cells in the testa of the legumes have been observed many authors [34-36]. Büyükkartal et al. [18] suggested that the macrosclereid and osteosclereid layers contribute to the mechanical strength of the seed coat in hard and soft seeded lines of *Vicia sativa*. An electron-translucent cap (light line) has been observed in the outer wall of macrosclereid cells in investigated *Astragalus* species. Manning and Van Staden [8] proposed that the light line in *Eryhrina lysistemon* was an optical phenomenon due to a structural discontinuity at the boundary between lignified and unlignified parts of cell walls.

Corner [7] stated that hourglass cells in legumes are tall, resembling pillared crypts, and intermediate in position and form between palisade-celled and stellate-celled mesophyll. In all Fabaceae, parenchymatic region derived from the crushed epidermis of the testa and entire tegmen is considered to be the possible role in metabolite translocation [37, 38].

ACKNOWLEDGEMENTS

We would like to thank Gazi University Research Fund (05/2010-54) for its financial support.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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