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INVESTIGATION OF CELL WALL  
THICKENINGS AND CRYSTALS IN THE  
PERICARP OF *PHASEOLUS VULGARIS* L.

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## Introduction

Cell wall thickenings can sometimes be found in plants of some families. Most common thickenings are semi-spherical in shape, occasionally incrustated with calcium carbonate. According to some authors such thickenings of cell walls represent an early stage of cystoliths development (Penzig 1881, Molisch 1882, Dubravec 1967/68). In vegetative and less commonly in generative organs of some families such as *Moraceae*, *Oleaceae*, *Cannabimaceae*, *Cucurbitaceae* etc. cystoliths can be found (Zimmermann 1891, Küster 1956).

Calcium oxalate crystals in *Phaseolus vulgaris* L. have till now been found only in the seed coat (Wiesner 1920). Semi-spheric thickenings of cell walls and cystoliths were never recorded in the pericarp of *Phaseolus vulgaris* L. Therefore we decided to carry out this investigation.

## Materials and Methods

This investigation was performed on the material cultivated in the experimental field of the Institute of Horticulture (Zagreb). Investigations were done on 'Favorit' and 'Top-crop' variety. The legumes were sectioned near the basis, in the middle and at the top. Microscopic analyses were made on transversal and longitudinal slices of epidermis and other parts of pericarp. The presence of calcium carbonate was proved with acetic and sulphuric acids. Crystals of calcium oxalate were identified with hydrochloric and sulphuric acids (Strasburger et Koernicke 1921, Braune et al., 1971).

Optical characteristics of cystoliths and calcium crystals were investigated with polarisation microscope.

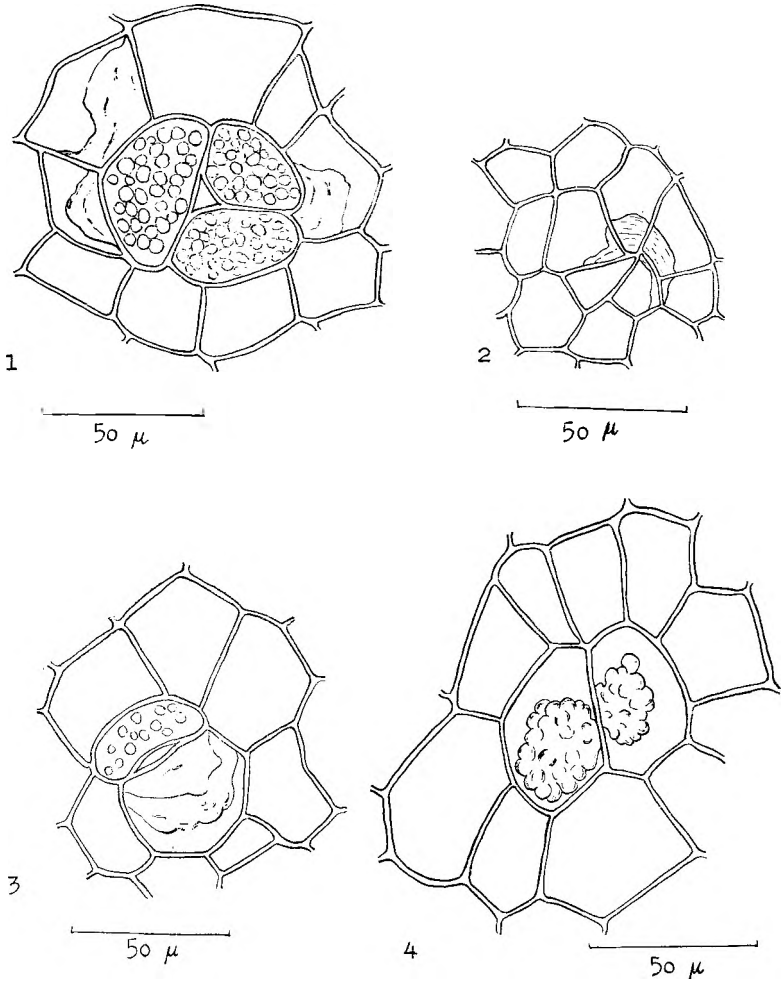


Fig. 1. Epidermis with giant stoma consisting of three guard cells and semi-spherical thickenings.

Sl. 1. Epiderma s orijaškom puči i polukuglastim zadebljanjima staničnih stijenki.

Fig. 2. Semi-spherical thickenings in epidermal cells of 'Favorit' variety.

Sl. 2. Polukuglasta zadebljanja u epidermi sorte 'Favorit'.

Fig. 3. Semi-spherical thickenings in epidermis of 'Top-crop' variety. One guard cell is dead, the other contains chloroplasts.

Sl. 3. Polukuglasta zadebljanja staničnih stijenki u epidermi sorte 'Top-crop'. Jedna stanica zapornica je mrtva, a druga sadrži kloroplaste.

Fig. 4. Cystoliths with verruciform surface in legume epidermis of 'Favorit' variety.

Sl. 4. Cistoliti s bradavičastom površinom u epidermi mahuna sorte 'Favorit'.

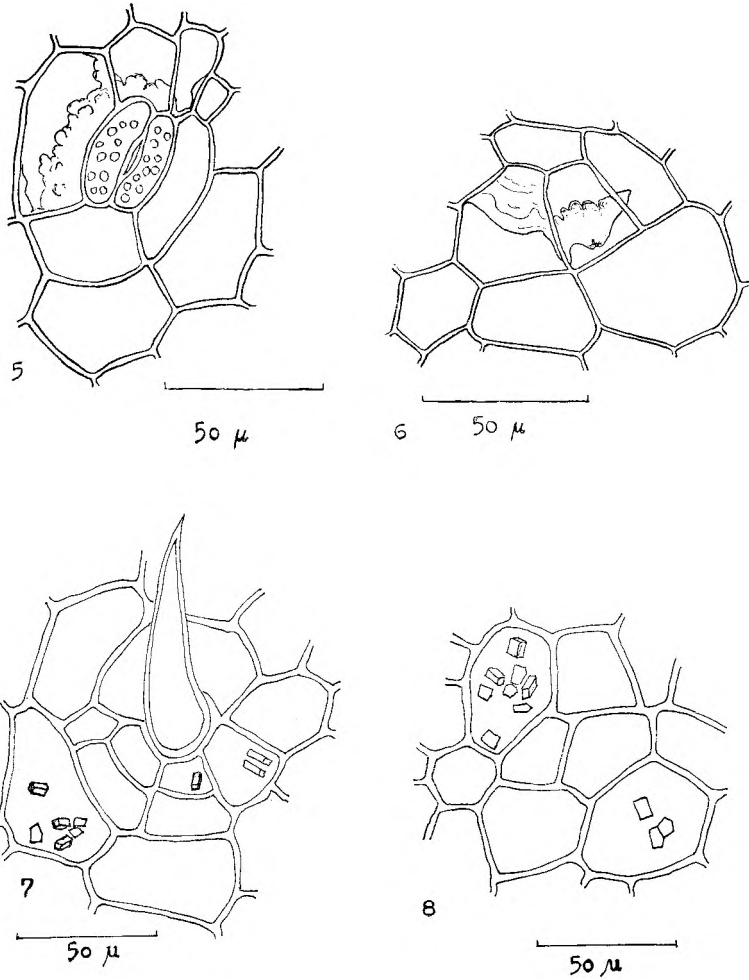


Fig. 5. Cystoliths in two cells around stomata.  
Sl. 5. Cistoliti u dvije stanice oko puči.

Fig. 6. Cystoliths and half-spheric thickenings in epidermal cells.  
Sl. 6. Cistoliti i polukuglasta zadebljanja staničnih stijenki u epidermalnim stanicama.

Fig. 7. Calcium oxalate crystals around basal hair cells of 'Top-crop' variety.  
Sl. 7. Kristali kalcijevog oksalata oko bazalnih stanica dlaka.

Fig. 8. Calcium oxalate crystals in legume epidermis of 'Favorit' variety.  
Sl. 8. Kristali kalcijevog oksalata u epidermi mahune sorte 'Favorit'.

## Results and Discussion

Continuing anatomical investigations (Plavšić-Gojković and Dubravec 1975) in epidermal cells of 'Favorit' variety we found semi-spherical thickenings of cell wall and cystoliths. In both varieties crystals of calcium oxalate were found in exocarp (epidermis, subepidermis) and mesocarp.

*Semi-spherical thickenings of cell walls*

While studying the anatomy of pericarp in the epidermis of 'Favorit' legumes 12—14 cm long, semi-spherical thickenings of cell wall were noticed. These structures were formed around certain centres, such as basal cells of hairs, necrotic epidermal cells, normally and anomalously formed stomata. The anomalous stoma consisted of three guard cells and was bigger than other stomata (Fig. 1). The guard cells of giant stoma had much more chloroplasts than normal stomata.

Semi-spherical thickenings of cell walls are often stratified (Fig. 2, 3). Since these formations appear around certain centres, similarly as cystoliths, and are less densely incrustated with calcium carbonate, we can with presume great probability that they represent a certain development stage of cystoliths (cf. Penzig 1881, Molisch 1882, Dubravec 1967/68).

In epidermal cells of 'Favorit' legumes 12—14 cm long besides, semi-spherical thickenings cystoliths can also be found. Cystoliths are formed on the lateral epidermis and other cell walls. They are formed in one, two, or less commonly in several epidermis cells (Fig. 4, 5). Cystoliths occupy the majority of the cell lumen so that the protoplast is placed in the smaller part of cell (Fig. 4). Cystoliths have verruciform surface and it seems that they are densely incrustated with calcium carbonate, which was proved by intensive appearance of carbon dioxide bubbles after treatment with acetic acid. Cystoliths are more or less irregularly graped-shaped and sometimes cone-shaped (Fig. 6). Verruciform cystoliths in the epidermis of 'Favorit' variety legumes scintillate very intensively in polarised light, which means that they are strongly incrustated with crystalline calcium carbonate. Microchemical reactions with chlor-zinc-iodine and 1% solution of Congo red revealed that cystoliths walls consist of cellulose.

*Crystals*

Calcium oxalate crystals were noticed in the pericarp of *Phaseolus vulgaris* L. variety 'Favorit' and 'Top-crop'. They can be found in the exocarp (epidermis and subepidermis) and the mesocarp of 6—8 cm long legumes of both varieties. The number of crystals increase proportionally with the increase of the legume length (Scott 1941). Most of the crystals are situated in the epidermis of the middle of the legume while they were fewer towards the top and the basis of the legume.

Epidermal cells containing calcium oxalate crystals are usually similar to other epidermal cells (Fig. 8). Calcium oxalate crystals can frequently be found near the basal cells of hairs (Fig. 7).

In the subepidermis and the mesocarp, unlike the epidermis, crystals are situated in cells that differ from other cells in shape as well as

in size. The crystals in the mesocarp cells are much more numerous and much bigger than in the subepidermal ones. Sometimes in big mesocarp cells raphides can be seen. Some authors consider that through the formation of calcium oxalate crystals in cells the harmful influence of oxalic acid is eliminated (Haberlandt 1909, Frey-Wyssling 1930, 1938, Küster 1956 ect).

### Summary

In the epidermis of some bean legumes of 'Favorit' variety of *Phaseolus vulgaris* L. semi-spherical thickenings of cell walls are formed. Sometimes they are slightly incrustated with calcium carbonate. These thickenings are most frequently found around necrotised epidermal cells and anomalous stomata. In the epidermis of 'Favorit' legumes irregularly grape shaped and conical cystoliths appear only in the variety described and they might have a taxonomic role too.

Calcium oxalate crystals were found in the exocarp and the mesocarp of 'Favorit' and 'Top-crop'. They are much bigger and more numerous in the mesocarp than in the exocarp.

### References

- Braune, W., A. Leman and H. Taubert, 1971: Pflanzenanatomisches Praktikum. Gustav Fischer Verlag, Jena.
- Dubravec, K., 1967/68: Cistoliti i druga zadebljanja staničnih stijenki u vrste *Ecballium elaterium* (L.) Rich. Acta Bot. Croat. 26/27, 145—149.
- Dubravec, K., 1972: Cistoliti i polukuglasta zadebljanja staničnih stijenki u plodovima *Cucurbita pepo* L., *C. pepo* var. *ovifera* Bailey i *C. pepo* var. *verrucosa* Naud. Polj. znanstvena smotra, 8, 103—106.
- Esau, K., 1969: Pflanzenanatomie. Gustav Fischer Verlag, Stuttgart.
- Frey-Wyssling, A., 1930: Vergleich zwischen der Ausscheidung von Kieselsäure und Kalziumssalzen in den Pflanzen. Ber. d. Dtsch. Bot. Ges. 48, 184—191.
- Frey-Wyssling, A., 1938: Submikroskopische Morphologie des Protoplasmas und seiner Derivate. Verlag Gebrüder Bornträger, Berlin.
- Haberlandt, G., 1909: Physiologische Pflanzenanatomie. Leipzig.
- Küster, E., 1956: Die Pflanzenzelle. Verlag Gustav Fischer, Jena.
- Molisch, H., 1882: Über kalkfreie Zystolithen. Österr. Bot. Z. 32, 245—347.
- Penzig, O., 1881: Zur Verbreitung der Zystolithen in Pflanzenreich. Bot. Centralbl. 8, 393—401.
- Plavšić-Gojković, N. and K. Dubravec, 1975: The significance of anatomical structure of Green bean (*Phaseolus vulgaris* L.) in determining the harvest time. Acta Horticult. 52, 193—202.
- Scott, F. M., 1941: Distribution of calcium oxalate crystals in *Ricinus communis* L. in relation to tissue differentiation and presence of other ergastic substances. Bot. Gaz. 103, 225—246.
- Strasburger, E. and M. Koernicke, 1921: Botanisches Praktikum Gustav Fischer Verlag, Jena.
- Wiesner, J., 1920: Anatomie und Physiologie der Pflanzen, Wien—Leipzig.
- Zimmermann, A., 1891: Über die radialen Stränge der Zystolithen von *Ficus elastica*. Ber. d. Dtsch. Bot. Ges. 9, 126—128.

S A Ž E T A K

MEMBRANSKA ZADEBLJANJA I KRISTALI U PERIKARPU  
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Ovim istraživanjima utvrđeno je da se u epidermi mahuna vrste *Phaseolus vulgaris* L. sorte 'Favorit' stvaraju polukuglasta zadebljanja staničnih stijenki oko nekrotiziranih stanica epiderme i anomalno građenih puči. Ona su katkad inkrustirana kalcijevim karbonatom. U epidermi sorte 'Favorit' pronađeni su i cistoliti grozdasta ili čunjasta oblika. Kako se cistoliti pojavljuju samo u navedene sorte, oni bi mogli imati i taksonomsko značenje.

U perikarpu mahuna sorte 'Favorit' i 'Top-crop' utvrđeni su u egzokarpu i mezokarpu kristali kalcijeva oksalata. Oni su znatno veći u mezokarpu nego u egzokarpu.

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