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Morphometric evaluation of inclusion body-containing leucoplasts in leaf epidermal cells of *Origanum dictamnus* L.

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Abstract

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Epidermal cells of mature leaves of *Origanum dictamnus* significantly differ in size and number at the adaxial and abaxial leaf side, respectively. At both leaf surfaces, epidermal cells are characterized by the existence of globular, membrane-limited intraplastidal inclusions. Application of morphometric methods to 10 mm-long leaves (differentiated epidermal cells) showed that, on average, 92% of the intracellular space of an epidermal cell is occupied by the central vacuole, 2.4% by the nucleus and 5.6% by the parietal cytoplasm. Furthermore, plastids occupy 0.95% and intraplastidal inclusion bodies 0.51% of the cellular volume (inclusion bodies occupy about 54% of the plastidal volume). Cell numbers of the lower leaf epidermis were 2.6 fold those of the upper epidermis. In addition, cells of the upper epidermis are about 6.3 fold larger by volume than those of the lower epidermis. The globular bodies contained in the plastid matrix have an average diameter of 1.05 μm and their total volume in the entire leaf epidermis is 0.024 mm^3 (0.017 mm^3 at the adaxial and 0.007 mm^3 at the abaxial leaf side, respectively). The total surface area (boundary membrane area) of the intraplastidal inclusions, also corresponding to the entire leaf surface is 145.98 mm^2 (103.34 mm^2 at the upper leaf side and 42.64 mm^2 at the lower one). The possible function of the intraplastidal inclusions in the leaf epidermal cells of *O. dictamnus* is discussed.

Key words: morphometry, inclusion body, leucoplast, *Origanum dictamnus*

Introduction

Plastids of different kinds of plant tissues may often possess inclusions, which ordinarily exhibit a geometrical outline and an amorphous or crystalline internal structure (Ragetli et al. 1970, Milne 1972, Favali and Patrignani 1973). The chemical constitution of such inclusions has been reported to be proteinaceous (Wrischer 1967), lipoproteinaceous (Boasson et al. 1972) or carbohydraceous (Dolzmann 1964). Intraplastidal inclusions may either represent normal components of the organelle or they can be in-

duced by stress (De Greef and Verbelen 1973). With respect to their physiological role, inclusion bodies in the plastid stroma have been suggested to constitute energy sources for carotenoid synthesis (Simpson et al. 1975), special storage pools (Gailhofer and Thaler 1974), osmotic pressure regulatory factors (Dolzmann 1964), sites providing membrane material (Platt-Aloia and Thomson 1977) or formations induced by virus cytopathology (Milne 1972).

In paradermal- and cross-sections of mature leaves of *Origanum dictamnus,* epidermal cells at the adaxial and abaxial leaf side appear to differ significantly in number and size. Epidermal cells of both leaf sides are characterized by the existence within the plastids of globular, membrane-bound inclusions. In the present report, morphometric methods were applied to a series of light and electron micrographs in order to determine the number and volume of the epidermal cells on both surfaces of *O. dictamnus* leaves, the volume of the central vacuole, the nucleus and the plastids in each epidermal cell, and particularly the volume and surface area of the membrane-bound intraplastidal inclusion bodies.

Materials and methods

Small pieces of leaves and stem apices of dittany (*Origanum dictamnus* L., Lamiaceae) were double-fixed with Karnovsky's (1965) glutaraldehyde-paraformaldehyde mixture and osmium tetroxide in 0.05 M cacodylate buffer (pH 7.2). After washing in buffer, the specimens were dehydrated in an alcohol series and subsequently embedded in Spurr's (1969) resin. Sections were cut on a Reichert-Jung Ultracut E microtome. Semithin sections, 1 μm thick, were stained with 1% toluidine blue in 1% borax solution and examined in a Zeiss Photomicroscope III. Ultrathin sections were stained with uranyl acetate and lead citrate (Reynolds 1963) and viewed in a Jeol 100B electron microscope.

For the morphometric evaluation of the inclusion body-containing leucoplasts in *O. dictamnus* leaf epidermal cells, leaves 10 mm-long were collected from eight test plants. Four tissue blocks were obtained from each plant (different leaves of the same size) providing a total of 32 blocks. Three levels of sampling were employed: light micrographs at a final magnification of ×500 and electron micrographs at ×10,000 and ×50,000. The lowest level (×500) was used to calculate the number and volume of epidermal cells per unit leaf surface area. For this level, 45 micrographs were studied which were taken from leaf cross-sections and from leaf paradermal sections cut at the levels of the upper and lower epidermis. The second level (64 micrographs) was analysed with a square lattice of point arrays, 10 mm apart. At this level, the volume fraction of nuclei in epidermal cells, the volume fraction of central vacuoles in epidermal cells, the volume fraction of plastids in epidermal cell cytoplasm and the numerical density of plastids per μm^3 of epidermal cell cytoplasm, were determined. Finally, a square lattice of points with a 10 mm spacing and a pattern of parallel lines, 10 mm apart, were applied on 48 micrographs to analyse the third level (volume fraction of inclusion bodies in plastids, surface density of inclusion bodies in plastids and diameter of plastid inclusion bodies).

Since the diameter of the plastid inclusion body (1.05 μm) was found to be about 16 times the section thickness (50 to 80 nm, in average 65 nm), corrections for section thickness were made (James 1977, Weibel and Paumgartner 1978) in order to avoid overestimation of inclusion body volume and surface densities. Direct measurements with an ocular micrometer on leaf cross-sections before and after tissue embedment, demonstrated no noticeable volume changes in the treated material. This fact combined with the good preservation of the plastid inclusion bodies, which was ascertained by a series of electron micrographs, suggest that no detectable shrinkage of these structures occurs which could significantly affect morphometric estimations.

Results and discussion

The anatomy of mature leaves of *O. dictamnus* does not exhibit any peculiarities when compared with that of most other dicotyledonous plants (Fig. 1). There is a striking difference in the size of the cells at the upper and lower epidermis. In surface view, epidermal cells demonstrate an irregular outline in the form of extensive infoldings, which appear to be more numerous at the upper epidermis (cf. Figs. 2 and 3). Investigation of ultrathin sections corresponding to cross-sections of young and mature leaves of *O. dictamnus* has disclosed that epidermal cell plastids are characterized by the presence of singular inclusion bodies (Figs. 4 and 5). Such bodies were observed on both leaf sides and usually have a globular shape. They become compartmentalized within the plastid stroma by a single membrane and during the late developmental stages their matrix appears fine granular (Fig. 6).

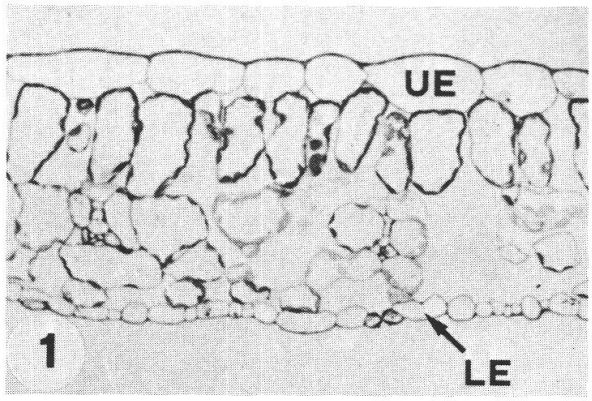
For the morphometric study of the intraplastidal inclusion-containing leaf epidermal cells of *O. dictamnus,* several parameters have at first to be determined (cf. Table 1). These parameters refer to fully developed epidermal cells in 10 mm-long leaves. The corresponding values have been corrected for section thickness. Since the cells of the upper leaf epidermis significantly differ in size from those of the lower epidermis, it is necessary for the corresponding calculations to be made independently of each other. Thus, calculations in brackets described below refer to the lower epidermis, those without brackets to the upper epidermis.

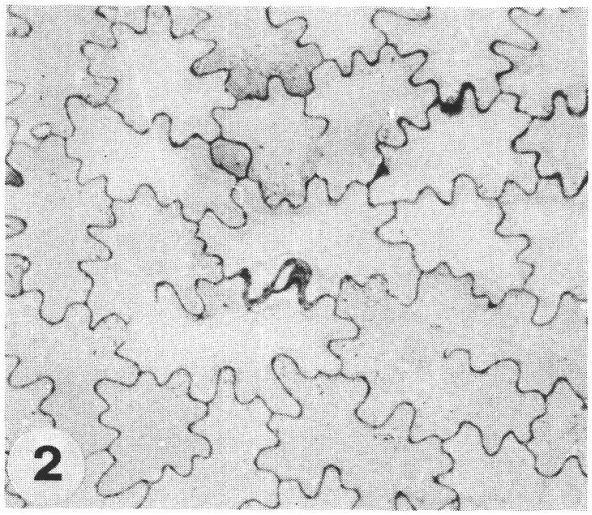
In order to determine the average volume of an epidermal cell, paradermal sections were obtained of leafes cut at the levels of the upper and lower epidermis, respectively (Figs. 2 and 3). In such sections it was found that there are 214.69 (556.83) cells in an epidermal area of 1 mm^2. Furthermore, in a 10 mm-long leaf, each surface (adaxial and abaxial) occupies an area of about 120 mm^2, as estimated by circumscribing such a leaf on a piece of paper with a square lattice of lines, 1 mm apart. Consequently, the upper epidermis of the entire leaf consists of $120 \times 214.69 = 25{,}762.8$ cells and the lower one of $120 \times 556.83 = 66{,}819.6$ cells. This further means that there are about 2.6 folds as many cells in the lower epidermis compared to the upper epidermis.

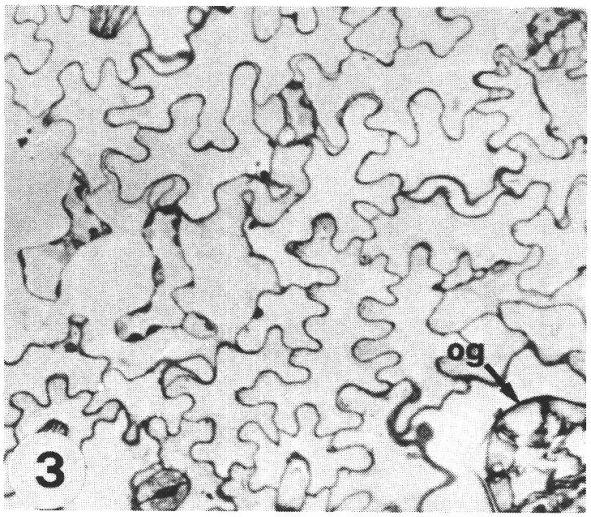
Measurements on cross-sections of leaves (Fig. 1) have revealed that the average thickness of the epidermis is 27.58 µm (11.38 µm). Each epidermal cell, hence, has an

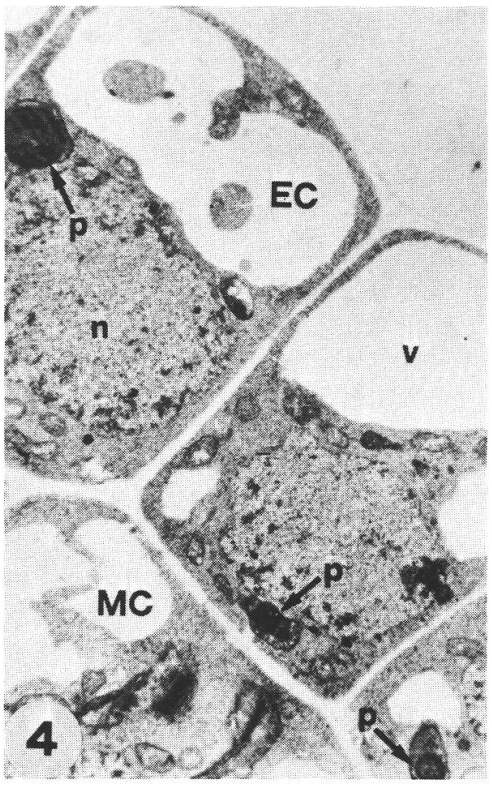
Table 1. Morphometric characteristics of intraplastidal inclusion-containing epidermal cells (fully-differentiated) in 10 mm-long leaves of *Origanum dictamnus* L.

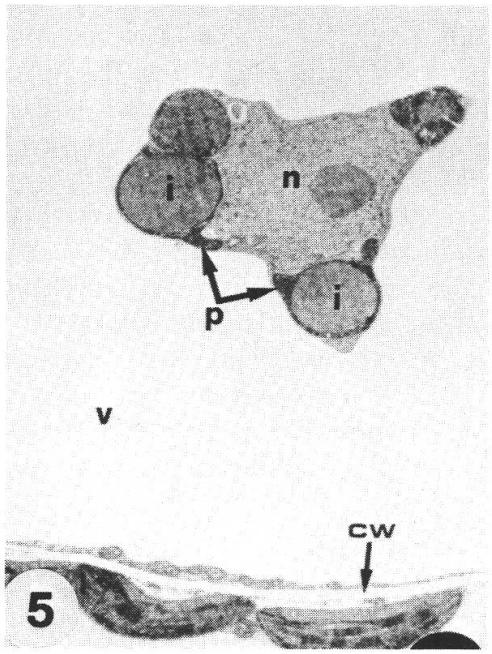
Parameters	Average values ± standard deviation
(1) Volume fraction of nuclei in epidermal cells	0.024±0.003
(2) Volume fraction of central vacuoles in epidermal cells	0.92 ±0.12
(3) Volume fraction of plastids in epidermal cell cytoplasm	0.17 ±0.03
(4) Numerical density of plastids per μm^3 of epidermal cell cytoplasm	0.16 ±0.01
(5) Volume fraction of inclusion bodies in plastids (estimated by point lattice overlay and phot. paper weighing)	0.54 ±0.08
(6) Surface density of inclusion bodies in plastids ($\mu m^2/\mu m^3$)	3.28 ±0.75
(7) Diameter of inclusion bodies, µm ($D = 4\,d/\pi$)	1.05 ±0.21











Abbreviations: cw, cell wall; EC, epidermal cell; i, inclusion body; LE, lower epidermis; n, nucleus; og, oil gland; p, plastid; UE, upper epidermis; v, vacuole.

Figs. 1–3. Light micrographs from 10 mm-long leaves of *Origanum dictamnus.*

Fig. 1. Leaf cross-section. Note the difference in size between the cells of the upper and lower epidermis. ×230.

Figs. 2 and 3. Surface views of the upper (Fig. 2, ×200) and lower (Fig. 3 ×270) leaf epidermis. Epidermal cells of both leaf sides exhibit an irregular shape with highly-infolded cell walls.

Figs. 4–6. Electron micrographs from primary and mature leaves of *Origanum dictamnus.*

Fig. 4. Young epidermal cells with dense cytoplasm and large nuclei. Plastids are few in number and contain globular, electron opaque inclusion bodies. ×5100.

Fig. 5. Partial view of a developed epidermal cell. The cytoplasm appears now dramatically restricted to a narrow parietal layer, while in an intravacuolar cytoplasmic filament, the small nucleus surrounded by several inclusion-containing plastids, can be seen. ×6200.

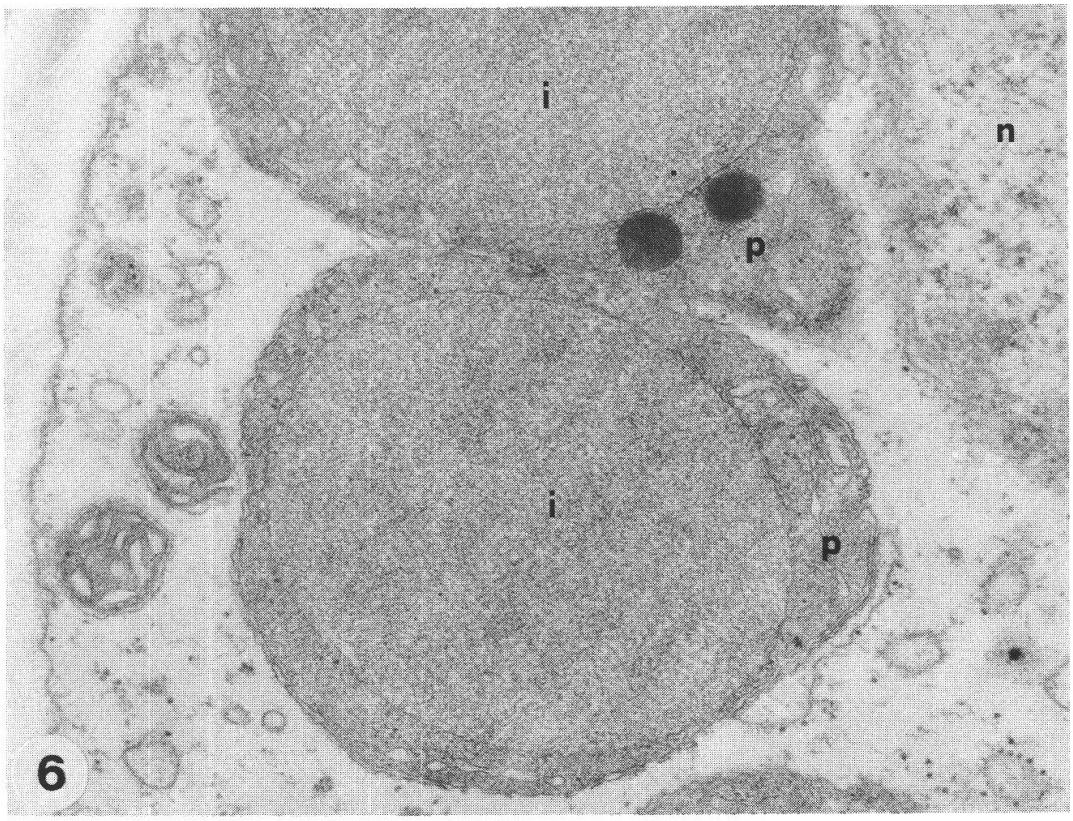


Fig. 6. Mature plastids in differentiated leaf epidermal cells. Note the fine-granular appearance of the intraplastidal globular inclusion and the presence of a single membrane that borders the inclusion. ×40500

average total volume of

$$27.58 \times 10^6 / 214.69 = 128{,}464.29\ \mu m^3\ (20{,}437.12\ \mu m^3)$$

From the comparison of the latter values it follows that the cells of the upper leaf epidermis are about 6.3 fold larger than those of the lower epidermis.

Combining the above values of epidermal cell volume with the parameters given in Table 1, we can further determine the actual volume and percentage volume values of the nucleus, the central vacuole and the cytoplasm in an epidermal cell at both leaf sides (Table 2). With respect to the plastids, which are of particular interest in the present study because of the inclusion bodies they contain, Table 3 exhibits the results of calculations concerning several parameters of leaf epidermal plastids. From these results it follows that plastids in epidermal cells of both leaf sides are of the same volume, though the cells of the upper and lower epidermis significantly differ in size. Furthermore, the average total volume of plastidal inclusions in each epidermal surface is

$$656.09 \times 25{,}762.8 = 16{,}902{,}715.0\ \mu m^3\ (6{,}974{,}629.8\ \mu m^3)$$
$$\text{or } 0.017\ mm^3\ (0.007\ mm^3)$$

A 10 mm-long leaf of *O. dictamnus,* therefore, contains in both the upper and lower epidermis a total volume of intraplastidal inclusions of

$$0.017 + 0.007 = 0.024\ mm^3$$

Table 2. Average actual volume (in μm^3) and percentage volume values of cell components per epidermal cell (10 mm-long leaves)

	Upper epidermis	Lower epidermis	%
Nucleus	3,083.14	490.49	2.4
Central vacuole	118,187.14	18,802.15	92.0
Cytoplasm	7,194.01	1,144.48	5.6

Table 3. Values of stereological parameters concerning epidermal cell plastids (10 mm-long leaves)

Parameters	Upper epidermis	Lower epidermis
Average total volume of plastids per epidermal cell (in μm^3)	1,222.98	194.56
Average number of plastids per epidermal cell	1,151.04	183.12
Average volume of a plastid (in μm^3)	1.06	1.06
Average total volume of plastid inclusion bodies per epidermal cell (in μm^3)	656.09	104.38
Average volume of inclusion body per plastid (in μm^3)	0.57	0.57
Average value of plastid inclusion body radius (in μm)*	0.52	0.52
Average total surface area of plastid inclusion bodies per epidermal cell (in μm^2)	4,011.37	638.16

* This value is in good agreement with that directly measured on a series of electron micrographs; cf. parameter (7) in Table 1.

In a parallel study conducted on the origin, differentiation and cytochemistry of these inclusions (Bosabalidis, 1987) it has been shown that they are constituted of protein. In view of this finding, the above determined volume value of 0.024 mm^3 undoubtedly represents a significant amount of protein, which could be isolated and biochemically analysed.

With respect to the value of the total surface area of the intraplastidal inclusions in each leaf epidermal surface, this is

$$4{,}011.37 \times 25{,}762.8 = 103{,}344{,}120\ \mu m^2\ (42{,}641{,}595\ \mu m^2)$$
$$\text{or } 103.34\ mm^2\ (42.64\ mm^2)$$

Both adaxial and abaxial surfaces of a 10 mm-long leaf, consequently, contain a total surface area of plastid inclusion-boundary membrane of

$$103.34 + 42.64 = 145.98\ mm^2$$

This value fits well with that resulting from calculations in which the inclusion surface area to inclusion volume ratio or the inclusion surface area to plastid volume ratio, were used. As to the possible functional role of the plastidal inclusions, the following interpretation could be given in correlation with parameter (5) of Table 1 ("volume fraction of inclusion bodies in plastids"). This parameter was morphometrically deter-

mined to be 0.54, which means that about 54% of the entire plastid volume is occupied by the inclusions. Such a high percentage suggests a storage role for the inclusions which to some degree could be compared to the large starch grains deposited in the amyloplasts. The presence of a membraneous sheath around the inclusions could further lead to the assumption that protein in the plastids of leaf epidermal cells is stored in a rather persistent form.

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