Subsequent development of microspores is accompanied by the disappearance of PAS-positive material present in the locule (Fig. 11). Callosic deposition also disintegrates from the peripheral microspore tetrads. But microspores do not separate and remain in tetrad condition. Subsequent to the loss of locular deposition of PAS-positive material and starch grains in the microspore, a distinct PAS-positive wall forms around each microspore (Fig. 11). But, as revealed by the calcocfluor-white test, microspore walls appear to be lacking in cellulosic deposition (Fig. 12). In the degenerating tapetum cell walls become less distinct before they disintegrate completely. The degenerating tapetal cells retain rich contents of RNA and proteins.

In the nearly mature anther, formation of exine wall is observed only in the peripheral pollen tetrads (Figs. 12, 13). Even in these pollen tetrads the exine is fragmentary and forms only on the outer face of the spores. The central pollen tetrads totally lack exine wall (Figs. 12, 13).

Exine wall of the peripheral pollen tetrads is autofluorescent in nature (Fig. 12). It is MBB-negative but azure-B positive (Fig. 13). With azure-B exine wall stains green indicating the presence of phenolic compounds and acid polysaccharides (Fig. 13). Even after the formation of exine wall, the degenerating tapetal cells continue to secrete the autofluorescent material on its inner tangential surface (Fig. 12).

Mature pollen grains, which remain in tetrads, possess rich contents of cytoplasmic polysaccharides (Fig. 11), RNA (Fig. 13) and total proteins. Pollen tetrads aggregate to form pollinium (Figs. 11, 12, 13). Prior to the anther dehiscence tapetum degenerates completely. Few cells of endothecium possess fibrous thickenings.

Discussion

Irregular synthesis of callose, presence of thick cell walls and starch storage in the meiocytes and tapetum, non-separation of microspores from tetrads and formation of fragmentary exine wall on the peripheral pollen tetrads are characteristic features of the anthers of S. plicata. These features are uncommon in the anthers that produce pollen grains in monads. Irregular synthesis of callose seems to be responsible for the origin of other unusual features listed above. Therefore, these unusual features are integrated and causal for the formation of pollinium.

Callose plays an important role in anther development

In the anthers, which produce pollen grains in monads, callosic wall is synthesized inside the primary walls of every pre-meiotic meiocyte. Temporal and spatial distribution of anther callose implicate its significance in the production of fertile pollen grains. In many male sterile plants pollen sterility is linked with the absence or poor synthesis of anther callose or failure of its degradation (Horner Jr. 1977, Theis and Robbelen 1990, Hegde and Isaacs 1992). According to Sakurai (1998) callose functions as an apoplastic molecule. So far 6 facets of anther callose functions are envisaged: (i) adhesion of meiocyte to meiocyte without allowing their fusion and cohesion (El-Ghazaly and Jensen 1987, Theis and Robbelen 1990, Bedinger 1992), (ii) skin to defend symplast from dehydration (Barskaya and Balina 1971, Vijayaraghavan and Shukla 1977), (iii) skeleton to establish microspore polarity (Blackmore and Barnes 1988), (iv) growth regulation by directing the orientation of cellulosic microfibrils (van Amstel and Keng 1996), (v) transportation route for selective nutrients by virtue of its ability to function as a ‘selective molecular filter’ (Heslop-Harrison and Mackenzie 1967, Knox and Heslop-Harrison 1970, Southworth 1971), and (vi) template or mold for exine wall formation (Vijayaraghavan and Shukla 1977).

Thick cell walls of meiocytes substitute for the absence of callose

Conversely, anthers of S. plicata defy the poor presence of callose and produce fertile pollen...
grains. It implies that the functional roles attributed to the callose are not universally applicable and plants have alternate mechanisms to overcome the deficiency of callose. In this context the formation of thick cellulosic and PAS-positive cell walls in meiocytes, a feature commonly not encountered in the majority of the angiosperm anthers, is very significant. It is important to note that the meiocytes become thick-walled, prior to meiosis, the stage at which callose synthesis begins in other angiosperm anthers. Therefore, it is cogent to assume that thick cell walls of meiocytes not only substitute for callose to maintain cell to cell contact but also prevent their cohesion and fusion. Since cell walls also constitute apoplast (Sakurai 1998), they presumably protect the symplast from dehydration and determine the microspore polarity.

Presence of thin cell walls and absence of storage carbohydrates are characteristic features of all meristematic and secretory tissues including meiocytes and tapetum. According to Sakurai (1998) presence of sucrose is a pre-requisite condition especially for gibberellin-mediated growth. Under normal circumstances, sustained growth of sporogenous cells/meiocytes and tapetum is largely due to unabated supply of sucrose to the anther by the green parts of the plants (Lawrence and Mayne 1991, Clement et al. 1994, 1996). EM studies in tomato have shown that the wall separating the adjacent tapetal cells and between tapetum and sporogenous cells are simple, fibrillar and includes a distinct middle lamella (Polowick and Sawhney 1993). During the period of callose deposition and meiosis, the wall fibrils loosen and become fibrous (Polowick and Sawhney 1993). Thus, by virtue of its extremely thin cell walls, tapetum functions as a secretory and nutritive tissue and regulates the supply of assimilate into the anther locule (Bedinger 1992, Loukides et al. 1995). Therefore, it is natural to assume that thick cell walls of meiocytes and tapetum might inhibit the transport of assimilates into the anther locule.

Meiocytes are self-sufficient during meiosis

On the contrary, in spite of having thick cell walls, meiocytes and tapetum in S. plicata show sustained growth. Possibly, the sustained growth of meiocytes during meiosis may be the result of occurrence of storage carbohydrates in them. It appears that the assimilates received by the sporogenous cells are not only consumed for their own growth but are also utilized for the biosynthesis of starch grains. Presence of storage carbohydrates and thick walls in meiocytes indicate their functional adaptation to serve as storage cells. Reduction in the storage carbohydrates at the conclusion of meiosis suggests the utilization of reserve metabolites by the meiocytes.

Invariably tapetum attains peak metabolic activity during meiosis. This is evidenced by its rich contents of RNA and total proteins (Present study) and also DNA, histones and ascorbic acid in other plants (Bhandari et al. 1976, Chewrot and Gorska-Brylass 1981, Chapman 1987, Sheel and Bhandari 1990). But occurrence of starch storage, prior to meiosis, in the tapetum is an unexpected

Figs. 1–13. Anther development in Spathoglottis plicata. 1) Azure-B test for RNA. Sporogenous cells are rich in RNA, 2) Mercuric Bromophenol Blue test for total proteins. Sporogenous cells are rich in total proteins, 3) PAS-test for total insoluble polysaccharides. Young meiocytes (M) and tapetal cells (T) are thick walled. Starch grains are seen in meiocytes, 4) Azure-B test showing rich content of RNA in meiocytes (M) and tapetum (T), 5) PAS-test revealing starch storage in late meiocytes (M) and tapetum (T), 6) Calcofluor-white staining for cellulose. Inner face of the tapetum (T) shows autofluorescent deposition (arrow), 7) Calcofluor-white staining. Autofluorescent deposition (arrow) surrounds the meiocyte mass (M), 8) Aniline blue test for callose. Callose deposition is confined to peripheral meiocytes, 9) PAS-test. Young microspore tetrads bathing in the locular PAS-positive material. Meiocytes are characterized by persistent primary wall (arrow), 10) Aniline blue test showing callose deposition only on the peripheral microspore tetrads, 11) Old anther tested with PAS reagent. Microspores remain in tetrads and possess PAS-positive walls, 12) Calcofluor-white test. Only peripheral pollen tetrads possess autofluorescent exine wall (arrow). Inner pollen tetrads lack exine wall, 13) Azure-B test. Peripheral pollen tetrads showing green-coloured exine wall. Pollen tetrads are rich in cytoplasmic RNA, Measurement bars are 15 μm (Figs. 1, 2), 12 μm (Figs. 6–8, 10, 11) and 10 μm (Figs. 3–5, 9, 12, 13).
novelty in S. plicata. Usually tapetal cells lack starch storage because proplastids present in them undergo a different programme of development from those present in the microspores (Pacini et al. 1992). Microspore plastids differentiate into amyloplasts whereas in the tapetal cells they differentiate into elaioplasts which are specialized type of chromoplasts. Tapetal cells of S. plicata are exceptional because proplastids differentiate into amyloplasts instead of elaioplasts. Thus, the temporary storage of carbohydrates in the tapetum is an example of its ability to elaborate the substances from the parent plant and to function as sink organ. The loss of starch storage in the tapetal cells during postmeiotic phase of the anther suggests its reabsorption needed for the maintenance of metabolic potential of the tapetum itself.

Presence of callose is not a pre-requisite condition for meiosis

In S. plicata, all the meiocytes, whether they are ensheathed in callosic wall or not, undergo normal meiosis and produce microspore tetrads. Apparently this contradicts the opinion of Heslop-Harrison and Mackenzie (1967), Knox and Heslop-Harrison (1970), Southworth (1971) and Shivanna and Johri (1985) who claim that the presence of callosic wall is a pre-requisite condition for the induction of meiosis. According to them callose functions as a ‘selective molecular filter’ and creates a congenial internal environment, that triggers the induction of orderly progress of meiosis, by preventing the transport of high Molecular weight substances. But this contention does not hold good for the anthers of Pergularia daemia (Vijayaraghavan and Shukla 1977), transgenic tobacco (Worrall et al. 1992) and Ceratophyllum (Takahashi 1995) where normal meiosis occurs in the absence of callose. Even, its role as a ‘selective molecular filter’ is doubtful because in Beta vulgaris cerium perhydroxide, a highly electron-dense material, is shown to pass through callose (Rodriguez-Garcia and Majewska-Sawka 1992). Delmer (1987) and Worrall et al. (1992) are of the opinion that in the absence of clear evidence about its role, anther callose is to be regarded as non-functional and ‘accidental’ product induced by the increased calcium ion concentrations associated with other cellular processes.

In spite of occurrence of normal meiosis in the absence of callose meiocytes, results of the present study on S. plicata does not support the views of Delmer (1987) and Worrall et al. (1992) that callose is totally non-functional in the anther. Presence of callose over the surface of meiocyte mass and formation of thick cell walls around each meiocyte suggest that isolation of meiocytes from the surrounding sporophytic tissues is indispensable requirement for the induction of meiosis. Thus, in S. plicata both callose and thick cell walls of meiocytes function as apoplast.

Formation of pollinium is multi-factorial

In S. plicata disintegration of callose does not result in the separation of microspores from tetrads. Thus, the pollinium in S. plicata consists of aggregation of microspore tetrads. Mechanism of pollen aggregation into pollinium involves several factors. In many pollinium producing plants the aggregation of pollen grains results from the synthesis of pollen coat molecules (pollenkitt, trypbine, elastoviscin) in the tapetum (Fitzgerald et al. 1994). In S. plicata also, at meiocyte stage, tapetum secretes an autofluorescent material on its inner tangential surface. Subsequently, this material extrudes from the tapetal surface and deposits on the surface of meiocyte mass completely surrounding it. This way, tapetally-derived material effectively holds the entire mass of meiocytes and their meiotic products together. Secretion of autofluorescent material continues even in the nearly mature anther after the formation of exine wall in the peripheral pollen tetrads. Although the nature of autofluorescent material is predicted to be sporopollenin (Audran and Willemse 1982), by its initiation and duration of its synthesis, presence of other compounds also has been envisaged. According to Cave and Bell (1974) the secretion consists of materials like sporopollenin, lignin, cutin or lipids. According to Fitzgerald et al. (1993, 1994) the pollen coat is primarily lipidic. While the sporopollenin precursors are utilized for the synthesis of exine wall, the other compounds con-
tribute to the formation of pollen coat that functions as a future pollen glue material. Because of continuous turnover of water (supplied by vasculature and removed by transpiration) in the anther locule, the glue passively oozes down between tetrads (Fitzgerald et al. 1994). When flower bud opens the pollen glue becomes hard by polymerization brought out by the exposure to air and light. Operation of similar mechanism can also be envisaged in S. plicata. As suggested by Fitzgerald et al. (1993, 1994) secretion of glue material prior to meiosis helps all the pollen tetrads to have pollen coat.

The PAS-positive material, present in the anther locule at young microspore tetrad stage, also serves as a temporary means to hold the microspores in aggregation. Since it appears subsequent to the loss of starch storage in the meiocytes, the locular PAS-positive material is not a product of tapetal secretion. The coincidence between disappearance of locular PAS-positive material and formation of microspore wall suggests that the former is chiefly nutritive in function rather than a pollen glue material. The persistence of meiocyte wall is another cause responsible for holding microspores together in tetrads. This supports the view of Worrall et al. (1992) that materials other than callose, such as cellulose, are also capable of holding the microspores in tetrad condition.

Exine formation is aberrant in the absence of callose

There are contradicting reports on the role of callose in the determination of exine formation. Generally, exine initiation occurs while the microspores are still encased within the callose. It is proposed that callose serves as a framework to provide a template or mold for exine wall formation (Shivanna and Johri 1985). According to Vijayaraghavan and Shukla (1977) callose serves as a source of glucose and/or as a stress factor needed for compression and flattening of the upper ends of the rod-like probaculae to form tacti. In Pergularia daemia failure or poor formation of microspore wall is linked to the absence of callose around tetrads (Vijayaraghavan and Shukla 1977). In Epacridaceae members, scanty exine, without any elaboration, develops where callose deposition is less (Ford 1971). On the contrary, in the anthers of space-flight grown Arabidopsis thaliana, well-developed, normal-looking exine is formed in spite of poor deposition of callose (Kuang et al. 1995). In Poinciana gilliesii (Skavarla and Rowley 1987), Hibiscus syriacus (Takahashi and Kouchi 1988), Caesalpinia japonica (Takahashi 1989, 1993) and Bougainvillea spectabilis (Takahashi and Skavarla 1991) it is implicated that exine pattern is determined, not by callose, but by the mosaic differentiation of the plasmamembrane. But the results of present study support the role of callose in the determination of exine. In S. plicata exine formation is observed only at the sites where callose is previously deposited. The central pollen tetrads lack callose and so exine wall.

Exine wall with its structures and surface substances functions as an adaptation when pollen is exposed to varied kinds of environmental factors. Exine possesses wide range of proteins and enzymes, glycoproteins, carbohydrates, lipids, pigments and allergens (Mascarenhas 1975, Shivanna and Johri 1985). These exine components along with intine-held materials play a role in the incompatibility phenomenon. Absence or poor development of exine, as in male sterile anthers, leads to pollen abortion (Horner Jr. and Rogers 1974, Horner Jr. 1977, Graybosch and Palmer 1987, Theis and Robbelen 1990, Hegde and Isaacs 1992, Katti et al. 1994). But the fragmentary exine wall in the peripheral pollen tetrads and its absence in the inner pollen tetrads in S. plicata does not affect the survival of pollen grains and the process of sexual reproduction. In fact the absence of sporopollenin in the walls of inner pollen tetrads is advantageous because, as suggested by Yeung (1987), such condition increases the chances of pollen germination and also helps in pollen aggregation. If all the pollen grains in the aggregation possess an elaborate exine it might disrupt the existing connections within the tetrads. Further, the formation of exine wall only on the outer surface of peripheral pollen tetrads satisfies the need for the compatibility and incompatibility part of pollen-pistil interaction, because on dispersal, it is only the peripheral pollen tetrads, not the inner ones, come in contact with stigmatic tissue.
Thus, irregular deposition of callose, starch storage in the thickwalled tapetum and meiocytes, non-separation of microspores from tetrads and restricted formation of exine, which are regarded as abnormal features in the majority of the angiosperm anthers, are adaptations associated with the formation of pollinium is *S. plicata*.

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