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ONTOGENY AND ORGANIZATION OF FEMALE GAMETOPHYTE IN *GOODYERA PROCERA* (KER-GAWL.) HOOK.

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Abstract

The development of female gametophyte of *Goodyera procera* (Ker-Gawl.) Hook. has been investigated. The ovule is anatropous, bitegmic and tenuinucellate. The inner integument alone forms the micropyle. The megaspore of triad nearest to the chalaza develops into 6-nucleate embryo sac. The mode of development of female gametophyte conforms to monosporic G3 type. The mature embryo sac contains an egg apparatus, secondary nucleus and one antipodal cell.

Introduction

THE GENUS *Goodyera* R. Br., of the subtribe Spiranthinae, tribe Neottieae belonging to the sub family Orchidoideae (Dressler and Dadson, 1960) comprising of about 160 species, is confined to the warmer parts except Africa. Twenty one species are known from India (Sathish Kumar and Manilal 1994). *Goodyera procera* (Ker-Gawl.) Hook. is the only species recorded from Karnataka (Ananda Rao and Sridhar,2007). It is a terrestrial leafy herb. The erect stem arises from the prostrate rooting base. *Leaves* are oblong-lanceolate. *Inflorescence* is a terminal spike. *Flowers* small, greenish-white. *Sepals* are oblong-lanceolate while the petals are spathulate. *Lip* is brownish-white and cymbiform when spread. *Column* short. *Anther* bears two pollinia. *Ovary* is inferior and small. *Fruit* is an ovoid capsule. Schnarf (1931), Swamy (1949), Wirth and Withner (1959), Davis (1966) and Abe (1972a, 1972b) summarized the previous embryological work on the family Orchidaceae. Treub (1879), Leavitt (1901) and Afzelius (1916) made preliminary developmental studies on *Goodyera repens, G. discolor, G. pubescens* and *G. tesselata*. Sharma and Vij (1984) examined *G. repens* and reported the presence of monosporic 8-nucleate embryo sac. Sood (1984) studied embryogeny in *G. biflora*. Embryology of *G. procera* has not been studied so far, hence, an attempt was presently made to study the ontogeny and organization of the female gametophyte.

Materials and Methods

The materials for this study include post-pollinated and matured ovaries collected from Bale honnur, Chikmagalure district, Karnataka, India, during the month of December. The placental columns were excised and fixed in formalin-acetic-alcohol, and preserved in 70% ethanol following thorough wash in running water after which conventional microtechnique was followed. The serial transverse and longitudinal sections of 10-12 μm were stained with Heidenhain's iron–alum and haematoxylin. Erythrosin in clove oil served as counter stain. Drawings were made using camera lucida and Meopta microscope.

Observations

A large number of finger-like ovular primordia arise from the three divided parietal placental ridges located on the inner wall of tricarpellary, syncarpous, inferior, unilocular ovary, following pollination. Each of them is made up of a central row of 6-7 nucellar cells surrounded by the nucellar epidermis. The uppermost cell of the row becomes densely protoplasmic and functions as the archesporial cell (Fig.1). It directly develops into a megaspore mother cell. Meanwhile, the two integuments are initiated in the ovular primordium, the inner of which is the first to form. The outer integument initiated later (Fig.2), outgrows the inner, only during post-fertilization stages of the ovule. The two integuments in the anatropous teniunucellate ovule remain two cell-thick. Occasionally, an axial cell below the megaspore mother cell becomes conspicuous by its large nucleus and dense cytoplasm and appears like the other megaspore mother cell (Fig.3). However, the fate of such cells could not be ascertained.

The nucleus of the megaspore mother cell undergoes meiosis-I, followed by cytokinesis, resulting in the formation of two unequal dyad cells (Figs. 4,5). The smaller micropylar dyad cell normally does not divide further but degenerates. The larger dyad cell after completing meiosis-II gives rise to a smaller megaspore towards the micropylar end and a larger one towards the chalazal region resulting in a triad (Fig.6). The

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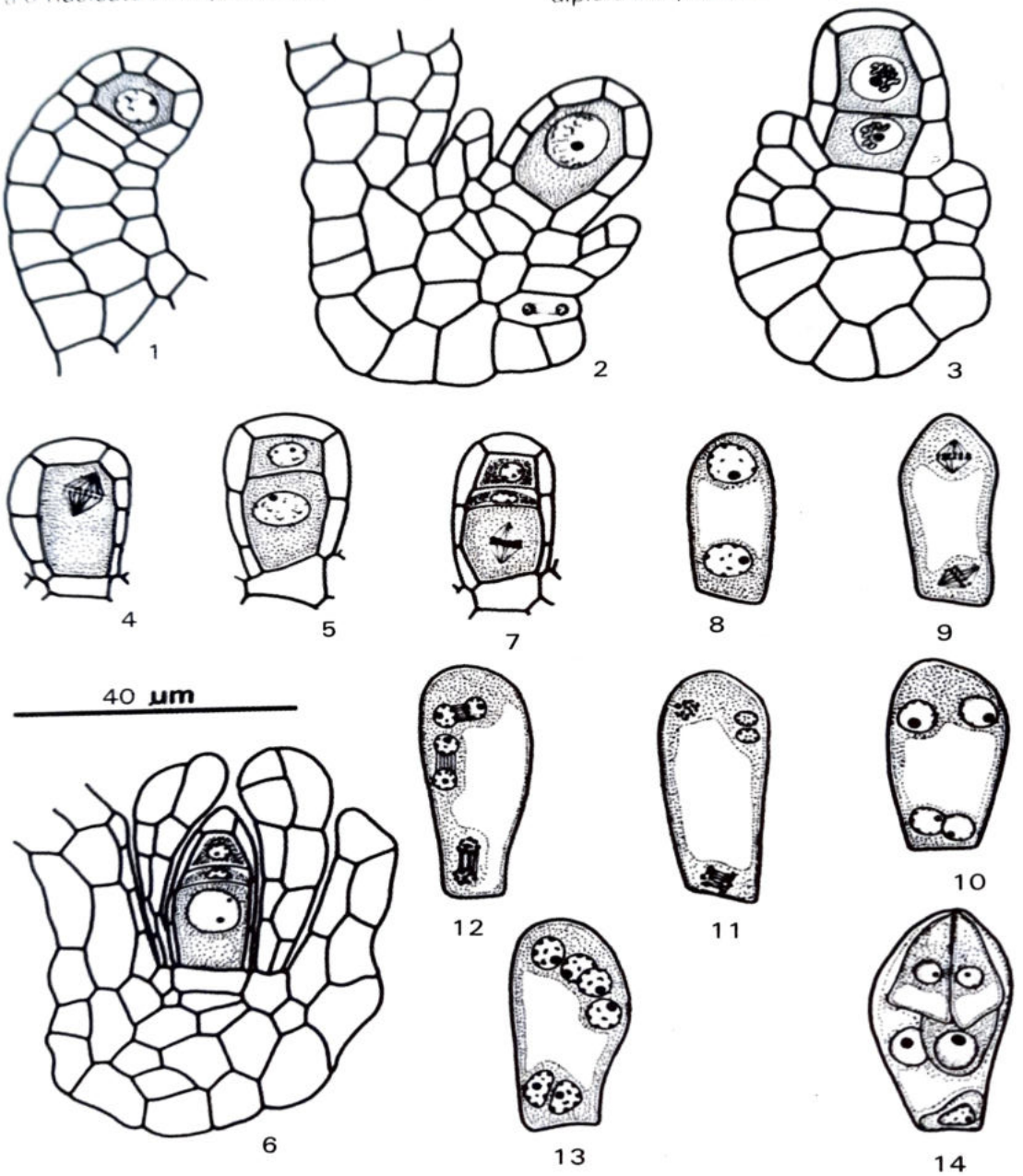
functional megaspore in the triad enlarges in size and its nucleus divides (Fig.7). The resulting daughter nuclei move apart to opposite poles as a central vacuole organizes. Thus, a 2-nucleate embryo sac is formed (Fig.8). It enlarges in size and its nuclei divide synchronously rendering the sac 4-nucleate (Figs.9,10). During the next synchronous division, the two micropylar nuclei engender four haploid daughter nuclei while the spindles of the two chalazal nuclei fuse together (Figs.11,12) and consequently two diploid daughter nuclei are produced at the end. Therefore, a 6-nucleate embryo sac with four haploid nuclei constituting the micropylar quartet and two diploid nuclei at the chalazal end results (Fig.13). This is followed by the organization of embryo sac. The micropylar quartet contributes to the egg apparatus and the micropylar polar while the chalazal pair contributes to a single diploid antipodal cell and a diploid chalazal polar. Later, the two polar nuclei fuse together to form a triploid secondary nucleus.

In the mature embryo sac, the egg apparatus is conspicuous and occupies a major part of the space (Fig.14). The secondary nucleus lies near the egg. The diploid antipodal cell stays intact.



Figs. 1-9. Ontogeny and organization of female gametophyte in *Goodyera procera:* 1, L.S. an ovular primordium with archesporial cell; 2, Young ovule with megaspore mother cell; 3, L.S. young ovule showing superposed double megaspore mother cell; 4,5,6, Stages of megasporogenesis; 7, Nucleus of functional megaspore in division; 8, 2-nucleate embryo sac; 9, Synchronous nuclear division in 2-nucleate embryo sac; 10, 4-nucleate embryo sac; 12, Nuclei of 4-nucleate embryo sac in division; note the fusion of spindle at chalazal end; 13, 6-nucleate embryo sac; 14, Organized embryo sac with a single antipodal cell.

Discussion

The ovary is tricarpellary, syncarpous and unilocular. The ovule initiation on the placenta is triggered after pollination. Finger-like ovular primordium consists of an archesporial cell. The archesporial cell enlarges in size and directly functions as megaspore mother cell (Abe, 1972a; Govindappa and Karanth, 1980; Sharma and Vij, 1987; Swamy,1949; Yeung and Law, 1989). The megaspore mother cell undergoes meiosis-I and gives rise to superposed dyad cells, the upper of which is smaller. The smaller upper dyad cell degenerates and lower one undergoes through meiosis-II, giving rise to a upper smaller non-functional and a lower functional megaspore. This leads to the organization of a triad. A similar triad formation is reported in several orchids (Abe, 1972a; Gurudeva, 2009; Gurudeva and Govindappa, 2008; Govindappa and Karanth,1980; Sharma and Vij, 1987; Sood and Mohana Rao, 1986; Swamy, 1949). In orchids, the formation of triad is more common and twice as frequent as the tetrads (Abe, 1972b). The chalazal megaspore becomes functional. The nucleus of the functional megaspore divides twice to form 4-nucleate embryo sac. The 4-nuclei take part in third free nuclear division. The nuclear spindles of the chalazal region fuse to produce two diploid nuclei instead of four haploid nuclei. This feature is occasionally observed in *Epipactis pubescens* (Brown and Sharp, 1911) and *Paphiopedilum insigne* (Afzelius, 1916). This is common in the present study and is in conformity with *Polystachya flavescens* (Ekanthappa and Govindappa, 1977). Thus, the embryo sac becomes 6-nucleate. Similar 6-nucleate embryo sac has earlier been reported in *Bulbophyllum neilgherrense* Swami (1949) and *Polystachya flavescens* (Ekanthappa and Govindappa, 1977). Of the two chalazal nuclei, one functions as the antipodal cell while the other behaves as the chalazal polar. The mode of development of the female gametophyte conforms to the monosporic G3 type of Abe (1972b).

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