Cytochemical study of nucleic acids, proteins and insoluble polysaccharides during microsporogenesis and pollen development in *Najas marina* L.

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Abstract. The present paper deals with quantitative estimations of DNA, RNA, total and -SH proteins during different stages of pollen development in *Najas marina* L. Attempts were also made to study insoluble polysaccharides qualitatively. The changes in the macromolecular substances were investigated and correlated with cell divisions, growth and differentiation during microsporogenesis and pollen development.

Keywords. *Najas marina*; microspore mother cell; pollen; nucleic acids; proteins.

1. Introduction

Meiosis is one of the most spectacular cellular activities consisting of a series of finely co-ordinated morphological, physiological, biochemical, autoradiographic and histochemical processes which involve the reduction of the diploid chromosomal set to haploid one (Moss and Heslop-Harrison 1967; Heslop-Harrison 1972; Sauter 1973; Reznickova 1979; Teizo et al 1980; Jain 1981; Moitra et al 1982). Meiotic cell differentiation shows changes in macromolecular substances like RNA, DNA, proteins and carbohydrates of the cell, nucleus and cytoplasm.

The present work was carried out to study the turn over and contributions of some cytochemical substances during different stages of microsporogenesis and pollen development in *Najas marina* L.

2. Materials and methods

Male flower buds of *N. marina* fixed in Carnoy’s medium were dehydrated in tertiary butyl alcohol (TBA) series and embedded in paraffin. Serial microtome sections of 10 μm thickness were cut and mounted using egg albumin adhesive (Jensen 1962). For histochemical localization, the standard procedures and their control methods were followed (table 1).

Extinction values (E values) of end products were measured with the cytophotometer. The relative contents were calculated by using the formula adopted by Pollister et al (1969). All values are expressed in arbitrary units (Au). For light microscopic study, PAS reaction of insoluble polysaccharides is effective and it is accessible for cytophotometry only after removing starch from the tissue (Jona and Foa 1977) and hence, only qualitative observations were made for insoluble polysaccharides.

For fine morphological study of pollen grains, samples were viewed and photographed in Cambridge Stereoscan S4-10 scanning electron microscope (for procedure refer Jain 1986).
Table 1. List of cytochemical methods used.

<table>
<thead>
<tr>
<th>Substances</th>
<th>Procedures</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA</td>
<td>Pyronin-G method (Tepper and Gifford 1962)</td>
<td>Enzymatic digestion with RNase (Jensen 1962)</td>
</tr>
<tr>
<td>DNA</td>
<td>Feulgen reaction (Jensen 1962)</td>
<td>Hydrolysis process was omitted (Jensen 1962)</td>
</tr>
<tr>
<td>Total proteins</td>
<td>Mercuric bromophenol blue (Mazia et al 1953)</td>
<td>Sections were treated with trichloroacetic acid (Pearse 1960)</td>
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<tr>
<td>-SH proteins</td>
<td>2-2'Dihydroxy 6-6. dinaphthyl-disulfide method (Barnett and Seligman 1952)</td>
<td>Sections were treated with trichloroacetic acid (Pearse 1960)</td>
</tr>
<tr>
<td>Insoluble polysaccharides</td>
<td>Periodic acid Schiff's (PAS) method (Jensen 1962)</td>
<td>Sections were brominated before oxidation (Pearse 1960)</td>
</tr>
</tbody>
</table>

3. Results

3.1 Micrometric changes

During microsporogenesis, changes in the cell area and nuclear volume manifest a parallel trend (figure 3). At the time of pollen development the cell area of microspore increases continuously up to 3 celled pollen formation, but the nuclear volumes of generative and vegetative cells decrease. The volume of vegetative nucleus is 5 times more than that of the generative one at bicelled pollen stage.

3.2 Histochemical changes

3.2a RNA: The RNA E value of microspore mother cell is double than that of the archesporial one (figure 2). With the onset of meiosis the E value decreases but prior to pollen mitosis it shows an increase. Content is at low ebb in the archesporial cells but shows a 5–6-fold increase at microspore mother cell stage (figure 3). RNA content is half in the dyad cell when compared to that of the microspore mother cell. After completion of meiosis, content restores in the cells and reaches to its peak at bicelled pollen stage.

A simultaneous increase in the cytoplasmic RNA and number of nucleoli is observed from archesporial to microspore mother cell formation. The sporogenous and microspore mother cell contain 2–3 nucleoli and high RNA in their cytoplasm (figure 1D), while the nuclei of archesporial, dyad and tetrad show only one or rarely two nucleoli (figure 1E).

3.2b Total proteins: E value (figure 2) and content (figure 3) increase from archesporial to sporogenous stage, where content reveals a 3-fold increase. The sporogenous cells are rich in cytoplasmic and nuclear proteins. Nucleoli also manifest high staining capacity. During further growth and differentiation into microspore mother cell the E value declines and maintained same level up to meiosis II. At the time of pollen formation the content shows a 2-fold increase from tetrad to mature pollen stage. In the 3 celled mature pollen the male gametes and the vegetative nucleus are stained more intensely than their cytoplasm.
Figure 1. Sections of the male flower buds at different stages of development. 

(BR, Bract; EPI, epidermis; PER, perianth; SPO, sporogenous cells).
3.2c -SH proteins: -SH proteins show a linear increase in their E value and content (figure 2) with the advancement of microsporogenesis, reaching their peak values at the microspore mother cell stage. There is a 5-fold increase in content from the archesporial to microspore mother cell stage which is later followed by a reduction up to tetrad stage. A gradual rise in E value and content is observed during first and second pollen mitosis.

3.2d DNA: The DNA content in microspore mother cell obtained cytophotometrically is two times more than in the dyad while 4 times more than in the tetrad (figure 3). At two and three celled pollen stage, the vegetative nuclei reveal faint stain and less E value (figure 2) as compared to those of generative and gamete nuclei (figure 1C) which are compact and stained deeply.

3.2e Insoluble polysaccharides: The cytoplasm of archesporial and sporogenous cells is uniformly and faintly stained with PAS reagent and shows no starch grains. However, their thick cell walls are stained deeply than the cytoplasm (figure 1A). In microspore mother cells the intensity of PAS reaction in cytoplasm increases and
remains higher up to the tetrad formation. Accumulation of starch starts with the onset of microspore mitosis and bicelled and mature pollen grains show a huge deposition of starch (figure 1B). Due to the absence of exine bicelled and mature pollen grains show evaginations on the intine wall (figure 5). These evaginations are formed due to pressure exerted by the densely accumulated starch grains from the inside (for detail refer Jain and Shah 1985; Jain 1986).

The regression graphs of RNA/total proteins (figure 4A) and RNA/-SH proteins (figure 4B) show positive correlation during entire period of microsporogenesis and pollen development. However, the points of experimental and calculated values lie near the central trend line reveal sharp positive correlation while those which are slightly away manifest weak correlation with respect to their stages. Correlation between DNA/RNA (figure 4C) is strongly positive during sporogenous, microspore mother cell, dyad and tetrad stages of development.

4. Discussion

A continuous increase in cell area and nuclear volume from archesporial cell to microspore mother cell formation is associated with increase in E value and content
of DNA. DNA content in the archesporial cell nuclei corresponded to 2C (18.14 Au) and nearly doubled in the microspore mother cell (4C, 32-60 Au).

According to Hertwig's hypothesis (Khesin 1967), the increase in cell and nuclear volume of sporogenous cells is an indication of conversion of mitotic to meiotic one. Results of Reznickova (1979) on Lilium candidum L. also support this view. Present result however, appears to rule out the view because of increase in nuclear volume is followed by increase in DNA content. Price et al (1973) observed that the meristematic cells of many herbaceous angiosperms show that increase in nuclear volume during cell division is only due to increase in DNA content per cell.

The number of nucleoli varies in each fertile cell. Archiesporial cell shows one nucleolus while sporogenous and microspore mother cells contain 2-3 nucleoli. Before the onset of meiosis, there is an increase in the levels of cytoplasmic RNA and total proteins. This upsurge is not accompanied by a decline in the levels of these substances in the nucleolus. Thus relative increase of these substances does not seem to be by leaching from the nucleolus. The increase in the number of nucleoli in the microspore mother cells correlate with the optimal synthesis of RNA (Vijayaraghavan and Cheema 1978).

The extinction values of RNA and total proteins are less in microspore mother cell than those of the sporogenous cell. This decline in the levels of both the substances may be due to either the dilution resulted from growth [note the increase in cell area from sporogenous (254-03 \( \mu^2 \)) to microspore mother cell (304-2 \( \mu^2 \))] or their low metabolic syntheses as reported by Rudramuniyappa and Panchaksharappa (1980) in Triticum durum.

During the meiosis of microspore mother cell, the contents of RNA, total and -SH proteins decrease to reach lowest value at tetrad stage. The autoradiographic study of Albertini (1967) on Rhoeo discolor has revealed that the proteins synthesised during premeiotic stage are involved in structural organization of chromosomes during meiosis. According to Rudramuniyappa (1973), the low syntheses of these substances during dyads and tetrads in Triticum may be due to the deposition of PAS positive substance (callose) around them. It is also possible
that the dividing cell may be constantly utilizing these substances with a consequent reduction in their contents.
DNA shows two peaks of the synthesis, one at microspore mother cell stage while other prior to microspore mitosis as also reported by Teizo et al. (1980) in *Pinus densiflora* and *P. thumbergii*. This increase in DNA level before meiosis and pollen mitosis prepares meioocytes and microspores for eventual divisions. After first microspore mitosis, generative nucleus becomes more pronounced and compact and shows more E value of DNA as compared to that of the vegetative nucleus. This high intensity of DNA in generative nucleus, which is destined to form gametes by mitosis is attributed to its higher metabolic activities. Similar results were also reported by Bolchovshikikh (1973) in *N. minor*, Bannikova et al. (1977) in *Nicotiana tabacum* and Thiebaud and Ruch (1978) in *Tragopogon paludosus*.

Increasing intensity of PAS reaction in the cytoplasm from microspore mother cell to tetrad indicates a continuous synthesis of PAS positive cytoplasmic substances (insoluble polysaccharides). This increase during meiosis is prerequisite for the synthesis of cell walls during dyad and tetrad formations. The huge deposition of insoluble polysaccharides in the form of starch grains during later stages i.e. bicelled and mature pollen, is utilized for pollen tube growth and tube wall formation during pollen germination.

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