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Research article/Araştırma makalesi

Calcium oxalate crystals in generative organs of *Astragalus hamosus* and *Astragalus glycyphyllos*

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Abstract

In this study, distribution of Calcium oxalate (CaOx) crystal types within generative organs of Astragalus hamosus L. and Astragalus glycyphyllos L. and crystal types of them were examined using light microscopy (LM). During cytoembryological studies on A. glycyphyllos, CaOx crystals have been observed in generative organs. Prismatic and druse type crystals were determined in different regions of flowers of A. glycyphyllos while were not observed in A. hamosus. This study represents additional data on the presence of CaOx crystals in examined Astragalus species.

Key words: Astragalus, Crystal, Generative organs

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Astragalus hamosus ve *Astragalus glycyphyllos* generatif organlarındaki kalsiyum okzalat kristalleri

Özet

Bu çalışmada, *Astragalus hamosus* L. and *Astragalus glycyphyllos* L. un generatif organlarındaki kalsiyum okzalat kristal tiplerinin dağılımı ışık mikroskobu kullanılarak incelenmiştir. *A. glycyphyllos* taki sitoembryolojik çalışmalar boyunca generatif organlarda CaOx kristalleri gözlenmiştir. *A. hamosus* ta gözlenmediği halde *A. glycyphyllos* un çiçeklerinin farklı bölgelerinde prizmatik ve druz tip kristaller gözlenmiştir. Bu çalışma incelenen *Astragalus* türlerinde CaOx kristallerinin varlığını göstermektedir.

Anahtar kelimeler: Astragalus, Generatif organlar, Kristal

1. Introduction

Astragalus L. (Fabaceae, Leguminosae) contains an estimated 2500 annual and perennial species and about 250 sections worldwide (Lock and Simpson, 1991; Podlech, 1998). *Astragalus* L. species are very old and well known curative plants with immunostimulant, hepatoprotective, antiperspirant, diuretic, and tonic properties (Tang and Eisenbrand, 1992). It is the largest genus of the family Leguminosae with more than 2000 species and also the largest genus with 450 species in flora in Turkey. About 48% of the Turkish species (218) are endemic (Akan et al., 2008; Pınar et al., 2009).

In Turkey, *Astragalus* spp. are often used for the production of gum tragacanth which has significant commercial value (Çalış and Sticher, 1996) and also for curative purposes (Bedir et al., 2001). Given the fact that the farmers usually have to use limited lands to produce products such as grain, industrial crops and fruits, the production of forage scrops are very limited. In addition to this, several species of *Astragalus* are used in folk medicine due to their hepatoprotective, antioxidative biological activities and their antiviral properties (Ríos and Waterman, 1997).

In Turkish folk medicine, the roots of *Astragalus* species are used for the treatment of leukemia and for the healing of open wounds (Yeşilada et al., 2005). Some *Astragalus* products, such as gum tragacanth, are widely used as the base product for certain pharmaceuticals and as thickening agents in certain foods (Zarre, 2000). Gođevac et al. (2008) investigated the antioxidant activity of methanol extract from the aerial part of *A. glycyphyllos* L. Although

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there are many systematic, anatomic, karyological and palynological studies on the *Astragalus* species, some taxonomic problems concerning this genus have not been resolved yet (Wojciechowski et al., 1999; Dane et al., 2007).

In recent years, there has been a significant increase in the number of anatomical studies (Khatun et al., 2011) and the studies of formation mechanisms of CaOx crystals and their taxonomic importance have been increased. CaOx crystals which are found in the structure of druse and raphide type are inclusions that are often seen in higher plants (Akan et al., 2008; Ekici and Dane, 2009). They can be observed in specific tissues of various plants. In significant amounts Ca-oxalate crystals are usually used as a taxonomical tool (Lernsten and Horner, 2000). However, their functional significance remains unclear, although various functions have been attributed to them (Dormer, 1961; Horner, 1977; Lersten and Horner, 2000; Franceschi and Nakata, 2005; Kuo-Huang et al., 2007).

In this study, *A. hamosus* and *A. glycyphyllos* species have been investigated for crystals in ovary, anther, filament, styles, corolla and calyx. This is the first report on these species based on *A. hamosus* and *A. glycyphyllos* for cryrstal types in European Turkey.

2. Materials and methods

The specimens of *A. hamosus* and *A. glycyphyllos* were collected from a natural population in Edirne in European Turkey. Voucher specimens were deposited in the Herbarium of Trakya University (EDTU).

Examined specimens are given as follows:

***Asragalus hamosus* L. ,** A1 (E) Edirne: Center, around Gullapoğlu Campus, 20.05.2002, coll. *F. Dane*, EDTU (8515) (Table 1, Figures 1b).

***Astragalus glycyphyllos* L.** A1 (E) Edirne: Center, Söğütlük forest, 10.08.1989, coll. *F. Dane*, EDTU (3826)!; Center, Izzet Arseven Forest, 20.07.2002, coll. *O. Dalgic*, EDTU (8516)! (Table 1, Figures 1a, 2 - 4)

For LM, materials were fixed in ethyl alcohol and glacial acetic acid (3:1 v/v) at room temperature overnight and transferred to 70% ethyl alcohol. Hand-sections were made from fixed calyx, corolla, filament and ovary. Anthers were hydrolyzed with 1 N HCl for 15 min. at 60^{o}C in an oven. They were stained with Feulgen reagent for 2 hours in darkness at 25^{o}C. Anthers were squashed and counterstained with aceto orcein. The slides were examined under an Olympus Photomicroscope and photographs were taken with the same microscope.

3. Results

In this study, CaOx crystals were investigated and their morphology and distribution determined by light microscopy in organs of *Astragalus glycyphyllos* and *Astragalus hamosus*. In recent years, studies of formation mechanisms of CaOx crystals and their taxanomic importance have been increased. During cytoembryologic studies on *Astragalus glycyphyllus*, CaOx crystals have been observed in generative organs. Both solitary and druse crystals were observed. Solitary crystals were type of prismatic. Taxa, crystal types and crystal's location were given in Table 1.

Table 1.

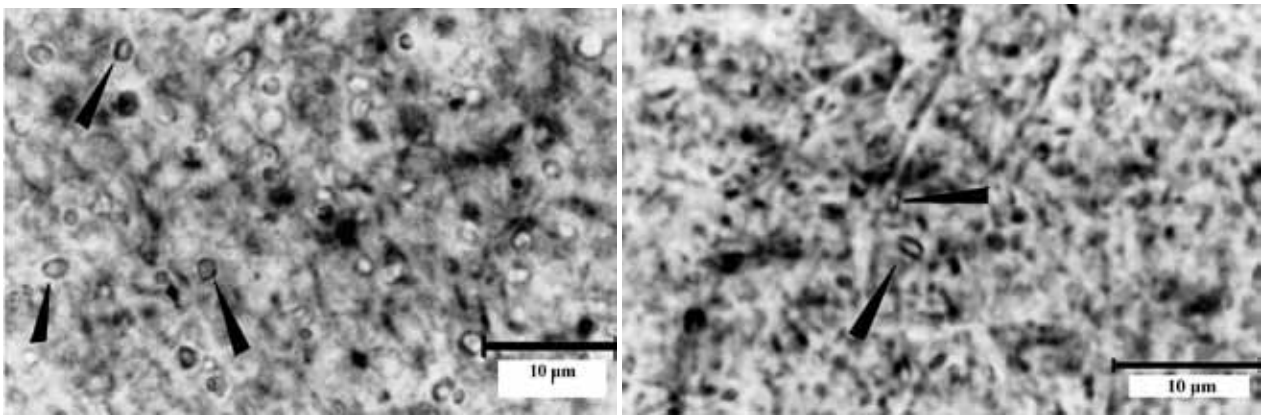
Location	**Taxa**	
	Astragalus hamosus	***Astragalus glycyphyllos***
Calyx	None	Druse (dense)
Corolla	None	prismatic (dense)
Anther tapetal cells	None	prismatic
Filament	None	None
Ovary	prismatic (few)	Prismatic (dense)
Style	None	prismatic (few)
Stigma	None	None

4. Conclusions

Druses were observed in calyx cells in *A. glycophyllos***.** However, no druse crystal was found in *A. hamosus*. The crystals were determined as prismatic in the ovaries of *A. glycophyllos* and *A. hamosus*. No crystals were found in stigma, style, filament and calyx in *A. hamosus* while few prismatic crystals were found in style of *A. glycyphyllos*. There were prismatic crytals found in tapetal cells of anthers of *A. glycophyllos.*

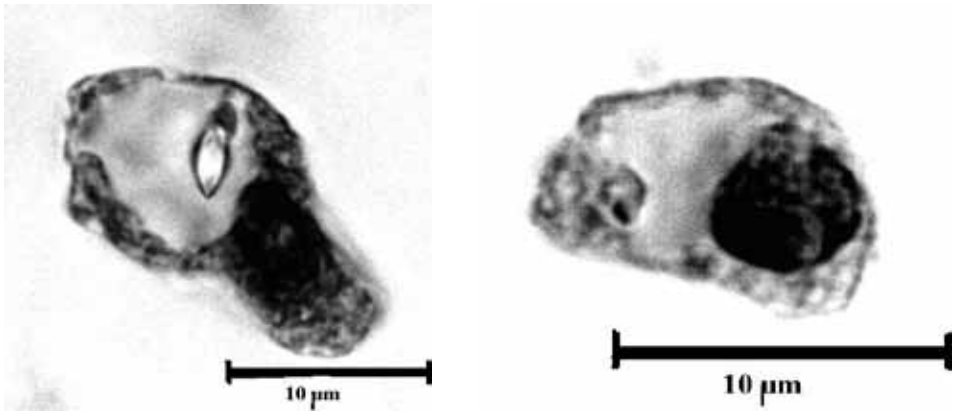
Meric studied CaOx crystals in Asteraceae. She thought that the presence of crystals in transitory floral organs such as the filament, anther and style was interesting. The function of crystals in those organs devoid of supporting

tissues might be to provide strength to the tissues of these floral organs, which were critical to pollination and fertilization for sexual reproduction (Meric, 2009).



a. **b.**

Figure 1. CaOx crystals in ovarium : a. *A. glycophyllos*; b. *A. hamosus*



a. **b.**

Figure 2. a. b. CaOx crystals are shown in tapetal cells of anthers in *A. glycophyllos*

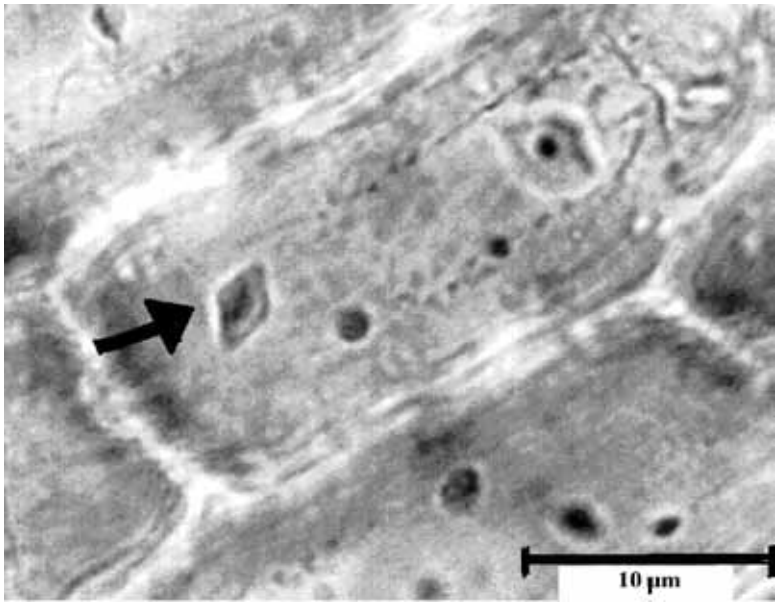
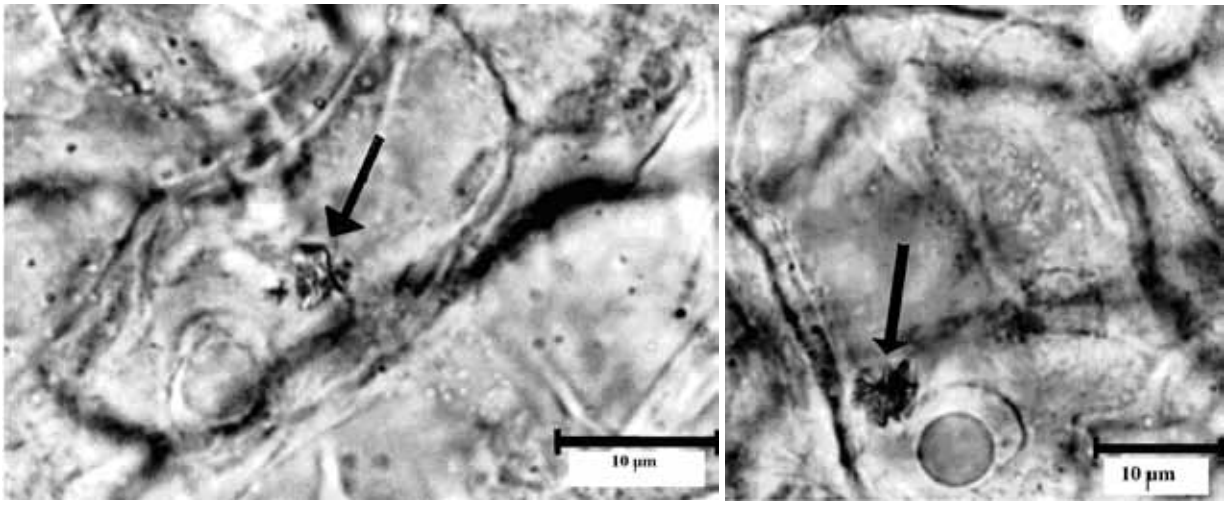


Figure 3. CaOx crystals shown in corolla of *A. glycophyllos*



a. **b.**

Figure 4. a. b. CaOx crystals shown in calyx of *A. glycophyllos*

Several studies have described about CaOx crystals (Dormer, 1961; Horner, 1977; Lersten and Horner, 2000; Kuo-Huang et al., 2007; Meric, 2009). CaOx crystals are found at all taxonomic levels in photosynthetic organisms, from small algae to higher plants (Franceschi and Nakata, 2005). The morphology and distribution of crystals is constant within a species. Because of that their presence was thought to be under genetic control (Ilarslan et al., 2001; Franceschi and Nakata, 2005).

Acknowledgements

The crystals are formed from endogenously synthesized oxalic acid and Ca taken from the environment, and they are produced and accumulated in species-specific morphologies. Thus the constancy of crystal type and distribution may be considered a taxonomic character for classification of species (Franceschi and Nakata, 2005).

In order to solve the taxonomic problems in *Astragalus* L. crystal types of *A. glycophyllos* and *A. hamosus* were determined.

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