Phyton (Austria)	**Vol. 26**	Fasc. 2	171–192	15. 4. 1987

Ultrastructure of the Callose and Cellulose Types of Crystal Envelopes in the Calcium Oxalate Idioblasts of *Abutilon pictum* WALP.[1])

By

Urs BRANDER*)

With 18 Figures (4 Plates)

Received November 11, 1985

Key words: *Abutilon,* anilin blue, calcium-oxalate crystal idioblast, callose, histochemistry, lipids, plant ultrastructure

Summary

BRANDER U. 1987. Ultrastructure of the callose and cellulose types of crystal envelopes in the calcium oxalate idioblasts of *Abutilon pictum* WALP. – Phyton (Austria) 26 (2): 171–192, with 18 figures (4 plates). – English with German summary.

Each crystal idioblast in the stem or petiole of *Abutilon pictum* contains a druse crystal generally enclosed in an envelope (= crystal sheath). Most sheaths cannot be stained with aniline blue and are mainly composed of two structural elements in a regular disposition: (1) Fibrillar, wall-like material forms the outer, peripheral sheath part and is mostly continuous with the cell wall. (2) Osmiophilic material accumulating near the crystals is at the origin of a faint sheath autofluorescence and can be stained with lipophilic fluorochromes.

About one per cent of the crystal sheaths, however, can be stained by aniline or resorcinol blue for callose. Idioblasts containing such sheaths are randomly distributed among the non-stained idioblasts. After standard fixation and heavy metal staining for electron microscopy, the periphery of their crystal sheath remains nearly unstained. Idioblasts fluorescing with aniline blue were identified in the electron microscope by consecutive sectioning from glycol methacrylate embedded tissue. Silver methenamine contrast after an oxidation with permanganate and periodate permits the ultrastructural delimitation of aniline blue positive sheath areas against empty spaces. This staining can no longer be produced on sections pretreated with

[1]) Part of a doctoral thesis submitted to the Faculty of Science, University of Fribourg, Switzerland.

*) Dr. Urs BRANDER, Obertor 9, CH-8400 Winterthur. For correspondence and reprints: Prof. J. WATTENDORFF, Institut für botanische Biologie und Phytochemie der Universität, Albert-Gockel-Straße 3, CH-1700 Freiburg, Switzerland.

(1→3)-β-D-glucanases. The possible relations between sheath composition and idioblast differentiation are discussed.

Idioblasts without crystal sheaths are supposed to represent early stages of development. Here, the crystals are present in a compartment resembling a vacuole. At the beginning of sheath formation, the crystals are covered by a cytoplasmic layer.

Zusammenfassung

BRANDER U. 1987. Feinbau der Kristallhüllen vom Callosetypus und Cellulosetypus in den Calciumoxalat-Idioblasten von *Abutilon pictum* WALP. – Phyton (Austria) 26 (2): 171–192, mit 18 Abbildungen (4 Tafeln). – Englisch mit deutscher Zusammenfassung.

Die Kristallidioblasten im Stengel und Blattstiel enthalten je einen Drusenkristall, der meist von einer Hülle (= Kristallscheide) umgeben ist. Die meisten Scheiden werden mit Anilinblau nicht angefärbt und weisen zwei strukturell verschiedene Komponenten in ähnlicher Anordnung auf: (1) Fibrilläres, wandartiges Material bildet die äußere, periphere Kristallscheide, die meist in die Zellwand übergeht. (2) Osmiophiles Material grenzt direkt an den Kristall und verleiht dem inneren Scheidenteil eine schwache Eigenfluoreszenz. Mit lipophilen Fluorochromen läßt es sich anfärben.

Etwa 1% der Kristallscheiden zeigen die Kallosefärbung mit Anilin- oder Resorcinblau. Idioblasten mit solchen Scheiden sind zwischen den ungefärbten Idioblasten zufällig verteilt. Der äußere Teil solcher Kristallscheiden kann mit Standardmethoden für die Elektronenmikroskopie kaum kontrastiert werden wie anhand eines Vergleichs von Folgeschnitten aus Glycolmethacrylat-Einbettung gezeigt wird. Silbermethenaminkontrast nach vorheriger Oxidation mit Kaliumpermanganat und Perjodat grenzt die mit Anilinblau fluoreszierenden Scheidenbezirke gegen leere, extraplasmatische Räume ab. Dieser Kontrast unterbleibt nach Vorbehandlung mit (1→3)-β-D-Glucanasen. Die möglichen Beziehungen zwischen dem Scheidenbau und der Kristallzelldifferenzierung werden diskutiert.

Idioblasten ohne Kristallscheiden stellen vermutlich frühe Entwicklungsstadien dar. Ihre Kristalle befinden sich in einem vakuolenähnlichen Kompartiment. Während der Scheidenbildung sind die Kristalle von einem Cytoplasmabelag überzogen.

Introduction

Calcium oxalate crystals occur in many species and in most organs and tissues of higher plants (NETOLITZKY 1929, ARNOTT & PAUTARD 1970, ARNOTT 1982, WEBB & ARNOTT 1982). The crystals are frequently found associated with membranes, chambers or sheaths (WATTENDORFF 1978, FRANCESCHI & HORNER 1980, WEBB & ARNOTT 1981). Crystals in a sheath (= envelope) fixed to the cell wall were discovered by ROSANOFF (1865) and are therefore often called Rosanoff crystals. Pecto-cellulosic crystal sheaths are common in many plant genera (SCOTT 1941, DENGG 1969, PRICE 1970, NANKO & al. 1976, THURSTON 1976). The sheaths may become lignified (FRANK & JENSEN 1970, PARAMESWARAN & SCHULTZE 1974, PARAMESWARAN & LIESE 1979, PARAMESWARAN & RICHTER 1984), suberized, or contain other lipidic material (SCOTT & al. 1948, WATTENDORFF 1969, 1976, 1978).

In *Abutilon pictum* the sheaths of some druse crystals react strongly for callose as revealed by its staining properties with aniline blue as a fluorochrome or with resorcinol blue as a diachrome (THALER & WEBER 1957). This was confirmed for *Abutilon megapotamicum* by HUGHES & GUNNING (1980). Callose has also been reported to occur in crystal envelopes in the primary cortex of *Salix fragilis* (GORSKA-BRYLASS and SMOLINSKY 1966), in a layer around crystals enclosed in the cell wall of *Dracaena fragrans* (THALER & WEBER 1960), and to be involved in other mineralization processes as in cystolith formation (ESCHRICH 1954) and in silicification (WATERKEYN & al. 1982).

Both the "filling cork" present in certain crystal sheaths and callose give a very poor or no reaction with conventional stainings for transmission electron microscopy. The present study seeks to find ultrastructures that only occur in those crystal sheaths which can be stained for callose in the light microscope and to see which structural element corresponds to callose.

Material and Methods

Cuttings of *Abutilon pictum* WALP. *"Thompsonii"* *VEITCH* (= *A. striatum* DICKS. *"Thompsonii"*) from plants of the Botanical Garden Geneva were grown in flower pots in the Botanical Garden Fribourg, Switzerland. Freshly rooted cuttings as well as plants which had attained full growth were chosen. A light microscopic comparison was made with *Abutilon molle* BAK. (SWEET), *Abutilon pictum* WALP. *"Spurium"* (= *A. striatum "Spurium"*), and the following *Abutilon* hybrids: *A.* × *hybridum* hort. and *A. darwinii* HOOK. × *A. pictum* "Golden Fleece".

For light microscopy, hand- and cryosections of fresh and formalin – acetic acid – alcohol fixed material as well as semithin sections from tissues embedded as for electron microscopy (BENNETT & al. 1976) were examined with the following techniques (in alphabetical order; percentages in wt/wt unless otherwise stated): (1) Aniline blue, water soluble (Wasserblau, Fluka, Buchs, Switzerland) Colour index (C.I.) 42755: 0.005% in a K_2HPO_4/K_3PO_4 buffer at pH 8.5 (ESCHRICH & CURRIER 1964). (2) Auramin O (Fluka, Buchs, Switzerland) C.I. 41000: 0.01% aqueous solution (HESLOP-HARRISON & HESLOP-HARRISON 1981). (3) 3,4-benzpyrene-caffeine: A saturated solution of caffeine containing 2 mg 3,4-benzpyrene (Fluka, Buchs, Switzerland) is diluted in the ratio 1 : 1 with water (1–10 min; JENSEN 1962). (4) Calcofluor white M2R new (American Cyanamid, Bound Brook, U.S.A.; HUGHES & MCCULLY 1975) and Calcofluor white ST (American Cyanamid, Bound Brook, U.S.A.; HERTH & SCHNEPF 1980): 0.01%–0.1% aqueous solution or in 10% ethanol. (5) Chelidonium fluorochrome for enhancing autofluorescence: An unrefined ethanolic extract from *Chelidonium majus* L. roots in a tenfold dilution with distilled water (15 min, WEERDENBURG & PETERSON 1983). (6) Chlorophyll for fluorescence with blue light excitation:

A crude ethanolic extract from leaves of *Dipsacus laciniatus* L. (WATTENDORFF 1969) and *Piper betle* L. (10–20 min). (7) Enzymatic hydrolysis of (1→3)-β-D-glucan as described for electron microscopy (5–60 min). (8) I_2KI-H_2SO_4: Sections are treated 5 min in a solution containing 1% of KI and 1% of I_2 in water, then 60–80% H_2SO_4 is added (SCOTT 1950). (9) Methylene blue-azure A, safranin: Methylene blue (Siegfried, Zofingen, Switzerland) C.I. 52015, 1%; azure A (Hopkin & Williams, Chadwell Heath, England) C.I. 52005, 0.02% in phosphate buffer (0.01 M, pH 6.9); safranin T (Fluka, Buchs, Switzerland) C.I. 50240, 0.1% in Tris buffer (0.2 M, pH 9.0) (WARMKE & LEE 1976). (10) Periodic acid-Schiff reaction (PAS): 1% periodic acid (20 min), Schiff reagent (10–30 min; LILLIE 1965). (11) PAS followed by aniline blue as under (1) or by calcofluor white as under (4). (12) Phloroglucinol-HCl: Sections are treated in a saturated aqueous solution of phloroglucinol (1–5 min), followed by 20% HCl (JENSEN 1962). (13) Phosphine (chrysaniline yellow, BDH Chemicals, Poole, England) C.I. 46045: 0.01% aqueous solution (15 min, BIGGS 1984). (14) Resorcinol blue: Preparation according to ESCHRICH & CURRIER (1964); in a dilution of 5–10 drops in 10 ml tap water or artifical, defined tap water (pH 7.6–8.0). (15) Silver nitrate-rubeanic acid for visualization of calcium oxalate (YASUE 1969, HORNER and ZINDLER-FRANK 1982 a): Removal of eventual calcium phosphate or carbonate by immersion in 5% acetic acid (30 min), then 5% aqueous $AgNO_3$ (10–20 min), followed by saturated rubeanic acid in 70% ethanol to which 2 drops of aqueous ammonia (25%) per 100 ml were added prior to use. (16) Solubility tests for calcium oxalate crystals (FRANK 1967, HORNER et al. 1983): Insoluble in 2% acetic acid or in glacial acetic acid, in 70% ethanol, in 5% potassium hydroxide and in 4% sodium hydroxide. Crystal druses are solubilized in 10% hydrochloric acid, in conc. potassium hydroxide, in 3% nitric acid without effervescence, and in 4 or 60% sulfuric acid with formation of gypsum crystals in or near the druses. (17) Sudan III (Ega-Chemie, Steinheim, FRG), C.I. 26100, Sudan IV (Fluka, Buchs, Switzerland) C.I. 25105; Sudan black B (Fettschwarz HB, Schuchard, München, FRG) C.I. 26150; in saturated 70% ethanolic solution (JENSEN 1962). (18) Toluidine blue (Sigma, St. Louis U.S.A.) C.I. 52040: 1% in 1% sodium borate. Counterstain with a mixture 1 : 1 (vol/vol) of 1% basic fuchsin (Merck, Darmstadt, FRG) C.I. 42510 and 0.06% sodium borate (FLORES & HOFFMAN 1981).

For observation in the polarized light and for the examination of autofluorescence, sections were mounted in water, glycerol or in the appropriate buffers. Fluorescence in transmitted light was observed in a Leitz Ortholux equipped with a Philips CS-200-W-4 ultra-high pressure mercury arc lamp. Two filters Leitz UG 1 (2 mm each) were used for UV-excitation and „UV-Abs" as a barrier filter. A filter BG 12 (5 mm) served for blue light excitation combined with a barrier filter „Blau Abs". A filter BG 38 (4 mm) for damping red light was always added. Epifluorescence was observed in a

Leitz Dialux with an Osram ultra-high pressure mercury arc lamp HBO 100 W and the Ploemopak 2.4 incident fluorescence device. Filter combinations Leitz A (UV-excitation with a band-pass 340–380 nm; suppression with a long-pass 430 nm) and Leitz D (blue light excitation with a band-pass 355–425 nm; suppression with a long-pass 460 nm) were used.

For electron microscopy, small pieces of stems, roots and petioles were removed a few cm behind the apices and leaf blades and preincubated in 0.4 M sucrose in phosphate buffer (30–50 min) or prepared directly in the first fixative. The following fixations were performed at room temperature: (A) 3.5–4.5% glutaraldehyde in 0.4 M sucrose in phosphate buffer (pH 6.8, 12–18 h). (B) As (A), followed by 1% OsO_4 in 0.4 M sucrose (2–5 h). (C) As (B), followed by 1% uranyl acetate (18 h). (D) $KMnO_4$ 4% in a solution according to ZETTERQVIST (1956) containing 0.5% NaCl, 0.03% KCl and 0.014% $CaCl_2$ (30–35 min), followed by 2% sodium(meta)periodate ($NaIO_4$) for up to 45 min (PICKETT-HEAPS 1967, fixation 4). Material fixed under A–C was washed, dehydrated through an acetone series and embedded in epoxy resin (Durcupan ACM, Fluka, Buchs, Switzerland).

Further tissue treated according to methods (A), (B), or (D) was dehydrated in an ethanol series and embedded in glycol methacrylate (SPAUR & MORIARTY 1977, Table II). For prepolymer preparation, permanently stirred batches of the complete embedding mixture (10–20 ml) were irradiated by visible and UV light (Osram Ultra Vitalux lamp 300 W, distance less than 10 cm) according to G. Schaffner, Kiel, FRG (personal communication). During this procedure the methacrylate was allowed to warm up to 30–50° C. Curing was done in the dark at 38° C.

The following procedures were performed at room temperature, if not mentioned otherwise, with thin sections picked up on formvar and carbon coated grids: (1) PATAg (THIÉRY 1967): 1% periodic acid (10–30 min), followed by 2% thiosemicarbazide in 10% acetic acid (12–15 h) and by 1% silver proteinate (Albumosesilber, Merck, Darmstadt, FRG) in the dark (30 min). Controls were effected by the omission of one or two of these steps (WATTENDORFF & SCHMID 1973). (2) Silver methenamine (SMA) contrast on glycol methacrylate sections obtained from fixation D (oxidation with $KMnO_4$ followed by $NaIO_4$): Silver nitrate, up to a final concentration of 0.1% is added to a 1% hexamethylene tetramine solution in a borax/boric acid buffer (0.025 M; pH 9.0) and allowed to react 10–70 min with the immersed grids in an open Petri dish in diffuse light at temperatures varying from 45° C to 62° C. After rinsing, nonreduced silver is removed with a 5% sodium thiosulphate solution (10 min) (PICKETT-HEAPS 1968). A section treatment with periodic acid preceding the silver solution results in an earlier appearance of the grains and reduces unspecific precipitates. Alternatively, glycol methacrylate sections obtained from the glutaraldehyde-osmium fixation B, and pretreated on the section with $KMnO_4$ (2%, 10 min) followed by periodic acid, were exposed to the same silver solution

(BRANDER & WATTENDORFF, in press). (3) 2% uranyl acetate in a 1 : 1 (vol/vol) acetone/water solution, 5 min, followed by lead citrate (REYNOLDS 1963; 10 min). (4) Exo-(1→3)-β-D-glucanase (EC 3.2.1.58) isolated by PILLONEL & al. (1980) from Basidiomycete sp. strain QM 806 and endo-(1→3)-β-D-glucanase (EC 3.2.1.39) isolated at this institute according to CLARK & al. (1978) from *Rhizopus arrhizus* (QM 6789). The enzymes (1–4 mg/ml) were incubated alone or together in a mixture 1 : 1 (vol/vol) in acetate buffer (20 mM, pH 4.8–5.0) at 37° C during 1–18 h. For control, sections were incubated on buffer only. The activities of the solutions were controlled by a simultaneous incubation of semithin sections. When a repeated aniline blue staining after the enzyme treatment did no more result in the characateristic fluorescence, a sufficient action of the enzyme was supposed.

Copper grids were used for reaction (3), gold grids in all other reactions. The sections were viewed in a Philips EM 300 transmission electron microscope at 60 or 80 kV.

Abbreviations used in the text: Cellin wall = pecto-cellulosic cell wall; PA = periodic acid; PAS = periodic acid-Schiff; PATAg = periodic acid – thiosemicarbazide-silver; SMA = silver methenamine.

Results

Light microscopy. Druse-crystal idioblasts occur throughout the stem (Fig. 1), leaf and root. Their shapes and distribution in the tissues are similar for all *Abutilon* species and horticultural forms studied. The crystals of *A. pictum* WALP. *"Thompsonii"* react for calcium oxalate as shown with the Yasue technique and with different solubility tests (given under 15 and 16 in materials and methods) (Fig. 2). A high birefringence points to the presence of the monohydrate (whewellite) form (FREY-WYSSLING 1981). The diameters of the crystal druses vary from a few μm to about 30 μm. The majority of the crystals can be classified into two size ranges of 5–10 μm and 15–30 μm respectively. The small class appears in the phloem part of vascular bundles (Fig. 1). Cells containing larger druses are found especially in the cortical parenchyma of the stem, in the mesophyll, and along the leaf veins. Idioblasts are also present among the subepidermal cells and in the pith where they occur preferably at its periphery. Crystals are observed a few cells behind the apical meristems of vegetative shoots as well as of flower buds, and are also seen in very young leaf primordia. In the root apices, however, crystal cells occur in a rather irregular pattern. A little farther from the apex, they are observed near young vascular elements.

In a few idioblasts the druse crystal appears to be localized in a central vacuole-like compartment (Fig. 4, asterisks). In most idioblasts (Fig. 5), however, the crystal is enclosed all around in a sheath of variable thickness covered by a cytoplasmic layer which sometimes is very thin. Cells with

sheathed crystals often contain a highly vacuolized cytoplasm; sometimes dead cells occur.

In most mature idioblasts the periphery of the crystal sheath is continuous with the cell wall or linked to it by a stalk. This is the peculiar disposition of Rosanoff crystals. Such sheaths give the same reactions as the cellin walls with PAS and with standard stains for semithin sections. A distinct fluorescence dichroism with calcofluor white (HUGHES & MCCULLY 1975, HERTH & SCHNEPF 1980) is observed in the cellin wall sheaths (Fig. 3, upper crystal). In a few cells, however, the corresponding region does not stain with PAS, and calcofluor white produces an enhanced fluorescence without a detectable dichroism (Fig. 3, lower crystal). These cells have been identified with the idioblasts containing callose and are described in the following paragraph.

In all species and cultivars of *Abutilon* some crystal cells are stained for callose by resorcinol blue and aniline blue (Fig. 6). This agrees with earlier observations in *A. pictum* WALP. *"Thompsonii"* (THALER & WEBER 1957) and in *A. megapotamicum* (HUGHES & GUNNING 1980). Stained cells are arranged in a random distribution among the other crystal idioblasts. In *A. pictum* WALP. *"Thompsonii"* about 1% of the crystal cells react for callose. The dyes emphasize the druse outlines (Fig. 6), and sometimes further inclusions react strongly. A previous treatment with (1→3)-β-D-glucanases abolishes aniline blue and resorcinol blue staining after a few minutes of incubation. The sheath material is not distinguishable histochemically from sieve pore callose and remains virtually unstained by methylene blue-azure A-safranin, a simultaneous PAS-reaction neither interferes with aniline blue (SMITH & MCCULLY 1978 b) nor with calcofluor white. If idioblasts are first stained with aniline blue and then with calcofluor white, the fluorescence changes from yellow to white. For the inverse sequence of dyes, HUGHES & MCCULLY (1975) observed an analogous change of fluorescence. The fluorescent material occurs irregularly in the sheath area (Fig. 3, lower crystal; Fig. 6). This form of idioblast is rich in cytoplasm, contrary to cells with sheaths of cellin wall material.

In cellin wall sheaths as well as in those racting for callose, a faint autofluorescence in the interior sheath parts, especially those filling the reentrant angles of the druses, is similar in colour to that of the cuticle and can selectively be enhanced by the *Chelidonium* fluorochrome method. The same region shows an affinity for lipophilic fluorochromes as examined with auramin O, 3,4-benzpyrene, chlorophyll, and phosphine (Fig. 11). Accumulation of Sudan III and IV, however, was not observed, and it was incertain for Sudan black B because of refraction phenomena near the crystals. The sheath autofluorescence is vigourously quenched by Sudan black B. BIGGS (1984) observed this quenching in suberin. The phloroglucinol-HCl test, however, is negative. The I_2KI-H_2SO_4-method (SCOTT 1950) often seems to dissolve the crystals beginning at distinct points of

attack. When the cellulose is dissolved, druse-shaped "ghosts" of yellow-brownish material sometimes appear, and can retain this aspect for several days.

Electron microscopy. Most idioblasts contain ensheathed crystals. In a few cells, however, naked crystals appear in a vacuole-like compartment (Fig. 15: left cell), but possibly coated by some granular or membranous material. Other crystals without sheaths are the small accessory ones seen near the druses with callose sheaths (e.g. Fig. 8 a).

The ultrastructure of an idioblast with a cellin wall sheath not fluorescent with aniline blue (as mostly occuring in the tissue) is shown in Fig. 10. The calcium oxalate crystal has disappeared during sectioning and staining without an important lesion of the adjacent plastic-embedded structures. Such sheaths appear to be rigid; calcium oxalate remnants adhering to the

---

Abbreviations in the figures and their legends:
AK = acessory crystal; Fix. = fixation; K = crystal or its place; OM = osmiophilic material; S = sheath; ST = section treatment; W = cellin wall.
All embeddings in glycol methacrylate except for Figs. 12 a, c, 14, 16, which are taken from araldite material. All magnification bars indicated in μm.

Plate I (Light microscopy)

Fig. 1. Distribution of calcium oxalate crystal idioblasts in the stem of *Abutilon pictum* WALP. "*Thompsonii*". Cross section of fresh material. Small druses in phloem part and sclerenchyma cap, larger druses in cortical parenchyma.

Fig. 2. Cryosection of fresh material treated with the silver-nitrate-rubeanic acid procedure for calcium oxalate (YASUE 1969). The reagent did not stain black a big crystal (▷), and only a partial reaction is visible in another cell (arrow).

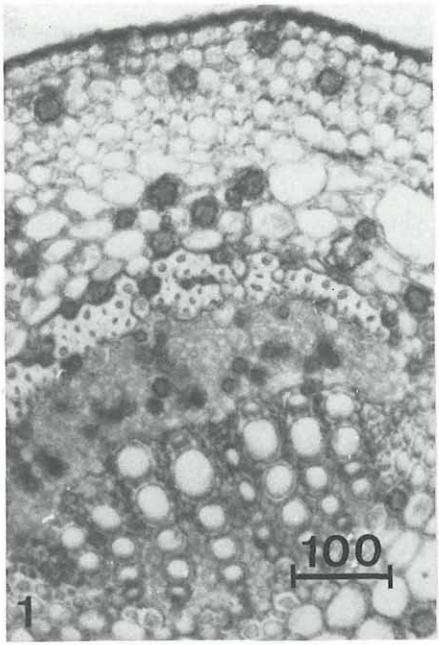
Fig. 3. Calcofluor white produces an enhanced fluorescence without dichroism in a crystal sheath which, on a consecutive serial section, had stained for callose with aniline blue. The cellin walls and a second sheath which had not stained with aniline blue (arrows) shows a clear dichroism. Fix. A: Glutaraldehyde 3.5%; semithin section.

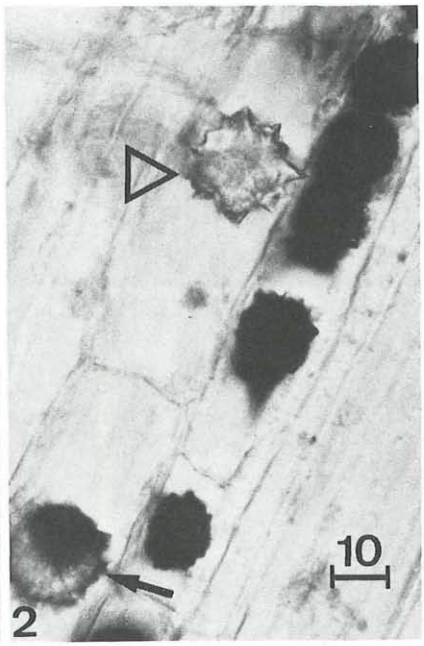
Figs. 4–5. Different idioblast shapes as seen on semithin sections in the light microscope. Fix. B: Glutaraldehyde 3.5%, OsO_4 1%; ST: Methylene blue-azure A -safranin.

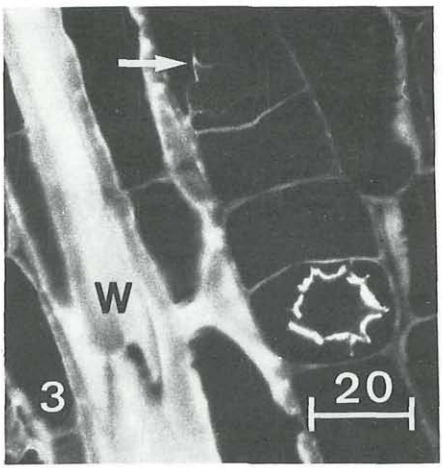
Fig. 4. Two cells with apparently sheathless crystals (asterisks) in a vacuole-like compartment and a third cell with a thin crystal sheath of cellin wall material coated with cytoplasm.

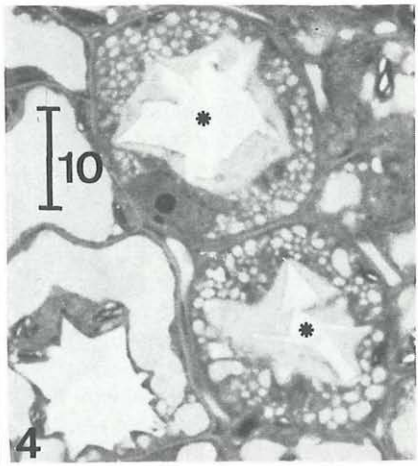
Fig. 5. Idioblast showing a crystal sheath with two structural components. The outer sheath part appears virtually unstained (▷◁) and is delimited towards the crystal faces with osmiophilic material. This darkish component accumulates especially at re-entrant angles of the sheath (small arrows). Two accessory crystals appear near the main crystal (large arrow).

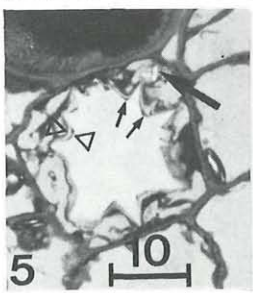
Fig. 6. Fluorescence pattern after staining with aniline blue on a semithin section. The sheath region stains irregularly, but in the same way as the callose of the sieve pores in the adjacent phloem (arrows). Fix A: Glutaraldehyde 3.5% ST: PAS followed by decolorized aniline blue.

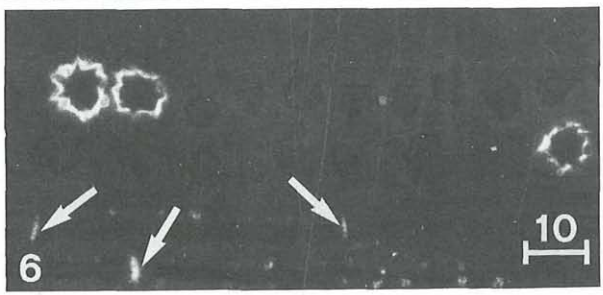


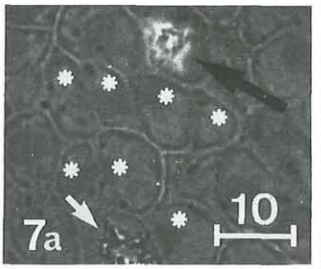


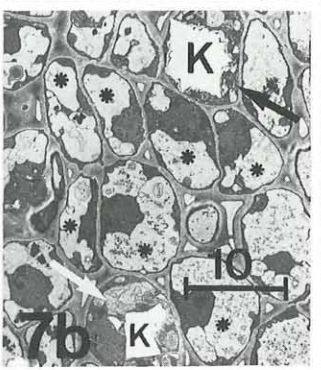


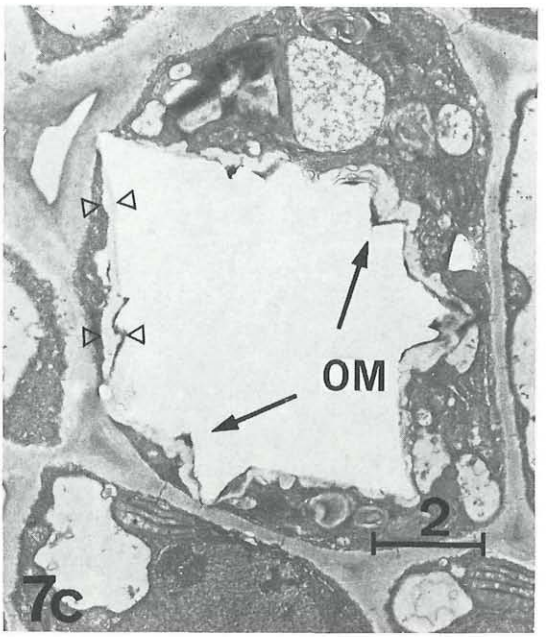


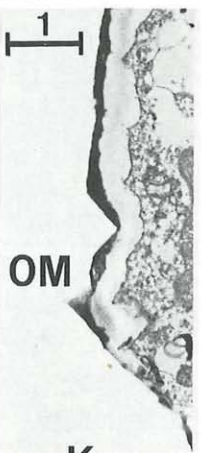


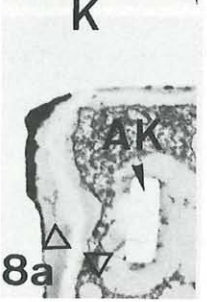


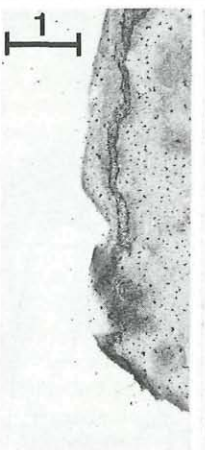


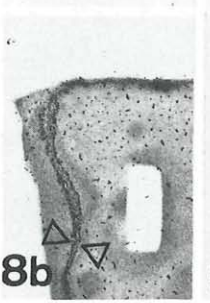


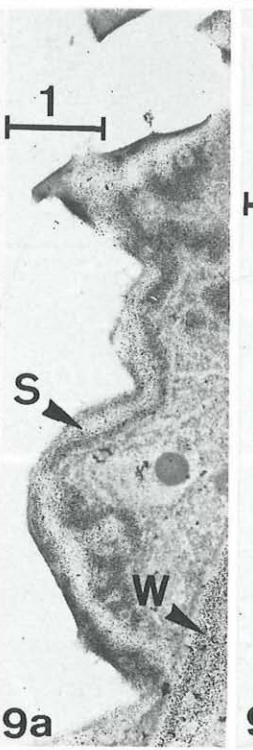












sheaths (WATTENDORFF & SCHMID 1973) indicate that during extraction of the crystal no sheath parts are lost. Such cells often contain a highly vacuolized cytoplasm. There is no difference between the outer sheath, the eventual stalks, and the cell walls, as to their reaction with either the PATAg-test for polysaccharides with vic-glycol groups or with uranyl acetate-lead citrate.

The osmiophilic interior sheath is shown in Figs. 8 a, 10. The dark material fills re-entrant angles of the crystal forming there sometimes large masses (Fig. 13) and occasionally continues into a narrow border along the crystals (Fig. 12 a). Sometimes, lamellae or myelin-like structures are visible (Fig. 12 a–c). In idioblasts with developing sheaths, the dark interior sheath part is seen connected with globular particles stained identically which seem to agglomerate and to condense at the crystal surface (Fig. 18). The formation of globules may therefore be one of the first steps of sheath formation (Fig. 16). In these young stages of development the exterior part of the sheath, if already present, appears to consist of callose. The cytoplasm adheres tightly to young crystal sheaths (Figs. 16, 18).

Callose-containing crystal idioblasts were identified by comparing consecutive serial sections of glycol-methacrylate-embedded material. A con-

---

Plate II

Fig. 7. Consecutive serial sections allowing an ultrastructural identification of aniline blue stained cells. Fix. B: Glutaraldehyde 3.5%, OsO_4 1%. Fig. 7 a. Fluorescent crystal idioblast (black arrow) beside an unreactive cell (white arrow). Asterisks indicate the reference cell pattern. ST: decolorized alcaline aniline blue. Fig. 7 b. The same tissue portion as Fig. 7 a on a thin section. The hard crystalline inclusions have been lost during processing. The crystal in the fluorescent cell is surrounded by an electron transparent space, the other crystal by a narrow cellin wall sheath. ST: Uranyl acetate followed by lead citrate. Fig. 7 c. At higher magnifications, a virtually unstained outer sheath (⋈) appears embedded in dense cytoplasm. A darkish component accumulates especially at re-entrant angles of the druse (arrows). One crystal edge (upper left) projects directly into the idioblast wall representing probably an preparation artefact. ST: As for Fig. 7 b.

Figs. 8–9. Ultrahistochemical characterization of sheaths staining with aniline blue on consecutive serial sections. Fix. B: Glutaraldehyde 3.5%, OsO_4 1%.

Fig. 8 a. Staining by uranyl acetate followed by lead citrate leaves the outer sheath portion unstained. A small, indistinct brighter zone (⋈) appears in this area.

Figs. 8 b, 9 a. A section treatment with KMnO and PA followed by SMA results in a granular silver contrast in the outer sheath. In 8 b, an especially dense silver deposition is visible over a region corresponding to the small brighter zone in 8 a. The inner, osmiophilic sheath component is unreactive to the silver stain.

Fig. 9 b. A prior treatment with exo-(1→3)-β-D-glucanase prevents any silver impregnation of the outer sheath part indicating thus sites of a sheath β-(1→3)-D-glucan (S, arrows).

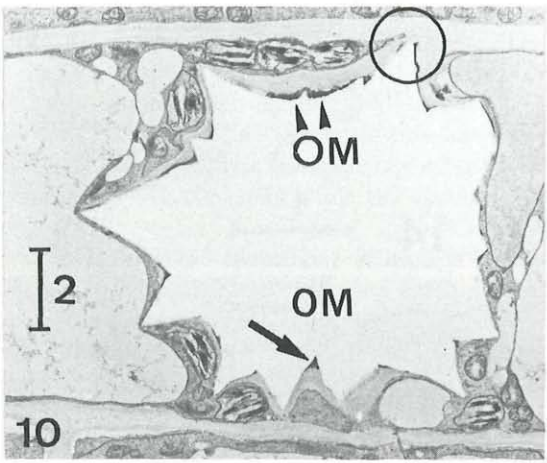
venient group of cells is selected by aniline blue fluorescence in a semithin section (Fig. 7 a; arrows and asterisks), and electron micrographs are prepared from a subsequent thin section (Fig. 7b, c). The site of the crystal is surrounded by an electron transparent zone of unequal thickness and bordered by a dense cytoplasmic coat. At higher magnifications (Fig. 7 c, 13) a sheath is observed. The outer sheath part may sometimes touch the cell wall (e.g. Fig. 13), but direct projections of such crystal sheaths into the idioblast cell wall, a frequent feature of cellin sheaths, are not observed. In a cloudy sheath zone with protrusions towards the cytoplasm, little or no contrast is obtained after fixation in glutaraldehyde and osmium, embedding in araldite or glycol-methacrylate, and section staining with uranyl acetate followed by lead citrate. In glycol-methacrylate embedded material, an indistinct, brighter zone occasionally appears in this area (Figs. 8 a, 17). After a section treatment with $KMnO_4$ and PA followed by silver methenamine, however, silver granules accumulate preferably over this zone as shown in Fig. 8b for a consecutive serial section, or develop less densely over the whole cloudy space (Fig. 9 a). The silver deposition is impeded when the section has been incubated previosly with exo- or endo-(1→3)-β-D-glucanases or with a mixture of them (consecutive serial sections in Fig. 9). This sheath material cannot be distinguished histochemically from the sieve pore callose in the adjacent phloem.

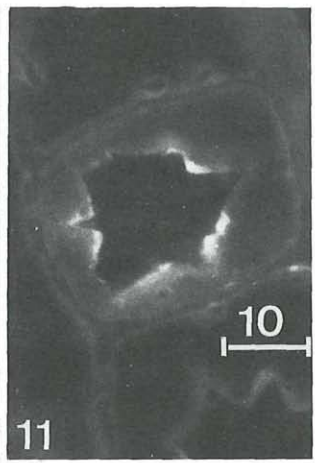
Plate III

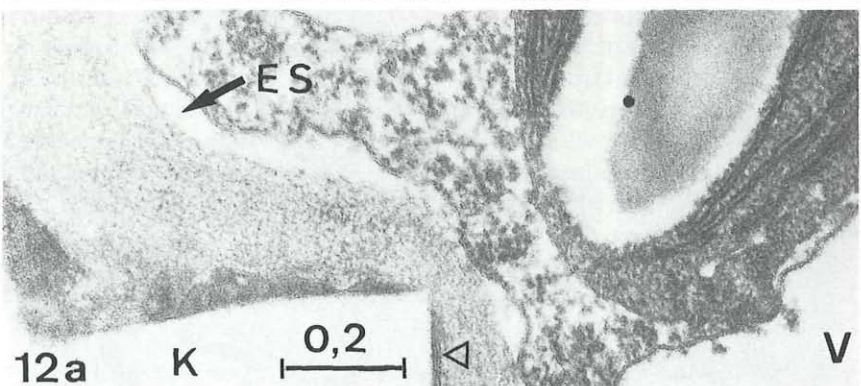
Fig. 10. Crystal idioblast with a cellin wall sheath. The major outer sheath part is linked with the cell wall forming there a stalk (○). The crystalline inclusion is incorporated into the apoplast by this sheath disposition. The wall-like sheath contains osmiophilic material (arrows) near the crystal surface. It accumulates especially at re-entrant angles of the druse. Fix. D: $KMnO_4$ 4%, $NaIO_4$ 2%; ST: Uranyl acetate followed by lead citrate.

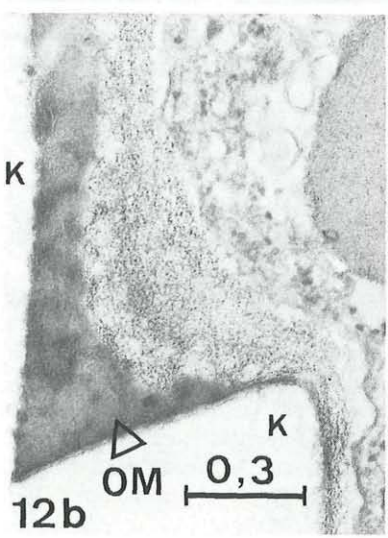
Fig. 11. Phosphine fluorescence emphasizes the inner sheath especially at re-entrant angles of the crystal. It was not examined if this idioblast contained callose. Fix. A: Glutaraldehyde 3.5%.

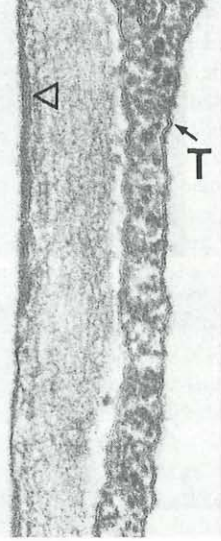
Figs. 12 a–c. Higher magnifications of a cellin wall crystal sheath. Fix. C: Glutaraldehyde 3.5%, OsO_4 1%, uranyl acetate 1%; except Fig. 12 c (Fix. B: Glutaraldehyde 3.5%, OsO_4 1%). ST: Uranyl acetate followed by lead citrate. Fig. 12 a. A thin cytoplasmic layer covers the fibrillar outer sheath part composed of cellin wall material. Small extraplasmatic spaces (ES) may be caused by slight plasmolysis. The inner, darkish sheath component seems to separate perfectly the crystal surface from the outer sheath. Sometimes, lamellae or myelin figures (▷) appear in this component. Vacuole V, delimited by the triple-layered tonoplast T. Figs. 12 b, c. Details of the osmiophilic sheath material filling re-entrant angles of the druses. Fig. 12 b. The interior part of the sheath appears more or less amorphous and adheres closely to the fibrillar outer part. Fig. 12 c. Myelin-like figures fill the interior sheath part at a re-entrant angle of the crystal.

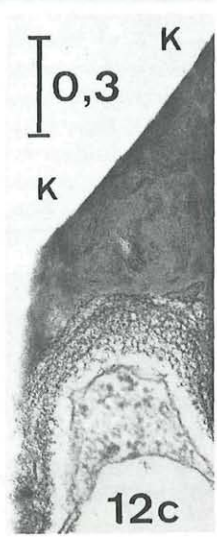


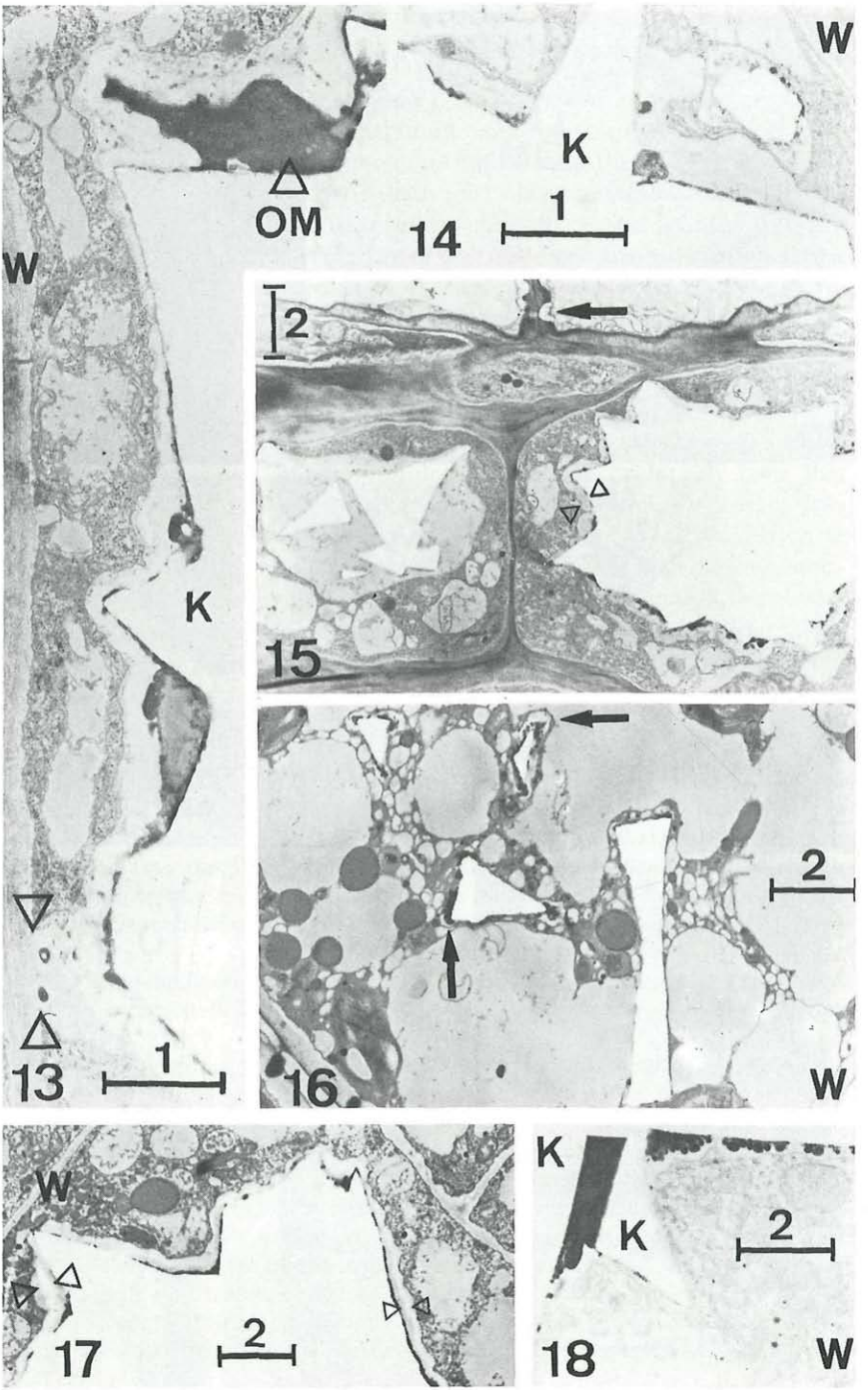












Discussion

The random occurrence of idioblasts with callose-containing crystal sheaths among other crystal idioblasts has been reported earlier (THALER & WEBER 1957), but their ultrastructure has not been examined. Callose has a characteristic electron translucency after conventional staining for electron microscopy (ESAU 1969), but no specific ultrastructural stain for it is known.

A lack in the specificity of aniline blue staining (SMITH & MCCULLY 1978 a) can be overcome by using combined histochemical procedures, and recent reports indicate that the fluorochrome contained in aniline blue is still appropriate for the cytochemical localization of this glucan with predominantly (1→3)-β-D-linkages (SMITH & MCCULLY 1978b, WATERKEYN 1981, STONE & al. 1984). When callose is suspected to occur at inhabitual places as in crystal sheaths, however, its ultrastructural discrimination from empty spaces becomes difficult. Staining methods based on the specific oxidation of vicinal OH-groups (ROUGIER & al. 1973, ROLAND 1978) must give negative results for pure (1→3)-β-D-glucans. Attempts to overcome this problem were made by using the silver methenamine (SMA) techniques after $KMnO_4$ and $NaIO_4$ oxidation (PICKETT-HEAPS 1968) by which a silver contrast can be introduced into sieve pore callose (BRANDER & WATTENDORFF, in press). In *Abutilon,* the adjacent phloem was used as a control reference in the same section (Fig. 15). Glycol methacrylate was

---

Plate IV

Figs. 13–18. Different callose-containing idioblasts. Fix. B: Glutaraldehyde 3.5%, OsO_4 1%; except for Figs. 14, 18 (Fix. C: Glutaraldehyde 3.5%, OsO_4 1%, uranyl acetate 1%). ST: Uranyl acetate followed by lead citrate.

Fig. 13. A cloudy callose sheath is in contact with the idioblast wall (▷◁) and encloses the interior sheath part containing masses of osmiophilic material.

Fig. 14. Largely unstained sheath. A small crystal hole in the upper right may be a side branch of the main crystal druse provided with its own sheath.

Fig. 15. Differently developed idioblasts adjacent to phloem (arrow). The left cell contains an unsheathed crystal surrounded by some granular material in a vacuole-like compartment. In the right cell an electron translucent outer sheath (▷◁) and osmiophilic globules in the interior part of the sheath are visible.

Fig. 16. Idioblast at a very early stage of sheath formation. A cytoplasmic layer, here and there very thin, is in close contact with the crystal. In several places (arrows) a sheath begins to form: osmiophilic droplets nearest to the crystal, callose-like material more peripherally near the crystal angles.

Fig. 17. Crystal idioblast with a callosic sheath and lipophilic material at its inner side. An indistinct brighter zone in the callosic sheath (▷◁) is visible as in Fig. 8 a.

Fig. 18. Early stages of sheath development. Osmiophilic globules accumulate near the crystal surface and aggregate (top). Dense uniform masses have developed in a re-entrant corner of the druse crystal (left).

found to be the embedding medium of choice for comparing aniline blue fluorescence, enzyme reactions, and ultrastructural silver reactions on semithin and thin sections from the same tissue block. The $KMnO_4$-$NaIO_4$-SMA-method produces well delimited areas in a previously unstainable region, while the embedding medium itself is not stained. A previous treatment with (1→3)-β-D-glucanases prevents such a silver deposition (consecutive serial sections see Fig. 9 a, b). These sites are supposed to represent the callose identified by aniline blue fluorescence. However, no holes or other signs of the disappearance of material are produced in the sections, even treated exhaustively, with the enzymes. This could have been caused by the high content of embedding medium in the callose due to very loose polysaccharide chains (HOROBIN & TOMLINSON 1976, BRANDER & WATTENDORFF, in press).

Sometimes, only very narrow zones within the sheaths are stained with $KMnO_4$-$NaIO_4$-SMA. Such zones could eventually be interpreted as dehydration or embedding artefacts (SITTE & FALK 1961) of a broader callose layer with a looser chain configuration. The composition of the SMA-unstained sheath parts is not known. A sheath component showing no contrast with the PATAg-reaction or with conventional section staining and giving no fluorescence with aniline blue was found in crystal idioblasts of *Acacia* and was supposed to be similar to "filling cork" or wax (WATTENDORFF 1978).

The composition of the interior, strongly osmiophilic structural sheath component is still unknown, although similarly located and stained layers have been reported by DENGG (1971), and WEBB & ARNOTT (1981). Even in osmicated material this part of the sheath was never seen to react with SMA after a previous $KMnO_4$ and PA-contact (Fig. 8b). Lignin, as detected by the phloroglucionol-HCl test (JENSEN 1962), was found in no part of the sheath. A silvery-white fluorescence with phosphine and a faint autofluorescence similar to that of the cuticle which can selectively be quenched by Sudan black B (BIGGS 1984), point to the lipidic nature of this component. The inner sheath probably acts as a penetration barrier causing the differential staining of some crystals (Fig. 2) in the procedure of YASUE (1969). This has also been observed by HORNER & ZINDLER-FRANK (1982a). Additional evidence for the lipidic nature of a sheath component has been provided by the standard I_2KI-H_2SO_4-method for cellulose (SCOTT 1941). When the blue-stained cellulose is dissolved during this test, sometimes brown druse-shaped "ghosts" appear to remain unaffected for several days. According to PETERSON & al. (1978) a resistance against digestion by concentrated sulphuric acid is one of several indications for cutin or suberin.

In callose sheaths osmiophilic material is often irregularly distributed (Figs. 13, 15, 16, 18) conferring to the sheath an immature aspect contrary to that in the idioblasts with cellin wall sheaths (Fig. 10). Although no confirmation by fluorescence microscopy was attempted in this special case, the exterior part of the sheath, in its youngest stage of development recorded

(Fig. 16), shows a callose-like ultrastructure. Some fibrillar material observed in an otherwise callosic sheath may indicate a transition stage towards a cellin sheath. WATERKEYN & al. (1982) have shown that callosic deposits form before, or during the silicification of cell walls preparing them possibly for mineralization. In the *Abutilon* idioblasts, the callose could also have a comparable function in regard to the synthesis of the cellin wall sheath. Since this glucan is considered as a possible intermediate in the synthesis of cellulose in cotton fibres (MEIER & al. 1981), its considerable occurrence in some idioblasts could also be caused by a disturbance of its further transformation into cellulose.

The sheath callose could also result from a mechanical perturbation (JAFFE et al. 1985) or represent wound artefacts (HUGHES & GUNNING 1980, GALWAY & MCCULLY 1984), but an increased percentage of fluorescent idioblasts near injured tissue was never recorded. Additionally, following HUGHES & GUNNING (1980), aniline blue positive material surrounding the crystals in *A. megapotamicum* is not induced by the fixative and is still present after a quick freeze-fixation.

Unsheathed crystals presumed to be relatively young have been always found in a compartment resembling a central vacuole (Fig. 15, left cell). Tubular, membraneous and vesicular elements as seen in the vacuoles of immature crystal cells of *Helianthus* (ARNOTT & PAUTARD 1970) and of *Canavalia* (FRANK & JENSEN 1970) are not observed. Idioblasts clearly showing the first stages of oxalate crystal formation with related cytoplasmic structures or an associated organic matrix (ARNOTT 1976, FRANCESCHI & HORNER 1980, HORNER & WAGNER 1980, ARNOTT 1982) were not seen. This could be explained by a very rapid transition from predetermined cells to the first stages of crystalline calcium oxalate accumulation. In the subsequent stages the unsheathed crystals are seen in close contact with portions of cytoplasm. Sheath formation seems to start with an aggregation of lipidic material in an electron translucent area (Fig. 16). Subsequently, an inner compact sheath condenses near the crystal surfaces (Fig. 18).

To conclude, cells with sheathed crystals fixed to the wall by a stalk are considered to represent advanced differentiation stages. This could be an advantage for the plant since crystals with sharp edges might be nocuous to the cell. Following HORNER & ZINDLER-FRANK (1982 b) the formation of cell wall sheaths may be similar to a wound response.

The possible functions of calcium oxalate idioblasts and crystals have been extensively reviewed by FRANCESCHI & HORNER (1980). In *Abutilon,* a majority of crystals is "morphologically exteriorized" resulting therefore in a granulocrine excretion (SCHNEPF 1969). An interesting observation is the abundant presence of markedly small idioblasts near the vascular tissues (Fig. 1) which may reflect an interrelation with the calcium transport (ZINDLER-FRANK 1980, BORCHERT 1984) or alternatively with the cell wall formation near thick-walled xylem (KOHL 1889).

Acknowledgements

The present study was carried out under the supervision of Professor J. WATTENDORFF. Much advice and suggestions as well as the critical reading of the manuscript are gratefully acknowledged. The author wishes to thank Professor H. MEIER for support, Drs. A. J. BUCHALA, C. PILLONEL and U. RYSER for stimulating discussions and for technical help. Thanks are also due to Mr. F. BÄTTIG, who cultivated the plant material.

Supported by the Swiss National Science Foundation.

References

ARNOTT H. J. 1976. Calcification in higher plants. In: WATABE N. & WILBUR K. M. (eds.), The mechanisms of mineralization in the invertebrates and plants, pp. 56–76. – University of South Carolina Press, Columbia.

— 1982. Three systems of biomineralization in plants with comments on the associated organic matrix. In: NANCOLLAS G. H. (ed.), Biological mineralization and demineralization (Dahlem Konferenzen 1982), pp. 199–212. – Springer, Berlin–Heidelberg–New York.

ARNOTT H. J. & PAUTARD F. G. E. 1970. Calcificaton in plants. In: SCHRAER H. (ed.), Biological calcification, pp. 375–446. – Appleton-Century-Crofts, New York.

BENNETT H. S., WYRICK A. D., LEE S. W. & MCNEIL J. H. Jr. 1976. Science and art in preparing tissues embedded in plastic for light microscopy, with special reference to glycol methacrylate, glass knives and simple stains. – Stain Technology 51: 71–97.

BIGGS A. R. 1984. Intracellular suberin: Occurrence and detection in tree bark. – IAWA Bulletin n. s. 5: 243–248.

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

BRANDER U. & WATTENDORFF J. (in press) Staining callose for electron microscopy: Experimental conditions. – La Cellule 74 (Louvain-La-Neuve).

CLARK D. R., JOHNSON J. jr., CHUNG, K. H. & KIRKWOOD S. 1978. Purification, characterization, and action-pattern studies on the endo-(1→3)-β-D-glucanase from *Rhizopus arrhizus* QM 1032. – Carbohydr. Res. 61: 457–477.

DENGG E. 1969. Zytologische Untersuchungen an Blattgallen von *Urtica dioica*. – Phyton (Austria) 13: 271–284.

— 1971. Die Ultrastruktur der Blattgalle von *Dasyneura urticae* auf *Urtica dioica*. – Protoplasma 72: 367–379.

ESCHRICH W. 1954. Ein Beitrag zur Kenntnis der Kallose. – Planta 44: 532–542.

— & CURRIER H. B. 1964. Identification of callose by its diachrome and fluorochrome reactions. – Stain Technology 39: 303–307.

FLORES T. R. & HOFFMAN E. O. 1981. Stain for epoxy resin sections. – RMS Proceedings (Oxford) 16: 163.

FRANCESCHI V. R. & HORNER H. T. jr. 1980. Calcium oxalate crystals in plants.-Bot. Rev. 46: 361–427.

FRANK E. 1967. Zur Bildung des Kristallidioblastenmusters bei *Canavalia ensiformis* DC. I. – Z. Pflanzenphysiol. 58: 33–48.

— & JENSEN W. A. 1970. On the formation of the pattern of crystal idioblasts in *Canavalia ensiformis* DC. IV. The fine structure of the crystal cells. – Planta 95: 202–217.

FREY-WYSSLING A. 1981. Crystallography of the two hydrates of crystalline calcium oxalate in plants. – Am. J. Bot. 68: 130–141.

GALWAY M. E. & MCCULLY M. E. 1984. A study of the deposition and subsequent disappearance of aniline blue-induced fluorescence in different tissues of wounded pea roots. – Am. J. Bot. 71 (5,2), Abstract 83 Ann. Meet. Bot. Soc. Am.

GORSKA-BRYLASS A. & SMOLINSKI M. 1966. Callose in the developing pericyclic and phloem fibres. – Acta Soc. bot. Pol. 35: 301–305.

HERTH W. & SCHNEPF E. 1980. The fluorochrome, Calcofluor white, binds oriented to structural polysaccharide fibrils. – Protoplasma 105: 129–133.

HESLOP-HARRISON Y. & HESLOP-HARRISON J. 1981. The digestive glands of *Pinguicula:* Structure and cytochemistry. – Ann. Bot. (London) 47: 293–319.

HOROBIN, R. W. & TOMLINSON A. 1976. The influence of the embedding medium when staining for electron microscopy: The penetration of stains into plastic sections. – J. Microscopy 108: 69–78.

HORNER H. T. jr., KAUSCH A. P. & WAGNER B. L. 1981. Growth and change in shape of raphide and druse calcium oxalate crystals as a function of intracellular development in *Typha angustifolia* L. *(Typhaceae)* and *Capsicum annum* L. *(Solanaceae)*. – Scanning Electron Microsc./1981/III: 251–262.

— TIFFANY L. H. & CODY A. M. 1983. Formation of calcium oxalate crystals associated with apothecia of the discomycete *Dasyscypha capitata*. – Mycologia 75: 423–435.

— & WAGNER B. L. 1980. The association of druse crystals with the developing stomium of *Capsicum annuum (Solanaceae)* anthers. – Am. J. Bot. 67: 1347–1360.

— & ZINDLER-FRANK E. 1982 a. Histochemical, spectroscopic, and X-ray diffraction identifications of the two hydration forms of calcium oxalate crystals in three legumes and *Begonia*. – Can. J. Bot. 60: 1021–1027.

— & — 1982 b. Calcium oxalate crystals and crystal cells in the leaves of *Rhynchosia caribaea (Leguminosae: Papilionoideae)*. – Protoplasma 111: 10–18.

HUGHES J. & MCCULLY M. E. 1975. The use of an optical brightener in the study of plant structure. Stain Technology 50: 319–329.

— & GUNNING B. E. S. 1980. Glutaraldehyde-induced deposition of callose. – Can. J. Bot. 58: 250–258.

JAFFE M. J., HUBERMAN M., JOHNSON J. & TELEWSKI F. W. 1985. Thigmomorphogenesis: The induction of callose formation and ethylene evolution by mechanical perturbation in bean stems. – Physiol. Plant. 64: 271–279.

JENSEN W. A. 1962. Botanical histochemistry. – Freeman & Co., San Francisco, London.

KOHL F. G. 1889. Anatomisch-physiologische Untersuchung der Kalksalze und Kieselsäure in der Pflanze. – Elwertsche Verlagsbuchhandlung, Marburg.

LILLIE, R. D. 1965. Histopathologic technic and practical histochemistry. Edn. 3.- McGraw-Hill Book Co. New York.

MEIER H., BUCHS L., BUCHALA A. J. & HOMEWOOD T. 1981. (1→3)-β-D-glucan (callose) is a probable intermediate in biosynthesis of cellulose of cotton fibres. – Nature (London) 289: 821–822.

NANKO H., SAIKI H., HARADA H. 1976. Cell wall development of chambered crystalliferous cells in the secondary Phloem of *Populus euramericana*. Bull. Kyoto Univ. For. 48: 167–177.

NETOLITZKY F. 1929. Die Kieselkörper. Die Kalksalze als Zellinhaltskörper. In: LINSBAUER K. (ed.), Handbuch der Pflanzenanatomie, Allg. Teil Cytologie, vol. III/1a. – Gebr. Borntraeger, Berlin.

PARAMESWARAN N. & LIESE W. 1979. Crystal-containing walls of spicular cells in *Welwitschia*. – IAWA-Bulletin/1979/4: 87–89.

— & RICHTER H.-G. 1984. The ultrastructure of crystalliferous cells in some *Lecythidaceae* with a discussion of their terminology. – IAWA Bulletin n. s. 5: 229–236.

— & SCHULTZE R. 1974. Fine structure of chambered crystalliferous cells in the bark of *Acacia senegal*. – Z. Pflanzenphysiol. 71: 90–93.

PETERSON C. A., PETERSON R. L. & ROBARDS A. W. 1978. A. correlated histochemical and ultrastructural study of the epidermis and hypodermis of onion roots. – Protoplasma 96: 1–21.

PICKETT-HEAPS J. D. 1967. Preliminary attempts at ultrastructural polysaccharide localization in root tip cells. – J. Histochem. Cytochem. 15: 442–455.

— 1968. Further ultrastructural observations on polysaccharide localization in plant cells. – J. Cell Sci. 3: 55–64.

PILLONEL Ch., BUCHALA A. J. & MEIER H. 1980. Glucan synthesis by intact cotton fibres fed with different precursors at the stages of primary and secondary wall formation. – Planta 149: 306–312.

PRICE J. L. 1970. Ultrastructure of druse crystal idioblasts in leaves of *Cercidium floridum*. – Am. J. Bot. 57: 1004–1009.

REYNOLDS E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. – J. Cell Biol. 17: 208–212.

ROLAND J.-C. 1978. General preparation and staining in thin sections. In: HALL J. L. (ed.), Electron microscopy and cytochemistry of plant cells, pp. 1–62. – Elsevier, Amsterdam.

ROSANOFF S. 1865. Über die Krystalldrusen im Marke von *Kerria japonica* D. C. und *Ricinus communis*. – Bot. Ztg. 23: 329–330.

ROUGIER M., VIAN B., GALLANT D. & ROLAND J.-C. 1973. Aspects cytochimiques de l'étude ultrastructurale des polysaccharides végétaux. – Ann. Biol. 12: 43–75.

SCOTT F. M. 1941. Distribution of calcium oxalate crystals in *Ricinus communis* in relation to tissue differentiation and presence of other ergastic substances. – Bot. Gaz. (Chicago) 103: 225–246.

— 1950. Internal suberization of tissues. Bot. Gaz. (Chicago) 111: 378–394.

— SCHROEDER M. R. & TURRELL F. M. 1948. Development, cell shape, suberization of internal surface, and abscission in the leaf of the valencia orange, *Citrus sinensis*. – Bot. Gaz. (Chicago): 109. 381–411.

SCHNEPF E. 1969: Sekretion und Exkretion bei Pflanzen. – In: Protoplasmatologia VIII, 8. Springer, Wien–New York.

SITTE P. & FALK, H. 1961. Artefakte in pflanzlichen Zellwänden durch Methacrylat-Einbettung. – Z. wiss. Mikroskopie 65: 26–30.

SMITH M. M. & MCCULLY M. E. 1978 a. A critical evaluation of the specificity of aniline blue induced fluorescence. – Protoplasma 95: 229–254.

— & — 1978 b. Enhancing aniline blue fluorescent staining of cell wall structures. – Stain Technology 53: 79–85.

SPAUR R. C. & MORIARTY G. C. 1977. Improvements of glycol methacrylate. I. Its use as an embedding medium for electron microscopic studies. – J. Histochem. Cytochem. 25: 163–174.

STONE B. A., EVANS N. A., BONIG, I. & CLARKE, A. E. 1984. The application of sirofluor, a chemically defined fluorochrome from aniline blue for the histochemical detection of callose. – Protoplasma 122: 191–195.

THALER I. & WEBER F. 1957. Kallosehülle um Kalziumoxalat-Kristalldruse. Phyton (Austria) 7: 8–10.

— & — 1960. Calciumoxalat-Kristalle fehlen in der Membran der Stoma-Zellen. – Phyton (Austria) 9: 24–26.

THIÉRY J. P. 1967. Mise en évidence des polysaccharides sur coupes fines en microscopie électronique. – J. Microscopie 6: 987–1018.

THURSTON, E. L. 1976. Morphology, fine structure and ontogeny of the stinging emergence of *Tragia ramosa* and *T. saxicola (Euphorbiaceae)*. – Am. J. Bot. 63: 710–718.

WARMKE H. E. & LEE S. J. 1976. Improved staining procedures for semithin epoxy sections of plant tissues. – Stain Technology 51: 179–185.

WATERKEYN L. 1981. Cytochemical localization and function of the 3-linked glucan callose in the developing cotton fibre cell wall. – Protoplasma 106: 49–67.

WATERKEYN L., BIENFAIT A. & PEETERS A. 1982. Callose et silice épidermiques. Rapports avec la transpiration cuticulaire. – La Cellule 73: 267–287.

WATTENDORFF J. 1969. Feinbau und Entwicklung der verkorkten Calciumoxalat-Kristallzellen in der Rinde von *Larix decidua* MILL. – Z. Pflanzenphysiol. 60: 307–347.

— 1976. Ultrastructure of the suberized styloid crystal cells in *Agave* leaves. – Planta 128: 163–165.

— 1978. Feinbau und Entwicklung der Calciumoxalat-Kristallzellen mit suberinähnlichen Kristallscheiden in der Rinde und im sekundären Holz von *Acacia senegal* WILLD. – Protoplasma 95: 193–206.

— & SCHMID H. 1973. Prüfung auf perjodatreaktive Feinstrukturen in den suberinisierten Kristallzell-Wänden der Rinde von *Larix* und *Picea*. – Z. Pflanzenphysiol. 68: 422–431.

WEBB, M. A. & ARNOTT H. J. 1981. An ultrastructural study of druse crystals in okra cotyledons. – Scanning Electron Microsc./1981/III: 285–292.

— & — 1982. A survey of calcium oxalate crystals and other mineral inclusions in seeds. – Scanning Electron Microsc./1982/III: 1109–1131.

WEERDENBURG C. A. & PETERSON C. A. 1983. Structural changes in phi thickenings during primary and secondary growth in roots. 1. Apple *(Pyrus malus)* Rosaceae. – Can. J. Bot. 61: 2570–2576.

YASUE T. 1969. Histochemical identification of calcium oxalate. – Acta Histochem. Cytochem. 2: 83–95.

ZETTERQVIST H. 1956. The ultrastructural organization of the columnar absorbing cells of the mouse jejunum. – Thesis Dept. Anat. Karolinska Institutet Stockholm.

ZINDLER-FRANK E. 1980. Changes in leaf crystal idioblast differentiation in *Canavalia* caused by gibberellic acid through an influence on calcium availability. – Z. Pflanzenphysiol. 98: 43–52.

Recensio

Ross Hans 1986. Potato Breeding – Problems and Perspectives. With the cooperation of Dr. habil. Werner HUNNIUS †. – In: HORN W. & RÖBBELEN G. (Eds.), Fortschritte der Pflanzenzüchtung. Beihefte zur Zeitschrift für Pflanzenzüchtung, Heft 13. – Gr. 8°, 132 Seiten, 22 Abbildungen; kart. – Verlag Paul Parey, Berlin und Hamburg. – DM 68,–. – ISBN 3-489-61110-1.

Das Heft enthält zusammenfassende Darstellungen über das Ausgangsmaterial für Kreuzungen, über die etwa 8000jährige Geschichte der kultivierten Kartoffel, über Genetik (tetrasome Vererbung) inkl. Fertilität und Heterosis sowie über Züchtungs-Ziele und -Methodik. An diese „klassischen" Abschnitte schließt sich ein Abschnitt über die neuen Züchtungsmethoden: Nutzung Dihaploider (n = 2x = 24) und Monohaploider (n = 1x = 12) und der Möglichkeiten der in vitro-Kultur (inkl. somaklonaler Variation; vgl. Rezension HOHN & DENNIS in diesem Heft). Auf einen kurzen Abschnitt über das Problem nichtspaltender Kartoffeln aus Samen folgt ein umfangreicher über Resistenzzüchtung gegenüber Viren, Älchen und Pilzen. Schließlich sind noch ein Beitrag über Züchtung auf Ertrag und Reifetypen sowie über Samenproduktion enthalten. Die berücksichtigte Literatur ist sehr umfangreich (Schriftenverzeichnis 22 kompakt bedruckte Seiten). Ein Hauptanliegen der Autoren ist es, zu besserem Verständnis zwischen praktischer Pflanzenzüchtung und reiner Forschung mit den an der Kartoffel besonders weit entwickelten „neuen Züchtungsmethoden" beizutragen. Aus der Sicht des Botanikers sei festgehalten, daß viele an der Kartoffel erforschte Einzelheiten auch von allgemein biologischem Interesse sind und daß die karyologischen Befunde – wie z. B. die Bildung unreduzierter Gonen und ihre genetische Bedingtheit – als Modelle beim Studium der Evolution anderer Pflanzen nützlich sein können.

H. TEPPNER