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Calcium oxalate crystals during development of male and female gametophyte in *Leucojum aestivum* (*Amaryllidaceae*)

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Abstract

In this study, calcium oxalate crystal content of *Leucojum aestivum* was investigated during its life cycle. Ca-oxalate crystals were observed in adventative roots and leaves in all life cycle of *L. aestivum* but they were seen in pedicel and tepals from budding to flowering stages. During sporogenesis, gametogenesis and mature embryo sac phase, crystals were observed in ovary, style and tepals. Ca-oxalate crystals were found only at pollen mitosis phase in anthers. It is established that calcium oxalate crystals were of the raphide type.

Key words: *Amaryllidaceae*, Ca-oxalate crystals, *Leucojum aestivum*, life cycle, generative organs, vegetative organs

INTRODUCTION

Ca-oxalate crystals which are found in the structure of druse and raphide type are inclusions that are often seen in higher plants [1] and in fungi [2]. Their occurence and abundance in specific tissues of various plants are so constant that they are used as a taxonomical tool [3]. The Ca-oxalate crystals were observed in the epidermis [4], mesophyll [5], cortex and pith of the stem [6], phloem, xylem [7], parenchyma and pericarp [8] of various plants by plant anatomists. Presence of the crystals in various tissues of laticifers [9; 5], corm [10; 11] and seeds [12] is known. In recent years it is also notable that these crystals are present in transitory floral organs such as stamens, gynoecia and petals [13]. They are quite prevalent in floral organs of many taxa, including *Dilleniaceae*, *Liliaceae*, *Palmae, Malvaceae*, *Cunoniaceae*, *Euphorbiaceae* [14], *Solanaceae* [15], *Leguminosae* [16], *Amaryllidaceae* [17; 18]. But there is no detailed description of the crystal content in life cycle of the plants.

In this study we described ca-oxalate raphide crystal content of *Leucojum aestivum* in some vegetative and generative tissues in different phases of its life cycle. Such information would greatly increase our understanding of their morphology and functional relationships.

MATERIAL and METHODS

In this study, *L. aestivum* plants were used as material. They were brought from Tavuk Forest- Edirne and grown in the Botanical garden of the Biology Department of Trakya University in 2005.

Materials were preserved in 70% ethyl alcohol after being fixed in Carnoy's fluid (3 ethyl alcohol: 1 acetic acid). Leaves, root tips and flower buds were dehydrated in ethanol and embedded in paraffin for microscopical preperations. The sections were stained with Haematoxylin and mounted in Entellan. Herr's (1971) clearing-squash technique was used to observe Ca-oxalate (CaOx) crystals in different tissues of *L. aestivum* [19].

RESULTS and DISCUSSION

Ca-oxalate crystals in raphide type were observed in some vegetative and generative tissues of *L. aestivum* in different phases during its life cycle. Table 1 shows distribution of these crystals in this species in different phases of its life cycle. Raphide crystals were seen in the adventive roots (Figure 1) and leaves (Figure 2). They were found in tepals (Figure 3), ovary (Figure 4) and style (Figure 5) during the megasporogenesis, megagametogenesis and mature gametophyte in the floral organs of gynaeceum. Raphide crystals were only observed in connective tissue of anthers at pollen mitosis phase (Figure 6).

Table 1: Distribution of raphide type CaOx crystals in *L. aestivum* in different phases of its life cycle.

Organs	Growth phase	Raphide existance
Leaves	All life cycle	+
Root	All life cycle	+
Pedicel	All life cycle	+
1mm anther	Sporogenous tissue	-
2mm anther	Dyad, tetrad, microspores with one nucleus (Microsporogenesis)	-
3mm anther	Pollen mitosis	+ (Connective tissue)
4-5mm anther	Mature pollen	-
6mm bud, style, ovary, tepal	Megasporogenesis	+
10mm bud, style, ovary, tepal	Megagametogenesis	+
15mm flower, style, ovary, tepal	Mature embryo sac	+

Figure. 1: Ca-Oxalate crystals in the root cells of *Leucojum aestivum* Scale Bar = 10µm.

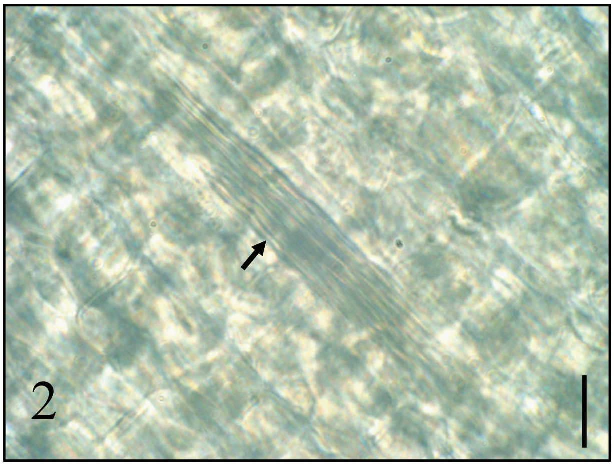

Figure. 2: Ca-Oxalate crystals in the leaf cells of *Leucojum aestivum* Scale bar = 20µm.

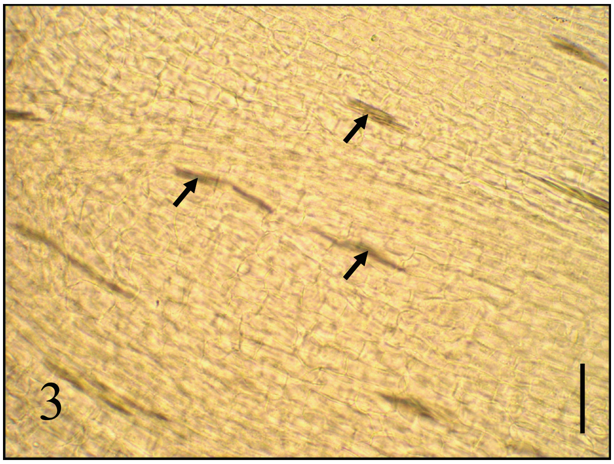

Figure. 3: Ca-Oxalate crystals in the tepal cells of *Leucojum aestivum* L. Scale bar = 100µm.

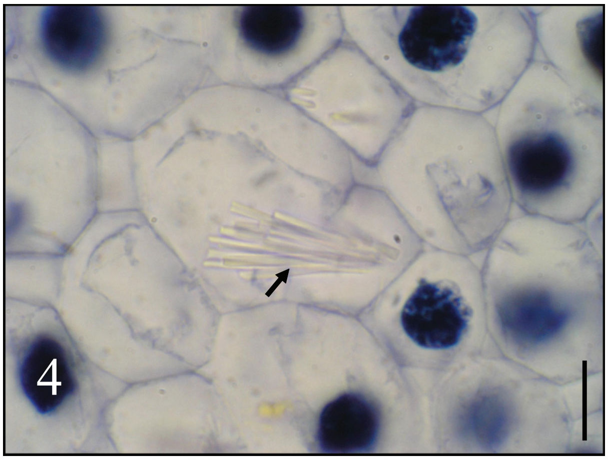

Figure. 4. Ca-Oxalate crystals in the ovary wall cells of *Leucojum aestivum*. Scale bar = 10µm.

Figure. 5. Ca-Oxalate crystals in the style cells of *Leucojum aestivum*. Scale bar = 30µm.

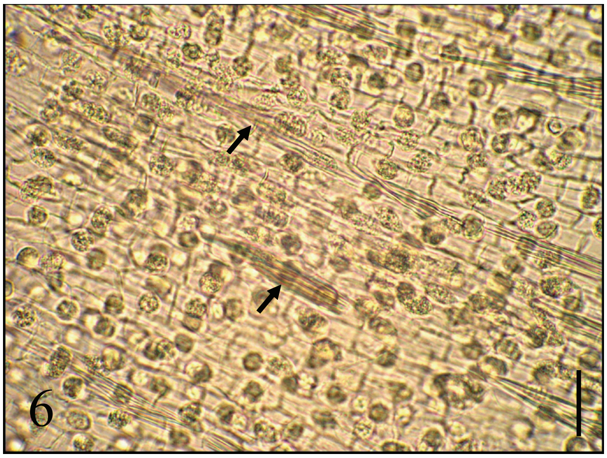

Figure. 6. Ca-Oxalate crystals in the connective tissue of anther cells in *Leucojum aestivum* Scale bar = 30µm.

Calcium oxalate (CaOx) crystals are distributed among all taxonomic levels of photosynthetic organisms from small algae to angiosperms and giant gymnosperms [20]. Calcium oxalate crystals have potential roles in plants as part of a defence mechanism against herbivores and/or in accumulating excess calcium [21]. Raphide type crystals were observed in some vegetative and generative tissues of *L. aestivum* in different phases of its life cycle. Existence of Ca-oxalate crystals in meristematic cells has been demonstrated in several studies [13;15; 22; 23; 24]. Mezophyll cells of plants in *Amaryllidaceae* contain raphide crystals [5]. These crystals were also seen in ovary wall of *Triteleia* (*Themidaceae*) [25], *Galanthus nivalis* [17], *Galanthus plicatus*, *G. gracilis* and *G. elwesii* (*Amaryllidaceae*) [18] like *L. aestivum*. In this study, raphide crystals were observed in all vegetative and generative tissues except anthers in *L. aestivum* at their flowering stage like *Galanthus sp*. which is the sister genus with *Leucojum*.

Physical and chemical conditions such as temperature, pressure, pH and ion concentration, may affect crystal development [26], however it is considered that formation of crystals within the cell is under genetic control [27]. Although some species of monocotyledones have different crystal types in adjacent cells but particular taxons can have specific crystal shape [28].

As a result of this study, Ca-Oxalate crystals of *L. aestivum* (*Amaryllidaceae*) are determined in different stages of its development. It's of interest because probably other members *Amaryllidaceae* can contain crystals in their floral organs. Thus, additional researches are needed in order to reveal the specific role of Ca-Oxalate crystals in plant metabolism and taxonomy.

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