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MEGASPOROGENESIS AND THE DEVELOPMENT OF EMBRYO SAC IN INDIGOFERA OBLONGIFOLIA

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Abstract. The investigations on megasporogenesis and megagametogenesis of *Indigofera oblongifolia* Forsk have revealed that the archesporium originates from a single subhypodermal cell and directly functions as megasporocyte. After reduction division, the megasporocyte forms a linear tetrad of four megaspores, however, triad formation is more frequent than the tetrads. The chalazal megaspore is functional while the the rest degenerate. The mature embryo sac is of the polygonum type.

After the differentiation of archesporial cell, the integuments initiate exogenously as annular rings. The inner integument remains two-cells thick, where as the outer one gradually becomes 4–6 cells thick especially near the micropylar end. In the course of embryo sac development most of the nucellar cells are being assimilated and the embryo sac at its maturity is circumscribed only by double layer of nucellus cells.

Present systems of classification of angiosperms are mainly based on the floral morphology; recently the interest in the embryological studies has been renewed and is becoming an important tool in the correct identification of the plants where morphological characters alone fail to decide the genuine identification and definite systematic position of the species. In interspecific hybridization, where sterility is very common, embryological data can be used successfully in determining the causes of sterility in them.

Papilionaceae, which was considered by Hutchinson[5] to be a separate family, occupies a place of special interest among the Dicotyledons. Most of its members are of great economic importance because of their value as fodder, food and being a source of many drugs. This family has been selected for a series of embryological works for the relative ease with which the material could be handled and the large diversification that it provides.

Guignard probably was the first who worked on the morphology of about 40 species of family Leguminosae.[13] Thereafter many workers like Young,[14] Martin,[8] Brown[1] and Reeves [11] studied the embryology of Papilionaceae.

Although hypodermal origin of the archesporial cell is a common rule,[2,6–8] cases of subhypodermal origin are also not uncommon.[9,12,13] Roy described the subhypodermal origin of archesporium in *Pachyrhizus*, *Cayanus*, *Dolichos* and *Pisum*, while in *Lathyrus* he noted a single hypodermal archesporium. Mohamed and Gould while working on the *Bouteloua curtipendula* complex observed the differentiation of the archesporial cell deep in the nucellar tissue prior to the development of integuments.[9] More recently, Rembert studied megasporogenesis in sixteen species of Papilionaceae and found that in fourteen species the archesporial cell is hypodermal but in two cases its inception was subhypodermal, the exceptions being *Sophora japonica* and *Desmodium paniculatum*.[12]

Material and Methods

The material was collected during the months of November, 1970 and February, 1971 from plants growing around the P.C.S.I.R. campus. Floral buds ranging from 0.5 to 5.0 mm in size were collected. These were killed and fixed in FAA (Formaline–acetic acid 50%–ethanol) for 48 hr and in Licent's solution for at least 36 hr. The buds were dipped in Carnoy's fluid for 20 sec before transferring to Licent's solution so as to facilitate rapid sinking. After fixation, the material was transferred to 70% alcohol. Of the two fixatives tried so far, the better results were obtained with Licent's solution. Vouchers of the species studied were deposited in the Herbarium of Botany Section, P.C.S.I.R., Karachi. All the material was dehydrated through graded series of ethanol, cleared in chloroform and embedded in paraffin. Over 300 ovaries were sectioned for this study. The longitudinal sections ranging from 5 to 10 μ in thickness were obtained and stained in Delafield's haematoxylin with safranin or light green. However, the combination of haematoxylin and safranin is found to be more valuable for this study.

Observations and Discussion

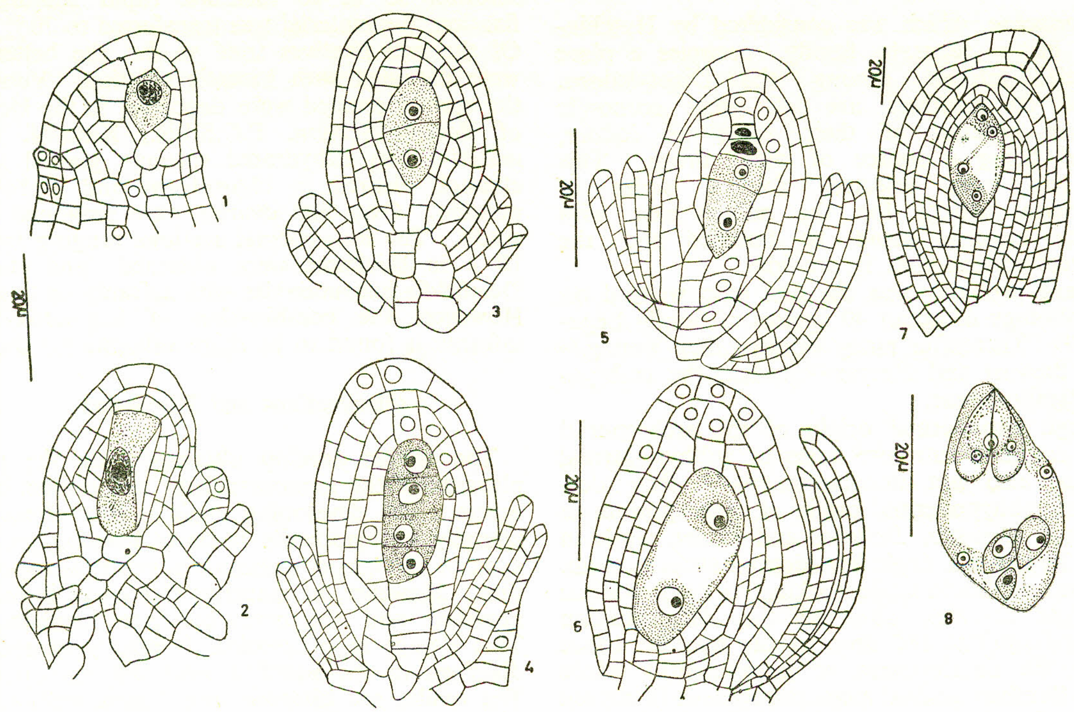
The ovules develop alternately on the marginal placenta of the monocarpellary and unilocular gynoecium. The developing ovule is first directed horizontally but gradually it starts bending toward the apex of the carpel assuming a campylotropous position. However, at maturity this curve is more pronounced and the ovule becomes hemianatropous. This change in orientation is mainly caused by the divisions in the nucellus near the micropylar end. The ovules are bitegmic and crassinucellate. These observations are in conformity with that of Martin,[8] Roy[13] and Paul and Datta.[10]

Integuments. The integuments originate soon after the differentiation of the archesporial cell although the initiation of the outer integument is slightly delayed. The primordia of both integuments are easily recognisable by the time the megaspore mother cell is fully enlarged. Roy[13] has observed the similar sequence in *Dolichos lablab*. Here the integuments arise exogenously as annular rings at the base of nucellar tissue in acropetal succession and appear to develop from the protoderm cells only. The configuration of the cells at the apices suggests that both integuments continue their growth in length by the elongation and division of the apical cells. The inner integument remains two-cells thick throughout its development and is more advanced in growth than the outer up to the megaspore tetrad stage (Fig. 4). Soon after, the growth of the outer integument accelerates and after overtaking the inner one, it forms the micropyle. Roy[13] and Paul and Datta[10] made similar observations working with *Pachyrhizus* and *Crotolaria*. Both integuments remain two-cells thick in the earlier stages of development, but later the outer one gradually becomes 4–6 layers thick at the micropylar end (Fig. 11). The formation of multilayered outer integument has also been reported by Paul and Datta.[10] At this stage the inner integument also increases in length and in few cases covers the nucellus.

Nucellus. In the earlier stages of development the ovule is composed of loose homogenous cells with no indication of a definite pattern of growth. After the differentiation of the megaspore mother cell, the nucellus expands by the divisions of hypodermal and subhypodermal cells. Simultaneous to this the ovule also increases in length mainly by the divisions of cells at the chalazal end. These cells divide in such a pattern that files of cells are formed radiating from the base of the developing embryo sac to the funiculus of the ovule (Fig. 7).

The archesporial cell makes its appearance very early in the development of the ovule. It is derived from a subhypodermal cell, an infrequent feature yet a fact which has previously been reported by Roy[13] in *Dolichos*, *Pachyrhizus*, *Pisum* and *Cayanus* and by Rembert[12] in *Sophora* and *Desmodium*. Although a multicellular archesporium is prevalent in the Papilionaceae,[13] in *Indigofera* single-celled archesporia have been observed (Fig. 1). The archesporium directly functions as the megaspore mother cell without cutting off any parietal cells. This is in conformity with the findings of Roy and Rembert.

Megasporogenesis. Enlargement of the megaspore mother cell marks the beginning of megasporogenesis, before undergoing the first meiotic division, the megaspore mother cell becomes four times as long as broad (Fig. 2). The cytopolasm of the megaspore mother cell appears to be granular and fills the entire cell. Nuclear division followed by wall formation results in a dyad (Fig. 3). The second division gives rise to a linear tetrad of four megaspores (Fig. 4). Although the occurrence of more than one tetrad and various pattern of tetrads have been reported for other members of this family,[2,10,12,13] in tthe material studied either a single linear tetrad or triad formation was observed triad formation being more frequent than the tatrad (Fig. 9). The chalazal megaspore is functional while the other spores degene-



Figs. 1–4. Megasporogenesis. Fig. 1. Young ovule with arcchesp Porial cell. Note the initiation of the inner integument on both sides and that of outer on the upper side only. Fig. 2. The megaspore mother cell. Fig. 3. A dyad. Fig. 4. A linear tetrad of megaspores. Fig. 5. Degeneration of the megaspores proceeds from the micropylar end downwards. Fig. 6. Two nucleate embryo sac. Fig. 7. Four nucleate embryo sac. Fig. 8. Eight nucleate embryo sac (polygonum type).

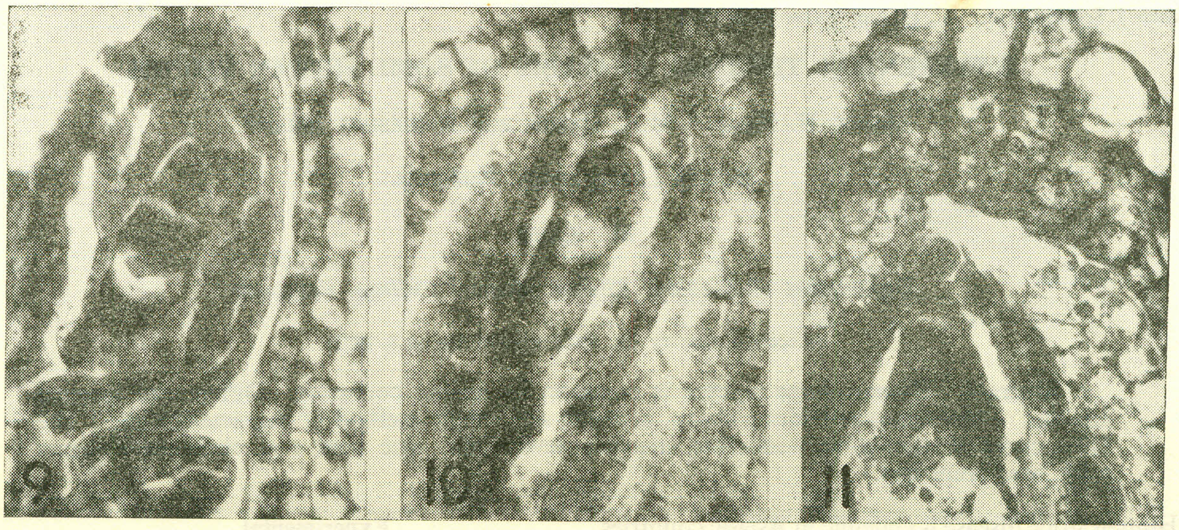


Fig. 9. A triad of megaspores. Fig. 10. Photomicrograph of binucleate embryo sac. Fig. 11. The multilayered outer integument at the micropylar end.

rate. The degeneration begins with the micropylar spore and proceeds progressively downwards (Fig. 5).

Embryo Sac Development. Embryo sac development in *Indigofera* conforms to the polygonum type and is similar to that described for many other genera of Papilionaceae by various investigators. The functional megaspore enlargement is accompanied by vacuolation. The vacuoles usually appear above and below the nucleus. The first division of the nucleus gives rise to two nuclei, which migrate to the opposite ends of the embryo sac. Each nucleus is surrounded by a thin layer of cytoplasm and separated by a large central vacuole (Fig. 6). The following two divisions of these nuclei produce four nuclei at each pole. Accompanying this the developing embryo sac also enlarges becoming two times as long as broad (Fig. 8). The cells of the micropylar quartet differentiate into a three-celled egg apparatus and the upper polar nucleus, while the chalazal quartet forms three antipodals and the lower polar nucleus. The egg apparatus consists of two synergids and one egg cell. The synergids are larger than the egg and appear identical, each possessing a vacuole in the chalazal end and a very prominent nucleus above the vacuole.

Unlike the observations of Martin[8] in *Trifolium pratense* and Roy[13] in *Cayanus indicum*, the synergids neither exhibit filiform apparatus nor are they hooked. The egg cell has a nucleus at its base and a vacuole in the centre. The antipodals are uninucleate and contain dense and granular cytoplasm. In the course of embryo sac development the surrounding nucellar cells are gradually being assimilated, with the result that the embryo sac approaching maturity is circumscribed by only two layers of nucellar cells. These observations are similar to that of Roy,[13] and Paul and Datta.[10]

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