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ONTOGENY AND ORGANIZATION OF FEMALE GAMETOPHYTE IN *DISPERIS NEILGHERRENSIS* WIGHT (=*DISPERIS ZEYLANICA* TRIMEN)

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Abstract

The development of embryo sac of *Disperis neilgherrensis* Wight was studied. The ovule is anatropous, bitegmic and tenuinucellate. The inner integument alone forms the micropyle. The megaspore of a triad nearest to the chalaza develops into 8-nucleate embryo sac. The mode of embryo sac development conforms to monosporic Polygonum and G1a type of Abe (1972b). The mature embryo sac contains an egg apparatus, two polar nuclei and three antipodal cells. Double fertilization occurs normally.

Introduction

THE GENUS *Disperis* Sw., subtribe Coryciinae, tribe Orchideae, subfamily Orchdoideae (Dressler and Dodson, 1960), comprises 74 species distributed in Subsaharan Africa, Madagascar, South India, Sri Lanka, Thailand, Philippines and New Guinea (Ananda Rao and Sridhar, 2007; Fernando and Ormerod, 2008; Seidenfaden,1969). Ten species are originally described in various parts of Asia. All Asian taxa were considered synonymous under *Disperis neilgherrensis* Wight (Kurzweil, 2005).

The family Orchidaceae has attracted the attention of several embryologists from time to time because of the diversity in the development and organization of female gametophyte, suspensor development, and embryogeny. The publications of Abe (1972a, b); Davis (1966); Schnarf (1931), Swamy (1949), Wirth and Withner (1959), summarized the previous embryological work on the family. Recent work includes the studies by Fredrikson (1991, 1992); Govindappa and Karanth (1980); Law and Yeung (1989); Sood (1985, 1986, 1989, 1992), and Sood and Mohana Rao (1986, 1988). Perusal of literature indicates that there is no information on the embryology of the genus *Disperis*. Hence, an attempt was made to study the ontogeny and organization of female gametophyte in *Disperis neilgherrensis* Wight.

Material and Methods

The material for this study includes post-pollinated and mature ovaries collected near Anemahal and Manjrabad Fort, Sakaleshpur, Hassan district, Karnataka, India, during the month of August. The placental columns were excised and fixed in formalin-acetic-alcohol, and stored in 70% ethanol, following a wash in running water for about 30 mins. Conventional methods of microtechnique were followed. The serial transverse and longitudinal sections were cut at 10-12 µm and stained with Heidenhain's iron-alum and haematoxylin. Erythrosin in clove oil was used as counter stain. Drawings were made using Camera lucida and a Meopta microscope.

Observations

Disperis neilgherrensis Wight is a slender, erect and sparingly branched tuberous terrestrial herb, variable in height from 12-20 cm. *Leaves* are 1-3 in number, cordate-amplexicaul and acute. *Inflorescence* is a 1-2 flowered cluster. *Flowers* are bracteate and pale pink. *Dorsal sepal* is fused with the broad sepals to form a hemispherical hood. *Lateral sepals* are fused and each has a depression. *Lip* is located under the hood. It has three lobes and lateral ones are horn-like (Fig.1 a, b). *Column* is short with two spiral lateral lobes. *Pollinia* 2, each with double row of ovate lobes, are attached to the caudicle and gland. *Capsule* is narrowly elliptical and ribbed.

The *gynoecium* is inferior, tricarpellary and syncarpous and the ovary is unilocular. Before pollination, three longitudinally oriented placental ridges arise from the inner wall of the ovary and become bipartite along their length. The superficial cells of the placental ridges multiply and produce many finger-like processes. Their ultimate branches organize the ovular primordia. Each ovular primordium consists of an axial row of 5-7 cells and a sheath of epidermis. The uppermost cells of the column becomes an archesporial by acquiring a large nucleus and dense cytoplasm (Fig. 2a). After pollination it enlarges in size and directly functions as the megaspore mother cell (Fig. 2b). By this time, the inner integument arises as an outgrowth below the level of megaspore mother cell in the ovular primordium. It develops rapidly and surrounds the nucellus and it alone forms the micropyle. The outer integument is initiated little later after the inner one. It gradually outgrows the

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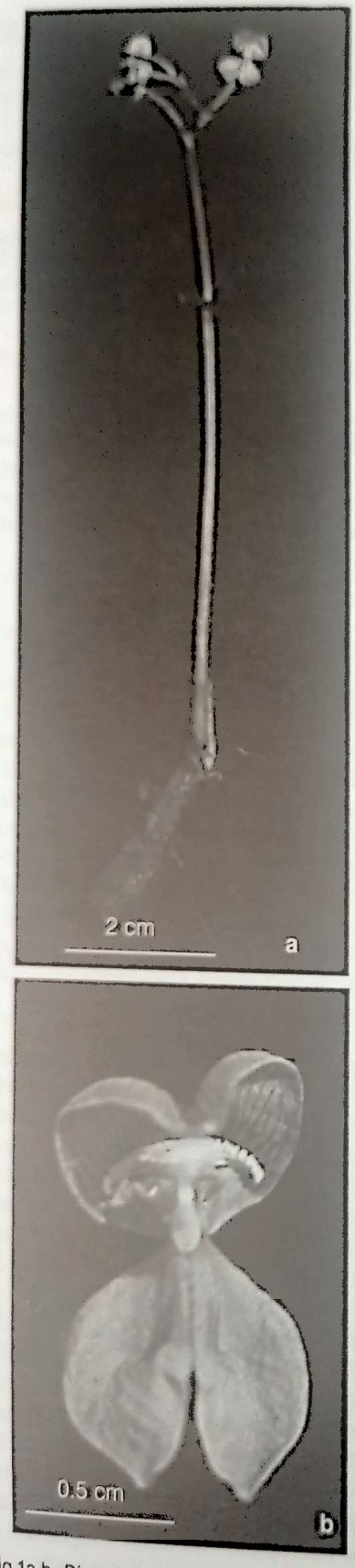

Fig.1a-b. *Disperis neilgherrensis*. Habit of the plant: a, Plant at flowering stage; b, A flower, enlarged.

inner integument by the time the megaspore mother cell completes meiosis-II. Both inner and outer integuments are bilayered.

The megaspore mother cell undergoes meiosis-I, forming two unequal dyad cells (Figs. 2c, d). The smaller micropylar dyad cell is non-functional, while the larger chalazal one completes meiosis-II (Fig. 2e) and give rise to two megaspores. Thus, a triad consisting of a micropylar dyad, a small lenticular megaspore below it, and a large chalazal functional megaspore is formed (Figs. 2 f, g). The nonfunctional dyad cell and the smaller megaspore degenerate gradually. The nucleus of the functional megaspore divides to form two daughter nuclei which become separated by a central vacuole (Figs. 2h, i). The two- nucleate embryo sac thus produced, elongates and its nuclei subsequently divides synchronously to result in a four-nucleate sac (Figs. 2j, k). Division of nuclei in this sac is simultaneous and leads to the organization of an eight-nucleate embryo sac. The eight-nuclei are disposed in two quartets (Fig. 2l). The micropylar quartet contributes to the organization of an egg apparatus containing an egg, two synergids and micropylar polar while the chalazal one gives rise to three antipodal cells and a chalazal polar. The two polars fuse together to form the secondary nucleus (Fig.2m). Double fertilization occurs normally (Fig.2n).

Discussion

It is consistently observed in a majority of orchids that the ovule initiation on the placenta is triggered after pollination (Brown,1833; Nimoto and Sagawa, 1961; Yasugi, 1983). According to Swamy (1943,1949), this trend is very true for monandrous orchids. However, in a few terrestrial monoandrous species, belonging to the sub tribe Habenarieae, the ovular primordium with only hypodermal archesporial is produced even before pollination and their further development depend upon pollination. A similar feature is noticed in the present investigation. Proliferation of ovule before pollination may be considered as a primitive feature contrast to the proliferation of ovule after pollination.

Inner integument is always the first to be differentiated, the first to be active and invariably first to degenerate unlike in *Vanilla planifolia* (Swamy, 1947), where it remained intact. The outer integument lags behind the inner, upto the organization of the embryo sac but subsequently, it outgrows the inner after fertilization, in a majority of orchids (Govindappa and Karanth, 1980; Swamy,1949; Wirth and Withner,1959). However, in the present study, it was observed that the outer integument outgrew the inner at triad stage. Similar observations were made in *Bulbophyllum mysorense* (Swamy,1949),

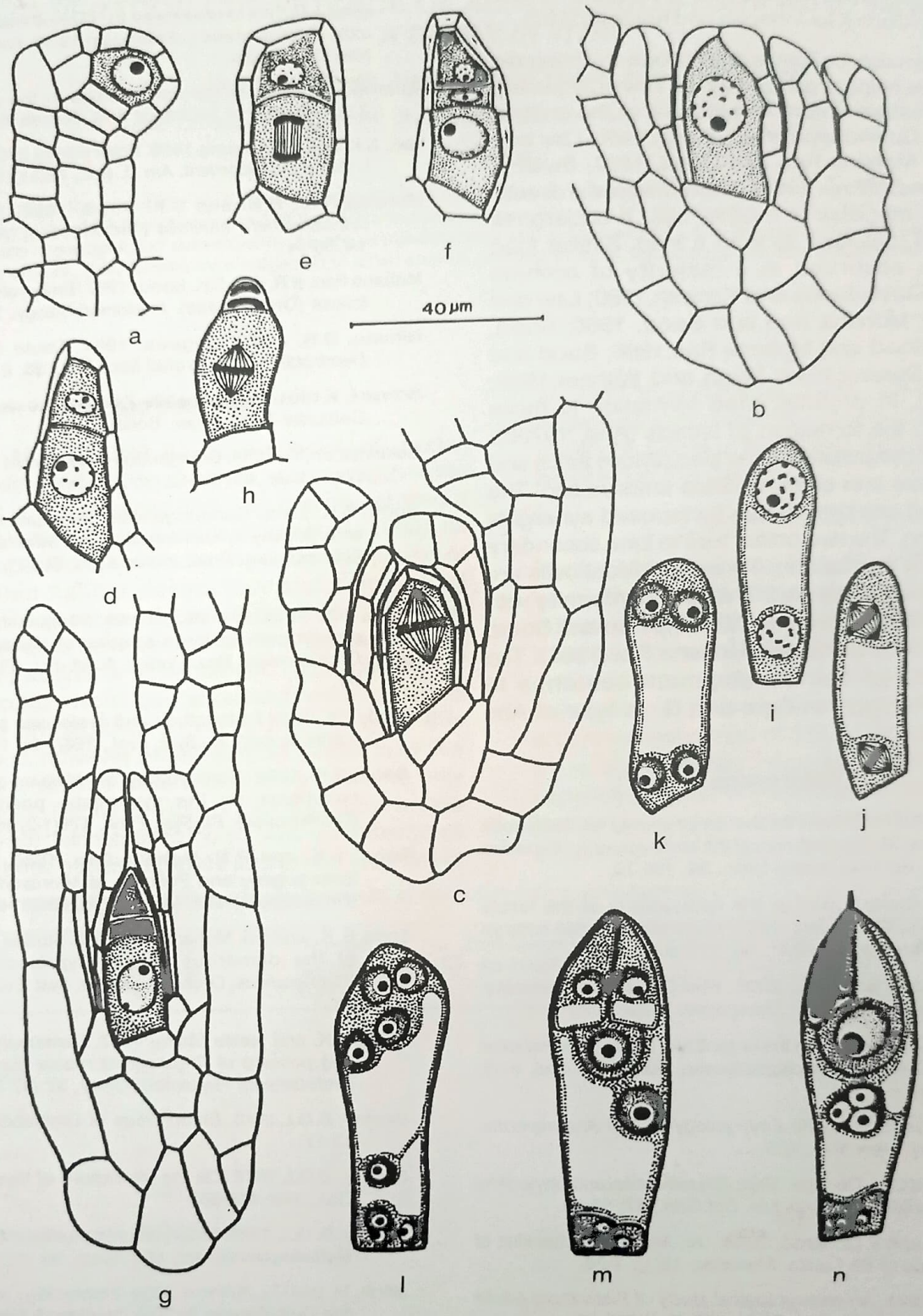

Fig. 2 a-n. Development of embryo sac in *Disperis neilgherrensis* : a, Longisection of an young ovular primordium showing hypodermal archesporial cell; b, Longisection of young ovule with megaspore mother cell; c, Longisection of young ovule containing megaspore mother cell at meiosis-I; d, Dyad cells; e, Nuclear division in the lower dyad cell; f, A triad, note enlarged functional megaspore; g, Longisection of ovule with a triad; note the outer integrument outgrows the inner integument; h, Nuclear division in the functional megaspore; note the degenerating nucellar cells; i, Two - nucleate embryo sac; j, Synchronous nuclear division in 2-nucleate embryo sac; k, Four - nucleate embryo sac; l, Eight - nucleate embryo sac; note quartet of nuclei at poles; m, Organized embryo sac; n, A stage in double fertilization.

Rhynchostylis retusa (Sood and Sham,1987), and *Zeuxine longilabris* (Govindappa and Karanth,1980).

The ovule ultimately becomes anatropous and bitegmic with micropyle being organized by the inner integument alone. This feature is common in most of the orchids (Abe,1972b; Govindappa and Karanth,1980; Law and Yeung,1989; Mohana Rao and Sood, 1987; Swamy, 1949; Wirth and Withner,1959). The archesporial directly functions as megaspore mother cell. It undergoes meiosis-I and meiosis-II to form a triad. Similar triad formation is observed in a majority of orchids (Abe,1972b; Govindappa and Karanth,1980; Law and Yeung, 1989; Mohana Rao and Sood, 1986; Sood, 1985, 1986; Sood and Mohana Rao,1986; Sood and Sham,1987; Swamy,1949; Wirth and Withner,1959; Yasugi,1983). In orchids, triad formation is more common than the formation of tetrads (Abe, 1972b). The functional megaspore of the triad divides thrice and finally organizes into eight-nucleate embryo sac. The egg apparatus consists of two juxtaposed synergids and a large egg. The two polars fuse to form secondary nucleus before fertilization. Three antipodal cells are located at the chalazal end. This is in conformity with the earlier findings in orchids (Mohana Rao and Sood, 1987; Sood,1985; Sood and Mohana Rao,1986). The mode of embryo sac development conforms to monosporic Polygonum type and G 1a type of Abe (1972b).

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