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Cytochemical Study of Male Gametophyte in *Ottelia alismoides* Pers.

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The nuclei of generative and vegetative cells of *Ottelia* pollen differ markedly in form as well as cytochemical set up. The vegetative nucleus is active with respect to RNA and protein syntheses until degeneration and shows a large nuclear area, diffuse chromatin and high amount of arginine-rich histones. The generative cell is lens-shaped having dense chromatin with deeply Feulgen-stained nucleus and shows the presence of lysine-rich histones. Factors responsible for the active state of vegetative nucleus are discussed.

Key Words: Pollen, Vegetative arginine, Generative lysine-rich histones

Introduction

The pollen grains of many angiosperms have been described (Poddubnaya-Arnoldi 1964) but limited information is available regarding the metabolic status. The DNA content of generative and vegetative nuclei has been studied in a few plants (Swift 1950, Woodard 1958, Taylor & Mc Master 1954 and Bannikova et al. 1977). Prior to mitosis the DNA content in the nucleus of the generative cell doubles and reaches 2C level (Bolchovshkikh 1973). The cytoplasm of the pollen grain has been analysed for RNA (Mascarenhas 1971) and protein (Woodard 1958) content. These reports indicate that the vegetative nucleus of the male gametophyte is active with respect to RNA and protein syntheses compared to the generative nucleus.

Material and Methods

Floral buds of greenhouse-grown *Ottella alismoides* Pers. were collected at different time intervals prior to anthesis and fixed in alcohol acetic acid (3:1) for studying nucleic acids, 10% neutral buffered formalin for basic proteins and formalin acetic acid alcohol for total proteins. The material was dehydrated, infiltrated and embedded in paraffin. Sections were cut uniformly (10μ thick.) The cytochemical detections of DNA, RNA, histones, total protein and insoluble polysaccharides were performed in a manner suggested by Gomori (1952), Tepper and Gifford (1962), Black and Ansley (1964), Mazia et al. (1953), and Mc Manus (1948) respectively. Control reactions were performed for all staining reactions.

The amount of metabolite present inside a cell or nucleus was scanned with the help of a cytophotometer assembled in our laboratory. It resolves close to the physical limit in the visible spectral region. For cytophotometry a Kohler illumination with a 15W 6V tungsten filament bulb is used as a light source. After the beam passes through the respective PBL interference filter, it is focussed on to a stained region. The transmittance of light is recorded by a sensitive light-dependent resistor (Phillips-Holland) connected to a microammeter (Shah et al. 1975). A large number of small individual spots, regarded as individually homogeneous are measured and the data are integrated for statistical analysis. For want of extinction co-efficient for the dye-metabolite complex, all the data are expressed as arbitrary units. Our results obtained from the above assembly rhyme with that of universal microspectrophotometer as opined by Evans Lance S (1979, pers. comm).

Results

The mitotic division in the highly-vacuolated microspore forms a vegetative and generative nucleus (figure 1B), which hardly differ from each other in their nuclear details. Both the nuclei show equal amount of DNA; number of nucleoli, size and area. The initial marked difference is the gradual disappearance of small vacuoles from the cytoplasm and concomitantly the generative cell moves from its original site and comes to lie in the centre of the pollen grain. (figure 1C). During this process the generative cell enlarges and assumes a crescent shape and in later stages extends the entire width of the pollen grain with pointed ends touching the intine.

As the pollen grain reaches maturity the cytoplasm becomes denser and there is less vacuolation at the shedding stage. Some striking differences are detected during the formation of male gametophyte. The generative nucleus becomes compact (nuclear area = 252.72μ^2) and increasingly Feulgen positive (0.0458 AU.). The vegetative nucleus, however, shows an increase in the nuclear area (484.38 μ^2) but less DNA stainability (0.0242 AU). Both these nuclei differ in other features too. The nucleolus of the generative nucleus is predominantly PAS-positive and gives a deep red colour with periodic acid Schiff's reagent. Such a response is lacking in the vegetative nucleus. Accumulation of RNA and total proteins is several times more in the vegetative cell than in the generative cell (table 1).

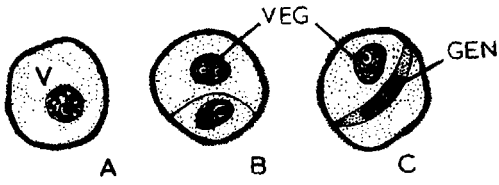

Figure 1 A–C *Ottelia alismoides*. Development of male gametophyte, *A*, Uninucleate pollen; *B*, Freshly formed two similar nuclei; *C*, Mature pollen before shedding (**GEN**, generative nucleus; **V**, vacuole, **VEG**, vegetative nucleus)(× 450)

Table 1 *Comparison of vegetative and generative cells with respect to their histochemical constitution*

Parameter	Vegetative cell	Generative cell
Nuclear area (μ^2)	484.38	252.72
DNA extinction (AU)	0.0242	0.0458
DNA 'C' value	1.01	2.1
RNA (extinction) (AU)	0.3011	0.2042
Total protein (extinction)	0.2799	0.1871
Histone type	Arginine rich	Lysine rich

Cytochemical detection of histones with ammoniacal silver nitrate reveals that the vegetative nucleus is profuse in arginine-rich histones (figure 2). The generative nucleus, on the contrary shows yellow end-product characteristic of lysine-rich histones.

During pollen ontogeny, the vegetative nucleus loses contour and shows signs of degeneration. At this stage it shows a very feeble response to the Feulgen reaction and a weak pyroninophilia.

Discussion

The vegetative nucleus is active with respect to RNA and protein contents till the time of its degeneration and disappears gradually during the later stages of pollen germination. Concomitantly there is an increase in DNA stainability in the generative nucleus. Higher levels of DNA in the generative nucleus have been reported in *Najas minor* (Bolchovshkikh 1973), *Nicotiana tabacum* (Bannikova et al. 1977), *Lilium longiflorum* (Taylor & Mc master 1954) and *Tradescantia paludosa* (Thiebaud & Ruch 1978). The DNA 'C' value was '2C' in *Nicotiana tabacum* (Bannikova et al. 1977) in the generative cell, which was ascribed to altered mitotic cycle parameter such as an increase in S and G_2 periods and the short G_1 period. The same regularity is found during spermatogenesis in animals (Dondua 1967). The generative nucleus in *Ottelia* shows dense Feulgen-stained nucleus with 2C DNA content as compared to the haploid vegetative nucleus (present work).

The vegetative nucleus contains several times more arginine-rich histone than that of generative nucleus (figure 2) and points to its active state. Sauter (1971) reported a very high content of DNA-associated histones which were lysine-rich in the generative nucleus while no lysine-rich histone in the vegetative nucleus. Further, lysine-rich histones that cross-link the chromatin fibrils are responsible for the formation of dense inactive chromatin as compared to arginine-rich ones (Sauter 1971 and Olszewska & Marciniak 1977). Since RNA synthesis proceeds mainly in the diffuse chromatin, the dense chromatin being inactive with respect to DNA-directed RNA synthesis (Sauter 1969), no RNA is stained in the generative

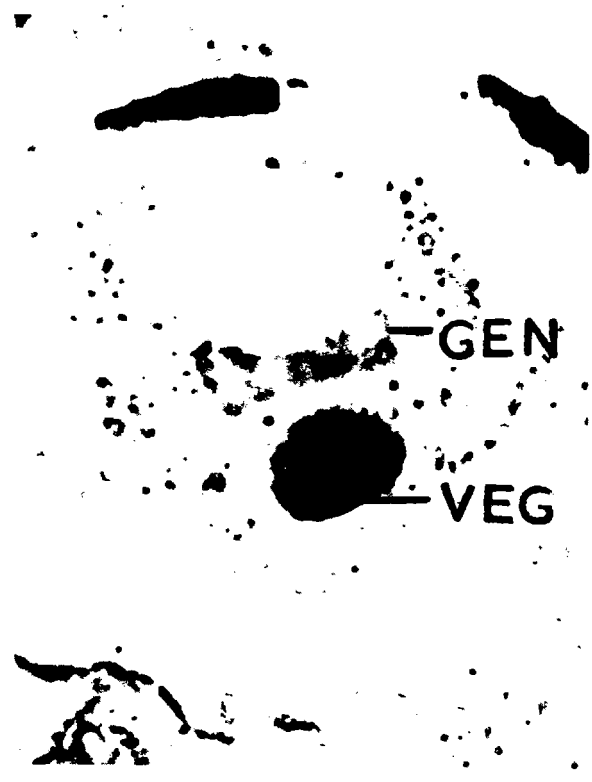

Figure 2 *Ottelia alismoides*. Mature pollen stained with ammoniacal silver nitrate. The generative nucleus (GEN) shows yellow end product, characteristic of lysine-rich histones. The black dye of the vegetative nucleus (VEG) indicates arginine-rich histones (× 790)

nucleus. On the other hand, the high distribution of arginine-rich histones in the vegetative cell accounts for the decondensed nucleus, with diffuse chromatin which is active as regards RNA and protein syntheses.

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