ACTA BIOLOGICA CRACOVIENSIA Series Botanica 39: 63–67, 1997 PL ISSN 0001-5296

POLYPLOIDIZATION OF STIGMATIC PAPILLAE IN *TRIGLOCHIN MARITIMUM* L. (JUNCAGINACEAE)

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Received March 4, 1997; revision accepted April 7, 1997

The cytological differentiation of the dry papillate stigma in *Triglochin maritimum* L. (Juncaginaceae) was studied. The polyploidization process started soon after the formation of unicellular stigmatic papillae. Later, huge, long papillae with single enlarged nuclei constituted the receptive surface of the maturing stigma. The nuclear DNA content of the polyploid papillae and of telophasic (2C) and prophasic (4C) cells of the ovule was measured cytophotometrically after Feulgen staining. Analysis of nuclear DNA content measurements permitted the degrees of ploidy reached by the papillae to be established. Nuclei with DNA content corresponding to levels of 4C, 8C, 16C, 32C and 64C were found in the mature stigma. The most common were nuclei with DNA content of 16C (29%) and 32C (24%). The absence of mitoses, rhythmical enlargement of the DNA content of the nuclei as well as their characteristic endochromocenters, pointed to endoreduplication as the mechanism of polyploidization of the stigmatic papillae.

Key words: *Triglochin maritimum* L., DNA cytophotometry, endoreduplication, polyploidization, stigma, stigmatic papillae.

INTRODUCTION

Multiplication of nuclear DNA content is one of the most common processes connected with cell differentiation in plants. Sometimes polyploidization occurs in almost 70–85% cells of one organism, both in tissues connected with sexual plant reproduction and in tissues functioning as vegetative (for review: NAGL, 1978; BRODSKY and URYVAEVA, 1985). Processes of polyploidization leading to particularly high levels of polyploidy are characteristic of trophic and secretory cells or tissues. Their lifetime is usually very short and limited to the period of performance of their peculiar function.

The stigma is one of the most short-lived structures of a plant. Studies on the structure and function of the stigma and stigmatic papillae show their secretory character, connected with pollination, hydration and germination of pollen grains (SEDGLEY and BUTTROSE, 1978; DUMAS et al., 1978; TILTON and HORNER, 1980; TILTON et al., 1984; KNOX, 1984; GAUDE and DUMAS, 1986). The literature on the stigma published up to now concentrates on the secretory aspects of its function as well as the chemical composition of the exudates produced by stigmatic cells (CRESTI et al., 1982; CIAMPOLINI et al., 1983, 1990, 1995; SHIVANNA et al., 1989; AMARASINGHE, 1990; GARG and BHATNAGAR, 1991), but little is known about the karyological status of the cells forming the stigma. Changes in the nuclear DNA content of secretory cells of the stigma have been reported only for *Vicia faba* (WRÓBEL and BEDNARSKA, 1992). The presence of a population of nuclei with an amount of DNA corresponding to about 2.5C has been found in this species.

The present study deals with the processes leading to multiplication of nuclear DNA content in cells of dry papillate stigmata in *Triglochin maritimum* L. and changes in the structure of their chromatin.

MATERIALS AND METHODS

The study materials were maturing inflorescences of *Triglochin maritimum* L. (Juncaginaceae) originating from plants growing in natural habitats on the Baltic Sea coast at Mrzezino near Puck. A few cytotypes of *T. maritimum* have been reported for Poland: 2n = 12, 24, 30 and 48 chromosomes (Piotrowicz, in: SKALIŃSKA et al., 1961). To determine which cytotype characterizes the studied plants, squash preparations made from young acetocarmine-stained anthers were analyzed. In all these specimens the haploid chromosome number n = 24 (2n = 48) was found.

Pistils excised from flowers were fixed for 4 h in ethanol/acetic acid (3 : 1) at room temperature and stored in 75% ethanol at 4°C. Stigmata and ovules isolated from the pistils were hydrolyzed for 1 h in 4N HCL at 20°C and stained by the Feulgen method. Squash preparations were made from the stigmata and ovules by the dry ice method. Nuclear DNA content was measured using an Amplival Photometrie MFV 4001 cytophotometer. The 2C and 4C values were established based on measurements of DNA content in telophasic and prophasic nuclei of ovule cells.

RESULTS

Triglochin maritimum L. flowers are bisexual and radial. There are six pistils, each terminating in a large hook-shaped stigma. Huge, long cells

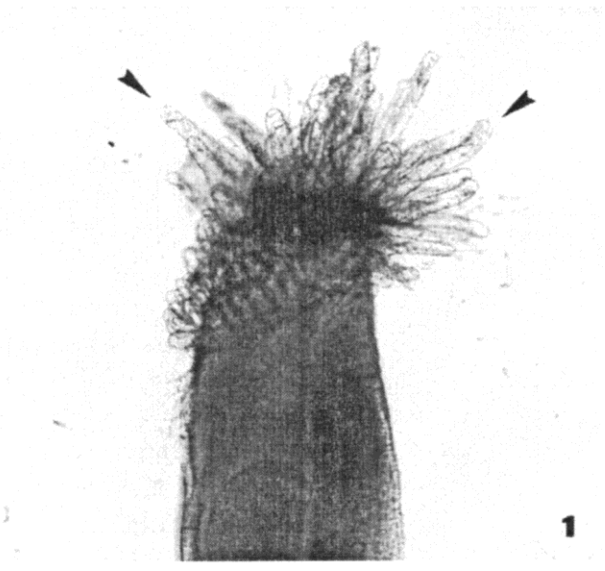

Fig. 1. Apical part of isolated pistil of *Triglochin maritimum* with huge, long stigmatic papilae (arrowheads). × 75.

TABLE 1. Frequency of nuclei with different DNA content in the papillae of mature stigmata in *Triglochin maritimum* pistils

DNA content		number of nuclei	% of nuclei
min.–max. (arbitrary units)	C values		
170–205	4	8	6.9
226–253	4–8	3	2.6
337–443	8	12	10.3
474–595	8–16	4	3.4
672–882	16	34	29.3
987–1249	16–32	19	16.4
1290–1690	32	28	24.1
2009–2449	32–64	5	4.3
2580–3450	64	3	2.6

with single enlarged nuclei form the surface of the mature stigma (Fig. 1).

The polyploidization process starts soon after the formation of unicellular papillae. The results of measurements of DNA content in the nuclei of papillae are presented in Table 1.

Comparison of nuclear DNA content in cells of mature stigma (polyploid) and meristematic cells of the ovule (di- and tetraploid) permitted the degrees of ploidy reached by the stigmatic cells to be established. Nuclei with DNA content corresponding to levels of 4C (Fig. 3), 8C (Fig. 3), 16C (Fig. 4), 32C (Fig. 5) and 64C were found. The presence of nuclei with DNA content corresponding to the 2C level was not noted. Such results suggest that all cells of the stigma in *T. maritimum* become polyploid during maturation. The highest nuclear DNA content, reached by 2.6% of the stigmatic papillae, corresponds to the level of 64C; however, the most common cells of the stigma (29.3% and 24.1%, respectively) attain levels of 16C (Fig. 4) and 32C (Fig. 5). The material studied contained a significant proportion (26.7%) of cells with intermediate levels of nuclear DNA (e.g., 16.4% of the cells had between 16C and 32C DNA; Tab. 1). This observation suggests that the mature stigmata are built not only from fully differentiated cells but also from maturing cells still undergoing polyploidization.

The structure of chromatin in the diploid cells of the ovule (Fig. 2) as well as the cells of the stigma in *T. maritimum* could be classified as chromocentric. With increasing levels of ploidy, the size of the chromocenters of stigmatic papillae increased, whereas their number underwent no significant changes (Figs. 3–5). There-

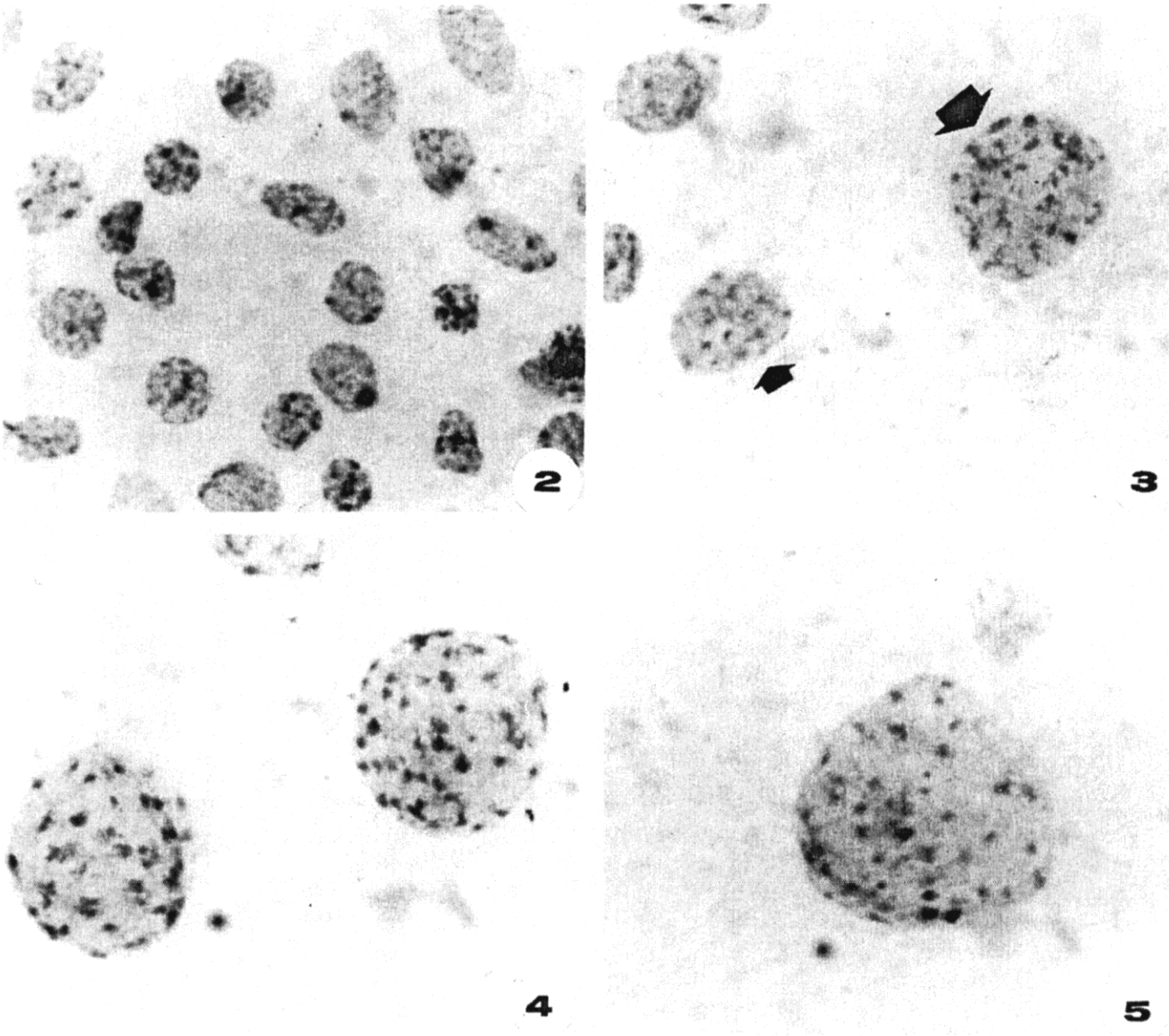

Fig. 2. Chromocentric, diploid nuclei from cells of young ovule. **Figs. 3–5.** Polyploid nuclei from stigmatic papilae. Fig. 3. DNA content corresponding to 4C and 8C (small and large arrows, respectively). **Fig. 4.** 16C. **Fig. 5.** 32C. The size of the endochromocenters increases with the increasing level of ploidy, wheres their number undergoes no significant changes. × 1500.

fore, in highly polyploid nuclei (16C, 32C and 64C) typical endochromocenters are present.

Analysis of the data on nuclear DNA content of polyploid stigmatic cells reveals their rhythmical growth, with the regular spherical or oval shapes of the nuclei retained. Moreover, neither endomitoses nor other processes causing similar effects were observed. All described changes in DNA content, size of nuclei, and chromatin structure point to endoreduplication as the mechanism of polyploidization in stigmatic papillae of *T. maritimum*.

DISCUSSION

The function of the stigma is collection, hydration and germination of pollen. The stigma develops a highly specialized receptive surface which fulfills these functions. Ultrastructural, histochemical and cytochemical studies yielded data and possible explanations of its role during pollination (SEDGLEY and BUTTROSE, 1978; TILTON and HORNER, 1980; CRESTI et al., 1982; CIAMPOLINI et al., 1983, 1990, 1995; TILTON et al., 1984; KNOX, 1984; GAUDE and DUMAS, 1986; KANDASA-

MY and KRISTEN, 1987; SHIVANNA et al., 1989; GARG and BHATNAGAR, 1991). The stigma in *Triglochin maritimum* is situated at the apex of the pistil and, according to the classification of HESLOP-HARRISON and SHIVANNA (1977, 1981), is included among the dry-surface stigmata. The receptive surface of the stigma is rather large and is composed of huge unicellular papillae containing single enlarged nuclei. The enormous size of the cells and their nuclei is obviously connected with multiplication of the basic nuclear DNA content during maturation of the stigma.

Polyploidization is the most common means of cytological differentiation in plant as well as animal cells and tissues during their ontogenesis (NAGL, 1978; BRODSKY and URYVAEVA, 1985). Higher ploidy level might be attained in different cellular processes: endoreduplication, endomitosis, disturbed mitoses or additional replication.

The most common mechanism of polyploidization resulting in the highest levels of ploidy is endoreduplication. The nuclear DNA content of endopolyploid cells is sometimes a hundred times higher than the DNA content of diploid cells of the same tissue (NAGL, 1978; BRODSKY and URYVAEVA, 1985). In many plant species, endoreduplication is intimately associated with differentiation of some secretory and/or nutritive cells and tissues inside the ovule (antipodals, synergids, endosperm, suspensor) as well as other secretory cells of the flower (corolla hairs, anther hairs, nectaries) (for review: NAGL, 1978; D'AMATO, 1984). However, the process of multiplication of basic nuclear DNA content in secretory cells of the stigma has not been described till now. A small increase of DNA content in the nuclei of stigmatic secretory cells was revealed in *Vicia faba* only (WRÓBEL and BEDNARSKA, 1992); in about 30% of these cells, the maximum nuclear DNA content went to the level of 2.5C. According to the authors, this result suggests that part of the genome of stigmatic cells may undergo additional replication (amplification). In contrast, all papillae of the stigma in *Triglochin maritimum* underwent regular endoreduplication during maturation. The highest ploidy level determined in the nuclei of the cells studied in the present work was 64C DNA content, corresponding to five endoreduplication cycles. A similar level of 64 n has been reported in, for example, cells of anther hairs in *Cucumis sativus* (TURAŁA, 1960).

Changes in the structure of chromatin of polyploid nuclei compared to that of diploid nuclei are effects of polyploidization very often described. Both diploid nuclei of the ovule and polyploid nuclei of stigmatic papillae in *T. maritimum* could be classified as chromocentric nuclei. In the papillae, however, the size of the chromocenters grows and endochromocenters are formed as a result of endoreduplication cycles. This is the most frequently occurring structure of chromatin at these middle levels of polyploidy. They have been described many times in many tissues and cells, for example in anther hairs of *Cucumis sativus* (TURAŁA, 1960). Endoreduplication as a means of polyploidization may lead to the formation of polytene chromosomes in highly polyploid cells occurring not only in the ovule. This kind of chromosomes was distinguished in the basal cell of anther hairs in *Bryonia dioica* (BARLOW, 1975), attaining a level of 256 n.

Triglochin maritimum flowers are anemophilous. They contain pistils with dry, relatively large stigmata. Polyploidization of stigmatic cells in *T. maritimum* is probably connected with their function. The increased size of the stigma and their papillae appears to facilitate the settling of wind-transported pollen grains on the stigma. The receptive cells of a dry stigma are usually covered by a protein-containing pellicle produced by the secretory cells. It is well known that the protein synthesis activity of polyploid cells is higher than that of diploid cells. Changing environmental factors can delay pollination, especially in anemophilous flowers. Stigmata, therefore, need good vitality to retain their function for some time. Probably to ensure this, fully differentiated papillae (ready to collect pollen) as well as maturing papillae (still undergoing polyploidization) are present in the mature stigma of *Triglochin*. Further studies, including analysis of ultrastructure, are needed to verify the above suppositions.

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