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Research Paper

Podostemaceae Embryological Question: Is the antipodal cell in the organized female gametophyte resolved?

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ABSTRACT: - *The female gametophyte presents unique embryological phenomena in the Podostemaceae. The megaspore mother cell undergoes meiosis – I to produce the two dyad cells. The micropylar dyad cell degenerates regularly and does not participate in the following development of the female gametophyte. After meiosis-ll, two megaspore nuclei are produced. This is the primary two– nucleate stage. The megaspore nucleus located at the chalazal end of the primary two-nucleate female gametophyte stage degenerates. The micropylar megaspore nucleus undergoes two successive mitotic divisions and four 'naked' protoplasts are produced. The micropylar quartet of nuclei alone participates in the cellular organization of the female gametophyte. There exist two synergids at the micropylar end, an egg cell in the middle region and a polar cell at the chalazal end. The ontogeny of the female gametophyte conforms to the monosporic category and the Apinagia type, form B. There are no antipodal cells; and the precursor primary chalazal megaspore nucleus is eliminated in the gametophyte through the "Strike" phenomenon. Single fertilization ensues in the Podostemaceae.*

Keywords:- *Podostemaceae, Megaspore mother cell, Micropylar and Chalazal dyad cells, Primary two-nucleate stage, Micropylar megaspore nucleus, Apinagia type form B, No antipodal cell, Syngamy.*

I. INTRODUCTION

Review of the female gametophyte ontogeny Battaglia (1971)[1] raised fundamental embryological questions in Podostemaceae. Studies of the Indian taxa of Podostemaceae (Arekal and Nagendran,1975a[2],b[3],1976[4],1977a[5],1977b[6]; Nagendran and Arekal,1976[7], Nagendran , Subramanyam and Arekal,1976[8]; Nagendran , Subramanyam and Arekal,1977[9]; Nagendran, Anand and Arekal,1980[10]) provided embryological data to resolve the issues. Battaglia (1987[11]) categorically stated that all embryological data not assigned to the Apinagia type are doubtful cases and therefore require reinvestigations. Cusset and Cusset (1988[12]) opine new classes of Podostemaceae based on the morphological, physiological, anatomical and embryological data as class Podostenopsida, Magnoliopsida and Liliopsida. Sikolia and Ochora (2008[13]) carried out female gametophyte reinvestigation in *Tristicha trifaria*. Further investigations were done in the *Inversodicraea bifurcata* and *I. keniensis* (Sikolia and Onyango, 2009[14]). However, the embryological questions regarding the status of the antipodal cell were not clearly given proper discussion. Thus, which course and causes results into the antipodal cellularization? Does the antipodal cell form in Podostemaceae? and what are the consequences of their formation? Furthermore, due to the endemism, availability of flowers and fruits only during a limited time and accessibility for collection, several taxa have not been investigated to resolve the antipodal cell question. Therefore, the present work to discuss, resolve and make some recommendations for future studies.

II. MATERIAL AND METHODS

Plant material collected was fixed in Formalin-Acetic-Alcohol and preserved in 70% ethanol. Fixed plant specimens were identified under binocular stereomicroscope. Individual flower buds of all stages of development were dehydrated in the ethanol-xylol series and imbedded in paraffin wax 52^0 C melting point. Serial microtome sections were cut at 7µmm-12µmm thickness. Sections were stained in Heidenhain's Iron Alum-Hematoxylin and counterstained in erythrosine or Fast Green in clove oil. DPX mountant was used to prepare permanent slides. Camera Lucida drawings of the female gametophyte stages were drawn at table level using a 'Leitz' monocular microscope at different magnifications.

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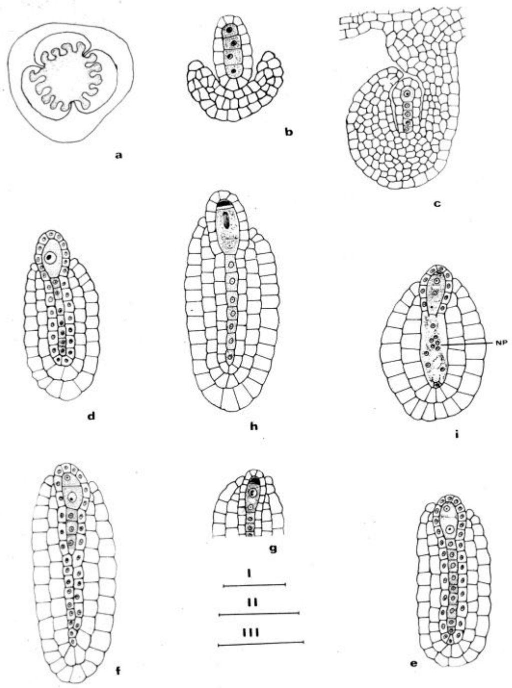
III. RESULTS

3.1 Female Gametophyte in *Tristicha trifaria*

Ovary is trilocular (Plate I, Fig. a). In the developing ovular primordial a central row of nucellar cells are seen (Plate I, Fig. b). A hypodermal cell in nucellus differentiates as a densely cytoplasmic, large nucleated cell (Plate I, Fig. c). This archesporial cell enlarges and forms megaspore mother cell (Plate I, Fig. d). The megaspore mother cell undergoes the first meiotic division resulting in two nuclei followed by wall formation (Plate I, Fig. e). This result in the formation of two dyad cells (Plate I, Fig. f). The micropylar dyad degenerates regularly and is visible as a crescent-shaped cap of dark mass in later stages of the developing embryo sac (Plate I, Figs. g-I; Plate II, Figs. a-e). Meanwhile, the chalazal dyad cell enlarges and completes meiosis-II. The resulting two nuclei are separated by a distinct vacuole (Plate I, Fig. g) of the two nuclei. The chalazal one degenerate without further division (Plate I, Fig. h) and in subsequent stages assumes a picnotic appearance. The other nucleus located at the micropylar end divides mitotically in a parallel to the long axis of the embryo sac (Plate I, Fig. e). The resultant two nuclei move apart (Plate I, Fig. i). Both these nuclei again divide mitotically and synchronously (Plate II, Fig. a) to produce four nuclei in the embryo sac Plate II, Fig. b) distributed cross-wise. At this stage, the remains of the degenerated chalazal megaspore nucleus is not observed. Now the embryo sac undergoes cellular organization (Plate II, Fig. c). Thus, the micropylar quartet of nuclei alone contributes to the organization of the mature embryo sac that consists of two juxtaposed synergids, an egg cell and micropylar polar cell. The present study filiform apparatus have been observed in both the synergids (Plate II, Fig. e). The development of the female gametophyte in this taxon conforms to the Apinagia type, form B of Battaglia (1971). Antipodal cell(s) have not been recorded.

The pollen tube enters the embryo sac overlapping one of the synergids (Plate II, Fig. e). In the protoplasmic mass of the pollen tube and degenerating synergid, two degenerating nuclei can be observed. One could be the undischarged second male gamete and the other one the synergid nucleus.

The fusion of the egg cell and a male gametes results in the formation of zygote (Plate II, Fig. e). The zygote is enlarged and extended towards the chalazal part and sideways in the central region of the embryo sac. No fusion of the polar cell and second male gamete was observed. Syngamy occurs.



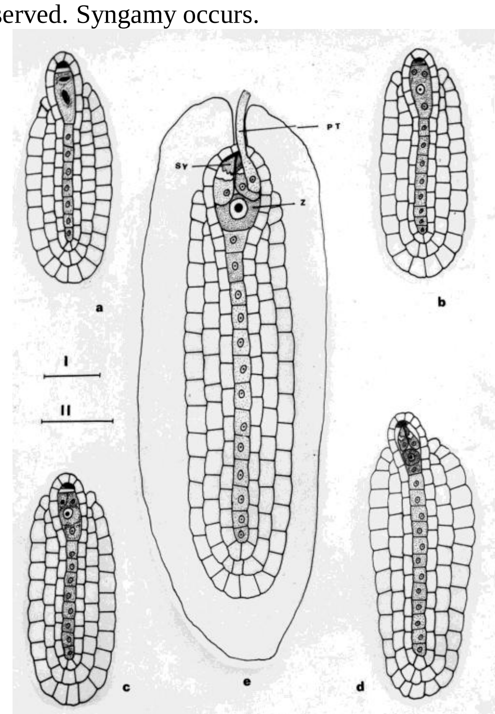


Plate I: Development of female gametophyte in *Tristicha* *Tristicha trifaria*

Plate II: Development of female gametophyte *trifaria* (continued)

3.2 Female Gametophyte in *Inversodicraea bifurcata*

Several ovular primordial arise from the placental hump in a young transverse section (Plate III, Fig. a). Later, inside the developing ovule, a densely cytoplasmic large nucleated hypodermal archesporial cell differentiates (Plate III, Fig. b). This cell without further division enlarges and transforms into megaspore mother cell (Plate III, Fig. c). It undergoes meiosis-I (Plate III, Fig. d) resulting two dyad cells. The micropylar one degenerates regularly and is recognizable as a crescent-shaped cap at the micropylar of the developing embryo sac (Plate III, Fig. e). The chalazal dyad cell undergoes the second meiotic (Plate III, Fig. f). The two resulting nuclei move apart towards the pole of the embryo sac. A prominent vacuole is observed between the two nuclei (Plate III, Fig. g). The chalazal megaspore nucleus commences to degenerates (Plate III, Fig. h). Meanwhile, the micropylar megaspore nucleus enlarges in size (Plate III, Fig. h). It undergoes the first mitotic division is free nuclear (Plate III, Fig. i) that results in a secondary two-nucleate embryo sac stage (Plate IV, Fig. a). The two nuclei undergo another free nuclear division (Plate IV, Fig. b), This results in a four-nucleate embryo sac (Plate IV, Fig. c). Wall formation results in the organization of four-celled embryo sac (Plate IV, Fig. d). The embryo sac consists of two synergids, a large central egg cell and polar cell. Each of the synergids possesses filiform apparatus (Plate IV, Fig. e). The development of the female gametophyte corresponds to the Apinagia type, form B of Battaglia (1971).The pollen tube enters the ovule through the micropyle and crushes one of the synergids (Plate IV, Fig. e). The zygote formed after syngamy enlarges in size considerably (Plate IV, Fig. d). The degenerated polar cell and remnants of the pollen tube can be seen at this stage. The second male gamete is never discharged but remains in the cytoplasmic mass at the tip of the pollen tube (Plate IV, Fig. e) No fusion of the second male gamete and a polar cell was seen. Syngamy occurs. The nucellar situated below the megaspore mother cell in the developing embryo sac become densely protoplasmic (Plate IV, Figs. d-e), and elongate. When the female gametophyte is at the primary two-nucleate embryo sac stage, their walls breakdown and disappears.

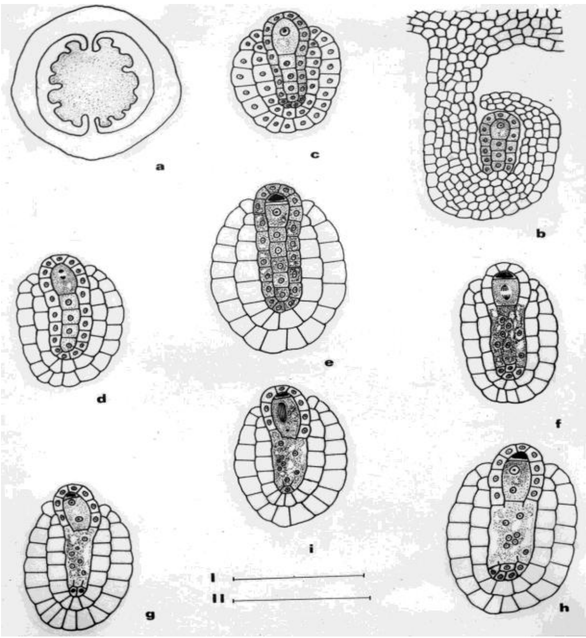


Plate III: Development of female gametophyte in gametophyte in *Inversodicraea bifurcata*

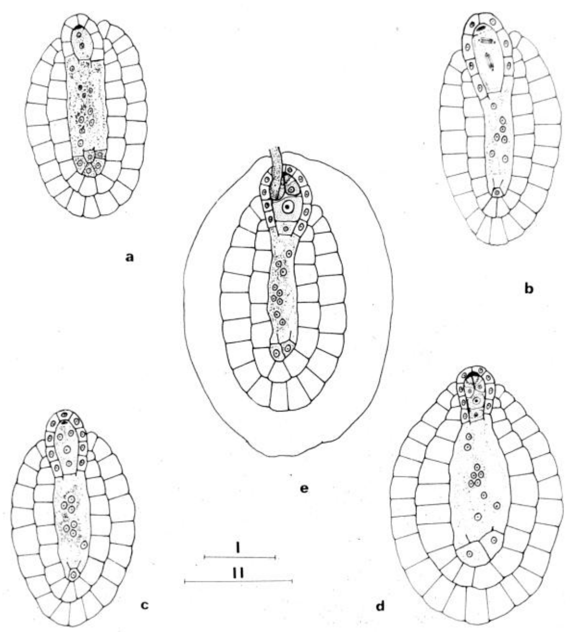


Plate IV: Development of female *Inversodicraea bifurcata* (continued)

This wall phenomenon of wall disintegration commence at the chalazal end and proceeds micropylar-chalazal axis. The naked protoplasts start coalescing in the long cavity formed. Their nuclei often gather at different loci, in numbers less than eight, in the vacuolated cytoplasmic mass in the cavity. This structure transforms a structure consisting multinucleate protoplasts in a long cavity and is referred to as the pseudo-embryo sac, observed below the embryo sac. It is organized before fertilization.

IV. DISCUSSIONS

4.1 Antipodal cell

The investigation clearly revealed that the primary chalazal nucleus fully degenerates and disappears in the organized female gametophyte [13, 14]. Went (1908, p.7 [15]) who first investigated the pair the female gametophyte ontogeny in *Oenone imthurnii* and *Mourea fluviatilis* of Podostemaceae observed a four–celled mature embryo sac stage; "… of these four two, the synergids, lie at the top, next to each other; then follow the other two, one under the other, the upper one of the pair being the egg and the lower one all that remains of the embryo sac with the upper polar". The interpretation [13] is accepted in this study, since only the micropylar megaspore nucleus of the primary 2-nucleate stage contributes to all four nuclei present in the organized female gametophyte [14]. Consequently, the micropylar quartet of nuclei can only organize into two synergids, an egg cell and the upper polar cell. This cellular organization has been reported in the female gametophyte in *Zeylanidium lichenoides* (Chaudhary, Kanduri, Tandon, Uniyal, Mohan Ram, 2014)[16] and is three–nucleate phase. The latter's nucleus, undergoes degeneration, and unlike in the present case, if the opposite mode of ontogeny happened, then the chalazal quartet of nuclei derived from the chalazal nucleus, could from three antipodal cells and the lower polar cell. But the disintegration of the primary chalazal nucleus commences after the disorganization of the nucellar cells below the embryo sac and completely disappears when a distinct vacuole separating it from the micropylar megaspore nucleus becomes invisible in the *Inversodicraea bifurcata* and *Inversodicraea keniensis* [14]. However, this occurs immediately after cellularization in *Tristicha trifaria* [13]. This disintegration phenomenon has been reported in all the investigated taxa of Podostemoideae and Tristichoideae showing the Apinagia type of ontogeny [9].

The 'Strike' phenomenon which completely eliminates the antipodal complement in the Podostemaceae has also been observed in *Epipogium roseum* (Arekal and Karanth, 1981) [17] and *Zeylanidium lichenoides* [16]. Davis (1966) [18] defines 'Strike' phenomenon as the failure of certain nuclei of the developing gametophyte to undergo some of or all of the post-meiotic mitoses. This is extreme in Podostemaceae because the functional nuclei in the female gametophyte are derivatives of the primary micropylar nucleus. To qualify this present observation, the chalazal megaspore undergoes further division either partly or wholly, resulting in gradual elimination of the antipodal nuclei in a similar biologically specialized family like Orchidaceae. Concomitantly, this gradual elimination of the formation of nuclei at the antipodal end of the embryo sac known as the 'strike' phenomenon is observed in the monosporic, bisporic as well as tetrasporic embryo sac throughout the family, Orchidaceae (Abe, 1972)[19].

Rodkiewicz (1970[20]), Rodkiewicz and Bednara (1976) [21] have reported, in those species in which the micropylar spore forms the female gametophyte, as in the present case, it is the micropylar pole which has less deposition of callose. Callose is known to reduce the permeability of cell wall to nutrients and other substances; and its deposition is part of the process by which the maternal genome suppresses somatic spores (Haig, 1986)[22]. This may shift nutrient supply to the advantageously placed megaspore nucleus as observed in *Spinacia* (Wilms, 1980) [23], during embryo sac ontogeny. Callose deposition in the stigmatic papilla below the incompatible pollen tube, inhibits the latter's growth (cf. Johri, 1984, pp. 250–252, 285–291)[24]. It is also deposited in sieve tubes in the abscission zone of a senescing leaf (Scott, Miller, Webster and Leopold, 1967) [25]. Given that, there is a high degree of self-pollination possibility in the taxa of Podostemaceae because the spathe usually encloses flowers, then, homozygosity is favoured. Consequently, deposition of callose in the wall of the megaspore mother cell is favoured as in *Oenothera hookeri* which possess complex homozygote [22] that disappears from the prophase resulting in the micropylar tip before the end of the first meiotic prophase resulting in the micropylar spore forming the female gametophyte. Then, the chalazal region of the developing embryo sac may be physiologically depressed or hypo functional [1,11]. This may explain the disintegration of the primary chalazal nucleus in the embryo sac of Podostemaceae because it is disadvantageously placed towards the direction of nutrient supply unlike the micropylar component.

Degeneration of the chalazal megaspore nucleus is evident in terms of its decrease in size which is a likely indication of reduction in physiological activities [1], and its division must be a quite an uncommon event. Then, how can antipodal cell(s) form, if its predecessor the primary chalazal nucleus completely disappears without further division in the female gametophyte? Concomitantly , many embryologists who investigated the female gametophyte of Podostemaceae accept that the cell in the chalazal region of the embryo sac as a proendospermatic cell with one polar cell contrary to view stated [1,11] as the antipodal cell. The former interpretation is more acceptable. While, Kapil and Bhatnagar (1978) [26] emphasized the need to re-evaluate antipodal cell in the family, [10] reported its complete absence in the organized female gametophyte consisting of two synergids, an egg cell and a polar cell, after investigating *Podostemum subulatus*. The concept that, "Since the days of Hofmeister and Strasburger … the antipodal cell (Strasburger's Antipoden oder Gegenfusslerinnen) is a cell situated at the chalazal end of the mature ES, usually 1-nucleate, regularly degenerating at the fertilization or, rarely, showing a mitotic activity (Phenomenon of Polyantipody)", [11]; could be accepted only if it is derivative of the primary chalazal nucleus during the female gametophyte ontogeny. Thus, the status of any component present in the organized female gametophyte must reflect its origin at the megagametogenesis, before its morphological location is taken into consideration. This point has been repeatedly stressed in all reviews on the angiosperm female gametophyte (Maheshwari, p., 1947[27], 1948[48]; Maheshwari, S.C., 1955[29]; Kapil and Tiwari, 1978[30] [26]).

In the Polyggonum type of female gametophyte reported by Strasburger (1877) [31], the chalazal quartet of nuclei form three antipodal cells and lower polar nucleus whereas the micropylar quartet of nuclei results in two synergids, an egg cell and upper polar nucleus. While justifying the presence of antipodals [1] opined that the free nuclei of the Polypleurum type attain their cellular morphology based their axial position along the female gametophyte, consisting of one synergid, an egg cell; both considered as sister cells, and two antipodals (Razi, 1949[32]; Mukkada, 1964[33]). However, [9] reinterpreted the Polypleurum type as an inverted female gametophyte consisting of two synergids, an egg cell, and polar cell. Thus, there are no antipodals in the Polypleurum type. Similar reports of reverse polarity in angiosperms have been reported [34], Tackholm, 1915[35]; Dutt and Subba Rao, 1933[36]; Narayanaswami, 1940[37]; Joshi and Venkateswarlu, 1941[38]; Thirumalachar and Khan, 1941[39]; Gopinath, 1943[40]; Swamy, 1946[41]). The 'Strike' phenomenon is completed so that the nucleus which is to degenerate is not produced at all, in both the Polypleurum type and the Podostemum type of female gametophyte ontogeny [9]. Furthermore, studies have reported the occurrence of both these female gametophyte types in a single taxon, *Hydrobryopsis sessilis* [3]. [9]) observed that the Polypleurum type of the female gametophyte need to be rationalized as unique in possessing, '.”…. the peculiar egg apparatus with a single synergid and egg both considered as sister cells, the absence of polar nuclei and the present of two (occasionally only one) antipodal cells are rare combination of characters not found in any other angiosperm – not even in any other member of Podostemaceae” [33].

It follows, and then the presence of two synergids, an egg cell is undisputed observation in an organized female gametophyte in the family of Podostemaceae. But the reasons [1, 11] has adduced in designating the fourth cell as 'antipodal' and not polar cell, are not cell, are convincing established because of the following objections:-

(i) Mere chalazal position of this cell in the specialized and most reduced female gametophyte found in the family cannot be the basis for considering it as an antipodal cell; and further. Location is bound by the function of the cells;
(ii) The smaller size of this cell should be evaluated and analyzed from the functional and spatial points of view;
(iii) Since triple fusion is absent in the family, the question of male nucleus penetrating this cell does not exist.

Embryological observations in the *Zeylanidium lichenoides* that the megagametophyte is monosporic and Apinagia B type, embryo sac is a three–celled and nucleate due to central cell generation; and that central cell degenerates before syngamy [13,14,16]) provide credible evidences on the ontogeny of the female gametophyte in the Podostemaceae. However, transmission electron microscope investigations on this cell in question need would be interesting and proof of the current status.

Fertilization in the family of Podostemaceae

Pollination was not studied [14]. Fertilization is porogamous. The pollen tube penetrates the embryo sac destroying one of the synergids on its pathway, contrary to Razi's (1955) [42] observation between the two synergids. The other synergid only degenerates during embryogenic stages [14]. This mode of pollen tube pathway has been recorded in *Dalzellia zeylanica* (Chopra and Mukkada, 1966) [43], *Indotristicha ramosissima* (Mukkada, 1969) [44], *Mourera fluviatilis* [15]. The contents of the pollen tube overlaps with the protoplasmic mass of the destroyed synergids and are often visible as a dark mass with two nuclei in a degenerated state [14]; and one of these nuclei may be the undischarged second male gamete. The observations confirm the reports of earlier investigators [15]; Walia, 1965[45];[43]; Mukkada, 1969) [46], that the second male gamete is not discharged, in the family. According to Mukkada (1969) [46], often the tip of the pollen tube and one of the synergids persist as late as quadrant and octant stages of the proembryo. At this time, the polar cell is degenerated of absent, a process that starts before the pollen tube enters the female gametophyte, as observed in other taxa of Podostemaceae [3, 6, 8]. The chances of the polar cell being fertilized by second male gamete do not arise because none of the preparations depicted the fusion of the second male gamete with the polar nuclei [14]. Further, there is no endosperm in the family and the primary endosperm nucleus never forms in the investigated taxa of Podostemaceae [3, 7, 14, 44]. The study [14] contrasts the reported fusion of the second male gamete with a polar nucleus [28]. The functions of the endosperm, a product of double –fertilization found in other families of angiosperms, are taken over by the nucellar plasmodium in Podostemaceae. The egg cell fertilized by a male gamete produces the zygote which increases in size considerably, extending towards the chalazal region of the female gametophyte as well sideways [14]). A similar type of fertilization has been reported in other investigated taxa of Podostemaceae [2, 5, 6, 7, 15, 43, 44]. Thus, only single –fertilization takes place in the family.

V. CONCLUSIONS

The micropylar dyad cell degenerates regularly and doesn't participate in subsequent stages of ontogeny. The primary chalazal nucleus degenerates, without division, referred as the 'Strike' phenomenon. The micropylar megaspore undergoes two successive mitotic divisions and four nuclei are produced. After cellularization the four-celled structure of two synergids, large central cell and polar cell forms. The course and causes of ontogeny does not reveal the presence of no antipodal cells. The following recommendations are urgently required. Study of the female gametophyte using transmission electron microscope to determine the fate of the micropylar dyad cell and primary chalazal nucleus degeneration and their final components. To investigate the possibility callose deposition around the walls of the megaspore mother cell, in cross-wall between the dyad cells in relation to the significance of the female gametophyte ontogeny and 'Strike' phenomenon in the family. Determine the general occurrence of selective callose deposition in wall surrounding megaspore mother cells, cross-walls within dyads and between dyads; the relative amount deposited in the walls, in different taxa of angiosperm showing monosporic, bisporic and tetrasporic female gametophyte types of development. To investigate the general relationship of selective callose deposition, nutrients supply shift, viability of micropylar megaspore nuclei, during ontogeny. Study the influence of external factors on the female gametophyte ontogeny through tissue culture techniques.

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