DEVELOPMENT OF EMBRYO SAC IN *ZEUXINE GRACILIS* (BREDA) BL. (ORCHIDACEAE)

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The development of embryo sac of *Zeuxine gracilis* (Breda) Bl. was studied. The ovule is anatropous, bitegmic and tenuinucellate. The inner integument alone forms the micropyle. The mode of embryo sac development conforms to bisporic and G3', type of Abe (1972b). The mature embryo sac contains an egg apparatus, two polar nuclei, and one antipodal nuclei. Double fertilization occurs normally.

Keywords: Angiosperms, bisporic, embryology, embryo sac, Orchidaceae.

Orchidaceae are embryologically interesting as they exhibit great diversity in the organization of embryo sac. Schnarf (1931), Swamy (1949), Wirth and Withner (1959) have reviewed the previous embryological literature on the family. Some of the recent works in the area includes those of Gurudeva and Govindappa (2008) and Gurudeva (2009).

The genus *Zeuxine* Lindl. has 70 species, about 15 species are reported from India and only 3 of them are occurring in Karnataka (Ananda Rao and Sridhar 2007). *Z. strateumatica* (L.) Schlt. (=*Z. sulcata* Lindl.) was studied embryologically by Seshagiriah (1932a,1932b,1941), Swamy (1946) and Vij et al.(1982). While, *Z. longilabris* (Lindl.) Benth. Ex Hk.f. was studied by Govindappa and Karanth (1980). Perusal of literature indicates that no work on the embryology of *Z. gracilis* (Breda) Bl., a third taxa occurring in Karnataka, hence, an attempt was made to study the development of embryo sac.

MATERIALS AND METHODS

The material for this study includes post-pollinated and mature ovaries collected near Abbey falls, Madikeri, Kodagu district, Karnataka, India, during the month of December. The placental columns were excised and fixed in formalin-acetic- alcohol, and stored in 70% ethanol following thorough wash in running water, followed by conventional microtechnique The serial transverse and longitudinal sections at 10-12µm were stained with Heidenhain's iron alum and haematoxylin. Erythrosin in clove oil was used as counter stain. Drawings were made using camera lucida and Meopta microscope.

OBSERVATIONS

Ovary is inferior, tricarpellary and syncarpous. Numerous ovular primordia develop from three parietal placental ridges. Each primordium consists of an axial row of cells surrounded by an epidermis. The hypodermal archesporial cell directly functions as megaspore mother cell (Figs. 1,2). Two integuments are initiated in the ovular primordium (Figs. 2,3,5) and only the inner integument organizes the micropyle (Fig .9). The tenuinucellate ovule becomes anatropous by the time it lodges the dyad cells and continues to remain as such even at the organized embryo sac stage. Both the integuments remain 2-cell layered throughout.

The megaspore mother cell undergoes

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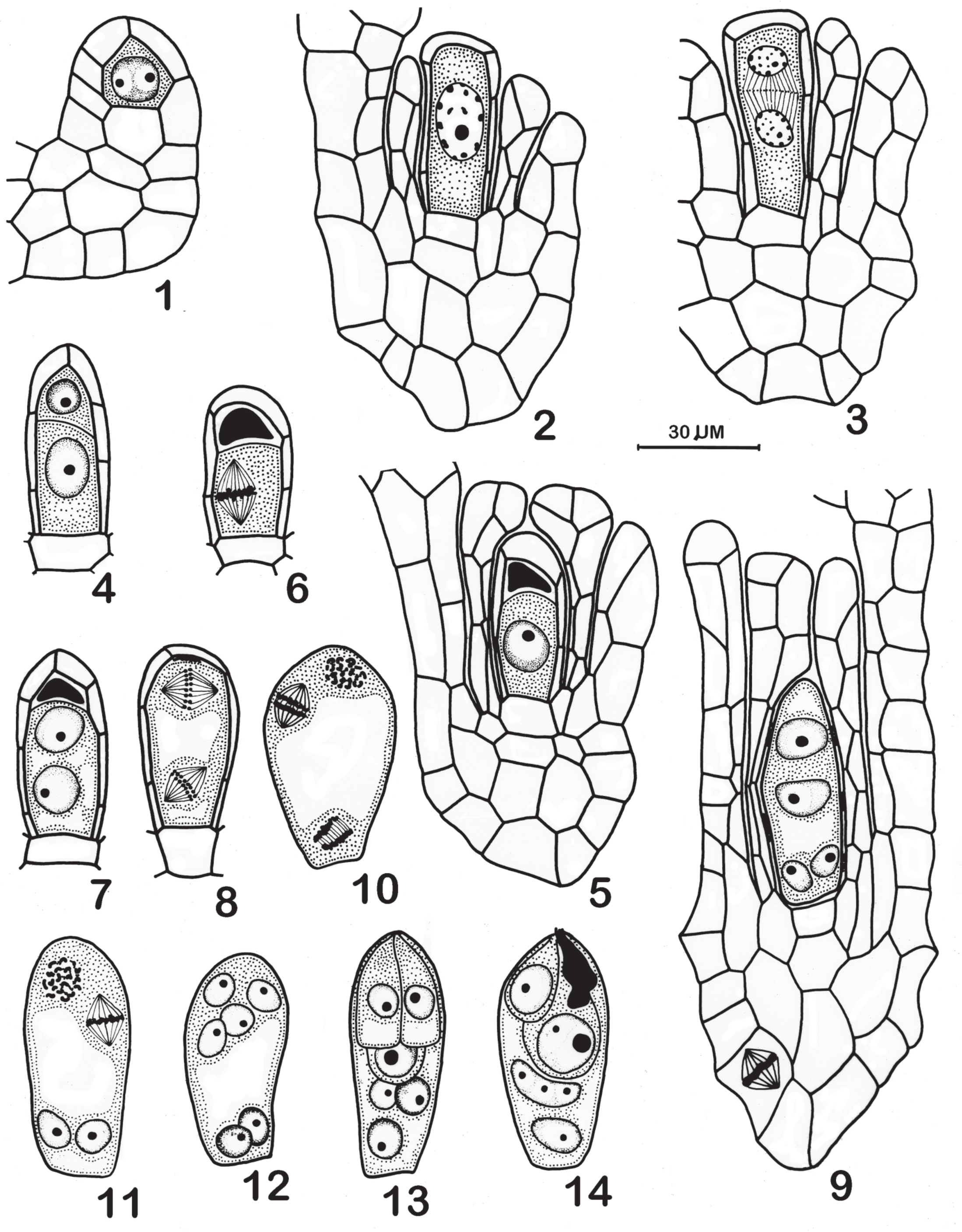

Figure 1-14

1.Longisection of ovular primordium showing single archesporial cell. 2. Longisection of young ovule with megaspore mother cell., 3. Megaspore mother cell at meiosis-I. 4. Dyad cells. 5. Longisection of ovule with dyad cells; note degenerating upper dyad cell. 6. Lower dyad cell in meiosis-II. 7. 2-nucleate embryo sac. 8. Synchronous division of nuclei in 2-nucleate embryo sac. 9. 4-nucleate embryo sac; note inner integument alone forms micropyle and outer integument extend beyond the inner integument. 10. Nuclear division in 4-nucleate embryo sac; note fusion of spindle at chalazal end. 11. 4-nucleate embryo sac; note only micropylar nuclei undergoing division. 12. 6-nucleate embryo sac. 13. Organized embryo sac. 14. Embryo sac soon after fertilization.

the first meiotic division to form a dyad with a large chalazal cell (Figs. 3,4). The micropylar dyad cell degenerates (Fig. 5) while the chalazal undergoes the second meiotic division to form two megaspores which are separated by a central vacuole (Figs. 6,7) resulting in a bisporic 2-nucleate embryo sac. Its nuclei undergoes a simultaneous division leading to the formation of a 4-nucleate embryo sac which destroys the surrounding nucellus and come in contact with the inner integument (Figs. 8,9). The four nuclei of the sac begin their synchronous division. The two micropylar nuclei completes this division producing four daughter nuclei, while the spindle of the chalazal ones fuse together and give rise to two large diploid nuclei (Figs. 10,12). Rarely, these two chalazal nuclei of the 4-nucleate embryo sac do not participate in the subsequent divisions (Fig.11). In either case, the female gametophyte becomes 6-nucleate with a 4+2 arrangement of nuclei. In the former, the chalazal nuclei remain diploid and haploid in the latter. The micropylar quartet organizes the egg apparatus consisting of an egg, two synergids and a micropylar polar nucleus. Of the two chalazal nuclei, one functions as the chalazal polar and the other represents the antipodal nucleus. The mature embryo sac has a slightly broad upper part which lodges a conspicuous egg apparatus. The two polar nuclei move towards each other, meet below the egg but do not fuse together. At the chalazal end, the antipodal nucleus remain as such without being enclosed by a cell wall (Fig. 13). Double fertilization occurs normally. A zygote and primary endosperm nucleus is formed. The single antipodal nucleus persists even after fertilization (Fig. 14).

DISCUSSION

The gynoecium is inferior, tricarpellary, syncarpous and ovary is unilocular, with numerous anatropous ovules on the divided parietal placentae. The finger like ovular primordium consisting of an axial row of cells which is covered by a layer of epidermis. The ovule is bitegmic with the micropyle being organized by the inner integument alone. The hypodermal archesporial cell directly functions as the megaspore mother cell as in other investigated species (Abe 1972a, Gurudeva and Govindappa 2008, Gurudeva 2009). The megaspore mother cell undergoes meiosis-I and gives rise to dyad cells, the upper of which is smaller and degenerates while the lower one passes through meiosis-II and gives rise to 6-nucleate embryo sac similar feature has been reported in *Z. longilabris* (Govindappa and Karanth 1980). There is a tendency towards the reduction of nuclei at the chalazal end from four to two. It may occur either by fusion of the spindle as in *Z .longilabris* (Govindappa and Karanth 1980) or through the strike phenomenon as in *Cypripedium calceolus, C. spectabile* (Abe 1972a) and *Cirrhopetalum fimbriatum* (Ekanthappa and Govindappa 1977) or by both as in *Z. gracilis* (present study). It is interesting to note that different species of the same genus behave differently with regard to megesporogenesis. In *Z. strateumatica* (Seshagiriah 1941) the embryo sac is monosporic and apomictic (Swamy 1946), whereas in *Z. longilabris* (Govindappa and Karanth 1980) and *Z. gracilis* (present study) it is bisporic. Vij et al. (1982) opined that the plants studied by Seshagiriah (1941) and Swamy (1946) might be polyploids. The sexual plant of *Z. strateumatica* exhibit normal bisporic 6 and 8 nucleate embryo sac.

The egg apparatus consists of 2 juxtaposed synergids and a large egg located behind the synergids. The two polar nuclei remain for a while and fuse together at the time of fertilization.The nature of the antipodals in the embryo sac appears to be interesting. The presence of 3 antipodal cells as recorded in many species by Abe (1972a), and Gurudeva and Govindappa (2008) is the basic feature. The next step in simplification is the elimination of walls of antipodal cells as observed in *Rhynchostyli sretusa* (Sood and Neelu Sham 1987) and *Epidendrum radicans* (Gurudeva and Govindappa 2008). On the other hand, the number of antipodal cells was reduced from three to one in *Z .gracilis*. The process of simplification appears to extend to the number of antipodal nuclei from three to two, one and none, a feature which has been recorded for a few species (Swamy 1949, Govindappa and Karanth 1980, 1981). It is obvious, therefore, that functionally, the chalazal end of the embryo sac is not as important as the micropylar end.

The mode of embryo sac development conforms to bisporic and G3' type of Abe (1972b). Entry of the pollen tube into the embryo sac is porogamous.

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