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Microsporogenesis of *Polypleurum wallichi* (R. Brown ex. Griff.) Warm. (Podostemaceae) growing in northeast India

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Abstract

In *P. wallichii,* the flowers are cleistogamous, enclosed by a thin membranous spathe. The two anthers are borne on a forked filament placed adjoining to the stigmatic lobes. Ontogenetically the two anthers developed separately; because of intercalary growth, it becomes bifid. The archesporial cell comprises of uniseriate rows of cells, the anther wall consists of a very thin epidermis, unilayered endothecium with fibrous thickenings and the tapetum with uni- and binucleate cells. The anther wall development is reduced type, middle layers are absent. The connective tissues also acquire fibrous thickenings. Cytokinesis of microspore mother cell is successive type within a distinct callose wall. Pollen grains are dispersed in dyads with minute and dense granular exine.

Key words: *Polypleurum wallichii,* Podostemaceae, Anther Development, Meghalaya, India.

Introduction

Podostemaceae display many unique morphological, anatomical and ecological features, and stands clearly apart from all other angiospermous families.[1] The pioneering work on Indian Podostemaceae was carried out by Willis.[2-5] Most of the previous workers mainly confined their studies on female gametophyte.[6-9] Though Khosla *et al.*,[10-11] and Mukkada[12] studied the reproductive biology in some members of Podostemaceae, the ontogeny and development of anthers and microsporogenesis were studied only to a limited extent. Therefore, the present investigation describes the development of anther from the ontogeny till the pollen formation in *Polypleurum wallichii* (R. Brown ex. Griff.) Warm.

Materials and Methods

Flowers of *Polypleurum wallichii* at various developmental stages were collected from a stream at fossil park, Janiaw, Lawbah region; about 6 km away from Mawsynram, located in East Khasi Hills District, Meghalaya State, India (92.10° N – 25.25°E) at an altitude of 1300 m. For the light microscopy the materials were fixed in phosphate-buffer solution of 2-3 % glu-

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taraldehyde, dehydrated in propanol and embedded in glycol metacrylate (Technovit 7100). To ensure proper fixation, the air was removed before fixing by vacuum pump. A rotary microtome was employed to make 7-10 µm thick sections that were stained with safranine-fast green and erythrosine. Callose was stained with decolorized aniline blue.[13,14] Proteins were stained with mercuric bromophenol blue[15] and nucleic acid with Azure blue.[16] Photomicrographs were taken using Nikon E600 and Leitz fluorescence microscopes. For SEM preperation, Glutaral dehyde fixed and dehydrated flowers were dried in Tetramethylsilane (TMS) solution. Dried flowers were dissected with razor blades, sputter coated with gold in an Eiko ion-sputter, JFC-1100. External morphology of the stamen and pollen grains were observed by using Joel (JSM-6360) scanning electron microscope.

RESULTS

Development of Stamen

In *Polypleurum wallichii,* the flowers are bisexual enclosed within a distinct membraneous spathe. The stamens are bifid, each lobe contains tetrasporangiate anther (Figs. 1 & 2) The two stamen primordia developed prior to gynoecium. On initiation, the staminal primordium comprises a mass of undifferentiated meristematic cells and each primordium comprises of unilayered dermatogen that covers the multicellular hump-like tissues. This configuration conforms to tunica-corpus concept (Fig. 3). Ontogenenetically the bifid stamens are two independent units, because of intercalary growth at the base of stamen (andropodium) the filament becomes biforked or bifid (Fig. 4) and each fork

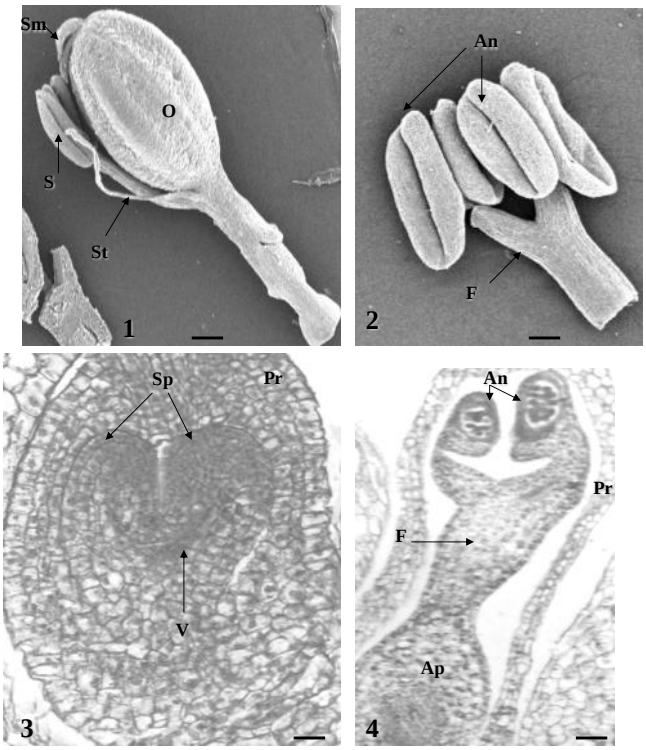

Figure 1. An entire bisexual flower showing Stamens (S), staminode (St), Stigma (Sm) and Ovary (O). Scale: 15 mm.
Figure 2. Bifid stamens (An) with forked filament (F). Scale: 25 mm.
Figure 3. Two anther primordia (Sp) enclosed by a distinct perianth (Pr). The differentiation vasculature (V) in an acropetal direction. Scale: 450 µm.
Figure 4. Longitudinal section of flower showing the bifid stamen (An), filament (F), Perianth (Pr) and intercalary growth of andropodium (Ap). Scale: 130 µm.

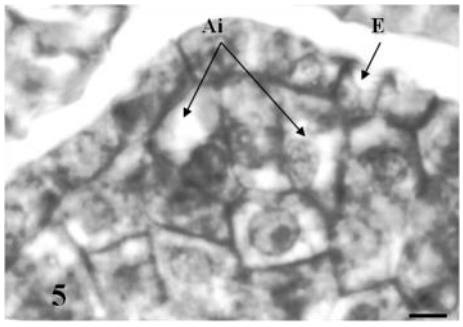

Figure 5. Transverse section of anther lobe showing a plate of archesporial initials (Ai) and epidermis (E). Scale: 900 µm.

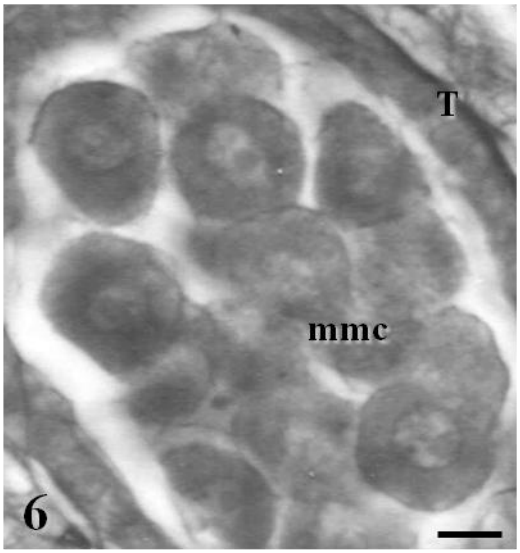

Figure 6. Transverse section of anther lobe with microspore mother cell (mmc) and Tapetum (T). Scale: 600 µm.

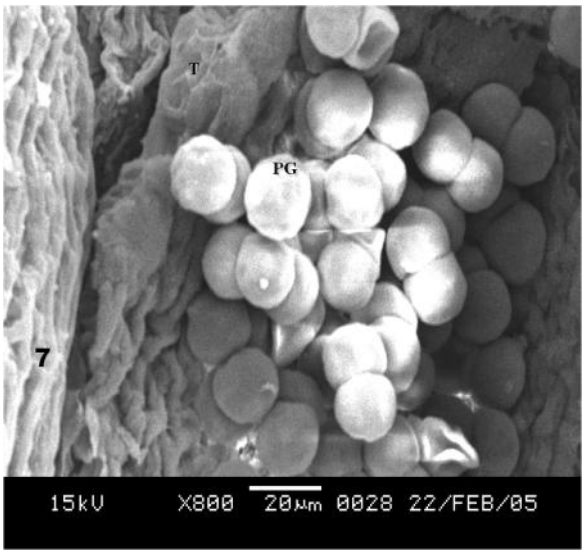

Figure 7. SEM photograph of Pollen grain (PG) and tapetum (T). note the hooked appearance at the distal end of dyad pollen grain and deposition of sporopollenin in a granular fashion.

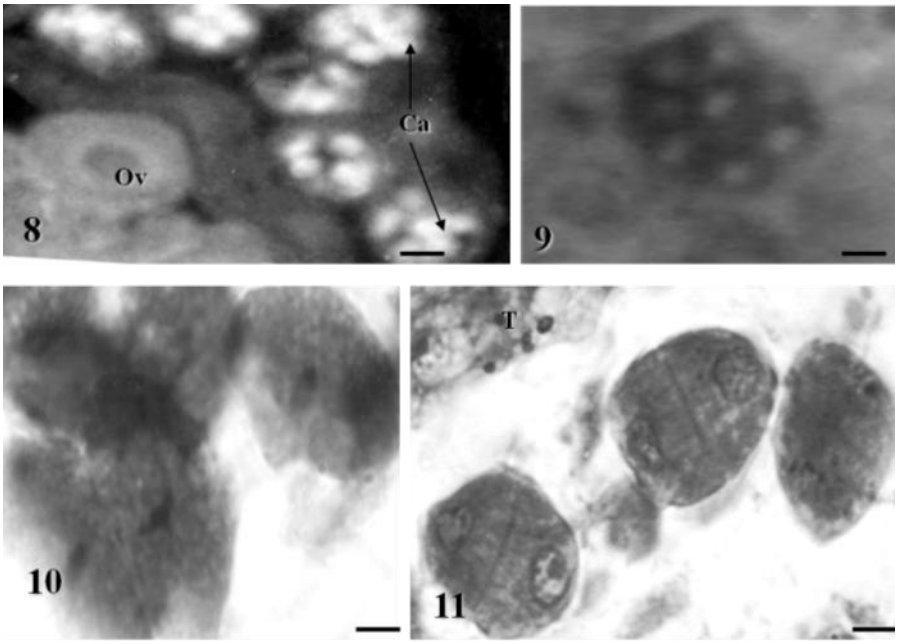

Figure 8. Transverse section of anther lobes stained with aniline blue showing and fluorescence of callose deposition(Ca) and ovule (Ov) . Scale: 130 µm.

Figure 9-11. Different stages of meiotic divisions: Figure 9. Prophase nucleus of microspore mother cell showing the organization of chromosome as rods. Scale: 2000 µm; Figure 10. Metaphase chromosome of meiosis-I. Scale: 1100 µm; Figure 11. Dyad stage after Meiosis-I. note the centrifugal formation of cell plate and the adjacent tapetum (T) showing granular appearances. Scale: 1100 µm.

bearing a single tetrasporangiate anther lobe. Each stamen primordium after reaching 15 µm in length, the vasculature differentiates in acropetal direction. The anther primordia are differentiated on the adaxial side. So that all the four lobes of each anther facing towards the stigma (as in Fig. 1).

Archesporial cell and Anther wall formation

A plate of 3-4 hypodermal cells differentiated into an archesporium in each anther lobe. Archesporial cells are radially elongated, densely cytoplasmic with a large, more prominent nucleus (Fig. 5). The entire archesporial initials divide periclinally, to form two layers which are of unequal in size; the inner row of cells is much larger than the outer ones. The former becomes primary sporogenous cell and the latter one primary parietal cells.

The primary parietal cells undergo only one periclinal division to form two-layered secondary parietal layer, which directly transformed into endothecium and tapetum respectively. The middle layers are absent. The cells of epidermis, endothecium and tapetum are fully laddened with starch grains. The starch grains in the endothecial layer start to disappear when the pollen mother cells enter into prophase-I. The endothecial layer as well as adjacent to the connective tissue also have fibrous annular thickenings which develops at the time of meiosis-II in the meiocytes .

Tapetum

Tapetum is secretory, unilayered in structure enclosing the sporogenous tissues. The cells are rectangular, both uni and bi-nucleate condition cells are seen. The cells are densely cytoplasmic with lots of starch and protein contents (Fig. 6). The entire tapetum is derived from inner layer of the secondary parietal layer. The tapetum releases granular substances that are similar to that of sporopollenin deposited on the inner tangential wall, facing towards the anther locule (Fig. 7). Under light microscope, these granular substances appear in the tapetal cells at the end of meiosis-I (see Fig. 11). The tapetal cells persist untill the release of pollen grains in pairs.

Meiosis and Cytokinesis

Pollen grains are arranged in isobilateral or decussate arrangement, minutely granulate with distinct projection at its distal end (Fig. 7). Prior to meiosis, the microspore mother cells becomes rounded and separated out. Meiosis is uniform in all the microspore mother cells in each theca, but not in adjacent anther locules. Meiosis is of successive type. The microspore mother cells are enclosed by a distinct callose wall (Fig. 6 & 8) that gets dissolved after the completion of meiosis-II and before the onset of pollen wall deposition. At the time of prophase-I, the chromosomes are distinctly organized as minute rods in the prophase nucleus (Fig. 9). After the first meiotic division a distinct cell plate is formed and the nuclear spindle disappeared. As soon as the first meiotic division is over, the nuclei of a daughter cells immediately undergo second meiotic division (homeotypic division) without any resting period in which the spindles of metaphase-II are either parallel or right angles to each other (Figs. 10 & 11).

During and after meiosis-II, the pro-orbicules (Ubisch bodies) are released from tapetum and deposited on the pollen exine (Fig. 7). Within the initial stages of microspore enlargement, the cytoplasm does not increase rapidly enough to fill the entire lumen of the microspore wall. Cytoplasmic vacoulation increases and when the microspores enlarge to their maximum size, the cytoplasm forms only a thin layer around nucleus with radiating strands. Subsequently the cytoplasm increases quickly and the nucleus shifts its position to the distal pole where it divides to form the vegetative and generative cells.

DISCUSSION

In P.*wallichii* the four microsporangia are protuberant on the adaxial surface; a similar situation is reported in Anonaceae, Degeneri-

aceae and Hemantandraceae except that the microsporangia are embedded and not protruberant.[17] Tetrasporangiate anthers have been reported in majority of angiosperm families.[18] In Podostemaceae the two staminal primordial arise independently because of the intercalary growth of andropodium, it becomes bifid or forked. The two forked stamen exceeding the ovary and stigma. Intercallary growth of andropodium has also been reported in N. *khasiana* and N. *lowii*.[19,20]

A plate of 3-4 cells, hypodermal in origin has been reported in many plants.[21,22] The anther development is of reduced type.[23] In P. *walichii* the entire tapetum arise as a concentric layer around the sporogenous cells from the parietal layer. A similar type of origin of tapetum from the parietal layer has been reported in *Triticale*.[17] The tapetum is of secretory type, unilayered structure; cells with distinct uni and two- nucleate condition and dense cytoplasm that persists until the formation of dyad microspores. The secretory type of tapetum has been observed in *Indotristica, Terniola and Dicraea stylosa*.[12] The sporopollenin deposited on the pollen grain as tiny granulation is mainly secreted by tapetum (see Fig. 7). Echlin[24] and Shaw[25] also reported the tapetal origin of sporopollenin in *Lilium henryi*.

In *Terniola,* Mukkada reported that the pollen mother cell undergoes simultaneous type of cytokinesis.[12] Rutihauser mentioned that dyad pollen grains are the characteristic feature of Podostemaceae.[26] In the present study on P. *wallichii* the pollen mother cell division is of successive type. The starch grains in the endothecial layer as well as adjacent connective tissues have been utilized for the development of pollen grains. Similar observation was observed in *Lilium*.[27]

Meiosis is not uniform in all the four thecae of an anther lobe. This feature has not been recorded so far in any member of Podostemaceae. The cell division in P. *wallichii* is successive type in which the meiosis-I is followed by centrifugal cell plate formation, after which the two daughter cells are enclosed by distinct callose wall that the resultant cells lie side by side in the common callose wall. The callose wall acts as a barrier or semi-molecular filter to allow only some macromolecules as well as providing genetic autonomy to each developing sporocytes.[28] Meiosis-II takes place in the resultant daughter cells so that two dyads are formed. After which, callose wall starts to disappear. In P.*wallichii,* meiosis-I and II are two different phenomena, not interconnected one another, so that two genetically different dyads are formed.

Perisamy and Swamy[21] emphasized that the terminology succesive and simultaneous types should not only represent temporal relationship, but two different cytological processes; the former is affected by cell plate while the latter by furrows. In successive type, the dissolution of callose wall after the formation of four microspores has been reported in *Pooea purpuria* and *Laventera trimestris*.[29] The parenchymatous cells adjacent to the anther locule to acquire endothecial thickening is also seen in *Chelone globra*.[8] Subramanyam and Shreemadhavan reported that anthers dithecous and anther wall four layered in both the Podostemacaee and Tricticha-caeae.[30] The present investigation did not confirm this view.

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