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THE MEGASPOROGENESIS AND THE DEVELOPMENT OF EMBRYO SAC IN WITHANIA SOMNIFERA

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Abstract. The archesporium is hypodermal in origin, being distinguished by having a polygonal cell of large dimension with granular, dense cytoplasm and large nucleus. The single archesporial cell which is usually present directly functions as megasporocyte. After meiosis the megasporocyte produces a solitary linear tetrad of megaspores. The chalazal megaspore develops into a polygonum type of embryo sac, while the other three spores disintegrate. The surrounding nucellar cells break down and are being assimilated by the developing embryo sac, as a result the later comes in contact with the inner epidermis of the integument which functions as a nutritive layer. A single massive integument originates as a ring of meristematic tissue at the base of the nucellus.

The embryological observations can be of considerable significance in a number of doubtful cases, where only morphological characters have proved inadequate to determine the definite systematic position of the species, the importance of this consideration has been apprehended by many workers, notable among whom are Maheshwari[6] and Davis.[3]

This data can be utilized not only for the development of new genetical varieties but is also becoming an important tool in determining the causes of sterility found commonly in interspecific hybridization.

The family Solanaceae has been selected for a series of embryological studies as it is a large, widely spread and economically one of the most important family. The available information concerning the megasporogenesis and development of female gametophyte are very limited. Therefore, it is proposed to study this aspect also with that of embryogenesis in various species of family Solanaceae and finally discuss the bearing of these data on their interrelationship and phylogenetic trends within the family.

Material and Methods

The floral buds of *Withania somnifera*, ranging from 1.5 mm to 5 mm in size, were collected from PCSIR Campus, Karachi.

The material was fixed either in F.A.A. (formalin–acetic acid–alcohol) or in Licent's solution for at least 48 hr, but better results were obtained with F.A.A. After washing, the material was dehydrated, cleared and finally embedded in paraffin-wax. Longitudinal sections were obtained at thicknesses varying from 5 to 10 μ, mounted serially and stained with Delafied's haematoxylin and counter stained with safranin or light green.

Observations and Discussion

Ovule. The ovule initial differentiate from the enlarged placenta in the form of small, somewhat conical protuberances; the ovule primordium which increases in size by the division of cells in the epidermal and subepidermal layers. It consists of a group of homogeneous cells in the earlier stages but soon one and occasionally two hypodermal cells near the apical region, begin to distinguish from the rest of the cells in having polygonal shape, large size, denser cytoplasmic content and a prominent nucleus (Figs. 1 and 2). However, only one of the archesporial cell is functional and in no case more than one megasporocyte is recorded. The occurrence of more than one functional archesporial cell has been reported by Rees-Leonard,[7] Bhaduri[1] and Rybchenko.[8] The single massive integument initiates at the base of nucellus by the periclinal divisions of one to two epidermal cells (Fig. 2). Its further development is similar to that described by Rees-Leonard[7] for *Solanum tuberosum* and illustrated in Figs 3–6.

The micropyle, which at first is in the form of a broad continuous passage, gradually becomes narrower and in few cases completely obliterated due to the massive growth of the integument.

The nucellar tissue being ephemeral begins to degenerate, soon after the differentiation of archesporial cell, as a result it is only represented by a single layer of cells, i.e. nucellar epidermis during the megasporogenesis (Figs. 4 and 5) and later on completely disintegrates (Figs. 6 and 7). Accompanying its degeneration the epidermal cells on the inner side of the integument become radially elongated and contain a dense cytoplasmic content to form an 'integumental tapetum'. A similar nutritive layer was observed in *S. tuberosum*.[9]

Subsequent differential growth on the basal portion of the ovule, causes it to become hemianatropous in most cases. However, in few cases anatropous position has also been observed.

The single archesporial cell, which is usually present, functions directly as the megaspore mother cell without differentiating into primary parietal cell and primary sporogenous cell. This has been reported for most of the species of the Solanaceae so far investigated.

The megaspore mother cell prior to the division enlarges in size significantly and its cytoplasm becomes vacuolated (Fig. 4).

The four daughter cells resulting from the meiotic

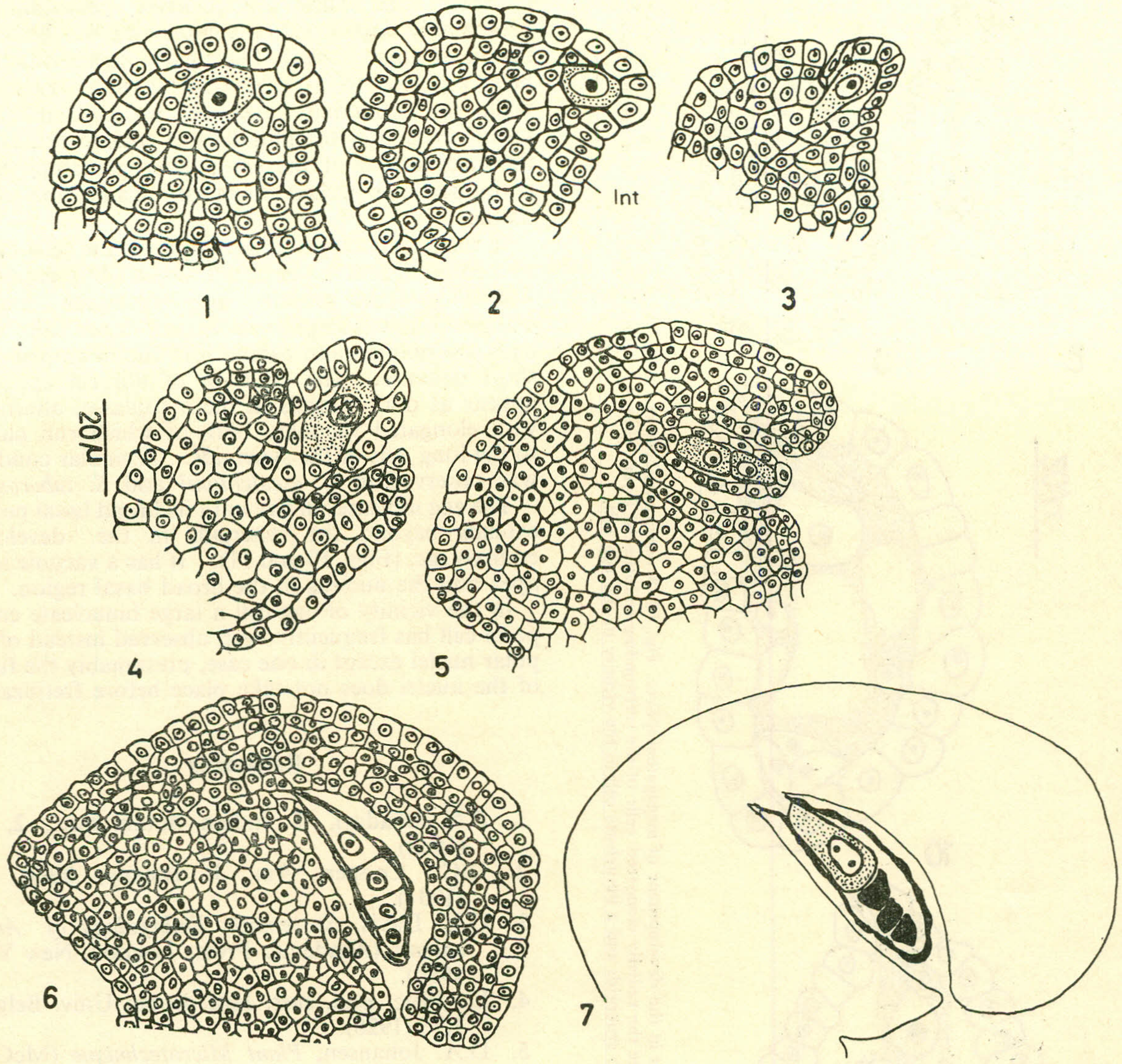


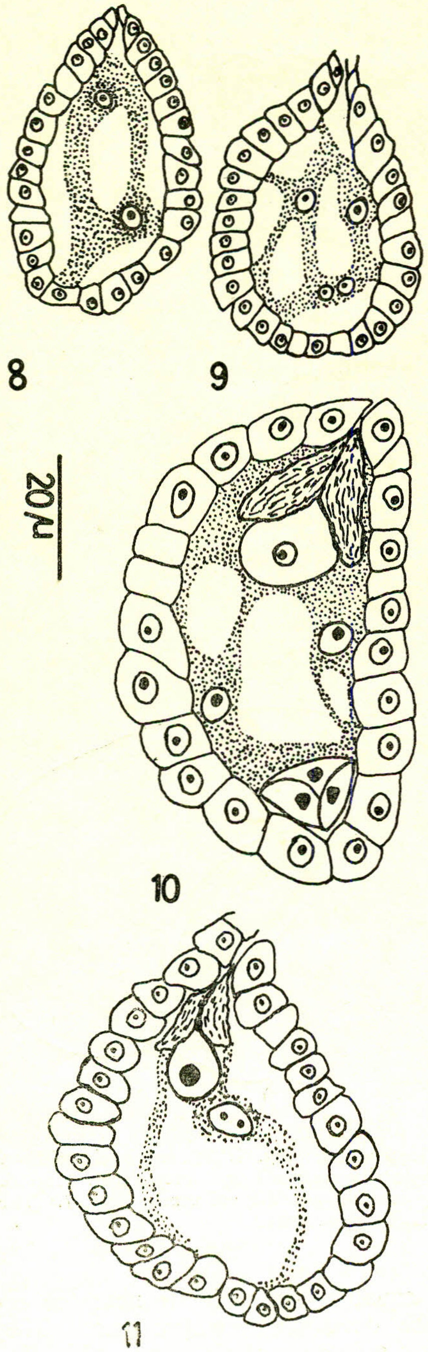
Fig. 1–6. Stages in the megasporogenesis and the differentiation of the integument. Fig. 1. Young ovule with a hypodermal archesporial cell. Figs. 2–5. The origin and the pattern of development of the integument. Fig. 6. Ovule with a linear tetrad and a well-developed integument, note the degenerating nucellar epidermis. Fig. 7. The functional chalazal megaspore has enlarged, while the rest are in the process of degeneration

divisions of megaspore mother cell form a linear tetrad. The latter consists of one chalazal cell which is larger in size and becomes the functional spore and the three cells nearly equal in size which ultimately disintegrate. Not more than one megaspore tetrad was observed in any ovule. These observations are substantiated by the findings of Bhaduri[1] in *Solanum melongena* and Rees-Leonard[7] in *S. tuberosum.* However, occurrence of more than one tetrad has been reported by Cooper[2] and Rybchenko.[8]

Megagametogenesis. The functional chalazal megaspore gradually increases in size, and becomes almost double in length at the time of its first nuclear division. By the time this division is completed, the three micropylar megaspores are degenerated and their remains are detectable as dark staining bodies only (Fig. 7). The resulting two daughter nuclei, approximately equal in size, migrate near the opposite poles of the developing embryo sac. They are separated by a large central vacuole (Fig. 8). The cytoplasm surrounding the nuclei is denser than in the rest of the cell. The young embryo sac generally increases in size before the four nucleate stage is reached.

The two daughter nuclei divide twice resulting in the formation of eight nucleate embryo sac. The quartet being noticed at each pole with numerous vacoules in the centre.

Following the third division of the neuclei the cell plates are laid down delimiting one egg and two synergid cells at the micropylar end and the three antipodal cells at chalazal end. The remaining fourth nucleus at each pole behaves as one of the polar nuclei.



Figs. 8 and 9. Stages in the development of megagametophyte. Fig. 10. A mature embryo sac with an egg apparatus two polar nuclei and three antipodals, note the radially elongated cells of the surrounding endothecial layer. Fig. 11. The longisection of a mature embryo sac with an egg cell, two synergids and a binucleate cell in the vicinity of the egg cell.

After cell formation the antipodals slightly increase in their size. They are relatively persistant and presumably degenerate only after and not before the fusion of polar nuclei. Their early degeneration has been reported in *S. tuberosum*[9] and *S. melongena*[1] while the antipodals have been observed in *Datura metal*[4] even after the initiation of endosperm. During the enlargement of the young megagametophyte the antipodals are pushed downward against the ovular tissue.

In their early stages of development the cells of egg-apparatus appear to be small and slightly elongated. Following some growth, the synergids become elongated and triangular in shape. Their micropylar ends are pointed and extend into the micropyle for a short distance. The striation of filiform apparatus appear as dark strands in the cytoplasm, alternating with elongated vacuoles, both of which run parallel to the long axis of the synergids. A similar condition was observed in *L. eseulentum*[2] and *S. tuberosum*.[7]

The egg increases in size until its broad basal portion extends beyond the synergids in the developing embryo sac. (Figs. 10 and 11). It has a vacuole at the apex and the nucleus in the broad basal region.

In the vicinity of egg cell a large binucleate endosperm cell has frequently been observed instead of two polar nuclei except in one case, presumably the fusion of the nuclei does not take place before fretilization.

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