NUCLEAR DNA CONTENT AND ULTRASTRUCTURE OF SECRETORY CELLS OF *VICIA FABA* L. STIGMA

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ABSTRACT

The object of study was the level of nuclear DNA and the ultrastructural transformations in the secretory cells of the stigma in *Vicia faba* L. It has been found that the stigmal cells which are active in biogenesis and exudate secretion are diploid cells whose differentiation starts from 2C DNA level. The presence of a population of nuclei with an amount DNA of about 2.5 C suggests that the metabolic activity of those cells may be regulated through supplementary incomplete replication. The ultrastructural transformations of secretory cells point to three stages of biogenesis and secretion of exudate. Stage I, before the start of the cell's secretory functions, is characterized by the development of the protein synthesizing apparatus and the activity of dictyosomes. In development stage II vesicular electron-transparent exudate is secreted. Stage III of exudate biogenesis is production of lipids. They form mainly in the plastids and are secreted with the involvement of the cell's vacuolar system.

KEY WORDS: *Vicia faba*, stigma, secretory cells, nuclear DNA content, ultrastructure.

INTRODUCTION

The development of a specialized stigmal surface, which after pollination, would made possible the recognition of pollen, its hydration and germination is one of the important elements constituting the fertility of plants. In the course of development of the dry type stigmas, their surface is covered with a membrane-like (Gaude and Dumas 1986), protein-lipid pellicle. During maturation of wet stigmas exudate is being produced, which contains different amounts of lipids, carbohydrates and proteins including enzymes (Knox 1984), depending on the plant species. In most plant species the exudate, after breaking the cuticle, pours out on to the surface of the mature stigma. In some *Leguminosae* the exudate remains closed between the cuticle and the epidermal cells (Kreitner and Sorensen 1984, 1985, Lord and Heslop-Harrison 1984). The components of the secretion are produced by specialized cells; those may be epidermal cells and/or the layer of subepidermal cells constituting the secretory tissue (Knox 1984).

The cells functioning as trophic and secretory cells, both in animals (Anisimov 1976; Kiknadze et al. 1975,) and in plants (Nagl 1978, Kowles and al. 1990, Marciniak 1991, 1993)), are characterized by the capability of complete or partial multiplication of genome by means of endoreplication. In plants that happens e.g. in the endosperm (Nagl 1978, Turala-Szybowska 1966, Kowles and al. 1990, Marciniak 1991, 1993) and in the secretory tapetum, in which the DNA level rises most frequently to 4n-8n, and in some species even to 32n (Chapman 1987). Endoreplication may – but does not have to – lead to intensified transcription and translation (Kononowicz and Janick 1988). On the other hand it is commonly

accepted that endoreplication is the most economical mechanism of obtaining by secretory cells a high level of productivity. It is not known whether a similar mechanism functions in the secretory cells of the stigma, as the DNA level in those highly specialised cells has not been studied so far.

Wet stigmas have been studied in more than 100 plant species (Kandasamy and Kristen 1987). The studies concerned mostly mature stigmas observed in a light microscope. The ultrastructure of the secretory tissue in the course of successive maturation stages of the stigma has been explored in only a few plant species (e.g. *Lycopersicon peruvianum* – Dumas et al. 1978, *Trifolium pratense* – Heslop-Harrison and Heslop-Harrison 1983, *Nicotiana glauca* – Kandasamy and Kristen 1987). The results obtained by the above authors show various ways of biogenesis and secretion of the exudate. That is why the understanding of the secretory processes in maturing stigma is still incomplete.

The object of the present work was to study 1) the level of DNA in the nuclei of secretory cells in the stigma of *Vicia faba*, 2) the ultrastructural transformations of those cells during pistil maturation.

MATERIAL AND METHODS

The material for study were maturing stigmas of *Vicia faba* var. Nadwiślański. Their maturity was estimated by the colour and size of the flower bud and by the morphology of the pistil viewed in a stereoscopic microscope (x 40). The experiments were carried out in three development stages of the pistil. Stage I – pistils straight-shaped, 3-5 mm long, the surface of stigma without papillae. Stage II – pistils 5-15 mm long isolated from green flower buds, elongate style bent to-

wards the ovary, on the surface of stigma papillae of different lengths, and sterile hairs at its base. Stage III – pistils 15-20 mm long with bent style isolated from white flowers, the surface of the stigma covered with papillae, long sterile hairs at its base.

Cytophotometric analysis

Pistils in development stages II and III and root meristems were fixed for 1 h in ethanol/acetic acid (3 : 1), hydrolized for 1 h in 4N HCl at 20 °C and stained by Foulgen's method. Smear preparations were made from the root meristems and the secretory tissue of the stigma, removing the cover glasses by the dry ice method. The nuclear DNA content was measured using a microdensitometer GN 5 (Bar and Struid). Values 2C and 4C were established in arbitrary units basing on measurements of DNA content in 50 prophase and telophase nuclei of meristematic cells.

Ultrastructural study

For ultrastructural studies stigmas were fixed for 6 h at +4°C in 2.5% glutar aldehyde in Na-cacodylate buffer pH 7.2, then for 12 h in 1% OsO₄ in the same buffer. The material was dehydrated in a series of ethyl alcohol and embedded after Spurr (1969). The semi-thin sections were stained with a mixture of Sudan III and IV and viewed in a light microscope. Ultra-thin sections were contrasted after Reynolds (1963) and viewed in the electron microscope Tesla BS 500.

RESULTS

The analysis of semi-thin preparations revealed the involvement of a 4-5-layer thick subepidermal secretory tissue in the secretion of the exudate. The presence of the exudate in the intercellular spaces of the tissue is observed starting from development stage II of the pistil. The exudate first accumulates in the intercellular spaces between the longitudinal walls of secretory cells, then between their transverse walls. In mature stigmata the individual cells of the secretory tissue are entire-

ly separated from each other by a large amount of exudate. In most mature stigmata the exudate remains closed between the cuticle and the epidermal cells. The exudate stains with Sudan III and IV, which indicates its lipid character.

DNA content in secretory cells

DNA content in the cells of the stigmal secretory tissue in *Vicia faba* in stages II and III of pistil maturation is present in histogram form in Figs 1 and 2 respectively. In pistil development stage II the nuclear DNA content in about 70% of the limits including 2C. In the remaining 30% cells the amount of nuclear DNA is higher, but never exceeds the value of 4C. Maximum DNA content can go up to about 2.5 C.

The presence of two classes of nuclei differing in DNA content has been also found in the secretory cells of the mature stigma. The frequency of nuclei with amount of DNA 2C or more is similar to that in the preceding development stage of the pistil. DNA content in any nucleus does not reach the value 4C.

The ultrastructure of secretory cells

In the first development stage of the pistil, the stigmal cells transforming into secretory ones contained cisternae of rough endoplasmic reticulum, numerous polysomes, active dictyosomes (Fig. 3) as well as mitochondria, oval electron-opaque bodies, and vacuoles containing fibrillar and electron-dense material (Fig. 4). The cells were connected via plasmodesmata (Fig. 5). In the plastids were present scattered starch grains. Sometimes, between the membranes of the thylakoids and in the external membrane of the plastid there appeared expansions with electron-transparent contents (Fig. 6). Similar structures occurred in the cytoplasm, mainly near the plasma-lemma (Fig. 5).

In the subsequent developmental stage of the pistil (stage II) an accumulation of the exudate was observed between the cells of the secretory tissue. It was changing in character as the stigma was maturing. The original secretion consisted of electron-transparent vesicles surrounded with an osmiophilic

Figs 1-2 Frequency of nuclei with different DNA content the cells of secretory layer of the stigma in *Vicia faba* pistil

Fig. 1 – development stage II,

Fig. 2 – development stage III (100 nuclei in each of 10 pistils).

Figs 3-6 Subepidermal cells of stigma in pistil development stage I (er – endoplasmic reticulum, P – plastid, M – mitochondrion, W – cell wall, Va – vacuole, pd – plasmodesmata, L – electron-opaque body, CF – fibrillar body, G – Golgi apparatus).

Fig. 3 – In cytoplasm there are numerous cisternae of rough endoplasmic reticulum, polysomes, Golgi apparatus, mitochondria and fibrillar body; x 32 000,

Fig. 4 – In the cells there are also electron-opaque body and vacuoles filled with fibrillar and electron-dense material x 38 000,

Fig. 5 – In cytoplasm of cells interconnected with plasmodesmata there can be seen accumulations of electron-transparent material; x 38 000,

Fig. 6 – In plastids containing small starch grains there can be seen accumulations of electron-transparent material between the thylacoid membranes and the membranes surrounding those organelles; x 25 000

Fig. 7 – Stigma epidermal cell surrounded with cuticle (C) adhering to the wall (W). In the cytoplasm there are vacuoles (Va) with fibrillar and electron-dense material and electron-opaque bodies (L); x 18 600.

Figs 8-9 Stigmat secretory cells while secreting electron-transparent vesicular exudate (E – exudate, V – vesicles, N – nucleus, mb – multivesicular body, P – plasmalemma, W – cell wall, Va – vacuole); x 25 000

Fig. 8 – Exudate consisting of electron-transparent vesicles present between the cells; in the cytoplasm there can be seen numerous vesicles, some of which become fused with plasmalemma;

Fig. 9 – Exudate accumulated between longitudinal walls of secretory cells, the transverse walls are contiguous to each other; in the right cell there can be seen a multivesicular body.

Fig. 10 – „Maturation” of exudate – among secretory cells the exudate (E) can be seen consisting of electron-transparent vesicles and osmiophilic bodies; in the cell cytoplasm lipid producing plastids (P) are present; W – cell wall, N – nucleus; x 25 000

Figs 11-14 Stigmat secretory cells while osmiophilic material is being synthesized (er – endoplasmic reticulum, V – vesicles, pd – plasmodesmata, W – cell wall, Va – vacuole, E – exudate, P – plastids, G – Golgi apparatus).

Fig. 11 – In cytoplasm present are expanded cisternae of endoplasmic reticulum and plastids surrounded with lipid drops. The plastids are in contact with the vesicles, into which penetrates the osmiophilic material; x 25 000.

Fig. 12 – In expanded cisternae of endoplasmic reticulum can be seen a drop of osmiophilic material; x 18 000,

Fig. 13 – Osmiophilic material leaking out of ER is in contact with plasmalemma (arrow); x 15 000,

Fig. 14 – In cytoplasm can be seen a lipid producing plastid and a Golgi apparatus producing electron-dense vesicles; one of them is in contact with plasmalemma (arrow); x 28 000

Figs 15-16 Stigma epidermal cells while the exudate is being accumulated between the cuticle and the cell wall (E – exudate, W – cell wall, C – cuticle, P – plastid, N – nucleus, L – electron-opaque body) x 25 000

Fig. 15 – In the space between the cuticle and the epidermal cell wall there are osmiophilic bodies and membrane-like structures; in the cytoplasm there are lipid producing plastids, electron-opaque bodies and vacuoles,

Fig. 16 – "Maturation" of exudate – among osmiophilic droplets there are electron-transparent bodies

border (Figs 8-9). In the cytoplasm of the cells surrounding the original secretion there occurred numerous vesicles, often in contact with the plasmalemma (Fig. 8). Between the plasmalemma and the cell wall the presence of multivesicular bodies was noted (Fig. 9).

In the following development stage the electron-transparent vesicles of the exudate transformed into electron-dense bodies (Fig. 10). In the cells of the "maturation" zone of the secretion intensive production of osmiophilic material, probably fats, was observed. In that process mainly plastids were involved. That process was associated with disorganization of the thylakoids and the appearance of osmiophilic drops on the membranes surrounding the plastid (Fig. 11). Lipid producing plastids occurred in contact with the vesicles, into which the osmiophilic material seemed to penetrate (Figs 11, 13). Electron-dense material also occurred inside the cisternae of the smooth ER (Fig. 12) and in Golgi vesicles (Fig. 14). Osmiophilic Golgi vesicles and electron-dense bodies from the endoplasmic reticulum were often seen in contact with plasmalemma (Figs 13, 14).

In the mature stigma electron-dense secretion mass separated the secretory cells from each other and was present between the cuticle and the wall of epidermal cells. The secretory cells were vacuolized with a few cisternae of the expanded endoplasmic reticulum.

In the epidermal cells similar ultrastructural transformations to those in the cells of the secretory tissue were observed. Before the start of the secretory activity of stigma there occurred in them cisternae of rough endoplasmic reticulum, polysomes and vacuoles containing fibrillar and electron-dense material (Fig. 7). In the period of filling the subcuticular space with exudate, lipids were produced by the plastids in the epidermal cells, and around the plastids osmiophilic bodies appeared (Fig. 15). In the space between the cuticle and the cell wall there were osmiophilic and electron-transparent bodies and membranelike structure (Fig. 15, 16).

DISCUSSION

The biological function of stigma is the reception and selection of pollen and making it possible for the pollen tube to germinate and grow. The fulfilment of those functions is conditioned by the development of a highly specialized surface of stigma. In *Vicia faba* the surface of the mature stigma is covered with cuticle underlain by a thick layer of lipid-protein secretion. The secretion is produced by specialized cells of the secretory tissue. A similar stigma has been described in another species of *Leguminosae*, *Trifolium pratense* (Heslop-Harrison and Heslop-Harrison 1983).

Like the cells of other specialized plant tissue (Heslop-Harrison 1967), the differentiated secretory cells do not exhibit mitotic activity. The results of cytophotometric measurements of the nuclei of stigmal secretory cells in *Vicia faba* revealed that their high metabolic activity connected with the production and secretion of exudate is not associated with polyploidization. The maximum nuclear DNA content in stigmal secretory cells can go up to 2.5 C. That makes them different from many other cells performing similar functions. In cells functioning as secretory cells intensive metabolic activity is often associated with multiplication of the genome to values exceeding the diploid value up to several thousand times (Rasch 1970, Nagl 1978, Chapman 1987).

Most secretory cells of the stigma of *Vicia faba* pistil contain 2 C DNA. That indicates that they stopped at G₁ phase of their life cycle. However, in about 30% cells the level of

DNA is higher than 2C. That suggests that part of their genome may have undergone additional replication. Such a possibility seems highly probable, since both in plants and animals incomplete multiplication of nuclear DNA content through differentiated replication is a common phenomenon (Nagl 1978). The number of cells with DNA content higher than 2 C remains at the same level through stigmal development stages II and III. That coincidence suggests some relationship between increased DNA content and the high secretory activity of those cells revealed in ultrastructural studies. Studies on that matter should be continued to detect the mechanism regulating the secretory activity of the cells of the stigmal surface layer in the pistil of plants.

Studies on *V. faba* have revealed that the process of biogenesis and secretion of the components of the stigmal exudate proceeds through several stages reflected in the varying ultrastructure of the secretory cells.

Stage I, before the exudate appears outside the secretory cells, is characterized by intensive synthesis of proteins and probably carbohydrates. The occurrence of electron-transparent material between the external membranes of plastids and in the expansions of thylakoids may suggest that those organelles are also involved in the production of non-osmiophilic secretory material.

Stage II of stigmal maturation is characterized by the presence of secretion among the cells of the secretory tissue. Electron-transparent material is secreted by exocytosis. Outside the plasmalemma, besides typical electron-transparent vesicles of secretion there occur multivesicular bodies. Griffing and Fowke (1985) associate their presence with the secretion of peroxidase to the cell walls. That enzyme occurs in stigmal cell walls (Bredenmeijer 1984). It cannot therefore be precluded that this is the way of that enzyme's secretion outside the secretory cells of *Vicia faba* stigma.

Stage III of stigmal development is associated with biogenesis and secretion of lipids. Primary biogenesis of lipids takes place in the plastids. It seems that in the secondary metabolism as well as in the transport and secretion of lipidic constituents of the exudate vacuolar cell structures are involved. That way of transport and secretion of lipids is not universal. The presence of lipid drops in ER has been found in the stigmal secretory cells in *Citrus limon* (Cresti et al. 1982). The most commonly described way of biogenesis and secretion of osmiophilic material is accumulation in the cytoplasm of secretory cells of lipid drops, whose contents directly penetrate through the cell membrane (Konar and Linsken 1966, Dumas et al. 1978, Kreitner and Sorensen 1984).

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ZAWARTOŚĆ DNA JĄDROWEGO I ULTRASTRUKTURA KOMÓREK WYDZIELNICZYCH ZNAMIENTA *VICIA FABA* L.

STRESZCZENIE

Badano poziom DNA jądrowego i przemiany ultrastrukturalne w komórkach wydzielniczych znamienia *Vicia faba*. Stwierdzono, że aktywne w biogenezie i sekrecji eksudatu komórki znamienia są diploidalne, a ich różnicowanie rozpoczyna się z poziomu 2C DNA. Obecność populacji jąder o ilości około 2.5 C DNA sugeruje, że aktywność metaboliczna tych komórek może być regulowana na drodze dodatkowej, niepełnej replikacji. Przemiany ultrastrukturalne komórek wydzielniczych wskazują na 3 etapy biogenezy i sekrecji eksudatu. Etap I – przed podjęciem funkcji wydzielniczych komórki charakteryzuje rozbudowa aparatu syntetyzującego białka oraz aktywność mitochondriów. W II etapie rozwojowym odbywa się sekrecja pęcherzykowej elektronowo-przeźrystej wydzieliny. III etap biogenezy eksudatu to produkcja lipidów. Powstają one głównie w plastydach i są wydzielane przy udziale systemu wakuolarnego komórki.

SŁOWA KLUCZOWE: *Vicia faba*, znamię, poziom DNA jądrowego, komórki wydzielnicze, ultrastruktura.