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ARTICLE

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 *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.
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KEY WORDS

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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 2003).

CaOx crystals are widely distributed in plant and found in over 215 plant families (Franceschi and Horner 1980; Molano-Flores 2001). The distribution and shapes of these crystals have been used as taxonomic characters for a number of plant families (Molano-Flores 2001). The shapes of CaOx crystals vary differently and they commonly described as raphides, druses, styloids, prisms and crystal sand (Ilarslan et al. 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 dicotiledons (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 embriyological study on *H. annuus.* Horner have indicated also exist of CaOx crystals in tapetum 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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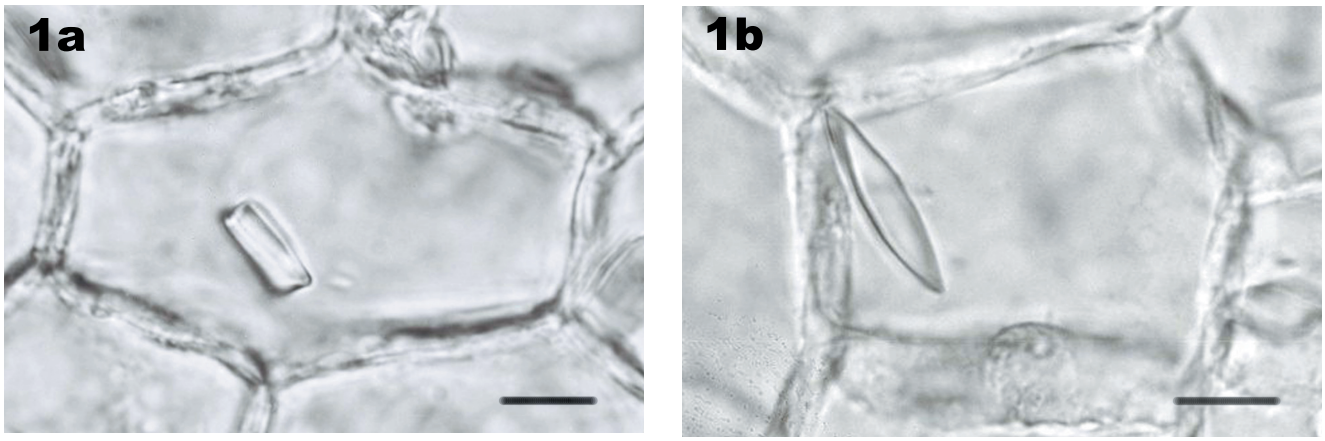

Figure 1. CaOx crystals in ligulate corolla of *H. annuus.* a, prismatic crystal; b, styloid crystal. Bar = 10 µm

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, calyxes, 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 tetraoxide (OsO_4) 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.

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

Location	Taxa	
Organs	*H. annuus*	*H. tuberosus*
bract	---	---
calyx	---	---
ligulate corolla	styloid, prismatic	styloid, prismatic
tubulate corolla	styloid, prismatic	styloid, prismatic
anther – endothecium	styloid	styloid
anther – tapetum	styloid	styloid
anther – trichome	druse	druse
filament	styloid, prismatic	styloid, prismatic
ovary	---	---
style	styloid,	styloid
stigma- trichome	druse	druse

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 prismatics (Fig. 1a) and styloids (Fig. 1b). Whereas in tubulate flowers they are equally distribute in all corollas and present as prismatics and styloids. In stamens they were found in both of anthers and filaments. Also in filaments crystals were determined as prismatics and styloids. In endothecial 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

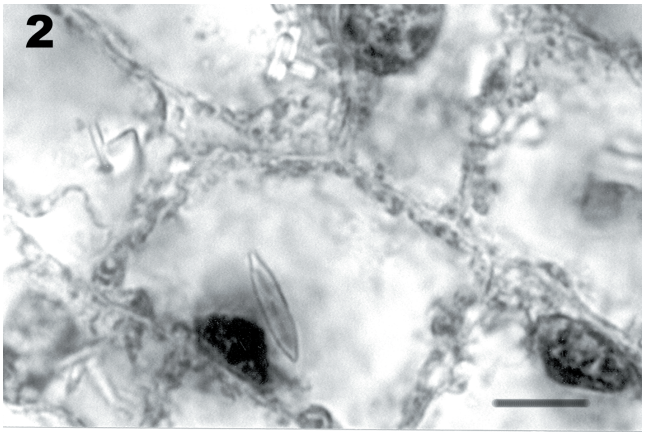

Figure 2. Styloids in endothecial cells of *H. annuus.* Bar = 10 µm

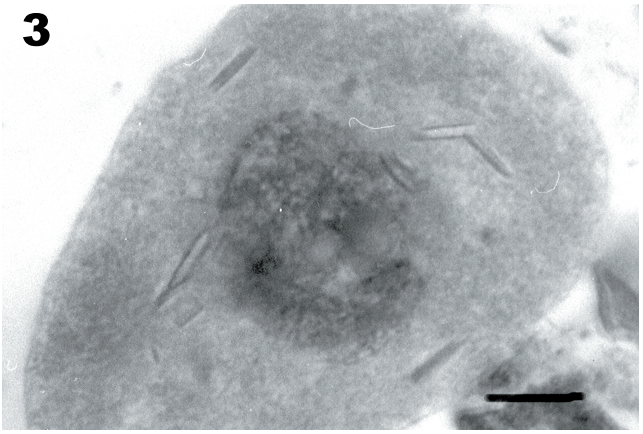

Figure 3. Styloids in tapetal cells of *H. annuus.* Bar = 10 µm

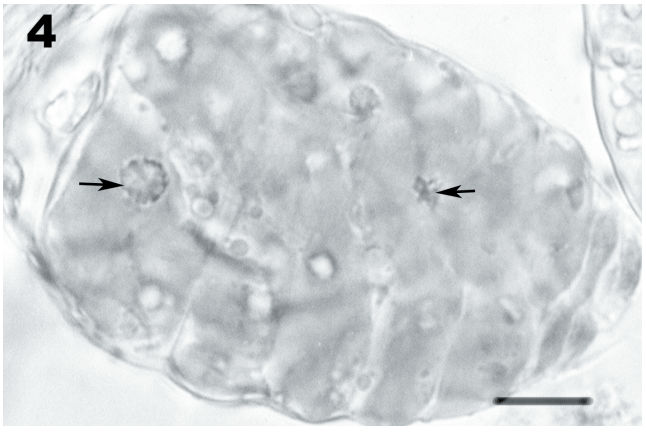

Figure 4. Druses in glandular trichomes at tip of anthers of *H. annuus* (arrows). Bar = 10 µm

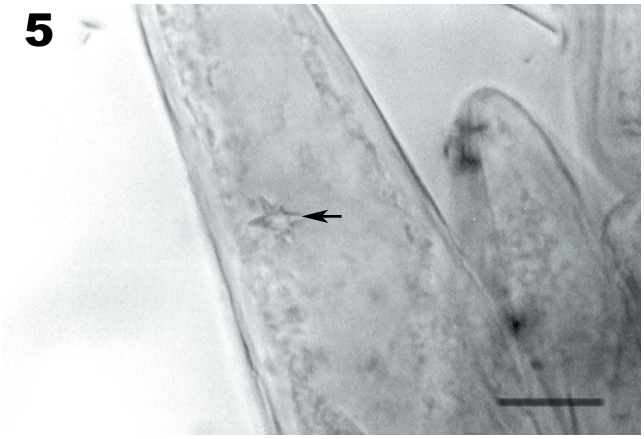

Figure 5. Druse in stigma trichomes of *H. annuus* (arrow). Bar = 10 µm

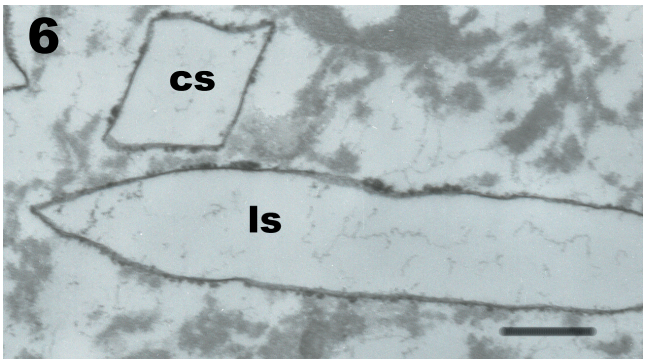

Figure 6. TEM photographs of styloids in tapetum of *H. annuus* (cs, cross section; ls, longitudinal section). Bar = 1 µm

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

Helianthus tuberosus L.

Distribution and existence of CaOx crystals in *Helianthus tuberosus* were similar with *Helianthus annuus.* Also calcium oxalate crystals were observed in stamen, style, stigma, ligulate petal and tubulate petal of *H. tuberosus* and they were not observed in sepals and bracts as *H. annuus*. Crystals in corolla of ligulate flowers were present as prismatics and styloids shapes and they are dense in basis of the corolla. In tubulate flowers they were observed as prismatics and styloids. In stamens they were found in both of anthers and filaments. In filaments crystals were determined as prismatics and styloids. Styloid type CaOx crystals were observed in endothecial cells

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 prismatics 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 plasmodial 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 joullensis* 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 or lead to in the anther for dehiscence (Horner and Wagner 1980). Besides CaOx crystals were suggested that they serve as a storage source for Ca (Ilarslan et al. 1997, 2001). This information is very important, because it is widely assumed that mitosis and cytokinesis are regulated by Ca^{2+} (Hepler and Wayne 1985). The existence of CaOx crystals in tapetum and endotecium cells may also affect microsporogenesis and microgametogenesis. The level of Ca^{2+} would control the assembly-disassembly of spindly microtubules and directly regulate both formation and function of the mitotic apparatus and phragmoplasts (Hepler and Wayne 1985). Besides synthesizing callose (β 1,3 glucan) during microsporogenesis is required high concentration of Ca^{2+} (Katti et al. 1994). Many studies already cited indicate the importance of Ca^{2+}, both as a structural and a physiological entity.

Tilton and Horner reported that crystals within the carpels are completely solved by the time the carpels dehisce in *Ornithogalum caudatum* Ait. They suggested that in *Ornithogalum* carpels may represent mobilization of Ca reserves from the carpels to the developing seeds.

Physical and chemical conditions such as temperature, pressure, pH, and ion concentration, may affect crystal growth, location, and properties (Franceschi and Horner 1980), however it is considered that crystal formation within the cell is under genetic control (Ilarslan et al. 2001). Although some species have different crystal types in adjacent cells, a particular taxon can have specific crystal shape (Prychid and Rudall 1999). In present study, similar results in both taxa were observed except for small differences.

In this study we aimed to determine CaOx crystals in *H. annuus* and *H. tuberosus* 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

Borchert R (1984) Functional anatomy of the calcium-excreting system of *Gleditsia triacanthos* L. Bot Gaz 145:474-482.

Buss PA, Lersten NR (1972) Crystals in tapetal cells of the Leguminosae. Bot J Linn Soc 65:81-85.

Dane F, Huseyinova G, Meric C (2000) Some ultrastructural observations on calcium oxalate raphide crystal idioblasts and meristematic cells of the adventive root tips of *Sternbergia lutea* (L.) Ker-Gawl. Ex Sprengel (Amaryllidaceae). Turk J Bot 24:71-80.

Franceschi VR (1989) Calcium oxalate formation is a rapid and reversible process in *Lemna minor*. Protoplasma 148:130-137.

Franceschi VR, Horner HT (1980) Calcium oxalate crystals in plants. Bot Rev 46:361-427.

Grimson MJ, Arnott HJ (1983) An ultrastructural study of druse crystals in the abscission zone of *Phyllanthus niruri* L. Scan Electron Micros IV: 1771-1778.

Hienrich G, Pfeifhofer HW, Stabentheiner E, Sawidis T (2002) Glandular hairs of *Sigesbeckia jorullensis* Kunth (Asteraceae): Morphology, Histochemistry and composition of essential oil. Ann Bot 89:459-469.

Hepler PK, Wayne RO (1985) Calcium and plant development. Ann Rev Plant Physio 36:397-439.

Horner HT (1977) A comparative light and electron microscopic study of microsporogenesis in male-fertile and cytoplasmic male-sterile sunflower (Helianthus annuus). Am J Bot 64:745-759.

Horner HT, Wagner BL (1980). The association of druse crystals with the developing stomium of *Capsicum annuum* (Solanaceae) anthers. Am J Bot 67:1347-1360.

Horner HT, Wagner BL (1992) Association of four different calcium crystals in the anther connective tissue and hypodermal stomium of *Capsicum annuum* (Solanaceae) during microsporogenesis. Am J Bot 67:1347-1360.

Horner HT, Zindler-Frank E (1982) Histochemical, spectroscopic, and x-ray diffraction identifications of the two hydration form of calcium oxalate crystals in three legumes and *Begonia*. Can J Bot 60:1021-1027.

Horner HT, Kausch AP, Wagner BL (2000) Ascorbic acid: a precursor of oxalate in crystal idioblasts of *Yucca torreyi* in liquid root culture. Int J Plant Sci 161:861-868.

Hurkman WJ, Tanaka CK (1996) Effect of salt stress on germin gene expression in barley roots. Plant Physiol 110:971-977.

Ilarslan H, Palmer RG, Horner HT (2001) Calcium oxalate crystals in developing seeds of soybean. Ann Bot 88:243-257.

Ilarslan H, Palmer RG, Imsande J, Horner HT (1997) Quantitative determination of calcium oxalate and oxalate in developing seeds of soybean (Leguminosae). Am J Bot 84:1042-1046.

Katti RY, Giddanavar HS, Naik S, Agadi SN, Hegde RR (1994) Persistence of callose and tapetum in the microsporogenesis of genic male sterile *Cajanus cajan* (L.) Millsp. with well formed endothecium. Cytologia 59:65-72.

Kinzel H (1989) Calcium in the vacuoles and cell walls of plant tissue. Flora 182:99-125.

Kupicha FK (1975) *Helianthus* L. In Davis PH, ed., Flora of Turkey and the East Aegean Island. Edinburgh Univ. Press, pp. 44-45.

Lersten NR, Horner HT (2000) Calcium oxalate crystals types and trends in their distribution patterns in leaves of *Prunus* (Rosaceae: Prunoideae). Plant Syst Evol 224:83-96.

Molano-Flores B (2001) Herbivory and calcium concentrations affect calcium oxalate crystal formation in leaves of *Sida* (Malvaceae). Ann Bot 88:387-391.

Nakata PA (2003) Advances in our understanding of calcium oxalate crystal formation and function in plants. Plant Sci 164:901-909.

Prychid CJ, Rudall PJ (1999) Calcium oxalate crystals in monocotyledons: a review of their structure and systematics. Ann Bot 84:725-739.

Robertson BL (1978) Raphide-sac as epidermal appendages in *Jubaeopsis caffra* Becc. (Palmae). Ann Bot 42:489-490.

Schmid R (1976) Filament histology and anther dehiscence. J Linn Soc London Bot 73:303-315.

Seiler GJ (1997) Anatomy and morphology of sunflower. In Schneiter AA, ed., Sunflower technology and production. American Society of Agronomy, Inc. Wisconsin, pp. 67-111.

Tilton VR, Horner HT (1980) Calcium oxalate raphide crystals and crystalliferous idioblasts in the carpels of *Ornithogalum caudatum.* Ann Bot 46:533-539.

Webb MA, Arnott HJ (1982) A survey of calcium oxalate crystals and other mineral inclusions in seeds. Scan Electron Micros III:1109-1131.

Webb MA, Arnott HJ (1983) Inside plant crystals: a study of the noncrystalline core in druses of *Vitis vinifera* endosperm. Scan Electron Micros IV: 1759-1770.

Zindler-Frank E (1987) Calcium oxalate crystals in legumes. In Stirton CH. ed., Advances in legume systematics, Part 3. Royal Botanic Gardens, Kew, London, pp. 279-316.