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Nuclear structure and DNA content in glandular hairs of *Salvia officinalis* L.

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The differentiation model of the structures which secrete pharmacologically active substances appears a particularly interesting field of study, since it can be related to the type and way of secretion. Four different types of hair of *Salvia officinalis* L. were examined in this light concerning their nuclear structure and the quantity of DNA, both of which are very important parameters in differentiation. Results indicated quite intense metabolic activity in all types of hair with fairly high levels of endopolyploidy, the probable presence of polyteny, and probable differential DNA replication. The peltate glandular hairs proved to be the most active ones. In the long-stalked capitate hairs, the distal cell of the pedicel also appeared to have a particularly active metabolism, suggesting that it is in some way involved in the secretory process.

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A morphological and histochemical study (BINI MALECI et al. 1983) has indicated that in *Salvia officinalis* L. glandular trichomes (the site of production and/or accumulation of pharmacologically active substances) show greatly variable patterns: (1) short-stalked capitate glandular hairs showing a big one- or two-celled head (*a* type); (2) long-stalked capitate glandular hairs, with a small one-celled head (*b* type); and (3) peltate hairs, having heads up to 12-celled with subcuticular space (c_1 type) and without (c_3 type).

Histochemical differences occurring with the above morphological ones suggest that, in most cases, different secretion products are present in the various glandular types.

The present research concerns the nuclear structure and the quantity of DNA (measured by cytophotometric methods) of these different types of glandular hairs. The aim was to contribute to the understanding of their differentiation model, always related to the type and way of secretion. As far as the glandular hairs are concerned, some data are available in literature on their nuclear structure (TSCHERMAK-WOESS and KASITSCHKA 1953, 1954; TURALA 1960, 1962; RENAUDIN 1967; LANDRÉ 1976a; GOSTEV and ASKER 1978; FRANCESCHI and GIAQUINTA 1983).

Cytophotometric data on the quantity of DNA are limited to those reported by LANDRÉ (1976a, b) on *Solanum nigrum*.

Materials and methods

All work was carried out on the same specimens of *Salvia officinalis* L. as those used by BINI MALECI et al. (1983) and cultivated at the Pisa University Botanical Gardens (PI). Leaf and calyx hand made sections about 30 μm thick and squashes prepared by macerating the calyxes with 5 % pectinase (Nutritional Biochemical Corp. Cleveland) at pH 4.0 and at 40°C for 1 hour were fixed in ethanol/acetic acid 3:1 (v/v) for 40 min, hydrolysed for 8 min in 1N HCl at 60°, stained in Schiff reagent for 90 min and then dehydrated and mounted in Canada balsam.

For *a* and *b* type hairs (cf. BINI MALECI et al. 1983) the quantity of DNA was measured for cells from both the pedicel and the secreting heads. For c_1 and c_3 type hairs only the quantity of DNA from the head was measured. This was because cytophotometric measurements were technically only possible in the calyx squashed, and in this sort of preparation the short pedicel remained covered by the secreting cells, placed like a shield, making it impossible to measure.

Hairs type c_2 (cf. BINI MALECI et al. 1983) were not considered in the present approach, since they seem to be more a functional moment of the c_1 type than a separate entity according to their morphology and localization in the plant.

As control, epidermic nuclei of calyx and leaf were also measured.

The cytophotometric measurements were carried out following the method suggested by MC LEISH and SUNDERLAND (1961) using the Deeley type cytophotometer produced by Barr and Stroud, Glasgow G.B. (integrating microdensitometer type GM5) at 565 μm wavelength. For each type of nucleus, measurements were taken on 5 different samples, but since no significant differences were noted, data were unified.

Results and discussion

Nuclear structure

In all the glandular hairs examined, the greatest nuclear dimensions were reached in the cell or cells of the secreting head. Nonetheless, in the pedicel cells too, the nuclei were larger than in the epidermis and when the pedicel is pluricellular, their size increases constantly from the proximal cells towards the distal. Among the nuclei of the secreting head of the various glandular types no uniformity in size was seen; in the *c* type, in fact (Fig. 1), they are smaller with a more compact structure than in types *a* and *b* (Fig. 2 and 3).

Both epidermic and glandular hair nuclei present typical chromocenter structure. Whereas in the epidermal nuclei the chromatin appears low and not very concentrate (Fig. 1, arrow), in the pedicel nuclei and even more in the large nuclei from the secreting heads, the chromocenters increase considerably in dimension, being very large and intensely stainable with the specific techniques for DNA (Fig. 2 and 3). Their number 14 (the same as the somatic chromosomes) and size indicate endopolyploidy with presence of polytene chromosomes. A similar situation has been reported for glandular hairs with pluricellular pedicel and monocellular head (corresponding to our type *b*) in *Salvia horminum* (L.) Briq. (GOSTEV and ASKER 1978). On the other hand it is known that endopolyploidy and often polyteny (which is merely a special morphological state of the former) are present in cells with high metabolism such as glandular cells (NAGL 1978). Furthermore, endomitotic phenomena have already been reported for various types of trichome, in particular in their secretory cells (D'AMATO 1952; TSCHERMAK-WOESS and KASITSCHKA 1953, 1954; TURALA 1960, 1962; LANDRÉ 1976a, b).

The degree of polyteny in all the types of hair examined increases in the pedicel from the proximal part towards the distal one and reaches its maximum in the cells of the secretory head. The highest degree of polyteny at a heterochromatic level (shown by the greater size of the chromocenters) is reached in the types *a* and *b*. Polytenization at a heterochromatic level has been suggested for *Salvia horminum* (L.) Briq. (GOSTEV and ASKER 1978). In *b* type hairs, the distal cell of the pluricellular pedicel (Fig. 3) seems very similar in its nuclear structure to the cell of the secretory head.

Cytophotometric analysis

Results are reported in Table 1 and Fig. 4. It can be seen clearly that both type *a* and type *b* hair pedicel cells proved to be 4C like epidermal cells. The same result was obtained by LANDRÉ (1976b) for secretory hairs of *Solanum nigrum*.

However, if the histograms (Fig. 4) are considered, it will be seen that, particularly in type *b* hair, pedicel cells with a higher degree of ploidy are present. This is in total agreement with the morphological observations, which indicate an increase in nuclear volume and chromocenter size in the long pedicels of this type of hair, progressing towards the secreting head, but chiefly in the cell nearest to it.

The cells in the secreting head always appear polyploid and the degree of ploidy reached in the various types of hairs is only slightly different. In the types *a* and *b*, endopolyploidy remains at rather low levels (8C), and slightly higher levels are reached only occasionally. The types c_1 and c_2, which appear similar in this respect, show a more marked tendency to displace ploidy to higher levels.

Endopolyploidy at the 8C level is reported for glandular hairs similar to our type *b* by LANDRÉ (1976b) for *Solanum nigrum*. As in *Solanum nigrum* (LANDRÉ 1976b), the disparity in DNA levels brings up the question of whether all types of trichomes are involved in identical secretory phenomena.

Fig. 4 indicates that the distribution of nuclear DNA is continuous. Although this is partly to be explained by late replication of the heterochromatic portions of the genome, the hypothesis of genic amplification phenomena in co-existence with already evident phenomena of endopolyploidy cannot be excluded.

The same hypothesis, based on an identical continuous distribution of DNA, has been put forward

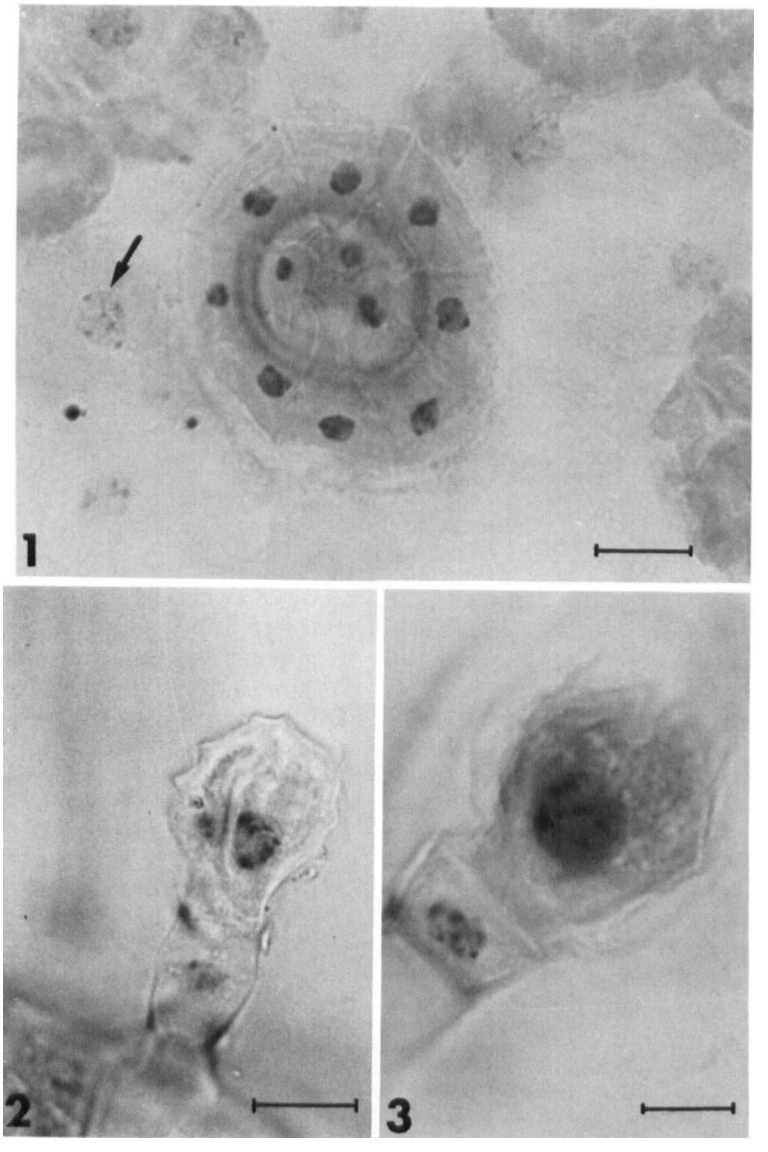

Fig. 1–3. Fig. 1. Peltate glandular hair (c_2 type) and epidermic cells in the calix (Feulgen). Arrow: an epidermic nucleus with not much concentrated chromatin. **Fig. 2.** Short-stalked capitate glandular hair (*a* type) (Feulgen). **Fig. 3.** Long-stalked capitate glandular hair (*b* type). In evidence, the distal cell of the pluricellular pedicel and the cell of the secretory head (Feulgen). (Bar = 10 μm).

by LANDRÉ (1976b) for secretory hairs of *Solanum nigrum*. In this author's opinion, genic amplification could reflect a functional state of secretory activity, in the sense that it arises during or slightly before secretion. The same should be true for *S. officinalis*, but naturally the question of the various types of hair will have to be looked into, and further investigations in this direction are already planned.

On the other hand it is already known that endopolyploidization, a manner by which the cell increases its capacity to synthesize proteins (NAGL 1978), and amplification, a way by which the

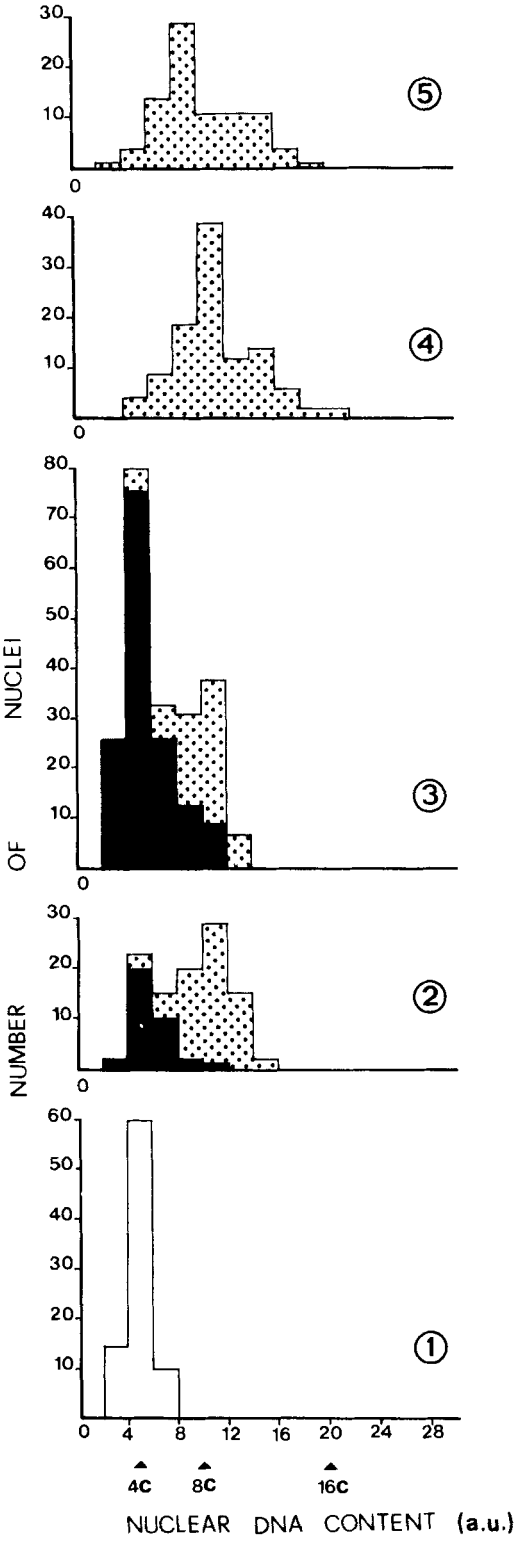

Fig. 4. Relative amounts of nuclear DNA (arbitrary units) in ① = epidermis, ② = *a* type glandular hair, ③ *b* = type glandular hair, ④ = c_1 type glandular hair, ⑤ = c_3 type glandular hair.

Open bars = epidermis nuclei
solid bars = pedicel nuclei
dotted bars = head nuclei

The 4C value is based on measurements of early root tip prophases.

Table 1. DNA content (arbitrary units; mean ± standard error) of epidermic and glandular hair nuclei

Type of cell	Number of nuclei	DNA content
Epidermis	85	5.03±0.37
a type hair, pedicel	28	5.67±0.07
head	56	10.64±0.58
b type hair, pedicel	156	5.58±0.53
head	64	9.93±0.69
c_1 type hair, head	106	11.47±0.29
c_3 type hair, head	87	12.45±0.35

amount of DNA regulator is varied and therefore a certain passage in differentiation is induced (NAGL 1978), are able to co-exist.

Conclusions

On the basis of the parameters considered, all the glandular hairs examined present a rather, although not extremely, intense metabolic activity. In type *a* and *b* hairs, the levels of polyteny-endopolyploidy found are more or less the same as these found in glandular hairs from other entities (LANDRÉ 1976b). They are higher in type *c* hairs.

Intense metabolic activity appears limited to the head. It does not concern the pedicel except for the distal cell of type *b* hairs which, for this reason, it is suggested, is in some way involved in the secretory process. The most active hairs seem to be the peltate. In all the types of hair examined endopolyploidy seems to co-exist with differential replication of DNA.

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