Histochemical Studies on the Female Gametophyte of *Argemone mexicana* L.

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Recently, there has been a great upsurge in the amount of information dealing with the physiological intricacies of pollen development (see Heslop-Harrison 1971). Contrastingly, the female gametophyte has received comparatively much less attention. One of the earliest study was that on *Zephyranthes drummondii* (Coe 1954). In *Stellaria media* (Pritchard 1964) and *Vanda* (Alvarez and Sagawa 1965), histochemical aspects of embryo sac development have been investigated. Recently Panchaksharappa and Hegde (1972) in *Cicer arietinum*, Mogensen (1973) in *Quercus gambelii*, Malik and Vermani (1975) in *Zephyranthes rosea* and *Lagenaria vulgaris*; Bhandari and Natesh (1976) in *Nigella damascena* and Prasad (1977) in *Farsetia hamiltonii* and *Eruca sativa* have investigated some histochemical aspects of ovules and embryo sac.

The present paper deals with histochemical study on the female gametophyte in *Argemone mexicana* L. Embryologically it has been studied by Sachar (1955) who reported a monosporic, Polygonum type of embryo sac with prominent and persistent antipodal cells.

Material and methods

Flower buds at different stages of development were fixed in 10% neutral formalin, Carnoy's fixative and FAA. Following conventional methods of dehydration and paraffin embedding, sections were cut at 6 μ. For histochemical localization of various metabolites, the following techniques were adopted: i) insoluble polysaccharides—PAS method (Jensen 1962); ii) RNA—pyronin Y (Tepfer and Gifford 1962); iii) total proteins—mercuric bromphenol blue (Mazia, Brewer and Alfert 1953), starch—IKI test (Jensen 1962). The qualitative difference in the intensity of colour reaction of the metabolite thus localized has been taken as indications of its quantity.

Observations

The ovules in *Argemone mexicana* L. are crassinucellate, bitegmic and anatropous. The hypodermal archesporium in an ovular primordium comprises of one or two cells. Only one of these develops further and divides periclinally to form an outer parietal cell and an inner megaspore mother cell (MMC). The MMC undergoes meiosis normally and results in a linear tetrad of megaspores.
The chalazal megaspore functions, and organises a Polygonum type of embryo sac. The egg cell resembles a synergid in having its nucleus at the micropylar end and a prominent vacuole at the chalazal end. The antipodals are large and persistent.

**Histochemistry**

*Insoluble polysaccharides:* In a young ovular primordium at the archesporial cell stage, all the cells show more or less uniform distribution of polysaccharides. However, as the archesporial cell develops into a megaspore mother cell, and during late dyad and tetrad stages, the surrounding cell wall becomes highly intense (Figs. 1 and 2). The degenerating megaspores particularly show the maximum intensity (Fig. 3). As the functional megaspore shows a multifold increase in volume, and enters the coenocytic phase, a prominent vacuole develops in the centre (Fig. 4). Throughout the coenocytic stages, not much variation is noticed in the distribution of insoluble polysaccharides.

In the mature embryo sac, the wall of the egg cell shows a weak affinity for the PAS reaction. The intensity of PAS reaction is more at the micropylar part of the egg (Fig. 5). Egg cytoplasm is totally devoid of insoluble polysaccharides (Fig. 5). In the synergids, the wall in the micropylar region shows a maximum stain intensity, while it diminishes gradually towards the chalazal end (Fig. 5). The filiform apparatus stains deeply indicating its polysaccharide nature. The antipodal cells possess a PAS positive cell wall all around their cytoplasm. The cytoplasm of the antipodals displays negligible, nevertheless positive staining (Fig. 6).

*Starch:* In a young ovular primordium the cells of the nucellus adjoining the megaspore mother cell begin accumulating starch grains. The number and size of starch grains in these cells increases through the progressive development and enlargement of the megaspore. Finally at the mature embryo sac stage, these grains exhibit a regular distributional pattern. The starch grains are larger in the nucellar cells close to the embryo sac. The size of grains and their number per cell, both show a decline towards the peripheral cells of nucellus. In the early stages of development, the nucellar cells at the micropylar region and the parietal cells also contain starch grains, which however, disappear later. Starch grains are also present in the outermost layer of the outer integument, while there are none in the inner layers of the outer integument and the inner integument.

*Total proteins:* The total proteins exhibit a uniformly moderate distribution in a young ovular primordium. The archesporium (1 or 2 cells) is soon demarcated from the surrounding nucellar cells by a denser intensity of proteins. But as the
MMC is differentiated, a decline in the intensity is noticed (Fig. 7). The stainability increases slightly at the dyad stage, and does not vary much in a tetrad (Fig. 8). The functional megaspore again increases in the intensity of protein staining, while the three degenerating megaspores show even more intense staining (Fig. 9). There is no notable change during the coenocytic phase, except that the proteins show a very polarized distribution at the micropylar and chalazal ends. The nucleoli exhibit very intense reaction (Fig. 10). Throughout gametogenesis, a gradual increase of protein content is evident in the chalazal nucellar cells (Figs. 11 and 12), which lasts up to the mature embryo sac stage.

The cytoplasm of the egg and the zygote presents a moderate staining (Fig. 13), while in the synergids both the cytoplasm and the nucleoli stain quite intensely. Among the various components of the embryo sac, the antipodals (Fig. 14) and the secondary nucleus exhibit maximum concentration of proteins.

**RNA:** The archesporial cell shows a moderate staining for RNA, the intensity being more or less equal in the nucleolus and cytoplasm. Thereafter, a decline in basophilia occurs, which becomes more conspicuous in the cytoplasm of the MMC. Nucleolar RNA content of the megasporocyte is moderate. Nucellar cells next to the megasporocyte show a weak RNA staining, while those which lie closer to the integument show higher intensity. This situation persists till the mature embryo sac stage. The dyads and tetrads display a moderate intensity and there is no conspicuous difference between the four megaspores of the tetrad.

However, with the degeneration of the three micropylar megaspores, a sharp difference in staining is noticed. The coenocytic phase is characterized by very intensely staining nucleoli, though the cytoplasm shows a weaker basophilia. All through the free nuclear stages, the chalazal nucellar cells show an increase in RNA content.

In the mature embryo sac, the egg cytoplasm shows weakly staining basophilia, while nucleolus stains quite deeply. The synergids are characterized by a moderate staining, except in the region of filiform apparatus, which completely lacks it. Antipodal cells possess the maximum RNA concentration in the embryo sac. The cytoplasm surrounding the secondary nucleus also shows a high content of RNA.

**Discussion**

The archesporial cell in *Argemone mexicana* L. possesses a moderate concentration of RNA and total proteins (present observation). Prasad (1977) reported a similar situation in *Farsetia hamiltonii* and *Eruca sativa*. However,

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Figs. 7–10. L.S. ovules at different stages of megasporogenesis stained with bromphenol blue for localising total proteins. 7, MMC showing a lower intensity of proteins than the surrounding nucellar cells. ×240. 8, linear tetrad of megaspores showing an increased intensity of stain. ×240. 9, tetrad showing densely stained degenerating megaspores and functional megaspore ×240. 10, 4-nucleate embryo sac (2 nuclei seen in this section) showing intensely stained nucleoli. ×240.
Figs. 11–14. L. S. ovules stained with bromphenol blue for localising proteins. 11 and 12, ovules showing a higher protein content in the chalazal nucellar cells at the 2-nucleate and post fertilization embryo sac. ×150 and ×75 respectively. 13, egg apparatus showing the densely stained synergids. ×160. 14, antipodal cells revealing the maximum concentration of proteins. ×160.
after the division phase has set in, there is a notable fall in these substances. Prasad (1977) in *Farsetia hamiltonii* and *Eruca sativa*, Bhandari and Natesh (1976) in *Nigella damascena*, Pritchard (1964) in *Stellaria media* and Miki-Hirosige (1964) in *Lilium longiflorum* also noticed a similar phenomenon. Since the meiocyte is undergoing a premeiotic differentiation, an increased rate of utilization of metabolites is to be expected. The rate of dilution of metabolites in the growing megaspore mother cell is attributed to the lack of synthesis at a rate sufficient to maintain its previous concentration (Alvarez and Sagawa 1965), the view to which we also subscribe. Heslop-Harrison (1972) described a temporary suspension of RNA synthesis in the meiocyte. Moreover, Newcomb (1973) in *Helianthus annus* and Israel and Sagawa (1965) in *Dendrobium* reported lesser number of organelles in the early phases of meiosis, a factor which can be correlated with the decreased metabolic content.

The meiocyte in *Argemone mexicana* is invested with a thick cell wall which stains intensely for PAS reaction. This thick wall continues to be present in the dyad and tetrad stages also (present report). The polysaccharide material which forms the coating is identified in several plants including members of the family Papaveraceae, as callose (Rodkiewicz 1969), which provides for metabolic isolation from the surrounding nucellus (Heslop-Harrison 1972) and creates a microenvironment for sporogenesis to take place.

No starch grains have been observed either in the meiocyte or in the megaspores (present observation). However, starch grains are found in the megaspores of *Paphiopedilum spicerianum* (Corti and Cecchi 1970) and *Epilobium* (Bednara and Rodkiewicz 1973). Bednara and Rodkiewicz (1973) reported a polarized distribution of starch grains in the MMC through meiosis I and II which also corresponded to the distribution of plastids.

Daughter cells of the first and second meiotic divisions gradually increase in RNA and protein concentration (present observation). Bhandari and Natesh (1976) have also noticed a secondary increase of metabolites in the dyads and tetrads of *Nigella damascena*.

The functional megaspore exhibits a high rate of metabolism as evidenced by its intense protein and RNA concentration (present observation). Similarly, Pritchard (1964), recorded a rapid synthesis of RNA by the functional megaspore in *Stellaria media*. An increased rate of synthesis of RNA and proteins in the functional megaspore have also been observed by Prasad (1977) in *Farsetia hamiltonii* and *Eruca sativa* and Alvarez and Sagawa (1965) in *Vanda*. Non-functional megaspores are characterized by a very intense colouration for all the active substances localized (present observations). This may be due to rapid disintegration of all organelles in these cells and, therefore, loss of specific sites of localization.

In *Argemone mexicana*, the cytoplasm of the embryo sac in the coenocytic phase stains moderately for RNA and proteins (present report), while the nucleoli show a higher intensity. Prasad (1977) reported a gradual decline in these two metabolites in *Farsetia hamiltonii* and *Eruca sativa* during gametogenesis.

In the mature embryo sac, the synergids stain intensely at the micropylar
region, for insoluble polysaccharides, because of the presence of the filiform apparatus (present observations). Prasad (1977) reported a high concentration of insoluble polysaccharides in the synergids of Farsetia hamiltonii and Eruca sativa. Malik and Vermani (1975) reported an intensely PAS positive filiform apparatus in the synergids of Zephyranthes rosea and Lagenaria vulgaris. They also found starch grains in these cells, clustered mainly around the filiform apparatus. The filiform apparatus in Vanda is also highly PAS positive (Alvarez and Sagawa 1965). The staining intensity decreases towards the basal region of the synergids. Ultrastructural studies on a large number of plants (see Jensen 1965, Maze and Lin 1975) have revealed the absence of a true cell wall at the chalazal part of the synergid. Our observations tend to support these observations. The presence of high concentration of RNA and total proteins in the synergids of Argemone mexicana (present observations) as also in Nicotiana synergids (Bannikova 1971) points out the fact that the cells are metabolically active and contribute by way of synthesis at the time of fertilization. This is in agreement with Alvarez and Sagawa (1965) who reported a similar nature of the synergids in Vanda. Although starch grains are absent within the synergids, small grains are seen in the adjacent nucellar tissue. The number of these grains decreases as the embryo sac matures (present observation), indicating that they are being utilized by the synergids. This was also indicated by Buell (1952) in Dianthus.

Compared to the synergids, the egg cell of Argemone mexicana shows a lesser staining for both RNA and proteins (present investigation). This has been observed in a number of other plants that have been studied histochemically (Pritchard 1964, Alvarez and Sagawa 1965, Malik and Vermani 1975). Bannikova (1971) found in Nicotiana that the cytoplasm of the egg is markedly pyroninophilic. In Plumbago (Cass 1972), the egg cell seems to be physiologically quite active and possesses a filiform apparatus as there are no synergids (Cass 1972). It appears to have taken up the twin function of the egg as well synergids.

In Argemone mexicana a high nucleolar RNA content of the secondary nucleus along with the total proteins in the surrounding cytoplasm indicate a high rate of synthetic activity of the central cell (present observations). Similar situation has been observed in Vanda (Alvarez and Sagawa 1965) and Farsetia hamiltonii and Eruca sativa (Prasad 1977).

The size of the antipodal cells is remarkably large in Argemone mexicana, a feature that has already been reported earlier by Sachar (1955), and is also common to Papaver (Hasitchska 1956), Paphiopedilum insigne (Zinger and Poddubnaya Arnoldi 1964) and Aquilegia vulgaris (Rifot 1971, 1973). The PAS reaction reveals a true cell wall of insoluble polysaccharides all around the cytoplasm of the antipodals. Malik and Vermani (1975) have observed the same in Zephyranthes rosea and Lagenaria vulgaris. Rifot (1973) found that each antipodal in Aquilegia vulgaris has a thin wall around, except at the upper region. She (1971) observed that the basal walls of the antipodals proliferated extensively and that this made them haustorial in function. She views them as transfer cells, helping in the nutrition of the embryo sac. Intense pyroninophilia, correlated
with an intense staining for proteins in *Argemone mexicana* (present observations) is indicative of the fact that these cells are physiologically the most active cells in the embryo sac. However, a low metabolic state of antipodals has been reported by other workers (Prasad 1977, Bannikova 1971, and Alvarez and Sagawa 1965). Rifot (1973) found that the nuclei of antipodals formed RNA particles, which later moved into the cytoplasm.

Associated with the embryo sac, is the storage of starch grains in the nucellar cells. Starch storage has been localized in the upper region of the nucellus and the integuments in *Cicer arietinum* (Panchaksharappa and Hegde 1972). In *A. mexicana* (present observations), starch grains are present mainly in the ovary wall, placentae, funiculus and the integuments. After the differentiation of MMC, they are seen particularly in the nucellar cells adjacent to the MMC. As the embryo sac matures, the starch content of the nucellus increases to a maximum and many small grains, subjacent to the antipodals are seen (present report). This is probably due to the increased rate of translocation from the storage zones like ovary wall, placentae etc., through the outer integument. This storage starch serves as a temporary pool for a quick translocation and absorption into the embryo sac. The antipodals, because of their high rate of metabolism, are probably involved in this process. Blankovskaya and Mironchak (1969) observed starch grains in the integuments in ovules of sunflower. The integumentary epidermis possessed a single starch grain, whereas the number increased in the other layers of the integument, although the cells closer to the embryo sac had smaller grains.

**Summary**

A qualitative histochemical analysis of insoluble polysaccharides, starch, RNA and total proteins during the development of female gametophyte of *Argemone mexicana* L. has been conducted. Ontogenetically, the plant exhibits a monosporic, Polygonum type of embryo sac.

Cytoplasmic polysaccharides and starch grains exhibit considerable changes during megagametogenesis. At the archesporial stage, the nucellus stains uniformly. From the MMC to the tetrad stage, the walls of these cells take a deeper stain than the cytoplasm. The degenerating megaspores stain very intensely. Wall of the egg is weakly PAS positive in the chalazal region, but stains strongly in the micropylar region. Synergids also stain intensely in the micropylar region. Antipodals have a PAS positive wall all around, but the cytoplasm is weakly positive.

Nucellar cells under the MMC start accumulating starch grains and the number increases till the formation of functional megaspore. In the coenocytic stages and the mature embryo sac, larger grains are found in cells closer to the embryo sac and smaller grains in cells away from the embryo sac.

RNA and total proteins parallel each other in their distribution. The archesporial cell stains moderately for them. There is a decline in the staining intensity in MMC. There seems to be no conspicuous difference in the distribution
of RNA and total proteins in the four megaspores in a tetrad. Nucellar cells near the MMC and subsequent stages of megasporo- and megagametogenesis show lesser staining for these metabolites as compared to the cells away from MMC. This trend continues till the mature embryo sac stage, when the chalazal nucellar cells stain deeply for RNA and total proteins. Among the constituents of the embryo sac, the antipodals appear to be the most metabolically active, as is revealed by the staining intensity.

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