

Calcium oxalate crystals in *Aster squamatus* and *Bellis perennis* (Asteraceae: Astereae)

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Abstract. Calcium oxalate crystals in the tissues and organs of *Aster squamatus* and *Bellis perennis* (Asteraceae: Astereae) were investigated and their morphology and distribution were determined with a light microscope. Crystals in various sizes in the stem pith cells of *A. squamatus* were observed as styloids and bipyramids. No crystals were found in the epidermis and cortex cells of the stem. While druses were found in the leaf mesophyll layers, they were not observed in the leaf epidermis cells. Druse crystals were also present in each petal cell of *A. squamatus*. Styloids were seen in the anther endothelial cells and in the ovary cells of *A. squamatus*. Small prismatic crystals were present in the style cells of this species. *Bellis perennis* had only small prismatic crystals in its ovary. No crystals were observed in the other tissues of *B. perennis*. This study presents further data about the existence of calcium oxalate crystals in Asteraceae.

Key words: *Aster squamatus*, Asteraceae, Astereae, *Bellis perennis*, calcium oxalate crystals

Introduction

Calcium oxalate (CaOx) crystals occur in different plant tissues, including leaves, stems, roots, seeds and floral structures (Dormer 1961; Franceschi & Horner 1980; Horner & Wagner 1980; Tilton & Horner 1980; Horner & al. 2000; Lersten & Horner 2000; Ilarslan & al. 2001). Although their value in plant development is largely unknown, various functions have been attributed to them. They provide protection against herbivory (Molano-Flores 2001), represent storage forms of calcium and oxalic acid (Prychid & Rudall 1999), regulate Ca levels in plant tissues and organs (Franceschi & Nakata 2005), involve in the photosynthetic process (Kuo-Huang & al. 2007), give strength to the tissues (Franceschi & Horner 1980) and ensure detoxification of heavy metals (Nakata 2003). The ability to precipitate calcium salts without osmotic implications is also important in some environments (White & Boadly 2003).

CaOx crystals are found in various shapes and sizes in plants and are usually described as raphide,

druse, styloid, prismatic and crystal sands (Franceschi & Horner 1980; Nakata 2003). The crystal type in the particular species is determined genetically by the cells of the species (Franceschi & Nakata 2005), but the amount of crystals can increase owing to environmental factors, such as light density (Kuo-Huang & al. 2007), herbivory and Ca existence (Molano-Flores 2001). Thus the distribution and shape of these crystals have been used as taxonomical characters for a number of plant families (Molano-Flores 2001).

Aster squamatus (Spreng.) Hieron. and *Bellis perennis* L, which belong to the tribe Astereae (Asteraceae), are mainly distributed in tropical and subtropical areas, with over 170 genera and 3000 species (Bremer 1994). The Asteraceae, the only family in the order Asterales, is classified as “potassium plants”, which typically contain little soluble Ca, are calcifuge, and have high shoot K:Ca ratios (Kinzel 1982; Write & Broadley 2003). Crystals in Asteraceae were shown in some earlier studies (Dormer 1961; Horner 1977; Meric & Dane 2004; Meric 2008). The current study is a part of a project aiming to bring

to light the CaOx crystals in *Asteraceae* and to define the characteristics of the distribution and morphology of CaOx crystals in *A. squamatus* and *B. perennis*.

Materials and methods

The plants investigated in this study were collected from natural habitats in Edirne province of Turkey. For light microscopy, the materials were fixed in ethyl alcohol and glacial acetic acid (3:1 v/v), at room temperature overnight and changed to 70% ethyl alcohol. The hand-sliced sections of fixed stems and leaves were examined. Corollas, stamens and pistils were dissected out of florets under a stereo microscope. The samples were treated with 2.5% Clorox (sodium hypochlorite) for 4 h. After graded ethyl alcohol series (about 10 min in each step), the samples were infiltrated with xylene, mounted in entellan on slides and covered with cover slips (Ilarslan & al. 2001). In cleared tissues, crystals were viewed using an Olympus microscope (Tokyo, Japan) and images were taken with an Olympus digital camera (Camedia C5060). Selected images were processed in PhotoShop 7.0 (Adobe, San Jose, California).

The histochemical revealing of CaOx crystals was carried out by Yasue (1969) procedure for tissue clearings. Cleared samples were immersed in 5% aqueous AgNO₃ (Merck) for 15 min, and thoroughly rinsed in distilled water. The samples were stained with saturated rubeanic acid (Dithiooxamide, Sigma) in 70% ethanol for 1 min. Control samples were treated with 5% acetic acid, 10% hydrochloric acid, 3% nitric acid, and 4% sulfuric acid (Molano-Flores 2001). Measurements of the crystals were taken with Image-Pro Plus, version 5.1 (Media Cybernetics, Silver Spring, MD). The diameters of the druses, the lengths of the styloid and prismatic crystals, and the side lengths of the bipyramidal crystals were measured for the analysis. One hundred crystals for each tissue and each crystal type were measured from randomly chosen 10 regions. The averages and standard deviations of data were calculated.

Results

The clearing technique removed all cytoplasm, except for cell walls and calcium oxalate crystals, and the crystals were observed easily under light

microscope. CaOx crystals displayed a different pattern in tissues and organs of *Aster squamatus* and *Bellis perennis*. Their morphology and distribution in tissues are shown in Table 1.

Crystals were stained brownish-black after treatment with silver nitrate and rubeanic acid. This reaction indicated the existence of oxalate. Crystals in stem pith cells of *A. squamatus* exhibited two types of morphology. One was typical of the tetragonal-bipyramidal type. The other crystal was of styloid type (Fig. 1). The lengths of styloids were $14.07 \pm 4.6 \mu\text{m}$ and the side lengths of bipyramidal crystals were recorded as $5.73 \pm 0.75 \mu\text{m}$ for this species. No crystals were found in the epidermis and cortex cells of the stem. In the mesophyll cells of the leaves, crystals were present as druses $3.45 \pm 0.38 \mu\text{m}$ in diameter (Fig. 2). No crystals were found in the leaf epidermis cells, such as stem epidermis cells. Each corolla cell had a single druse crystal and the diameters of these crystals were measured as $6.24 \pm 1.41 \mu\text{m}$ (Fig. 3). Styloids were observed in each cell of the anther endothelial layers (Fig. 4). The cells had single styloid crystal, or a cluster (3–5) of them. The lengths were determined as $6.03 \pm 1.08 \mu\text{m}$. No crystals were found in the filament tissues. In ovary cells, crystals were observed as styloids $5.97 \pm 1.08 \mu\text{m}$ in length (Fig. 5). A few of these crystals in cells, or ovary wall cells were single crystals. At the bottom of the ovary, druses were densely present, while the ova-

Table 1. Morphology and location within tissues of calcium oxalate crystals in *A. squamatus* and *B. perennis*.

Location	<i>Aster squamatus</i>	<i>Bellis perennis</i>
stem – epidermis	---	---
stem – cortex	---	---
stem – pith parenchyma	styloid, bipyramid ($14.07 \pm 4.61 \mu\text{m}$, $5.73 \pm 0.75 \mu\text{m}$)	---
leaf – epidermis	---	---
leaf – mesophyll	druse ($3.45 \pm 0.38 \mu\text{m}$)	---
corolla	druse ($6.24 \pm 1.41 \mu\text{m}$)	---
anther	styloid ($6.03 \pm 1.08 \mu\text{m}$)	---
filament	---	---
ovary	styloid, druse ($5.97 \pm 1.08 \mu\text{m}$, 7.00 ± 0.64)	prism ($4.76 \pm 0.62 \mu\text{m}$)
style	prism ($3.00 \pm 0.34 \mu\text{m}$)	---

ry wall had styloid type crystals (Fig. 6). The diameter of these druses was $7.00 \pm 0.64 \mu\text{m}$. Small prismatic crystals were observed in the style cells. Their agglomeration resembled the druse structure (Fig. 7). Their lengths were measured as $3.00 \pm 0.34 \mu\text{m}$.

Bellis perennis exhibited a distinctly different pattern from *A. squamatus*. Crystals were present only on the ovary wall of *B. perennis*. The morphology of these crystals had atypical prismatic shape and they were distinctive from the crystals in the tissues of *A. squamatus*. The length of these prismatic crystals were measured as $4.76 \pm 0.62 \mu\text{m}$ (Fig. 8).

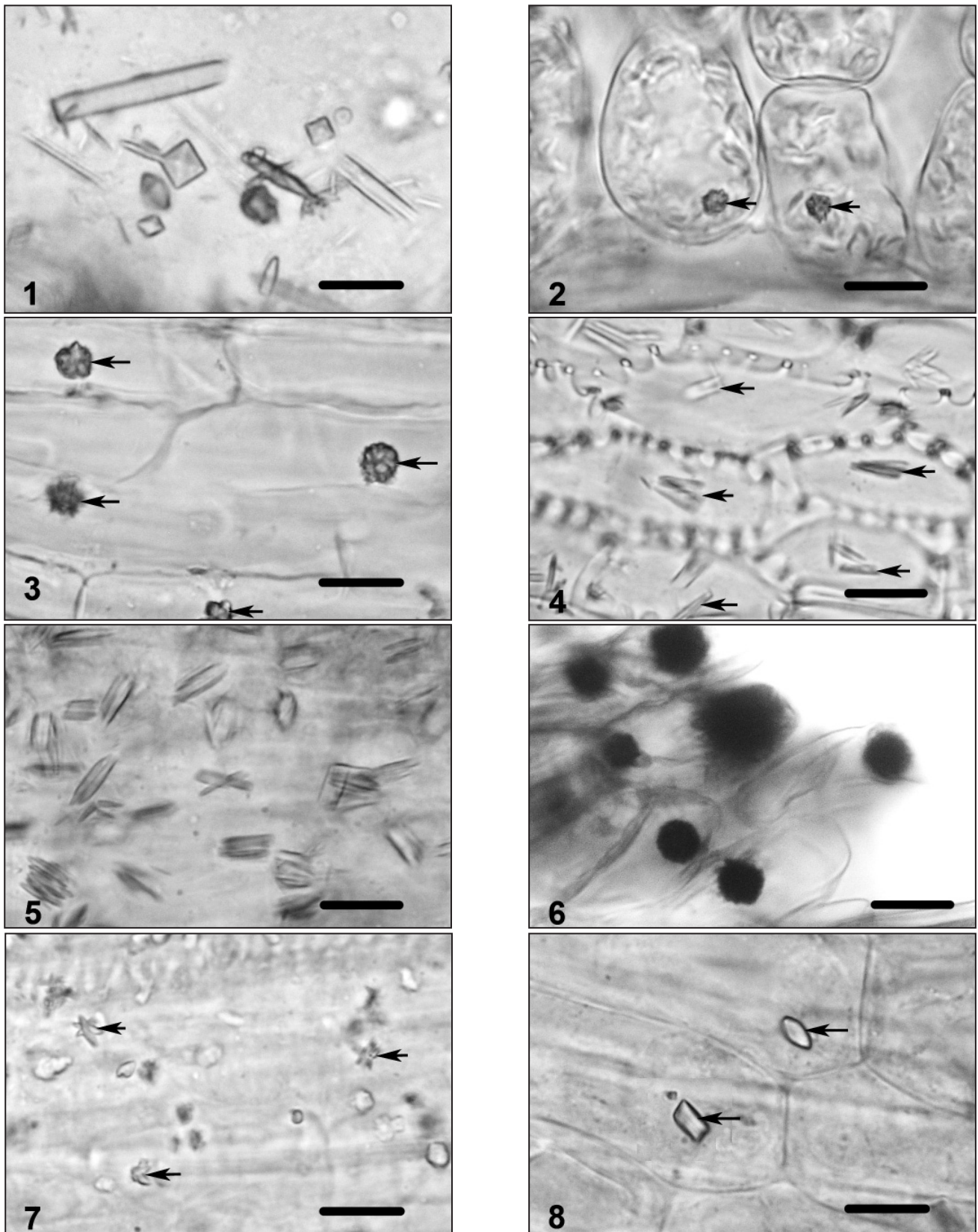
Discussion

Calcium oxalate crystals are found in over 215 plant families and they occur in almost every part of both vegetative and reproductive organs in plants (Franceschi & Horner 1980; Prychid & Rudall 1999). Their type, presence and absence represent some useful taxonomic characters. Unfortunately, only few studies have been conducted into the existence of crystals in *Asteraceae* (Dormer 1961; Horner 1977; Meric & Dane 2004; Meric 2008). Crystals in the stem of *Aster squamatus* occur only in pith parenchyma, as styloids and bipyramids. Their pattern differs from *Conyza* which is in the same tribe (Meric 2008). While *Conyza* species contain druses in the epidermis and cortex cells of the stem, no crystals are found in these tissues of *A. squamatus*. *Conyza* species have needle-shaped and bipyramidal crystals in the stem pith parenchyma cells (Meric 2008). Crystal types in the stem pith cells of *A. squamatus* are styloids and bipyramids. The reason of the presence of needle-shaped and styloid crystals in the stem pith of these species is probably defence against herbivory. Thus they are apt to puncture the mouth and throat tissues of animals (Vogel 2004). Prismatic and druse crystals within cells may contribute to the strength of tissues and/or act as storage mechanism for Ca and oxalate (Franceschi & Horner 1980).

Crystals are more common in the leaves than stems and there are many papers related to the presence of crystals in leaves (Wu & Kuo-Huang 1997; Lersten & Horner 2000; Lersten & Horner 2006; Demiray 2007). They often form in the epidermal, mesophyll and vascular tissues of the leaves. Druses are present in the mesophyll cells of *A. squamatus*. They occur in both

epidermis and mesophyll cells of *Conyza* spp. of the *Astereae* tribe (Meric 2008). Druse crystals provide structural support to the tissues and regulate Ca levels in plant tissues and organs (Nakata 2003). Furthermore, they are involved in dispersing light to the chloroplasts in the photosynthetic parenchyma cells of the leaves (Kuo-Huang & al. 2007). CaOx crystals appear also in the floral organs (petal, anther, ovary and style) of *A. squamatus*. In addition to the presence of CaOx in long-living organs, such as stems and leaves, these crystals are notably present in the temporary floral organs, such as petals, stamens and pistil. They are very common in the floral organs of many taxa (Franceschi & Horner 1980; Tilton & Horner 1980; Meric & Dane 2004). Only a few studies have been related to the presence of crystals in petals (Robertson 1978; Meric & Dane 2004; Meric 2008). In these studies, various crystal types are reported as raphides (in *Jubaeopsis caffra*), prismatics (in *Helianthus* spp.) and druses (in *Conyza* spp.). The existence of crystals in petals, a transitory organ, is unclear at present. Probably, crystals accumulate the excess Ca in tissues. In anthers, crystals are reported in the endothelial layers (Meric & Dane 2004), tapetal cells (Buss & Lersten 1972; Horner 1977), connective tissues (Horner & Wagner 1980) and trichomes (Meric & Dane 2004). In ovaries, crystals are widely found as raphides, styloids and prismatics (Dormer 1961; Tilton & Horner 1980; Meric 2008). In ovaries of *A. squamatus*, two types of crystals have been observed as styloids and druses. Druses appeared at the bottom of the ovary, while styloids were present in the other parts of the ovary. Earlier studies have shown that druses give strength to the tissues, and crystals with pointed ends, such as styloid, help keep herbivory at *Sida* (Franceschi & Horner 1980; Molano-Flores 2001). Carpels and ovules are rich in cytoplasm, and maturing seeds are rich nutrients. During seed maturation, such protection against herbivores is very important.

This study shows that there are major differences in CaOx distribution patterns among the species belonging to the tribe *Astereae*. Morphology and distribution of crystals are quite different among the genera, whereas they are similar among the closely related species. Crystal morphology within a particular species is constant. Crystal morphology and distribution have been indicated as genetically controlled by the cell. This phenomenon can be useful in classification of some taxa. Additional researches are required in order to use the



Figs 1-8. Calcium oxalate crystals in the tissues of: *A. squamatus* – 1, Styloid and bipyramidal crystals in the pith parenchyma cells of the stem 2, Druses in the leaf mesophyll cells (arrows); 3, Druses in the corolla cells (arrows); 4, Styloid crystals in the anther androthecial cells (arrows); 5, Styloids in the ovary wall cells; 6, Druses in the cell bottom of ovary of *A. squamatus* (stained with Yasue procedure); 7, Agglomeration of prismatic crystals in the style cells (arrows); *B. perennis* – 8, Prismatic crystals in the ovary cells (arrows). Scale bar = 10 μ m.

morphology and distribution of crystals in *Asteraceae*, along with the morphological characters, as an anatomical feature in the classification of *Asteraceae*.

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