A Histochemical Study of Floral Nectaries in *Dipcadi montanum* Dalz. and *Allium cepa* L.¹

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The occurrence of floral nectaries is a common feature in many angiosperms. The cells framing these nectaries produce a secretion, the nectar, which is of much importance in resulting entomophilous type of pollination. Such floral nectaries are also found in *Dipcadi montanum* and *Allium cepa*, and these glands are situated in the septa of the ovaries in them. Following is an account of qualitative histochemical investigation on the constitution of the septal nectaries in *D. montanum* and *A. cepa* from the time of their inception till their disorganization with an aim to correlate the cytochemical changes with their functioning and to understand the significance of their presence in the flower.

Materials and methods

The flower buds and fruits of *D. montanum* and *A. cepa*, at different developmental stages, were fixed in Carnoy's fluid (6 parts of absolute alcohol + 3 parts of chloroform + 1 part of glacial acetic acid) for 1-2 hours and washed in 80% alcohol. The materials were dehydrated using alcohol-butanol grades. Paraffin of 56°C was used for infiltration and embedding. Serial sections of 10 microns thickness were subjected to qualitative histochemical methods listed in Table 1.

Table 1.

Cytochemical substance	Tests (from 'Botanical Histochemistry', Jensen 1962)	Indication
1. DNA and insoluble polysaccharides	azure A and periodic acid Schiff's (azure A-PAS) combined method (Himes and Moriber, 1956)*	DNA-blue polysaccharides-magenta
2. Nucleic acids	methyl green pyronin (MGP) method	RNA-deep purple DNA-green or deep blue
Control	differential extraction of DNA or RNA by perchloric acid method and testing with MGP method	
3. Proteins	mercuric bromophenol blue (MBB) method (Mazia, Brewer and Alfert, 1953)*	deep blue

* Referred from original.

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Observations

The ovaries, in both the plants studied, are tricarpellary and syncarpous. The three carpels arise free on the thalamus as horse-shoe shaped structures, very early in the ontogeny of the flower. Later, the margins of each carpel grow sideways and inwards to meet the growing margins of adjacent carpels. At archesporium level, the margins of the neighbouring carpels begin to coalesce resulting in a common septum and because of the incomplete union of the carpellary walls at certain regions a cavity (septal nectary) is formed in the septum (Figs. A and B). This cavity is bordered by the outer epidermis of the incurved carpel. The line of demarcation evident between the carpels at this level becomes indistinguishable with the complete coalescence of the margins of the neighbouring carpels. The cells of the septal nectary attain conspicuousness with a steady increase in their basiphilic cytoplasm which is gradually lost during the post-fertilization stages of development.

At the time of the origin of septal nectary when the ovular primordium is at archesporial level, the cells bordering the nectary cavity are not distinguishable from the other cells of the carpel as they too lack PAS-positive cytoplasm (Fig. 1) and

Figs. A and B. Diagram of ovary showing the location of septal nectaries. A, ovary (diagrammatic). B, cross section of the ovary at a-a level of Fig. A. Ax: axis, L: locule, SN: septal nectary, VS: vascular strand.

starch grains but rich in RNA and proteins like the other cells of the carpels. With the growth of the ovary, the layer of cells bordering the nectary cavity and 2-3 layers around the nectary enlarge a little and starch grains accumulate in them in *D. montanum* (Fig. 2) while no starch deposition is noticed in these cells of *A. cepa* at 4-nucleate embryo sac stage. The cytoplasmic RNA and proteins in the nectary cells are at a higher level than in the cells of the carpels neighbouring to them even at 4-nucleate embryo sac stage.

At 8-nucleate embryo sac stage, the cells of the septal nectary in *D. montanum* (Fig. 3) show a moderate concentration of cytoplasmic polysaccharides while those in *A. cepa* (Fig. 6) possess negligibly low concentration of the substance. The walls of the nectary cells bordering the nectary cavity are thickened and are PAS-positive.

Figs. 1-5. Sections of septal nectary in *D. montanum*. Figs. 1-3, sections tested with azure A periodic acid Schiff's (azure A-PAS) method for DNA and total insoluble polysaccharides. 1, septal nectary at the time of its inception when the ovule is at archesporium stage. The septal nectary is formed due to the incomplete union of the neighbouring carpels. The line of demarcation (LD) between the neighbouring carpels is evident. The cells of the septal nectary (SN) have very low level of cytoplasmic polysaccharides. $\times 310$. 2, septal nectary at 4-nucleate embryo sac stage. The cell layers bordering the nectary have starch grains. $\times 450$. Figs. 3-5, sections of

septal nectary at 8-nucleate embryo sac stage. $\times 450$. 3, the cells of the nectary have moderately PAS-positive cytoplasm. The walls of the cells bordering the nectary are thick and PAS-positive. 4, methyl green pyronin (MGP) method for nucleic acids. The cells of the nectary have a higher level of RNA than the other neighbouring cells of the septum. The nucleoli in the nectary cells are intensely stained for RNA. 5, mercuric bromophenol blue (MBB) method for proteins. The cytoplasmic proteins are at a higher level in the cells of the septal nectary than in the other cells of the septum. Figs. 6-8, sections of septal nectary in *Allium cepa*; all at mature embryo sac stage. $\times 80$. 6, azure A-PAS method. The cells of the septal nectary have PAS-negative cytoplasm. 7, MGP method. The cytoplasmic RNA in the nectary cells is at a higher level than in the other cells of the carpel. 8, MBB method. The cytoplasmic proteins in the nectary cells are at a higher level than in the other cells of the carpel.

The cytoplasmic RNA (Figs. 4, 7) and proteins (Figs. 5, 8) are at a higher level in the nectary cells than in the other cells of the carpel. The slightly enlarged nuclei in the cells of the nectary appear densely azure A positive at this stage.

After pollination and fertilization, during the post-fertilization stages of fruit development, the cells of septal nectary gradually lose all their cytoplasmic RNA as well as proteins and in the older fruits the nectary continues to remain but with its cells depleted of all their cytoplasmic contents.

Discussion

At the time of inception of septal nectary, its cells are meristematic like the other cells of the carpel in having low cytoplasmic polysaccharides but rich RNA and proteins. With the growth of the ovary, the cells of the septal nectary possess a higher level of RNA and proteins even with a limited enlargement whereas the enormously enlarging cells of the carpel surrounding the septal nectary lose their rich RNA and protein contents. The starch grains accumulated in the differentiating nectary cells are degraded as the nectary assumes its complete secretory activity subsequently, in the older flower. As the flower opens and the ovular primordium is at mature embryo sac stage, the septal nectary cells are at the peak of their secretory activity, as evidenced by their rich cytoplasmic RNA and proteins and slightly enlarged densely azure A positive nuclei. Following pollination and fertilization the cells of the septal nectary in the developing fruit lose their cytoplasmic RNA and proteins gradually but completely indicating the slackening leading to the final cessation of the secretory activity of these cells. Eventhough the present study reveals the presence of a direct relationship between the actively developing female reproductive structure and an increase in the secretory activity of the septal nectary to a certain extent, the actual physiological relationship between the development of the former and the secretory activity of the latter is still to be ascertained by improved techniques. Any way, the hypothesis that the functioning of nectaries is mainly to induce insect pollination appears to be merely speculative and as suggested by Helder (1958) it is perhaps a mere biological coincidence with no physiological significance.

Summary

The ontogeny of the septal nectary in *Dipcadi montanum* and *Allium cepa* is studied employing histochemical staining techniques for the localization of polysaccharides, nucleic acids and proteins. Morphologically, the septal nectary makes its appearance because of the incomplete union of the adjacent carpels at this region. To begin with, the nectary cells resemble their neighbouring cells of the septum in having low level of polysaccharides, but rich RNA and proteins. The starch grains accumulated in the nectary cells, at the 4-nucleate embryo sac stage, of *D. montanum* get degraded as the female gametophyte grows and attains maturity. The cytoplasmic RNA and proteins are at a higher level in the nectary cells than in their neighbouring cells, at 4-nucleate, 8-nucleate and mature embryo sac stages. These substances are diminished and lost completely in the cells of the septal nectary of the

developing fruit. Thus the increase in the activity of the septal nectary seems to have a direct relation with the development of the female gametophyte and decreases gradually to cease finally in the post-fertilization stages of development. The secretion of nectar from the septal nectary and insect pollination are apparently biological coincidents.

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Literature cited

- Helder, R. J. 1958. The excretion of carbohydrates. *Encyclopaedia of plant physiology* **6**: 978–998.
- Himes, M. and Moriber, L. 1956. A triple stain for desoxyribonucleic acid, polysaccharides and proteins. *Stain Tech.* **31**: 67–70.
- Jensen, W. A. 1962. *Botanical Histochemistry. Principles and Practice.* Freeman and Co. London.
- Mazia, D., Brewer, P. A. and Alfert, M. 1953. A cytochemical staining and measurement of protein with mercuric bromophenol blue. *Biol. Bulletin* **104**: 57–67.
- Syamasundar, J. 1974. *Histochemical and embryological studies in monocotyledons: Pre- and Post-fertilization studies in some members from Karnatak.* Ph. D. Thesis, Karnatak University, Dharwar, India.