Calcium oxalate crystals in floral organs of *Helianthus annuus* L. and *H. tuberosus* L. (Asteraceae)

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**ABSTRACT** *Helianthus annuus* L. and *Helianthus tuberosus* L. belong to Asteraceae that is one of the greatest families of plant kingdom. Calcium oxalate crystals are found in most organs and tissues of many plant species. The type, morphology and distribution of calcium oxalate crystals in floral organs of *H. annuus* and *H. tuberosus* were studied. Crystals were investigated at light and electron microscopy levels. CaOx crystals in calyx and bracts both of both *H. annuus* and *H. tuberosus* were not observed. The ligulate and tubulate corollas of *H. annuus* had styloid and prismatic crystals. Also in both of the ligulate and tubulate corollas of *H. tuberosus* were observed prismatic and styloid crystals as similar with *H. annuus*. Styloid and prismatic types of CaOx crystals in filaments of *H. annuus* and *H. tuberosus* were determined. In endothecial layer and tapetum cells of anthers of both of taxa only styloid type crystals were observed. The ovary was not contains CaOx crystals in *H. annuus* and *H. tuberosus*, Style of both of taxa had styloid shape crystals. But in stigma trichomes of *H. annuus* and *H. tuberosus* druses were found. The raphides were not observed in both of taxa. This study provides additional knowledge about the presence of CaOx crystals in Asteraceae.

**KEY WORDS**

Asteraceae calcium oxalate crystals floral organs *Helianthus annuus* L. *Helianthus tuberosus* L.

Calcium oxalate (CaOx) crystals are found in many plant species (Franceschi and Horner 1980; Prychid and Rudall 1999). They occur in different plant tissues including leaves (Horner and Zindler-Frank 1982; Lersten and Horner 2000), stems (Grimson and Arnott 1983), roots (Dane et al. 2000; Horner et al. 2000), seeds (Webb and Arnott 1982, 1983; Ilarslan et al. 1997, 2001). CaOx crystals also occur in floral organs including ovaries (Tilton and Horner 1980), anthers (Buss and Lersten 1972; Horner 1977; Horner and Wagner 1980, 1992) and petals (Robertson 1978). There are not only a few taxa including Brassicaceae, Campanulaceae, Papaveraceae, Saxifragaceae and Equisetaceae (Kinzel 1989). However, their functional significance remains unclear, although various functions have been attributed them. CaOx crystals give protection against foraging animals (Molano-Flores 2001), bind toxic oxalate (Borchert 1984), involved in in-plant Ca regulation (Franceschi 1989), salt stress and homeostasis (Hurkman and Taraka 1996) and detoxification of heavy metals (Nakata 1997). Prychid and Rudall (1999) reported that there are three main types of CaOx crystal as raphids, styloids and druses in monocotyledons. Druses are relatively rare in monocotyledons than dicotyledons (Prychid and Rudall 1999).

Besides existence of CaOx crystals in long-living organs such as roots, stems and leaves, it is also notable that these crystals are present in transitory floral organs such as stamens, gynoecia and petals. They are quite prevalent in floral organs of many taxa including Dilleniaceae, Liliaceae, Palmae, Malvaceae, Cunoniaceae, Euphorbiaceae (Tilton and Horner 1980), Solanaceae (Horner and Wagner 1980, 1992), Leguminosae (Buss and Lersten 1972).

Our interest in CaOx crystals began with observations of crystals in tapetal and endothecial layers during the embryological study on *H. annuus*. Horner have indicated also exist of CaOx crystals in tapetal cells of *H. annuus* (Horner 1977). But other floral organs of *H. annuus* were not reported. This conducted us to investigate types and distributions of CaOx crystals in floral organs of *Helianthus* species growing in Turkey. There are two species of *Helianthus* genus in Turkey: *H. annuus* and *H. tuberosus* (Kupicha 1975). We aimed to determine types and distributions of CaOx crystals in floral organs of *H. annuus* and *H. tuberosus* in the study.

**Materials and Methods**

Plants of *Helianthus annuus* L. and *Helianthus tuberosus* L. were grown in the Greenhouse of Department of Biology, Trakya University. The buds and opened flowers were col-

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lected from *H. annuus* and *H. tuberosus*.

**Light Microscopy**

Florets were at different development stages belonging to *H. annuus* and *H. tuberosus* were fixed in mixture ethyl alcohol and glacial acetic acid (3:1) at room temperature overnight and changed to 95% ethyl alcohol. Bracts, calyces, corollas, stamens, ovary, style and stigma were dissected out of florets. The samples were treated with 2.5% Clorox (sodium hypochlorite) for 4 h. After graded ethyl alcohol series, the samples were infiltrated with xylene, mounted in entellan on slides, and covered with cover slips (Ilarslan et al. 1997). Photographs were taken with an Olympus Photomicroscope.

**Transmission Electron Microscopy**

Florets were fixed in 3% glutaraldehyde in Milloning’s phosphate buffer at 4°C for 2 h. The floral organs were dissected and then placed in fresh fixative at 4°C for overnight. Fixed samples were passed though three buffer rinses, post-fixed in 1% osmium tetroxide (OsO₄) in the same buffer for 4 h at 4°C. Then the samples are rinsed several times in the buffer, dehydrate in a graded acetone series to propylene oxide, and embedded in Epon 812. The acid tests were used to determine the chemical composition of the crystals. Control samples were immersed in turn in 5% acetic acid, 10% hydrochloric acid, 3% nitric acid and 4% sulfuric acid (Molano-Flores 2001). All these tests confirmed that the crystals were calcium oxalate.

**Results**

In *Helianthus* L. genus the inflorescence is a capitulum and it consist of two types flowers; ligulate flowers and tubulate flowers (Seiler 1997). The ligulate flowers have pistils, but contain no stamens. The tubulate flowers have both of pistil and stamens. Calcium oxalate crystals are displayed a similar distribution in both flower types of two taxa. Results were shown in Table 1.

**Helianthus annuus L.**

Calcium oxalate crystals were observed in stamen, style, stigma, ligulate petal and tubulate petal of *H. annuus*. They are not observed in sepals and bracts. Crystals in corolla of ligulate flowers were dense in basis of the corolla and exist different shapes as prisms and styloids (Fig. 1a) and styloids (Fig. 1b). Whereas, in tubulate flowers they are equally distribute in all corollas and present as prismatic and styloids. In stamens they were found in both of anthers and filaments. Also in filaments crystals were determined as prismatic and styloids. In endothelial cells (Fig. 2) and tapetal cells (Fig. 3) of anthers CaOx crystals were observed as styloid type. In these tissues no other types of crystals were observed. Epidermal cells and middle layer cells of anther contain no crystals. Only druse type of crystals was observed in glandular trichomes.

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**Table 1. The types and distribution of CaOx crystals in *H. annuus* and *H. tuberosus***

<table>
<thead>
<tr>
<th>Location</th>
<th><em>H. annuus</em></th>
<th><em>H. tuberosus</em></th>
</tr>
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<tbody>
<tr>
<td>bract</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>calyx</td>
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</tr>
<tr>
<td>ligulate corolla</td>
<td>styloid, prismatic</td>
<td>styloid, prismatic</td>
</tr>
<tr>
<td>tubulate corolla</td>
<td>styloid, prismatic</td>
<td>styloid, prismatic</td>
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<tr>
<td>anther – endothecium</td>
<td>styloid</td>
<td>styloid</td>
</tr>
<tr>
<td>anther – tapetum</td>
<td>styloid</td>
<td>styloid</td>
</tr>
<tr>
<td>anther – trichome filaments</td>
<td>druse</td>
<td>druse</td>
</tr>
<tr>
<td>ovary</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
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</tr>
<tr>
<td>stigma-trichome</td>
<td>druse</td>
<td>druse</td>
</tr>
</tbody>
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**Figure 1. CaOx crystals in ligulate corolla of *H. annuus*. a, prismatic crystal; b, styloid crystal. Bar = 10 µm**

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CaOx Crystals in Helianthus L.

In Helianthus L., CaOx crystals (Fig. 4) were found at the tip of anthers. In style, a styloid type was found. In stigma, crystals were found only in trichomes and in druse shape (Fig. 5). CaOx crystals were not found in the ovary of *H. annuus*. Styloids in cross sections seemed cubical. In longitudinal sections, they were typically elongated and had pointed ends (Fig. 6).

**Helianthus tuberosus L.**

Distribution and existence of CaOx crystals in *Helianthus tuberosus* were similar to *Helianthus annuus*. Calcium oxalate crystals were observed in stamen, style, stigma, ligulate petal, and tubulate petal of *H. tuberosus* and were not observed in sepals and bracts as *H. annuus*. Crystals in corolla of ligulate flowers were present as prisms and styloids, and they are dense in the basis of the corolla. In tubulate flowers, they were observed as prisms and styloids. In stamens, they were found in both anthers and filaments. In filaments, crystals were determined as prisms and styloids. Styloid type CaOx crystals were observed in endothecial cells at the tip of anthers (Fig. 4). In style, a styloid type was found. In stigma, crystals were found only in trichomes and druse shape (Fig. 5). CaOx crystals were not found in the ovary of *H. annuus*. Styloids in cross sections seemed cubical. In longitudinal sections, they were typically elongated and had pointed ends (Fig. 6).
and tapetal cells of anthers. In epidermal cells and middle layer cells no crystals were found. In glandular trichomes at tip of anthers druse crystals were determined. In style they were observed styloid type. In stigma crystals were found only in trichomes and they were druse type. Also CaOx crystals were not found in ovary of *H. tuberosus* as *H. annuus*.

**Discussion**

In this study CaOx crystals in floral organs of *H. annuus* and *H. tuberosus* was revealed. Two types of the calcium oxalate crystals were common; styloids and prismatic in both of taxa. Druses were observed rarely in glandular hairs of anthers of and hair trichomes of stigmas in both of taxa. Only styloid crystals in the tapetum and endothecium cells of anthers were observed and while plasmoidal tapetum degenerated they disappeared. Both of styloid and prismatic crystals in the corolla and filament were located.

In the Asteraceae crystals were shown by a few previous studies (Horner 1977; Heinrich et al. 2002). Horner (1977) reported that styloids occur in tapetal cells of *H. annuus*. Crystals is not only in tapetum cells but also in endothecial cells and in glandular hairs of anther. Heinrich et al. (2002) have observed CaOx crystals in glandular hairs of *Sigesbeckia joulensis* Kunth such as glandular hairs of anther of *H. annuus*. But researchers did not determined calcium oxalate crystal types in glandular hairs. Calcium oxalate crystals have been shown to occur within the anthers of other higher plants (Schmid 1976; Horner and Wagner 1980). Primarily researchers have suggested that CaOx crystals in the anther may supply for pollen against predators, are metabolic waste products and hair trichomes of anther. Heinrich et al. (2002) reported that styloids occur in tapetal cells of *H. tuberosus* as *H. annuus* and *H. annuus* being a member of the Asteraceae. It is probably that also the other members may Asteraceae are contain crystals. The most comprehensive review of crystal types and distribution for a single family (Zindler-Frank 1987, Leguminosae) lacks substantial documentation (Lersten and Horner 2000). Thus additional research is needed to better determine CaOx crystals in other taxa belonging Asteraceae.

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**References**


