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"Research Note"

DIFFERENTIATION OF CRYSTAL-CONTAINING CELLS DURING ANTHER DEVELOPMENT AND CRYSTAL TYPES IN THE ANTHER OF *PETUNIA HYBRID GRANDIFLORA* (SOLANACEAE)*

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Abstract – Calcium crystals are evident in many parts of anther. There is little literature about differentiation of crystal-containing cells and crystal formation during anther development. *Petunia hybrid grandiflora* Flower buds collected at different developmental stages were fixed and studied. The results revealed that young anthers are tetrasporangiate and in each of the four corners, the primary parietal layer externally and primary sporogenous cells internally, arise from periclinal divisions of the archeosporial cells. In the young anther, septum and connective cells contain calcium crystals as druse. At stomium, there is an arrangement of a band of 8-14 cells just beneath the epidermis which densely stain and lack visible vacuoles. During the division of sporogenous cells and the formation of crescent shaped tissue, no crystals are seen in hypodermal cells. In this stage, druse crystals become larger in connective tissue. While meiosis is occurring, the hypodermal cells of stomium are elongated, yet crystals are not observable. During pollen development, hypodermal cells degenerate and sand crystals appear. It seems that these crystals result from druse crystal.

Keywords – Anther development, crystal-containing cells, hypodermal cells, *Petunia hybrid grandiflora*

1. INTRODUCTION

Calcium oxalate crystals are widespread in plant and animal cells and their occurrence in plants was surveyed by Kuster, Horner and Wagner and Smith [1, 2, 3]. The first note on crystal accumulating tissue in anther is attributed to Hegemaier in Lemnaceae [4]. It was later recorded in the Ericaceae [5, 6, 7]. Namikawa described it in anthers of the Solanaceae and identified its contents as calcium oxalate [8]. Most of these researchers have referred to the hypodermal layer at the stomium as the best known configuration of crystals. They are found in a special structure commonly located beneath the epidermis at the stomium and running lengthwise around the anther with longitudinal dehiscence. It is usually a row of cells that are filled with fine calcium oxalate crystals [9]. Except in *Deprea*, crystals almost abruptly demarcated from the septum [10]. Iwano observed them as crystal-like grains in some connective tissue cells and in cells beneath the stomium in the anther of *Petunia* by scanning electron microscopy [11]. Studies of Iwano using an X-ray microanalysis system fitted with SEM showed that many calcium crystals are accumulated under stomium in the anther of *Petunia*. They adhered to pollen grains so that they could supply calcium ions for pollen germination. In this paper, the histological changes of crystal bearing cells during anther development and the different types of crystals were studied.

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2. MATERIALS AND METHODS

The young *petunia* buds were collected at close intervals until flowering (anthesis) time. Samples fixed in FAA (20 ml formalin, 10 ml acetic acid and 70 ml 96% ethanol) were dehydrated in a series of graded ethanol solution and embedded in paraffin. Transverse serial sections were cut at a thickness of 8-12 μm using a rotary microtome. Hematoxiline and Eosine were used for staining. Observations were made by Light Microscopy (LM).

3. RESULTS

As seen in Fig. 1A, young anthers are tetrasporangiate. In each of the four corners of anther, the primary parietal layer externally and primary sporogenous cells internally arise from periclinal divisions of the archeosporial cells (Fig. 1B). In this stage, septum and connective cells contain calcium crystals as druse (Fig. 1A, B). At stomium, during the division of sporogenous cells and formation of crescent shaped tissue surrounded by the tapetum, a band of 8-14 cells are arranged beneath the epidermis (Figs. 1C-F). These cells are densely stained and lack visible vacuoles, suggesting a high metabolic activity. They do not contain any crystal. In this stage, in connective tissue, druse crystals increase in number and become larger (Figs. 1C-F). At about the time of meiosis, hypodermal cells of stomium elongate, yet crystals are not observed (Figs. 1G-I). During microspore development, these hypodermal cells are vacuolated and crystal-like grains (sand crystals) are observable (Figs. 2A-F). In these stages, druse crystals located in connective tissue have the largest size (Figs. 2A-D). Later, at maturity, hypodermal cells are degenerated to leave a mass of calcium oxalate crystals that do not enlarge further (Figs. 3A-D). Finally, during epidermis dehiscence, pollen grains are exposed to crystal-like grains (calcium package) (Fig. 3E).

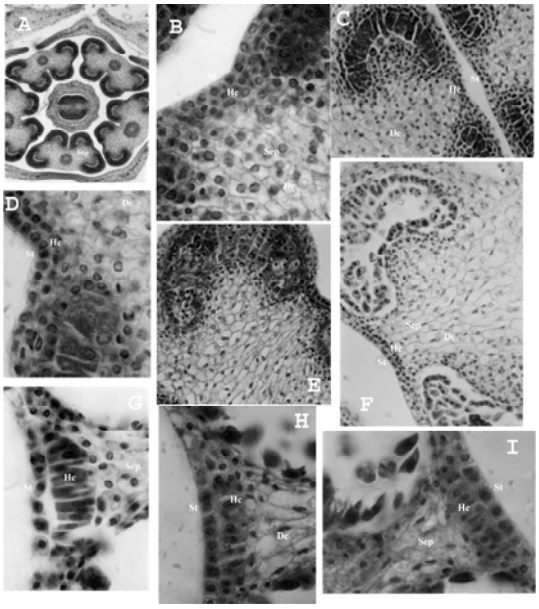

Fig. 1. Light micrograph of cross sections of anther at different developmental stages showing oxalate calcium crystals formation. A: Young anther (X10). B: Wall layers and sporogenous tissues are forming; Druse crystals are differentiating in intersporangial septum, hypodermal cells beneath stomium are undifferentiated (X100). C-F: Druse crystals in intersporangial septum and connective tissue are obvious and numerous. Hypodermal cells in stomium are differentiating as a band of 8-14 cells (respectively X40, X100, X40, and X40). G-I: Differentiation of hypodermal cells as palisade cells and early organization of crystal like grains in these cells during meiosis, druse crystals are obvious (X100)

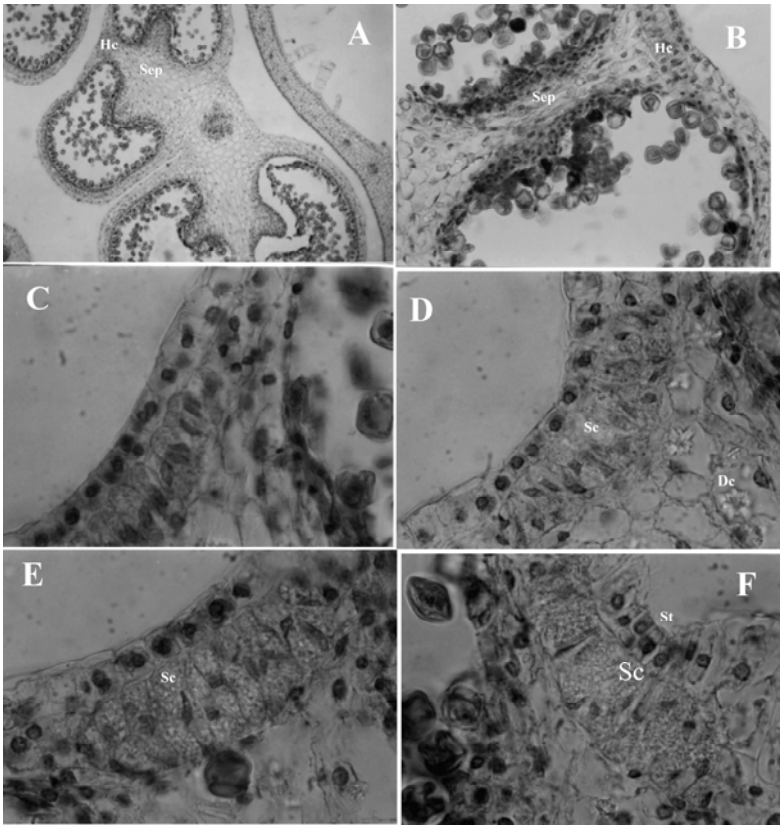

Fig. 2. Light micrograph of cross sections of anther at different developmental stages showing oxalate calcium crystals formation. A-F: Formation of crystals like grain (sand crysals) as well as gradual degeneration of crystal containing cells (A, B, respectively X10, X40; C-F, X100)

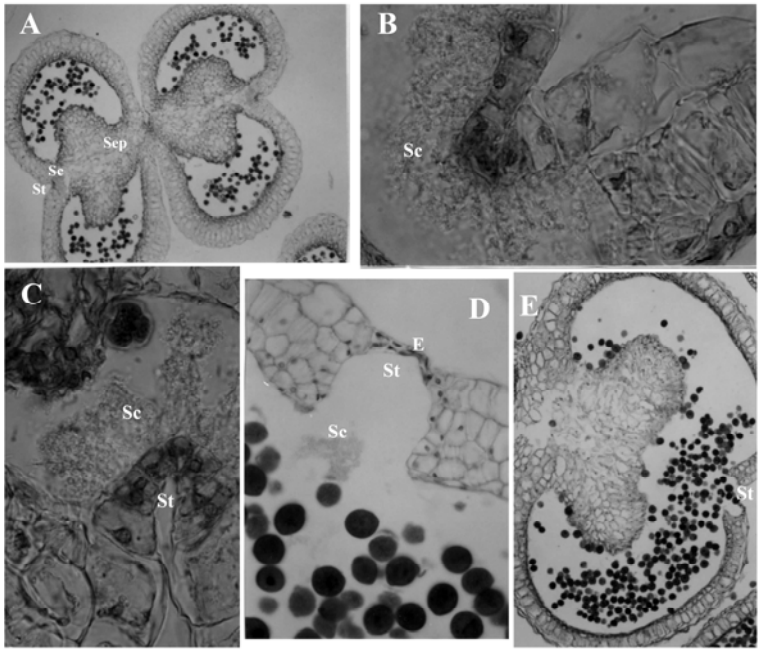

Fig. 3. Light micrograph of cross sections of anther at different developmental stages showing oxalate calcium crystals formation. A-C: Degeneration of crystal containing cells and formation of oxalate calcium package, respectively X10, X100, X100. D, E: Break down and anther dehiscence; release of pollen grains and oxalate calcium package, respectively, X40, X10

4. DISCUSSION

Two kinds of calcium crystals were accumulated in the anthers of *Petunia*: 1- Druse crystals were observed in some connective tissue cells and septum. These crystals appeared in the early stage of anther development, at the time of sporogenous tissue and anther wall formation. They were abundantly accumulated in intersporangial septum. During development of anther, they grew in size and finally disappeared during anther dehiscence. 2- Crystal like grains (sand crystals) were observed in cells beneath the stomium. The differentiation of crystal bearing cells started just before meiosis. At maturity, these crystals appeared in palisade cells beneath the stomium. Finally, when anther was dehiscing, these crystal bearing palisade cells degenerated and the calcium package was formed. It is concluded that crystal-like grains (crystals sand) result from break down and reorganization of druses. D' Arcy *et al*. presented a calcium oxalate package in the anthers of solanaceae [10]. They reported that these crystals serve to enhance pollinator attraction through visual or chemical stimuli or both. Iwano et al. observed that many crystals accumulate under the stomium in the anther of *Petunia* [11]. They stated that when the anther dehisces and pollen grains are released from the stomium, the calcium crystals adhere to the pollen and dissolve in the aqueous drop under the exudates on the stigma and supply calcium ions for pollen germination [11]. Ca is one of the main factors in pollen growth and pollen tube growth [12]; therefore the results show that these crystals induce pollen germination and pollen tube growth.

NOMENCLATURE

Dc	Druse crystal
E	Epidermis
Hc	Hypodermal cells
Sc	Sand crystal
Sep	Septum
St	Stomium

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