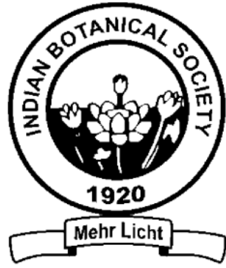

CALLOSE: DISTRIBUTION, CHEMICAL STRUCTURE, BIOSYNTHESIS AND ROLE IN REPRODUCTION

S.V.S. CHAUHAN

Academy of Life Sciences, 8/13 I Kaushalpur
Bye Pass Road, Agra-282005
e-mail : svs350@rediffmail.com;
svsc16@gmail.com

Respected Dr. Singh, senior botanists of the country and young participants, At the very outset I wish to express my sincere thanks to the Executive Council of Indian Botanical Society for electing me the President of this oldest plant science society of the country.

Callose (cal·lose -kal′ōs′) is wide spread as an important cell wall constituent in a wide variety of plants. It is a plant polysaccharide present in the form of polymer of β-1, 3-glucan linkages with some β-1, 6-branches and it is different from cellulose. Callose has been reported on algae; in the trumpet, the Laminariaceae; in stem hairs of species of *Borago, Vitis, Geranium* and of *Osmunda* and *Blechnum*; in *Cuscuta* haustoria and in penetrated host cells; in root hairs; in cystoliths in species of the Urticaceae, Moraceae, and Ulmaceae; in pollen-mother cell walls and parenchyma cells in irregular accumulations in an apparently unorganized manner, often result of parasitic attack; in pits of young tracheids; in laticifers of *Allium cepa* and *Hevea brasilliensis;* in the endodermis of Equisetaceae and Filicineae; and in pollen grains, pollen tube walls and as plugs in pollen tubes. A substance having the properties of callose was demonstrated under certain conditions in the pits of parenchyma cells. A special callose wall around the zygote functions to preserve the genetic isolation of this cell from maternal and endosperm tissues of different genotype. The callose wall is also surrounding the generative cell in a transitory manner, which breaks down and the generative and vegetative cells are separated by membranes of these cells. Callose is a multiple player in plant growth and development and plays an essential role during pollen development, exine formation, pollen germination, pollen tube growth, fertilization, plasmodesmetal regulation and cell plate formation. The most important function of callose is the formation of exine. Absence or premature dissolution or persistence (lack of dissolution) of callose surrounding the pollen mother cells (PMCs) leads to pollen sterility. Callose surrounding the PMCs provides a template or mould for the formation of the species-specific exine sculpturing pattern. In several species exhibiting sporophytic self-incompatibility, there is complete inhibition of pollen germination on the stigmatic surface. The inhibition or rejection is caused by the deposition of callose in the germ-pore or at the tip of the pollen tube. The stigmatic papillae also produce a lenticular plug of callose at their tips. Abnormalities in pistil development causing low seed-set are known to be due to abnormal patterns of callose deposition.

Callase is synthesized during a variety of processes in plant development and/or in response to multiple biotic and abiotic stresses. It is now generally believed that callose is synthesized by a class of enzymes, termed callose synthases and that it is degraded by β-1, 3-glucanases. Molecular and genetic studies in Arabidopsis have been used to identify a set of genes that are involved in the biosynthesis and degradation of callose. The Arabidopsis genome contains 12 callose synthase (CalS) genes, also known as glucan synthase-like (GSL) genes which encode the catalytic subunit of callose synthesis. In this mini review an attempt has been made to highlight the callose distribution, chemical nature, functions and biosynthesis

Callose was first detected almost 125 years ago on sieve plates of phloem elements, around pollen mother cells, in pollen grains, and in pollen tubes on the basis of its specific staining with aniline blue. The chemical structure of callose was characterized by Aspinall and Kessler (1957). Callose is wide spread in higher plants, in which it is a component of specialized cell walls or cell-wall-associated structures at particular stages of growth and differentiation (Stone and Clarke 1992).

Interest in callose was displayed by the students of phloem (Esau 1939, 1950, Crafts 1951) since sieve tubes contain an abundance of the substance. The mycelial walls of many fugal attacks are known to induce callose formation in host cells (Thomas 1928, Rawlins and Takahashi 1952). In addition to its presence

Presidential address delivered at the 34th annual conference of the Indian Botanical Society held at Lucknow University, Lucknow, during Oct. 10-12, 2011

in sieve tubes, callose has been reported on algae (Kuster 1903); in the trumpet, the Laminariaceae (Oliver 1887); in stem hairs of species of *Borago, Vitis, Geranium* (Mangin 1892) and of *Osmunda* and *Blechnum* (Gardiner and Ito 1887); in *Cuscuta* haustoria and in penetrated host cells (Thoday 1911); in root hairs (Ridgway 1913); in cystoliths in species of the Urticaceae, Moraceae, and Ulmaceae (Mangin 1889); in pollen-mother cell walls and parenchyma cells in irregular accumulations in an apparently unorganized manner, often result of parasitic attack (Mangin 1890); in pits of young tracheids (Gardiner 1885); in laticifers of *Allium cepa* (Rendle 1889); and of *Hevea brasilliensis* (Spencer 1939); in the endodermis of Equisetaceae and Filicineae (Poirault 1891); and in pollen grains, pollen tube walls and as plugs in pollen tubes (Mangin 1892). A substance having the properties of callose was demonstrated under certain conditions in the pits of parenchyma cells (Currier and Strugger 1956).

Distribution: Currier (1957) has reviewed the reports on the distribution of callose in plant cells. Callose has been reported in:

1. Algae (Kuster1903).
2. Trumpet (Laminariaceae) (Oliver 1887).
3. Stem hairs of species of *Borago, Vitis, Geranium* (Mangin 1892) and of *Osmunda and Blechnum* (Gardiner and Ito 1887).
4. *Cuscuta* haustoria and in penetrated host cells (Thoday 1911).
5. Root hairs (Ridgway, 1913).
6. Cystolith in species of Urticaceae, Moraceae and Ulmaceae (Mangin 1889).
7. Pits of young tracheids (Gardiner 1885).
8 Sieve tubes contain an abundance of callose (Esau 1939, 1950; Crafts 1951).
9. In laticifers of *Allium cepa* (Rendle 1889) and *Hevea brasilliensis* (Spencer 1939).
10. Endodermis of Equisetaceae and Filicineae (Poirault 1891).
11. In zygote wall of *Chlamydomonas monoica* (Bai and VanWinkle-Swift 2000).
12. The presence of callose was reported for the first time (Schuette *et al.* 2009) in the spore exine of moss *(Physcomitrella patensi).*

Chemical Nature: The chemical nature of callose was earlier disputed. Most workers considered it to be related to cellulose. Analyses by Mangin (1910) of mycelium of *Bornetina corium*, a root parasite of *Vitis,* revealed glucose as the hydrolytic product of callose; analysis of *Ficus* cystolith callose by Eschrich (1954) similarly indicated a d-glucose polysaccharide. Sykes (1908) considered it a hydrated form of cellulose. Earlier Nageli (1861) and recently Salmon (1947), thought sieve tube callose to be proteinaceous in nature on the basis of staining tests. According to Currier (1957) in view of the paucity of detailed analytical reports and the possibility that several kinds of "callose" exist, it was prudent at that time to speak merely of "callose substance". Now it is well known that it is a linear homopolymer made up of β-1, 3-linked glucose residues with some β-1, 6-branches, and is termed a β-glucan. It is thought to be manufactured at the cell wall by callose synthases using UDP-glucose as a substrate and is degraded by β-1, 3-glucanases. Currier and Strugger (1956) have made aniline blue fluorescence microscopy of callose in bulb scales of *Allium cepa.* The callose appeared yellow when stained with water-soluble aniline blue. Callose in sieve tubes and in other reported locations was clearly revealed, and it also appeared in primary pit fields of parenchyma cells. It was especially evident in the anticlinal walls of *Allium* epidermis. The formation of callose, as judged by intensity of fluorescence, increased by wound stimulation. Limited at first to pit fields, spreading occurred later. Once formed, the pit callose was stable to plasmolysis, boiling water, and chemical

fixation. A major function of callose in plants is considered to be a sealing or plugging action, although there appear to be other functions. The presence of callose in parenchymatous walls is further evidence of the close relationship between sieve fields and primary pit fields

The callosic wall which covers microsporocyte mother cells during meiotic division has been studied using different fluorochromes as alternatives to the widely used aniline blue by Alche and Rodriguez-Garcia (1997). They have confirmed that both acridine orange and 4′, 6′ diamidino-2-phenylindole (DAPI) produce a fluorescent response to callose which is comparable in specificity and intensity to that of aniline blue: therefore, they can be used to study callose wall formation. Staining properties of these fluorochromes, as well of those of curcumin and sirofluor, reported earlier as fluorescent stains for callose, are discussed. We also discuss the efficacy of the combined use of sirofluor and DAPI to study particular aspects of the deposition of callose.

Quantifying callose in the roots in forest soils is hampered by the presence of auto fluorescent materials in the roots that disturb the measurement of callose by fluorescence spectrophotometry. Tannins in the roots cause these measurement problems. Hirano and Brunner (2006) have measured the quantity of callose in the root apices of European chestnut *(Castanea sativa)* seedlings collected in an acidified forest soil. The callose was quantified with a modified protocol which included three washing steps with polyvinylpolypyrrolidone (PVPP) before the callose was extracted. This procedure reduced the autofluorescence by about 50%. With the use of water or ethanol alone, callose could be measured in only about 15% of the root samples, whereas with the use of PVPP callose could be determined in 95% of the samples. This improved method could help to evaluate the effects of aluminium toxicity on tree roots grown in forest soils, where callose is detected as a physiological indicator. Schildknech *et al.* (2004) examined histochemically the compounds present in the epidermal mucilage of *Zea mays* L. and *Triticum aestivum* L. Tests for lignin, lipophilic compounds, pectin, callose, and cellulose detected only the two latter compounds. Callose occurred sporadically as interrupted deposits in the epidermal mucilage and was detected in only a few plants. Cellulose was the main compound of this layer.

Function: Leubner-Metzger and Meins (1999) have reviewed the functions and regulation of plant ß-1, 3-glucanases (PR-2). In almost all higher plants each individual microspore of the tetrad is surrounded by a thick callose wall composed of a β-1, 3-glucan, which is laid down between the cellulose cell wall and the plasma membrane. At a critical stage of microspore development, the callose wall of the tetrads is degraded by callase activity, which is secreted by the tapetal cells. The microspores are then released into the anther locule where they develop into mature pollen grains. Although the callose wall is essential for production of fertile pollen, its function is unknown. Proposed functions include physical and chemical isolation of the developing gametes from sporophytic tissues, mechanical isolation of the meiocytes and tetrads, protection from environmental and osmotic stress, and formation of the pollen cell wall (Bucciaglia and Smith 1994).

Shivanna (2003) has described three important functions of callose wall separating MMC and microspores. These are: 1. Isolation is necessary for the MMC to embark on (a) transition from the suporophytic phase to the gametophytic phase and (b) expression of gametophytic genome without interference

either from other spores or from the parent sporophytic tissue. Callose wall also prevents the entry of some molecules e.g. thymidine (Heslop-Harrison and Mackenzie 1967), phenylalanine (Sowthworth 1971) and fluorescein diacetate (Knox and Heslop-Harrison 1970a) while it allows the entry of sucrose (Southworth 1971). 2. Isolation of young microspores by callose wall is essential for the formation of exine (Waterkeyn and Bienfait 1970). 3. The breakdown of callose wall provides soluble carbohydrates as the source of nutrients to the developing microspores.

Recently, Chen and Kim (2009) have highlighted the recent progress in understanding callose structure and callose synthase; role of callose in plant growth and development and the role of callose during pollen development; role of callose in cell plate formation; plasmodesmetal regulation; callose deposition in response to stress; and biosynthesis and degradation. They have also discussed the future challenges of unraveling the mechanism(s) by which callose synthase operate.

ROLE IN REPRODUCTION

Microspore mother cells (MMC): Formation of a synchytium of MMC in each anther locule is an important feature of meiosis. The syncytium develops during prophase and individual MMC and microspores subsequently become isolated by callose wall (Heslop-Harrison 1966, 1971, Whelan 1974). Callose is deposited around each MMC on the onset of meiosis and this followed by the dissolution of the primary cell wall. Callose wall has several gaps at the sites of plasmodemeta and these gaps enlarge to make big cytoplasmic channels joining with other MMC. These channels help in the exchange of cytoplasm and cell organelles and all the MMC of a syncytium forms a single entity. In subsequent stages of development, the callose wall around MMC becomes continuous and formation of microspore tetrads and each microspore is separated by callose wall. The severance of plasmodesmatal connections by deposition of a special wall is analogous to the blockage of cytomictic channels by deposition of the sporocyte special callose wall during the first meiotic prophase in microsporogenesis (Heslop-Harrison 1966a,b).

Bansal and Chauhan (In Press) have observed the presence of plasmodesmetal connections in the abnormally thick callose wall separating microspores of a tetrad in tomato *(Lycoperisicon esculentum)* plants grown under low temperatures. However, these plasmodesmetal connections fail to disappear and the microspores fail to show initiation of exine formation. On the other hand, in the anthers of plants grown at normal temperatures, the plasmodesmetal connections disappear and the exine formation on microspores in tetrads is initiated.

The role of callose during pollen development: Callose plays an essential role during pollen development, pollen germination, pollen tube growth and fertilization. Angiosperm pollen grains develop in close association with the tapetum, a layer of cells that lines the anther locule (Chasan 1992). Many details of the interaction between tapetal cells and developing pollen have yet to be elucidated, but it is clear that the tapetum is a source not only of nutrients for the microsporocytes but also of substances that aid pollen development in other, more specific ways (Pacini *et al*. 1985). For example, the tapetal cells secrete some of the substances that assemble into the outer wall (exine) of the pollen grain. The tapetum is also responsible for the degradation of the special callose (a p-1, 3-glucan) wall that lies beneath the cellulose wall of the microsporocytes. The microsporocytes synthesize the callose layer

before meiosis begins, and it surrounds them throughout meiosis. The subsequent dissolution of the callose wall is thought to be carried out by a tapetally secreted endo-p- 1, 3-glucanase enzyme known as callase, whose activity rises dramatically at the end of meiosis (Steiglitz *et al*. 1977). The functions the callose wall and its breakdown serve in microsporogenesis are not known, but the existence of several cytoplasmic male sterile (CMS) lines of *Petunia* in which the timing of callose breakdown is perturbed (Frankel *et al*. 1969) suggests that both the presence of the callose wall and its destruction at the appropriate time are essential for normal pollen development. Separation of MMC by callose wall is essential for normal development of pollen (Scott *et al*. 1991). Absence of callose or premature dissolution of callose surrounding the MMC leads to pollen sterility is well known in several plants. In several cytoplasmic male sterile (CMS) lines, there is abnormal development of callose wall around the MMC. In the anthers of CMS sorghum there is complete absence of callose wall and this results in the degeneration of MMC devoid of any wall (Overman and Warmke 1972). Izhar and Frankel (1971) and Bino (1985a, b) in CMS lines of *Petunia* have observed earlier or delayed activity of enzyme callase, responsible for callose degradation is either activated earlier or delayed as compared to that takes place in fertile anthers. In either case this untimed degradation of callose results in pollen abortion. The enzyme callase functions under the direct control of pH of the anther locule and in fertile anthers the pH is normally at 7, but when it drops to 6, the callase activity is optimal. In *Capsicum annuum,* sterility is apparently associated with the mistiming of or lack of callose dissolution. The callose wall continues to surround the microspores and even persists on the aborted pollen (Horner and Rogers 1974). Nanda and Gupta (1974) have recorded the absence of callose in the anthers of male sterile Allium cepa. According to them, it is perhaps due to excessive formation of callase. Ahokas (1980) in CMS anthers of barley has observed the deposition of thick sporopollenin on the tapetal cell surface and on the exine of aborted microspores.

In the anthers of *Petunia* and lily, expression and secretion of callase activity is under strict developmental control (Stern 1973, Frankel *et al*. 1969). The callase complex of lily consists of a 32 kDa endo-βGlu and a 62 kDa exo-type β-1, 3-glucanase (Steiglitz 1977). The endo-type enzyme seems to be most important for the degradation of the callosic walls, while the exotype β-1, 3-glucanase is involved in the further hydrolysis of released oligosaccharides. Alterations in the timing of β-Glu expression, or failure to express βGLU, leads to abnormal dissolution of the tetrad callose walls, which has been shown to be a primary cause of male sterility in cytoplasmic male-sterile lines of petunia (Izhar and Frankel 1971), sorghum, (Warmke and Overman1972) and soybean (Jin *et al.* 1997).

Stieglitz and Stern (1973) have observed the specialized walls consisting of callose, presumably a polymer of β-1, 3-glucose in *Lilium* microsporocytes. At the termination of meiosis, the walls undergo sudden and rapid dissolution resulting in the liberation of young microspores. This event is correlated with a sharp peak in activity of β-1, 3-glucanase. The activity is localized in the somatic region of the anther with less than 7.3% of the total activity associated with the microsporocytes at any time during meiosis. Thus, β-1, 3-glucanase appears to be an enzyme necessary to the development of meiotic cells but whose action is mediated by the surrounding somatic tissue

rather than by the meiotic cells themselves.

Light microscopic studies were carried out on the anthers of *Allium tuberosum* Rottl. and *Cyclamen persicum* Mill. by Bhandari *et al.* (1981). They observed that the callose deposition was initiated within the primary wall round each microspore mother cell during early prophase of meiosis I and was of maximum thickness at the tetrad stage. The original cellulosic wall, contrary to earlier reports for other species, persisted around the microspore mother cells until the late tetrad stage in both species. In *C. persicum*, it dissolved to release the callose-encased tetrads, and in *A. tuberosum,* it dissolved simultaneously with callose at the time of release of microspores.

Binh and Hendrychova-Tomkova (1982) have observed microscopically the squash preparations of anthers from pollen fertile and sterile plants of sweet pepper *(Capsicum annuum* L. cv. Severka) that callose envelopes of microsporocytes, stained specifically with resorcin blue. During normal course of microsporogenesis in fertile plants the envelopes remained intact up to the stage of microspore tetrads. Then callose begins to dissolve and that from individual microspores towards the envelope periphery. In sterile analogues of the same cultivar the callose breakdown occurred precociously, usually in the course of the second, but sometimes as early as the first meiotic division of PMCs. Having completed meiosis sporadic microsporocytes formed microspore tetrads. Most PMCs contained an undivided four-nucleate protoplast rimmed with a narrow or wider unstained zone of dissolved callose. In certain cases more condensed callose septa pointing to the furrows on the surface of the PMC protoplast were well-observable in this lytic zone, as a residuum of normal mechanism of tetradogenesis.

Jos *et al.* (1990) have recorded diverse types of male sterility in cassava. The male sterile clones were more frequent among the indigenous than exotic genotypes. Though the male sterile clones of indigenous origin showed empty anthers, those of exotic origin, except Ce 539, had 100% sterile pollen of small size. Male sterility among the indigenous strains resulted from non-separation of microspores from the tetrads, which may be due to the suppression of callase activity.

Worrall *et al.* (1992) confirms that premature callose dissolution in transgenic tobacco is, indeed, sufficient to cause male sterility. To investigate the role of callose in microsporogenesis, they have created transgenic tobacco whose tapetal cells secrete P-1, 3-glucanase much earlier than usual, i.e., when microsporocytes are just beginning to enter meiosis. The tapetally expressed callase was not isolated, but several other p-l, 3-glucanase genes have been cloned from tobacco (Ward *et al.* 1991). These genes show complex patterns of regulation: glucanases can be activated by developmental signals, hormones, and/or as part of the hypersensitive response to pathogen infection or wounding. Although some p-1, 3-glucanases are basic and others are acidic and their specific activities vary widely, all are able to cleave p-1, 3-linkages between glucose molecules. To construct their hybrid gene, Worrall and coworkers used the gene for a well characterized, pathogenesis-related basic glucanase (Shinshi *et al*. 1988). Evidence that this glucanase would be able to cleave callose came from R. Scott's unpublished observation that intercellular fluid from tobacco leaves treated with salicylic acid, which induces a hypersensitive response, is able to cleave the callose wall of tetrads *in vitro*. Because this particular glucanase is targeted to the vacuole rather than secreted, the authors created a

secretable form of the enzyme by removing sequences coding for the C-terminal peptide that, by analogy with other vacuolar proteins, directs vacuolar targeting. To achieve the inappropriate expression of this modified glucanase gene, the authors cloned it downstream of three different heterologous promoters: the cauliflower mosaic virus 35s promoter and A3 and A9, two *Arabidopsis* promoters that are activated in the tapetum of anthers containing microsporocytes that are just beginning to enter meiosis. Transgenic plants carrying constructs in which the A3 or A9 promoter drives the expression of the modified glucanase gene exhibit varying degrees of male sterility. The anthers of the most severely affected plants are small and apparently lack pollen grains entirely. By staining the contents of anthers at various developmental stages with aniline blue, which makes callose fluoresce, the authors have found that the callose walls of microsporocytes in transformed plants appear normal. By early meiosis, however, the microsporocytes are no longer surrounded by a callose wall. Despite the lack of callose, microsporogenesis proceeds on schedule, and microspores are released from the tetrads at the same time as in wild-type plants. Shortly after their release, most of the microspores rupture. Pollen development is normal in transformants carrying the 35s-glucanase construct, even though the modified glucanase accumulates to levels comparable to those in the A3 and A9 transformants. This result is in accord with previous observations that suggest that the 35s promoter has little or no activity in tapetal cells or microsporocytes (Plegt and Bino 1989). The loss of the callose wall is not the sole abnormality in the anthers of transgenic plants. The tapetal cells, too, appear highly unusual: they are larger than normal and extend into the locule. This observation suggests that tapetal hypertrophy, which is often observed in male sterile plants, can be a secondary effect of aberrant microspore development. The striking effect of abnormal microspore development on tapetal proliferation points out the intimate association of the two cell types and shows just how problematic it can be to interpret the primary defect in a male sterile phenotype. Why does the premature destruction of the callose wall so dramatically affect microspore development? Several functions have been proposed for this unusual cell wall, including keeping the microsporocytes or the microspores themselves separated from one another so they can disperse as single cells, holding the tetrads together, temporarily isolating the microsporocytes from the influence of sur rounding cells, and providing a surface upon which the outer wall, or exine, is assembled. Degraded callose could also provide a source of glucose for post-meiotic development. The results presented in this issue indicate that the loss of callose does not prevent the microspores from separating at the conclusion of meiosis, nor does it cause the tetrads to fall apart prematurely. Callose may, however, be important for exine wall formation. The smooth outer wall surface known as the tectum is absent, and the wall is very irregular. The lack of a properly formed outer wall may cause the microspores to burst after they are released into the hypotonic environment of the locule.

On the other hand, Periasamy and Amalathas (1991) have observed the absence of callose and tetrad in the microsporogenesis of *Pandanus odoratissimus* with well-formed pollen exine. According to them, in the microsporocytes of *Pandanus odoratissimus,* cytokinesis is successive with centrifugal cleavage in both the meiotic divisions. The dyads move apart from each other after the first division and the microspores likewise after the second division, so that only monads are formed at the end of meiosis. Although no trace

of callose wall is found at any stage around the microsporocyte or microspore, fertile, monocolpate pollen with well-developed, spinescent exine develops, and is shed at the two-celled stage.

Similarly, Anger and Weber (2006) are of the opinion that the lack of callose does not influence fertility in *Arum alpinum* but could be the reason for the formation of uncommon pollen wall, from where a sporopolleninous ektexine is missing. The outermost pollen wall layer is formed by the endexine which is covered by polysaccharidic ornamentation elements. An ontogenetical investigation was made by Anger and Weber (2006) in this species to clarify pollen-wall development, with special reference to callose and pollen-wall development. At all the stages of pollen-wall formation callose was missing. Microspores were released from the tetrad by invagination of the amoeboid tapetum. The polysaccharidic wall ornamentations were formed by the tapetum. According to Anger and Weber (2006) there appears to be no truth in the dogma that callose is essential for microspore separation and release from the tetrad.

Zygote: Deposition of new wall material around the zygote in the first 2 days after fertilization has been observed in ultrastructural studies of cotton embryogenesis by Jensen (1968, 1974). This wall was described as thickest at the micropylar end and thinnest at the chalazal end. Changes accompanying wall deposition included the shrinkage of the zygote to half the original egg volume, apparently by decrease in the vacuolar volume; appearance of prolific tubule containing endoplasmic reticulum; relocation of plastids; starch accumulation in the plastids; formation of giant polyribosomes and the appearance of large numbers of new ribosomes. These correlated changes were interpreted to indicate a period of activation or conversion in cell function. Synthesis of a new ribosome population has also been observed at the sporophyte/gametophyte phase change (Mackenzie *et al.* 1967; Dickinson and Heslop-Harrison, 1970; Williams *et al*. 1973). The presence of a continuous PAS-positive wall around the zygote has also been reported by Olson and Cass (1981) for *Papaver nudicaule.* Since it appears that the egg wall is likely to be discontinuous, some post-fertilization wall synthesis is implied. A special callose wall around the zygote may function to preserve the genetic isolation of this cell from maternal and endosperm tissues of different genotype during initiation of the new sporophyte development phase. In addition, it may function similarly to 'wound callose' to seal off the zygote from the adjacent degenerating synergid. Alternatively, as suggested by Jensen (1968), the special callose wall may be involved in determining the precise shape and volume of the zygote, and in controlling osmotic balance between this cell, the now developing endosperm, and other adjacent cells. An analogous alternative function for the microsporocyte callose special wall has been proposed by Knox and Heslop-Harrison (1970): apart from possible involvement in direct filtering out of macromolecules by virtue of low callose permeability, the wall may function to restrain cell expansion physically and therefore limit movement of materials into cells to that possible by exchange only. The assumption of a spherical shape by microsporocytes after callose wall deposition, and the immediate expansion of young microspores on release from meiotic tetrads, does suggest a degree of physical restraint imposed by the callose special wall. In developing pollen, the generative cell becomes temporarily isolated from the cytoplasm of the vegetative cell by a plasmodesma-free, callose wall (Gorska-Brylass 1967, Heslop-Harrison 1968, McConchie 1983). This wall is at first

hemispherical, cutting off a lens-shaped generative cell against the vegetative cell wall. Subsequently, as callose deposition continues to cover the entire generative cell surface, the cell assumes a spherical shape and separates from the vegetative cell wall. In parallel with possible functions suggested for microsporocyte and zygote special walls, the generative cell callose wall may isolate the future gamete genome from transient activating molecules in the highly metabolic vegetative cell cytoplasm (Heslop-Harrison 1968). A further possible function may also be envisaged: the physical restraint imposed by the wall during a period of osmotic expansion of the generative cell may cause it to become spherical and to aid the detachment from the pectocellulosic intine, to which it is initially attached. The early appearance of a special wall around a pro-embryonal cell may be a feature extending to certain instances of somatic embryogenesis *in vitro*. Street and Withers (1974), for example, described and illustrated the isolation of the basal pro-embryonal cell of induced embryoids of *Daucus carota* from surrounding cells by a thick, plasmodesma-free wall.

The occurrence of callose deposits in the unfertilized ovules of abscised pistils of *Rhododendron* indicates that the phenomenon may be more general, and may provide a useful guide for the occurrence of fertilization during breeding programmes. A zygote callose special wall therefore appears to be an important developmental marker in embryogenesis, adding a new and significant finding to the list of processes of reproduction in which callose is involved (Dumas and Knox 1983). In plant breeding, callose is also known as a useful marker of ovule viability in certain species. Callose deposits become general throughout the ovule when it becomes non-viable, presumably because of senescence. This has been demonstrated in cytological studies of the breeding systems of various stone fruits (see Anvari and Stosser 1978, 1981, Stosser and Anvari 1982, Martinez-Tellez and Crossa-Raynaud 1981).

Of particular relevance are the observations of Wilms *et al.* (1983) on development of special walls around the nucellar 'zygote-resembling' cells, which give rise to adventive embryos in *Citrus*. These cells lay down thick new walls within the original primary walls, severing plasmodesmatal connections and isolating themselves from neighbouring nucellar cells, which later degenerate. Although cytochemical tests were not applied, the ultrastructural image of the wall of these zygote-resembling cells is similar to that of other examples of callose deposits (Dickinson and Lewis 1975). Possibly, the special wall of *Citrus* zygote-resembling, embryogenic cells forms an isolating genetic screen that allows a phase change from the differentiated nucellar condition to the re-initiation of sporophyte morphogenesis. As suggested for the zygote wall in *Rhododendron* and *Ledum*, the special wall of *Citrus* adventive zygotes may also function to isolate these viable meristematic cells from adjacent degenerating cells.

Williams *et al*. (1984) have observed a callose wall around the zygote two days after fertilization in *Rhododendron* spp. and *Ledum groenlandicum*. According to them, the periodic acid/Schiff-positive, aniline blue-fluorescence (ABF) positive callosic wall is initiated adjacent to the degenerating synergid, extends to cover the entire zygote surface, and remains visible during the initiation of embryogeny as the zygote elongates before the first pro-embryonal division. Unfertilized ovules show eventual callose deposition in the ovule wall cells during senescence in undeveloped abscising pistils, but development of callose within the embryo sac

was not seen. Williams *et al*. (1982) in their previous work observed the fluorescent zygote wall in several incompatible interspecific crosses of *Rhododendron*, and interpreted at that time as a possible anomaly of the pollen-tube tip within the embryo sac, or a callose deposit within the ovum stimulated by incompatible pollen tube/embryo sac interaction. Subsequent work with a greater range of compatibly pollinated materials has shown this phenomenon to be characteristic of normal early post-fertilization development. Embryological studies on compatible and incompatible interspecific crosses in which pollen tubes enter the ovules (Kaul, Rouse and Williams, unpublished), have shown that abortion in incompatible crosses may occur after apparently normal fertilization and early pro-embryonal development. Williams *et al*. (1984) are of the opinion that, the angiosperm zygote after fertilization begins a new programme of sporophyte development based on the diploid genotype established at the time of gamete fusion. In close proximity to maternal and endosperm cells of different genotypes, this cell must initiate expression of a new genotype in a new developmental sequence. The process has conceptual similarities to the reciprocal sporophyte—gametophyte phase change, which occurs at the time of sporogenesis and is accompanied by deposition around the sporocytes of an aniline blue fluorescence (ABF)-positive wall of callose (De Sloover 1961, Waterkeyn 1961, 1962, 1964, Heslop-Harrison 1964, 1966a, Rodkiewicz 1967, 1970 Rodkiewicz and Gorska-Brylass 1968, Jalouzot 1970, Knox and Heslop-Harrison 1970, Russell 1979 and see review by Kapil and Tiwari 1978). This wall is composed predominantly of l, 3-/3-glucans or mixed 1, 3-/3- and 1, 4-jS-glucans (Clarke and Stone 1984). Temporary deposition of callose special walls around the sporocytes and their products, and the consequent severance of protoplasmic connections between them, has been suggested to confer some degree of genetic independence between haploid sibs and diploid parent tissues (Heslop-Harrison 1964, 1966a, Rodkiewicz 1967, 1970, Knox and Heslop-Harrison 1970). While enclosed within the callose special wall microsporocytes and spores are not readily penetrated by various molecules (Heslop-Harrison and Mackenzie 1967, Knox and Heslop-Harrison 1970, Southworth 1971). Thus, the presence of these walls may allow the initiation of a developmental phase change without macromolecular interference from neighbouring cells of different genotype. It is not altogether clear whether this isolating function of the callose special wall is imposed by virtue of impermeability or physical restraint to uptake by expansion (Knox and Heslop-Harrison 1970).

Generative cell: The callose wall is also known to surround the generative cell in a transitory manner (Gorska-Brylass 1970, Mepham and Lane 1970, Echlin 1972). The callose wall breaks down and the generative and vegetative cells are separated by the membranes of these cells (Shivanna and Johri 1985).

Achamma and Radhamany (200) have made differential recognition of self pollen and sterility in *Hibiscus rosa-sinensis* L. *(Malvaceae)*. According to them, *Hibiscus rosa-sinensis* (Malvaceae) exhibits significant variations in the percentage of sterility/fertility in all the cultivars studied in different seasons. The bacto-agar medium suggested by Cochis (1966) was found to be successful for in vitro pollen germination. The result obtained is in line with FCR test for viability. In vivo pollen germination and pollen tube growth studies carried out to locate the site of pollen recognition and rejection after self and cross pollination showed that recognition occurred at the stigmatic surface itself. But rejection may occur in the stigma, style or in the ovary as

indicated by irregular callose depositions in stigmatic papillae, pollen grains and pollen tubes. Callose occlusions in the ovarian tissues suggested the presence of sterile ovule may also contribute the sexual sterility in this plant. Therefore, it is assumed that the reason for lack of seed set in *H. rosa-sinensis* may be due to male/female sterility and self incompatibility.

Teng *et al.* (2005) have studied the microsporogenesis and pollen development in *Leymus chinensis* with emphasis on dynamic changes in callose deposition. In this economically and ecologically important plant there is low sexual reproductivity. A variety of histochemical stains were used including Heidenhain's hematoxylin, decolorized aniline blue, DAPI and acetocarmine along with a temporary mount method. Pollen shed at three-celled stage. Callose initially appeared in the centre of the anther locule at the pre-meiotic phase, and then gradually and unevenly deposited around MMC before the commencement of meiosis. At the onset of meiosis, the accumulation of callose enclosing the MMC peaked, accompanied by the disappearance of callose in the centre of the locule. At the end of dyad stage and tetrad stage, the dyads and tetrads were surrounded by callose wall and the microspores in the tetrads were isolated by a cross cell plate composed of callose. Microspores just released from tetrads were still enclosed in callose wall, and then callose gradually disappeared in the pollen wall. Ultimately, callose almost completely disappeared from the walls of mature pollen grains. In most of the cases of meiosis of the MMCs, pollen development, and callose dynamics were normal, with only a few abnormalities observed. The results suggest that microsporogenesis, male gaemetogenesis, and callose dynamics during these processes are generally normal in this species, and that the callose wall plays an important role in the production of functional pollen grains. The small number of abnormalities of these processes that occurred likely does not adversely affect the production of viable pollen grains.

Enns *et al*. (2005) in *Arabidopsis*, several studies demonstrated that multiple GSLs (glucan synthase-like) are involved in pollen development. They have reported that GSL1 and GSL5 are necessary and that they function redundantly in pollen development and fertility. GSL1 and GSL5 are responsible for the formation of the callose wall that separates the microspores of the tetrad; the genes also play a gametophytic role later in pollen grain germination.

Two other studies provided contradictory data about the function of the GSL2 (CalS5-callase synthase) gene in exine formation during microgametogenesis and pollen viability. Dong *et al.* (2005) identified two T-DNA knockout mutants of cals5 (cals5-1 and cals5-2) and found that the cals5 mutant displayed male sterility and lacked the normal callose wall which affected the exine pattern of the microspore. Tryphine, an extracellular pollen coat, was synthesized in the mutant, but was randomly deposited as aggregates on the surface of microspores. Upon release from the tetrad, mutant microspores did not survive and the pollen wall collapsed, demonstrating that callose is required both in the synthesis of pollen wall exine and in the viability of pollen. Contrary to the report described above, Nishikawa *et al.* (2005) have isolated three additional T-DNA alleles of cals5 (cals5-3, cals5-4 and cals5-5) that similarly altered the exine deposition pattern, but which actually produced fertile pollen. Furthermore, mutation of cals5-3 resulted in formation of pollen tubes that lacked callose walls and plugs, however, these tubes could still perform fertilization, demonstrating that a structured exine is not essential for pollen development, viability or fertility. Additionally, this study demonstrated

that callose is not required for pollen functions. Thus, together, these two studies highlight the importance of generation and analysis of an allelic series of a gene under study.

Mamun *et al.* (2006) have observed that chilling during male gametophyte development in rice inhibits development of microspores, causing male sterility. Changes in cellular ultrastructure that have been exposed to mild chilling include microspores with poor pollen wall formation, abnormal vacuolation and hypertrophy of the tapetum and unusual starch accumulation in the plastids of the endothecium in post-meiotic anthers. Anthers observed during tetrad release also have callose (1, 3-β-glucan) wall abnormalities as shown by immunocytochemical labelling. Expression of rice anther specific monosaccharide transporter (OsMST8) is greatly affected by chilling treatment. Perturbed carbohydrate metabolism, this is particularly triggered by repressed genes OsINV4 and OsMST8 during chilling, causes unusual starch storage in the endothecium and this also contributes to other symptoms such as vacuolation and poor microspore wall formation. Premature callose breakdown apparently restricts the basic framework of the future pollen wall. Vacuolation and hypertrophy are also symptoms of osmotic imbalance triggered by the reabsorption of callose breakdown products due to absence of OsMST8 activity.

According to Zhu *et al*. (2008), the tapetum plays important roles in anther development by providing enzymes for callose dissolution and materials for pollen-wall formation, and by supplying nutrients for pollen development in *Arabidopsis*. They have reported the identification and characterization of a male-sterile mutant, defective in tapetal development and function 1 (tdf1) that exhibits irregular division and dysfunction of the tapetum. The TDF1 gene was characterized using a map-based cloning strategy, and was confirmed by genetic complementation. It encodes a putative R2R3 MYB transcription factor, and is highly expressed in the tapetum, meiocytes and microspores during anther development. Callose staining and gene expression analysis suggested that TDF1 may be a key component in controlling callose dissolution. Semi-quantitative and quantitative RT-PCR analysis showed that TDF1 acts downstream of DYT1 and upstream of AMS and AtMYB103 in the transcriptional regulatory networks that regulate tapetal development. They have concluded that TDF1 plays a vital role in tapetal differentiation and function.

Popova *et al*. (2008) have studies the topography and content of callose in the process of microsporogenesis as well as the formation of pollen grain in dry-valley and air-aquatic *Sium latifolium* L. plants grown under the conditions of different water regimes. An increase in the content of callose in the microspore walls at the tetrad stage and in the pollen grains of dry-valley *S. latifolium* plants was discovered based on the intensity of fluorescence of the callose and a quantitative luminescence analysis. This result was obviously associated chiefly with the function of callose in maintaining moisture under conditions of water deficit.

Nutrition: Degraded callose could also provide a source of glucose for post-meiotic development. The loss of callose does not prevent the microspores from separating at the conclusion of meiosis, nor does it cause the tetrads to fall apart prematurely. Callose may, however, is important for exine wall formation. The smooth outer wall surface known as the tectum is absent, and the wall is very irregular. The lack of a properly formed outer wall may cause the microspores to burst after they are released into the hypotonic environment of the locule.

Exine formation: The callose wall present

around the microsporocytes is believed to act as barrier or molecular filter (Heslop-Harrison 1964) and according to Waterkeyn and Beinfait (1970) it also provides a template or mould for the formation of the species-specific exine sculpturing pattern seen on mature pollen grains. Vijayaraghavan and Shukla (1977) failed to localize callose in the walls of the microspore tetrad of *Pergularia daemia*. At anthesis, the exine is thin and sparsely deposited.

Callose (β-1,3 glucan) separates developing pollen grains, preventing their underlying walls (exine) from fusing. The pollen tubes that transport sperm to female gametes also contain callose, both in their walls as well as in the plugs that segment growing tubes. Mutations in CalS5, one of several *Arabidopsis* β-1,3 glucan synthases, are known to disrupt callose formation around developing microspores, causing aberrations in exine patterning, degeneration of developing microspores, and pollen sterility (Nishikawa *et al*. 2005). They have described three additional cals5 alleles that similarly alter exine patterns, but instead produce fertile pollen. Moreover, one of these alleles (cals5-3) resulted in the formation of pollen tubes that lacked callose walls and plugs. In self-pollinated plants, these tubes led to successful fertilization, but they were at a slight disadvantage when competing with wild type. Nishikawa *et al*. (2005) have concluded that contrary to a previous report, these results demonstrate that a structured exine layer is not required for pollen development, viability or fertility. In addition, despite the presence of callose-enriched walls and callose plugs in pollen tubes, the results presented here indicate that callose is not required for pollen tube functions.

Chauhan *et al*. (2009) have induced complete pollen sterility in cotton *(Gossypium arboreum)* by treatments with ethephon (2, chloroethyl phosphonic acid) and observed that sterility was associated with tapetal abnormalities. They have recorded that the callose wall surrounding some of the microsporocytes of an anther locule degenerated prematurely, while the callose wall surrounding other microsporocytes in the same locule degenerated normally. In both these cases, the resulting microspores were sterile. However, the exine of the microspores developed from the microspore tetrads showing premature dissolution of callose wall was thin and devoid of characteristic spines. On the other hand, the microspores released from the tetrads after normal callose wall dissolution were sterile but showed more or less normal exine with spines. These observations clearly indicate that the untimely dissolution or break down of callose wall surrounding the microsporocytes not only leads to pollen abortion but also influence exine formation. According to Chauhan *et al.* (2009), their findings strongly support the view that callose surrounding the microsporocytes acts as a barrier or molecular filter (Heslop-Harrison 1964) and provides a template or mould for the formation of the species-specific exine sculpturing pattern observed on mature pollen grains (Waterkeyn and Beinfait 1970).

Pollen Germination: Sedgley (1977) has observed pollen tube growth in the pistil of the male floral stage of the avocado. Self-pollination of the male stage occurred but pollen tubes did not reach the ovary. When female and male stages were hand pollinated, fewer pollen tubes were present in the pistil of the male than in the female stage. A tube reached the ovary in only 1 of 110 male stage pistils, as compared with all female stage pistils observed. Flowers pollinated in the female stage were retained on the plant for longer than flowers pollinated in the male stage. Aniline blue-positive and resorcin blue-positive

material (callose) was not present in the pistil of flowers in the female stage but occurred in 46% of the aniline blue-stained and 30% of the resorcin blue-stained pistils in the male floral stage. By 42 h after first opening, callose was present in every pistil observed, in association with the cell walls of the stigmatic papillae, the transmitting tissue, the vascular tissue or the cortex and epidermis or a combination of these tissues. Callose occurred earlier in pollinated than in unpollinated pistils, particularly in the transmitting tissue. It is suggested by Sedgley (1977) that reduced pollen tube growth in the pistil of the male floral stage of the avocado is associated with the presence of callose.

A detailed molecular characterization of one of the suppressor lines, cs5ds1 for cals5 dominant suppressor 1 mutant was made by Huang *et al.* (2009). Similar to cals5 mutant plants, the peripheral callose wall of tetrads in the cs5ds1 suppressor plants was depleted in callose, and the mature pollen grains lacked a regular reticulate network of exine patterning. Anatomic analyses of anther development revealed that the primary cell wall enclosing the tetrad was defective in cals5, but relatively intact in cs5ds1, suggesting possible down regulation of an enzyme responsible for removal of the tetrad primary cell wall in the suppressor. Microspores of cs5ds1 proceeded normally with gametogenesis, giving rise to the formation of viable pollen grains that developed pollen tubes even though lacking callose in the wall and callose plugs inside the tubes. A T-DNA insertional mutant (cs9) was characterized at the CalS9 gene and an *Arabidopsis* transgenic line expressing the CalS5 gene. In both lines, callose deposition during pollen development was abnormal and pollen grains germinated prematurely in closed anthers. The functions of other CalS genes in Arabidopsis were also investigated. Discovery of premature pollen germination prior to anthesis in two *Arabidopsis* lines with genetic alterations in callose synthase genes has been made. Prior to anthesis in *Arabidopsis*, pollen is fully mature but does not germinate inside the anther locule. How premature pollen germination is prevented at the molecular level has not been studied. It was discovered that pollen germination takes place in the anther locule before anther dehiscence in two *Arabidopsis* lines. One of them is a T-DNA insertional knockout mutant in the CalS9 gene (cs9 mutant) and the other is a transgenic line expressing Strep-tagged CalS5 (S-CS5). These discoveries provide a solid platform for further study on the molecular mechanisms involved in the control of pollen germination. Abnormal callose deposition in the callose wall has been seen during pollen development in the S-CS5 line. Callose is an important polysaccharide that is synthesized in specialized cell walls and in response to various environmental stresses and internal cues. Although both *Arabidopsis* lines exhibiting precocious pollen germination contained genetic alterations in the CalS genes, the molecular mechanisms underlying the precocious pollen germination phenotype appeared to be different. In the cs9 line, the callose wall of pollen mother cells and tetrads contained a uniform layer of callose as in the wild type control, indicating that CalS9 is not responsible for callose wall biosynthesis. Instead, CalS9 is required for synthesis of the transient callose present during pollen germ mitosis and plays a pivotal role in positioning the MGU inside the pollen. In the S-CS5 line, the peripheral callose wall surrounding pollen mother cells and tetrads was abnormal, containing irregular patches of callose deposits. The interstitial callose wall that separated the spores within a tetrad was not affected in S-CS5 plants. The irregular callose deposits on the pollen surface in S-CS5 plants could be a potential factor that triggered precocious pollen germination in this line.) Induction of precocious pollen germination in

WT Arabidopsis anthers. Under normal growth conditions, wild type *Arabidopsis* pollen does not germinate in the anther. When WT *Arabidopsis* unopened flowers were treated with water, 1.5 mM boric acid, 37ºC-heat stress, or *Agrobacterium* infection, no precocious pollen germination was observed, suggesting that humidity, ions and infection were not sufficient to induce pollen germination. Sucrose (18%) alone was found to be a strong inducer of pollen germination and could sustain pollen tube growth in the anther. All these results clearly indicated that certain environmental factors could mimic the function of stigma signals or genetic alterations such as cs9 and rtg mutations in promoting early pollen germination inside the anther.

According to Xie *et al*. (2009) although pollen grains of wild-type *Arabidopsis thaliana* do not germinate inside the anther under normal growth conditions but they have reported two *Arabidopsis* lines that produced pollen grains able to *in situ* precociously germinate inside the anther. One of them was a callose synthase 9 (cs9) knockout mutant with a T-DNA insertion in the Callose Synthase 9 gene (CalS9). Male gametophytes carrying a cs9 mutant allele were defective and no homozygous progeny could be produced. Heterozygous mutant plants (cs9/+) produced approximately 50% defective pollen grains with an altered male germ unit (MGU) and aberrant callose deposition in bicellular pollen. Bicellular pollen grains germinated precociously inside the anther. Another line, a transgenic plant expressing callose synthase 5 (CalS5) under the CaMV 35S promoter, also contained abnormal callose deposition during microsporogenesis and displaced MGUs in pollen grains. We also observed that precocious pollen germination could be induced in wild-type plants by incubation with medium containing sucrose and calcium ion and by wounding in the anther. These results demonstrate that precocious pollen germination in *Arabidopsis* could be triggered by a genetic alteration and a physiological condition

Self-incompatibility (SSI): There is complete inhibition of pollen germination on the stigmatic surface. The inhibition or rejection reaction is caused by the deposition of callose in the germ-pore or at the tip of the pollen tube. The stigmatic papillae also produce a lenticular plug of callose at their tips. It lies between the cell wall and the plasma membrane (Shivanna 2003). On the other hand, such plugs are not produced on compatible pollination. In the species exhibiting gametophytic self-incompatibility, pollen germination is normal but the pollen tubes are inhibited in the style. In some cases e.g. Papaveraceae, the inhibition takes place in stigma. Inhibition of pollen tube is caused by the deposition of excess quantity of callose at the tip. According to Shivanna *et al.* (1978b, 1982) excessive deposition of callose is a characteristic feature of self-compatible pollen tubes, but is not the primary event and therefore, not the cause of inhibition. Pectic material deposition at the tube tip is the earliest deviation recorded in selfed tubes. Callose is initially deposited behind the tip region and later may extend to the tip.

Pistil Development: Teng *et al*. (2006) observed abnormalities in pistil development resulting in low seed set in *Leymus chinensis* (Poaceae). Megasporogenesis and megagametogenesis and the callose deposition during these developmental processes were investigated. In addition, morphological and histochemical studies of pistils at anthesis and pollen behavior on the stigmas after pollination were examined. The results indicate that embryo sac development and callose deposition pattern of this grass follow the archetypal *Polygonum* type. Nearly half of the pistils developed abnormally in megagametogenesis, while only 8.6% of abnormalities occurred in megasporogenesis.

Over 47% of pistils at anthesis were abnormal in appearance. By 24 h after anthesis, many pollen grains had germinated on the stigmas of normal pistils. The high percentage of abnormal pistils and their low capacity to capture pollen grains may be the main factor in the low seed set of *L. chinensis.* Callose initially forms around the megaspore mother cell during the meiotic prophase. By the first meiotic metaphase, the entire megasporocyte is enveloped in a callose-containing wall. At the dyad stage, the dyad is completely enclosed by thick walls that exhibit strong callose fluorescence, and the two dyad cells are separated by a thick cell plate that also shows strong, fluorescence. However, when the mother cell reaches the tetrad stage, the callose is unevenly deposited in the walls of the tetrad. In general, the external transverse wall of the chalazal megaspore emits weak or no fluorescence, whereas the other three megaspores are still enveloped by a callose-containing wall. In addition, the transverse walls usually exhibit stronger callose fluorescence than the side walls adjacent to the somatic cells of the surrounding tissue. The callose around the tetrad degrades in the direction from the chalaza to the micropyle. By the time the chalazal megaspore has differentiated into the mother cell of the embryo sac, the other three megaspores have completely degenerated and very weak or no callose fluorescence is visible around the tetrad. Thus, at this stage, the wall of the mononucleate embryo sac is devoid of callose, and only slight callose fluorescence is detectable in the surrounding somatic tissues. During the development of the mononucleate embryo sac into a binucleate, then a tetranucleate, and finally an octanucleate embryo sac, callose does not appear. Therefore, callose only forms during megasporogenesis and begins to degrade at the tetrad stage, and no callose is synthesized during megagametogenesis. Many abnormal patterns of callose deposition were observed during pistil development. However, most of them took place during the late stages of pistil development, similar to those observed in abnormal pistils at anthesis, and only a few abnormal patterns of callose deposition were observed in megasporogenesis.

The role of callose in cell plate formation: During cell division in higher plants, the first visible evidence of the new cell wall is deposition of the cell plate in an equatorial plane between daughter nuclei. Cell plate development is initiated with the fusion of Golgi-derived vesicles into a continuous membrane network in the center of the phragmoplast; this process defines where the new cell wall will be assembled (Staehelin *et al.* 1996). Immunolabeling data indicate that the callose is the main luminal component of forming cell plate and it forms a coat-like structure on the membrane surface (Samuels et al. 1995). Based on electron micrograph studies, the maturation of tubular network into a fenestrated cell plate and then into a cell wall may be driven primarily by the synthesis of callose (Samulels *et al.* 1995, Staehelin *et al.* 1996). This driving force of callose on the membranes might be increased by the polymerization of phragmoplastin and sequeezing of phragmoplastin polymers (Verma *et al.* 2001), since one of the subunits of the callose synthase complex interacts with phragmoplastin (Hong *et al.* 2001). Callose deposition is followed by the deposition and organization of cellulose and other cell wall components; at the same time, cell plate callose is degraded by β-1,3-glucanase.

According to Hong *et al*. (2001) callose is synthesized on the forming cell plate and several other locations in the plant. They have cloned an *Arabidopsis* cDNA encoding a

callose synthase (CalS1) catalytic subunit. The CalS1 gene comprises 42 exons with 41 introns and is transcribed into a 6.0-kb mRNA. The deduced peptide, with an approximate molecular mass of 226 kD, showed sequence homology with the yeast 1, 3-ß-glucan synthases and is distinct from plant cellulose synthases. CalS1 contains 16 predicted transmembrane helices with the N-terminal region and a large central loop facing the cytoplasm. CalS1 interacts with two cell plate–associated proteins, phragmoplastin and a novel UDP-glucose transferase that copurifies with the CalS complex. That CalS1 is a cell plate–specific enzyme is demonstrated by the observations that the green fluorescent protein–CalS1 fusion protein was localized at the growing cell plate, that expression of CalS1 in transgenic tobacco cells enhanced callose synthesis on the forming cell plate, and that these cell lines exhibited higher levels of CalS activity. These data also suggest that plant CalS may form a complex with UDP-glucose transferase to facilitate the transfer of substrate for callose synthesis.

Callose is reported to be formed by enzymes located in the forming plasma membrane since callose is not found in cell plate-targeting vesicles. Through the use of BY-2 cells, it has been demonstrated that one callose synthase, At GSL6, is located at the developing cell plate where it interacts with two other cell plate-associated proteins, phragmoplastin and a UDP-glucose transferase. It is therefore possible that these three proteins form part of a larger cell plate callose synthase complex on cell plate (Hong *et al*. 2001a, 2001b). However, several T-DNA knockout mutants of gsl6 (cals1) exhibited no detectable cytokinesis phenotype and actually retained callose at the cell plate, suggesting that more than one callose synthase is involved in cell plate formation (Hong and Verma 2007, Chen et al. unpublished data).

More recently, Thiele *et al.* (2008) cloned a putative callose synthase gene, GSL8/MASSUE. Mutants in gsl8 were found to be seedling lethal, and had typical cytokinesis-defective phenotypes, such as bi- or multi-nucleate cells with cell wall stubs. This finding indicates that callose is indeed essential for plant cytokinesis by synthesizing callose at cell plate. This result is also consistent with recent finding from our lab (Chen *et al*. unpublished data).

Plasmodesmata (PD) regulation: In higher plants, almost all cells are symplasmically connected through PD (dynamic plasma-membrane and ER-based intercellular channels) which regulate the trafficking of nutrients, signal molecules etc. PD have several functional states, such as open, closed and dilated (Lucas and Lee 2004). The exact functional state of PD depends on what plants require to respond to developmental and/or environmental cues. Callose is frequently found to deposit at PD, where it is generally believed to control the movement of molecules through plasmodesmata as a developmental regulator of symplasmic continuity. Callose can also deposit at PD in response to abiotic and/or biotic stresses.

Callose plugs at PD have been implicated to function in the maintenance of dormancy by isolating the meristem from symplasmic continuity with surrounding tissues. Rinne *et al*. (2001) have found that a short photoperiod can induce the transition of two concentric symplasmic domains in the birch shoot apical meristem into completely symplasmically isolated cells by through the formation of a callose plug at corresponding PD. Interestingly, the same group also showed that the birch shoot apical meristem can restore its symplasmic organization to break bud dormancy after chilling treatment. And, furthermore, that this restoration is likely to be mediated by β-1,3-glucanase since β-1,3-

glucan degraded from PD during chilling. These findings led the authors to propose a model for "dormancy cycling" that depicts the meristem as passing through sequential states of cellular communication with characteristic sensitivities to distinct environmental cues (Rinne and School 1998, Rinne *et al.* 2001).

Another example of a callose-regulated symplasmic domain is the cotton fiber cell. Each cotton fiber is a single cell that forms from the epidermis of the outer integument of the ovules at or just prior to anthesis (Ruan *et al.* 2001). Previously, Ruan *et al.* (2001) demonstrated that the transient closure of PD facilitates elongation of the cotton fiber. The authors concluded that cotton fiber cells are symplamically isolated so as to maintain the high turgor pressure required for cotton fiber elongation. Furthermore, aniline blue staining and immunolocalization studies revealed that callose deposition and degradation at the cotton fiber base correlates with the closure and reopening of PD, respectively. In addition, the expression of a β-1, 3-glucanase, GhGluc1, could play a role in this process by degrading callose and opening the PD (Ruan *et al.* 2004). To date, however, no single callose synthase responsible for callose deposition at PD has been reported. It will be interesting to determine if blocking callose deposition at PD leads to developmental defects, in particular shoot apical meristem (SAM). Interestingly, it has been proposed that symplasmic domain formation in SAM is mediated by callose turnover at PD (Rinne and School 1998, Rinne *et al.* 2001).

Callose deposition in response to stress: Callose plays important roles in many aspects of plant growth and development. In addition, callose is deposited at the plasma membrane and cell wall interface as one of the many responses of plant tissue to a range of stresses e.g. temperature, toxicity, wonds caused by mechanical injuries or pathogens. Observable callose deposition occurs within minutes of damage by mechanical, chemical or ultrasonic treatments and in physiological or biotic stress induced by plasmolysis, raised or lowered temperatures and microbial infection, respectively (Stone and Clarke 1992).

According to Hamissou *et al.* (2003) when plant cells are wounded, they block the damaged sites and their plasmadesmata with a polysaccharide cement known as callose, a complex branched carbohydrate commonly associated with sieve areas of sieve elements. This helps prevent the loss of cytoplasmic contents from adjacent cells. Callose is a β-1, 3-glucan polymer of glucose, a major component of inducible plant cell wall, and a barrier against fungal infection.

Abscisic acid (ABA) plays an important role as a plant hormone and it has been shown to modulate plant responses to abiotic stress situations and in recent years, it has become evident that it is partaking in processes of plant defense against pathogens (Flors *et al*. 2005). According to them, abscisic acid and callose are team players in defense against pathogens. Although ABA's role in influencing the outcome of plant-pathogen interactions is controversial, with most research pointing into the direction of increased susceptibility, recent results have shown that ABA can also be involved in rendering plants more resistant to pathogen attack. In these cases, ABA interacts with callose deposition allowing an early and efficient build up of papillae at the sites of infection. The present review tries to shed some light on a possible interplay between ABA and callose in the protection of plants against invading pathogens.

The depression of carbohydrate translocation in petioles by low temperature is well known and it has been presumed that the depressive effect may be a consequence of the lowering of metabolic activities (Majumdar and Leopold 1967). It is known that callose plugs forming on

the phloem sieve plates are associated with the retardation of translocation in woody plants in autumn. Majumdar and Leopold (1967) carried out experiments to determine whether the response to periods of low temperature may involve the formation of callose plugs in the phloem. Their results indicate that in the bean plant in which translocation is depressed by low night temperatures, callose plug do form, whereas in tomato which may actually show improved growth under low night temperatures there is no stimulation of callose plug formation.

Several independent research groups have reported GSL5/PMR4 (powdery mildew resistant) / CalS12 to be responsible for callose synthesis in sporophytic tissue in response to wounding and/or pathogen. Loss-of-function mutants of GSL5/PMR4/CalS12 failed to synthesize callose at papillae. Unexpectedly, depletion of callose in gsl5 mutants rendered the plants more resistant, not more susceptible to pathogens. These data indicate that callose exerts a negative effect on plant defense against pathogen infection. One possible explanation for this is that callose could hinder the plants' defense machinery against pathogen; removal of callose in the gsl5 mutant could therefore activate defense systems (Jacobs *et al*. 2003). Alternatively, pathogen-induced callose could negatively regulates the SA signaling pathway of plants, and the lack of callose in the pmr4 mutant may enhance the SA signaling, which results in increased resistance to pathogen (Nishimura *et al*. 2003). These studies demonstrated that multiple mechanisms, in addition to callose, are involved in plant pathogen response (Jacobs *et al*. 2003, Nishimura *et al*. 2003, Dong *et al*. 2008). To investigate the impact of induced host defenses on the virulence of a compatible *Peronospora parasitica* strain on *Arabidopsis thaliana*, Donofrio and Delaney (2001) examined growth and development of this pathogen in nim1-1 mutants and transgenic salicylate hydroxylase plants. These plants are unable to respond to or accumulate salicylic acid (SA), respectively, are defective in expression of systemic acquired resistance (SAR), and permit partial growth of some normally avirulent pathogens. We dissected the *P. parasitica* life cycle into nine stages and compared its progression through these stages in the defense-compromised hosts and in wild-type plants. NahG plants supported the greatest accumulation of pathogen biomass and conidiophore production, followed by nim1-1 and then wild-type plants. Unlike the wild type, NahG and nim1-1 plants showed little induction of the SAR gene PR-1 after colonization with *P. parasitica*, which is similar to our previous observations. We examined the frequency and morphology of callose deposits around parasite haustoria and found significant differences between the three hosts. NahG plants showed a lower fraction of haustoria surrounded by thick callose encasements and a much higher fraction of haustoria with callose limited to thin collars around haustorial necks compared to wild type, whereas nim1-1 plants were intermediate between NahG and wild type. Chemical induction of SAR in plants colonized by *P. parasitica* converted the extrahaustorial callose phenotype in NahG to resemble closely the wild-type pattern, but had no effect on nim1-1 plants. These results suggest that extrahaustorial callose deposition is influenced by the presence or lack of SA and that this response may be sensitive to the NIM1/NPR1 pathway. Additionally, the enhanced susceptibility displayed by nim1-1 and NahG plants shows that even wild-type susceptible hosts exert defense functions that reduce disease severity and pathogen fitness.

Callose synthesis appeared as a rapid response to wounding and inoculation of slash pine seedlings with conidial suspensions of pitch

canker fungus (Valluri and Soltes 1990). Aseptically grown tissue culture seedlings showed greater hypersensitivity to pathogen inoculation by accumulating 15% more callose in infected tissue than greenhouse grown seedlings.

Jacobs *et al*. (2003) have transformed *Arabidopsis* with double-stranded RNA interference (dsRNAi) constructs designed to silence three putative callose synthase genes: GLUCAN SYNTHASE–LIKE5 (GSL5), GSL6, and GSL11. Both wound callose and papillary callose were absent in lines transformed with GSL5 dsRNAi and in a corresponding sequence-indexed GSL5 T-DNA insertion line but were unaffected in GSL6 and GSL11 dsRNAi lines. These data provide strong genetic evidence that the GSL genes of higher plants encode proteins that are essential for callose formation. Deposition of callosic plugs, or papillae, at sites of fungal penetration is a widely recognized early response of host plants to microbial attack and has been implicated in impeding entry of the fungus. Depletion of callose from papillae in gsl5 plants marginally enhanced the penetration of the grass powdery mildew fungus *Blumeria graminis* on the non host *Arabidopsis*. Paradoxically, the absence of callose in papillae or haustorial complexes correlated with the effective growth cessation of several normally virulent powdery mildew species and of *Peronospora parasitica*. Exposure to aluminum also induces callose production (Enns *et al*. 2005, Nishikawa *et al.* 2005).

Biosynthesis: As mentioned above, the cell wall polymer callose (1, 3-β-D-glucan) is normally synthesized at specific developmental events, like in the cell plate (Stone and Clarke 1992) and in pollen tube walls (Samules *et al*. 1995). Callose is also deposited at plasmodesmata (Hong *et al*. 2001 a, b, Iglesias and Meins 2000) to occlude plasmodesmata (Tollet *et al.* 2008, Huang et al. 2008) and at sieve plates (Bucher *et al*. 2001) to limit intercellular transport, often as a response to developmental cues or environmental signals, e.g., wounding and pathogen attack (Verma and Hong 2001, Brownfield *et al.* 2007, 2008). Callose deposition reinforces the cell wall at the site of the attack (Richmond and Somerville 2000, Jacobs *et al.* 2003), but callose can also be found at plasmodesmata in neighboring non-infected cells to limit spread of a fungal infection in resistant cultivars (Nishimura *et al*. 2003).

Over the last decade, most of our knowledge about callose in plants has been derived from analyses of a number of mutations that affect callose synthesis that were made by knocking out the individual callose synthase genes of *Arabidopsis* (Jacobs *et al*. 2003, Nishimura *et al*. 2003, Enns *et al*. 2005, Nishikawa *et al.* 2005, Toller *et al.* 2008, Huang *et al.* 2008, Thiele *et al*. 2008, Dong *et al*. 2008). Biochemical evidence and molecular studies in several plant species indicate that callose is synthesized by a class (Verma and Hong 2001, Brownfield *et al.* 2007, 2008) of enzymes, termed callose synthases. In the model plant *Arabidopsis thaliana,* twelve genes encoding putative callose synthase have been identified by two independent research groups (Verma and Hong, 2001; Richmond and Somerville, 2000). Accordingly, two different nomenclatures have been adopted for the *Arabidopsis* genes. The group of Desh Verma uses the CalS (Callose synthase) system to name the twelve genes: AtCalS1-AtCalS12 based on their relative similarity to AtCalS1 (Hong *et al.* 2001). The Somerville group refers to the twelve *Arabidospsis* genes as GSL (Glucan synthase-like) genes, and has designated them as AtGSL1 to AtGSL12 (Richmond and Somerville, 2000). Due to its wide usage by the callose synthase research community, we have adopted the GSL

nomenclature system (Jacobs *et al.* 2003, Nishimura *et al.* 2003, Enns *et al.* 2005, Nishikawa *et al.* 2005, Toller *et al*. 2008, Huang *et al.* 2008, Thiele *et al.* 2008). From *Arabidopsis* 12 callose synthase (CalS1-12) genes have been isolated by Dong (2005). Out of these 12 CalS gene family members, only one (CalS5) has been directly linked to pollen tube callose (Abercrombie *et al*., 2011). Knockout of the CalS5 gene by T-DNA insertion resulted in a severe reduction in fertility caused by the degeneration of microspores.

Biochemical evidence and molecular studies made by Verma and Hong (2001), Brownfield *et al*. (2007, 2008) in several plants have indicated that callose is synthesized by a class of enzymes, termed callose sythases. In the model plant *Arabidopsis* it is laid down at plasmodesmata, at the dividing cell plate and during pollen development. It is produced in response to wounding, infection by pathogens, aluminium and abscisic acid. Deposits often appear on the sieve plates at the end of the growing season (Bell and Allan 2000). Synthesis of callose (beta-1, 3-glucan) in plants has been a topic of much debate over the past several decades (Verma and Hong 2001). Callose synthase could not be purified to homogeneity and most partially purified cellulose synthase preparations yielded beta-1, 3-glucan in vitro, leading to the interpretation that cellulose synthase might be able to synthesize callose. While a rapid progress has been made on the genes involved in cellulose synthesis in the past five years, identification of genes for callose synthases has proven difficult because cognate genes had not been identified in other organisms. An *Arabidopsis* gene encoding a putative cell plate-specific callose synthase catalytic subunit (CalS1) was recently cloned. CalS1 shares high sequence homology with the well-characterized yeast beta-1, 3-glucan synthase and transgenic plant cells over-expressing CalS1 display higher callose synthase activity and accumulate more callose. The callose synthase complex exists in at least two distinct forms in different tissues and interacts with phragmoplastin, UDP-glucose transferase, Rop1 and, possibly, annexin. There are 12 CalS isozymes in *Arabidopsis*, and each may be tissue-specific and/or regulated under different physiological conditions responding to biotic and abiotic stresses.

Dong (2005) has made a functional investigation of *Arabidopsis* callose synthases and the signal transduction pathway. Callose synthesis occurs at specific stages of cell wall development in all cell types, and in response to pathogen attack, wounding and physiological stresses. He has isolated promoters of 12 *Arabidopsis* callose synthase (CalS1-12) genes and demonstrated that different callose synthases are expressed specifically in different tissues during plant development. That multiple CalS genes are expressed in the same cell type suggests the possibility that CalS complex may be constituted by heteromeric subunits. Five CalS genes were induced by pathogen *(Peronospora parasitica,* a causal agent of downy mildew) or salicylic acid (SA) treatments, while seven CalS genes were not affected by these treatments. Among the genes that are induced, CalS1 and CalS12, showed the highest responses. When expressed in npr1, a mutant impaired in the response of pathogen related (PR) genes to SA, the induction of CalS1 and CalS12 genes by the SA or pathogen treatments was significantly reduced. The patterns of expression of the other three CalS genes were not changed significantly in the npr1 mutant. These results suggest that the high induction observed of CalS1 and CalS12 is NPR1-dependent while the weak induction of all five CalS genes is NPR1-independent. In a T-DNA knockout mutant of CalS12, callose encasement around the haustoria on the infected leaves was

reduced and the mutant was found to be more resistant to downy mildew as compared to the wild type plants. *Arabidopsis* contains 12 callose synthase (CalS) genes that have evolved in order to catalyze callose synthesis in different locations and in response to biotic and abiotic cues. We demonstrate that one of these genes, CalS5 is responsible for the synthesis of callose deposited to the primary callose wall of meiocytes, tetrads and microspores, and is essential for the exine formation and pollen viability. CalS5 encodes a transmembrane protein of 1923 amino acid residues with a molecular mass of 220 kD. Knockout of the CalS5 gene by T-DNA insertion resulted in a severe reduction in fertility. The reduced fertility in cals5 mutants was attributed to the degeneration of microspores. However, megagametogenesis is not affected and the female gametes are completely fertile in cals5 mutants. CalS5 gene is expressed in several organs with the highest expression in meiocytes, tetrads, microspores and mature pollens. Callose deposition in these tissues in cals5 mutants was nearly completely depleted, suggesting that this gene is essential for the synthesis of callose in these tissues. The pollen exine wall was not formed properly in the mutant and tryphine appeared to be transported from the pollen outer wall into the central vacuole presumably via endocytosis. These data suggest that callose synthesis has a vital function in building the exine sculpture, integrity of which is essential for pollen viability. Using the cell plate specific CalS1 as a bait to screen an *Arabidopsis* cDNA library constructed in the yeast two-hybrid vector, we obtained two positive clones. One of these interacting clones, RLK1, encodes protein kinase and may play a role in the regulation of CalS1 activity during cell plate formation. Another clone, UGP1, encodes an UDP-glucose pyrophosphorylase and may act to provide alternative source of UDP glucose for the synthesis of callose. On the basis of these findings, Dong (2005) concluded that induction of callose synthase genes by pathogen infection or SA treatment involves both npr1-dependent and npr1-independent signaling pathways. CalS5 is required for exine formation during microgametogenesis and pollen viability in *Arabidopsis.*

Franco and Iriti (2007) have evaluated the effectiveness of chitosans with different molecular weights (MW 6-753 kD) to elicit callose synthesis in *Phaseolus vulgaris* and correlated with their capability in inducing resistance to tobacco necrosis virus (TNV). To rapidly screen the pattern and amount of callose apposition, leaf fragments were floated in a cell culture multi-wells dish, each well filled with a different chitosan dissolved at variable concentration (0.1-0.2%). Aniline blue staining, performed 12 h after treatment, showed that chitosans with MWof 76, 120 and 139 kD were the most effective in inducing callose synthesis in comparison with those having lower or higher MW. Callose appositions were randomly scattered in the mesophyll tissues, forming a homogeneous network of bright fluorescent spots. TNV inoculation of chitosan-treated bean plants showed that the efficacy of chitosans as resistance elicitors positively correlated with their ability in inducing callose apposition, with the 76-kD one being the most effective, with a 95% reduction of viral lesions. Microscopic and ultrastructural alterations in leaf fragments floated on chitosan and inoculated with TNV indicated that the mechanism of induced resistance involves, besides callose, a network of hypersensitive-like reactions, elicited by the compound, that impair virus spreading (Franco and Iriti 2007).

More recently, Töller *et al.* (2008) reported both gsl8 and gsl10 mutants to be male gametophytic lethal, and the authors failed to recover homozygous mutants for either genes.

Further analysis of gsl8 and gsl10 mutants during development revealed specific malfunctions associated with asymmetric microspore division as well as failed entry of mutant microspores into mitosis. These authors proposed that GSL8 and GSL10 might exert indirect regulatory functions through interactions with other proteins, rather than through their catalytic activity alone (Toller *et al.* 2008). Interestingly, through yeast two-hybrid screening, Dong (2005) and Dong *et al.* (2005) found that the amino terminus of AtGSL6 could interact with a novel lectin-containing receptor-like kinase (LecRLK1). It will thus be very interesting to test whether GSL8 and GSL10 can also interact with receptor-like kinases (RLKs) to regulate pollen entry into mitosis. Recently, as described in more detail below, we also obtained a similar result from GSL10, but Huang *et al.* (2008) successfully recovered homozygous gsl8 mutants.

According to Chen *et al.* (2009) a rapid progress has been made on the genes involved in cellulose synthesis in the last five years, identification of genes for callose synthases has proven difficult because there was no molecular probe for a plant callose synthase available to fish out the cognate gene(s) (Chen et al. 2009). Attempts have been made to study phragmoplastin-interacting proteins, we identified a novel UDP-glucose transferase (UGT1) encoded by a gene located on chromosome 1 of *Arabidopsis.* Upstream walk from this gene revealed an open reading frame which showed homology with the catalytic subunit of the yeast β-1, 3-glucan synthase. The encoded protein proved to be a catalytic subunit of callose synthase (CalS1). The CalS1 gene comprises 42 exons and is transcribed into a 6.0-kb mRNA. The deduced peptide contains 16 predicted transmembrane helices with the N-terminal region and a large hydrophilic central loop facing the cytoplasm. CalS1 interacted with a novel UDP-glucose transferase (UGT1) that copurified with the CalS complex. Following fusion with GFP, both CalS1 and UGT1 were colocalized at the growing cell plate. Expression of CalS1 in transgenic tobacco cells resulted in higher levels of CalS activity and enhanced callose synthesis on the forming cell plate. UGT1 also interacted with phragmoplastin and Rop1 that may regulate activity of the CalS enzyme. There are 12 CalS enzymes in *Arabidopsis* and each may be tissue specific and/or regulated under different physiological conditions. A multi subunit composition of callose synthase enzyme, coupled with association of UGT1 and possibly sucrose synthase, makes a large callose synthase complex which exists in two forms. The nature of these two distinct complexes, sedimenting at 45% and 55% sucrose, is not known. The association of sucrose synthase may facilitate transfer of UDP-glucose via UGT1 to the CalS catalytic site. In addition, other proteins such as annexin and Rop1 may provide further regulatory function to control the activity of different CalS complexes in response to biotic and abiotic stresses as well as under tissue-specific developmental control.

According to Aidemark *et al.* (2009) callose synthases are membrane-bound enzymes that have been relatively well characterized in vitro using isolated membrane fractions or purified enzyme. However, little is known about their functional properties *in situ,* under conditions when the cell wall is intact. To allow *in situ* investigations of the regulation of callose synthesis, cell suspensions of *Arabidopsis thaliana* (Col-0), and tobacco (BY-2), were permeabilized with the channel-forming peptide alamethicin. Nucleic acid-binding dyes and marker enzymes demonstrated alamethicin permeabilization of plasma membrane, mitochondria and plastids, also allowing callose synthase measurements. In

the presence of alamethicin, Ca^{2+} addition was required for callose synthase activity, and the activity was further stimulated by Mg^{2+} Cells pretreated with oryzalin to destabilize the microtubules prior to alamethicin permeabilization showed significantly lower callose synthase activity as compared to non-treated cells. As judged by aniline blue staining, the callose formed was deposited both at the cell walls joining adjacent cells and at discrete punctate locations earlier described as half plasmodesmata on the outer walls. This pattern was unaffected by oryzalin pretreatment, showing a quantitative rather than a qualitative effect of polymerized tubulin on callose synthase activity. No callose was deposited unless alamethicin, Ca^{2+} and UDP-glucose were present. Tubulin and callose synthase were furthermore part of the same plasma membrane protein complex, as judged by two-dimensional blue native SDS-PAGE.

β-1,3-glucan polymers are major structural components of fungal cell walls, while cellulosic β-1,4-glucan is the predominant polysaccharide in plant cell walls. Plant β-1, 3-glucan, called callose, is produced in pollen and in response to pathogen attack and wounding, but it has been unclear whether callose synthases can also produce cellulose and whether plant cellulose synthases may also produce β-1, 3-glucan (Østergaard *et al*. 2002). They have described here an *Arabidopsis* gene, AtGsl5, encoding a plasma membrane-localized protein homologous to yeast β-1, 3-glucan synthase whose expression partially complements a yeast β-1, 3-glucan synthase mutant. AtGsl5 is developmentally expressed at highest levels in flowers, consistent with flowers having high β-1, 3-glucan synthase activities for deposition of callose in pollen. A role for AtGsl5 in callose synthesis is also indicated by AtGsl5 expression in the *Arabidopsis* mpk4 mutant which exhibits systemic acquired resistance (SAR), elevated β-1, 3-glucan synthase activity, and increased callose levels. In addition, AtGsl5 is a likely target of salicylic acid (SA)-dependent SAR, since AtGsl5 mRNA accumulation is induced by SA in wild-type plants, while expression of the nahG salicylate hydroxylase reduces AtGsl5 mRNA levels in the mpk4 mutant. These results indicate that AtGsl5 is likely involved in callose synthesis in flowering tissues and in the mpk4 mutant.

Phylogenetic analsysis of the AtGSL family suggests that the GSL family can be classified into four main subfamilies. The first subfamily contains AtGSL1, AtGSL5, AtGSL8 and AtGSL10, the second subfamily contains AtGSL2, AtGSL3, AtGSL6 and AtGSL12, the third subfamily contains AtGSL7 and AtGSL11, and the last subfamily includes AtGSL4. According to previously characterized functions of GSL genes, members belonging to different subfamilies exhibit partially redundant roles during pollen development or fertilization. A single GSL gene can also have diverse functions; for example, GSL5 is responsible for the synthesis of wound-and pathogen-inducible callose in leaf tissue; it also plays an important role in exine formation and pollen wall patterning (Jacobs *et al.* 2003, Nishimura *et al.* 2003, Enns *et al.* 2005).

Based on gene structure modeling, most GSL genes have 40–50 exons; exceptions include GSL1 and GSL5, which have two and three exons, respectively. Most of the AtGSL genes encode proteins of around 2,000 amino acids, which are larger than most plant genes (Verma *et al.* 2001, Brownfield *et al.* 2007, 2008, Richmond and Somerville 2000, Jacobs *et al.* 2003, Nishimura *et al.* 2003, Enns *et al*. 2005). All *Arabidopsis* GSL proteins contain multiple transmembrane domains that are clustered into two regions (N-terminal and C-terminal), leaving a large hydrophilic central loop that

faces the cytoplasm. This loop contains the putative catalytic domain which has been further subdivided into two domains: the UDP-glucose binding domain and the glycosyltransferase domain. These domains are characterized by the presence of multiple aspartic acid triplets (D,D,D) and a QXXRW motif that is conserved in the CeSA superfamily (Verma *et al*. 2001, Thiele *et al*. 2008, Dong *et al*. 2005).

Aidemark *et al.* (2009) have studied the regulation of callose synthase activity *in situ* in alamethicin-permeabilized *Arabidopsis* and tobacco suspension cells. According to Aidemark *et al.* (2009) the cell wall component callose is mainly synthesized at certain developmental stages and after wounding or pathogen attack. Callose synthases are membrane-bound enzymes that have been relatively well characterized *in vitro* using isolated membrane fractions or purified enzyme. However, little is known about their functional properties *in situ,* under conditions when the cell wall is intact. To allow in situ investigations of the regulation of callose synthesis, cell suspensions of *Arabidopsis thaliana* (Col-0), and tobacco (BY-2), were permeabilized with the channel-forming peptide alamethicin. Nucleic acid-binding dyes and marker enzymes demonstrated alamethicin permeabilization of plasma membrane, mitochondria and plastids, also allowing callose synthase measurements. In the presence of alamethicin, $Ca2^{+}$ addition was required for callose synthase activity, and the activity was further stimulated by $Mg2^{+}$ Cells pretreated with oryzalin to destabilize the microtubules prior to alamethicin permeabilization showed significantly lower callose synthase activity as compared to non-treated cells. As judged by aniline blue staining, the callose formed was deposited both at the cell walls joining adjacent cells and at discrete punctate locations earlier described as half plasmodesmata on the outer walls. This pattern was unaffected by oryzalin pretreatment, showing a quantitative rather than a qualitative effect of polymerized tubulin on callose synthase activity. No callose was deposited unless alamethicin, $Ca2^{+}$ and UDP-glucose were present. Tubulin and callose synthase were furthermore part of the same plasma membrane protein complex, as judged by two-dimensional blue native SDS-PAGE. Alamethicin permeabilization allowed determination of callose synthase regulation and tubulin interaction in the natural crowded cellular environment and under conditions where contacts between the cell wall, the plasma membrane and cytoskeletal macromolecules remained. These results also suggest that alamethicin permeabilization induces a defense response mimicking the natural physical separation of cells (for example when intercellulars are formed), during which plasmodesmata are transiently left open.

Genes encoding callose synthases (GSL) (Thiele *et al*. 2008, Dong and Hong 2005, Dong 2005) have now been identified in several plant species. In *A. thaliana* as many as 12 callose synthase genes have been identified (Dong *et al*. 2005). Biochemical studies have indicated that at least some GSL genes can produce proteins capable of synthesizing callose (Staehlin and Hepler 1996).

Callose synthases use UDP-glucose as glucose donor to the growing polymer chain (Samules *et al.* 1995) similar to cellulose synthases (which form 1,4-β-D-glucan) although callose production appears to dominate in most in vitro experiments (Hong *et al*. 2001, Hong and Verma 2007). It was earlier believed that the two polymers were produced by one enzyme, which switched to callose synthesis in vivo upon wounding or during extraction to allow enzyme activity determinations (Iglesian and Meins 2000, Hong and Verma 2007). The

binding site for UDP-glucose for callose synthase (as well as cellulose synthase) is on the cytoplasmic side of the plasma membrane and is thus inaccessible to direct assays in intact cells. To overcome this permeability barrier, detergents have been added to cells or isolated plant plasma membranes.

Despite such problems, callose as well as cellulose synthesis have successfully been monitored with isolated proteins after solubilization of microsomal membranes with detergents e.g., digitonin, Brij 58, CHAPS or taurocholate (Lucas and lee 2004, Ruan *et al*. 2004, Dong *et al*. 2008). The use of sucrose rather than UDP-glucose as substrate, led to less callose and more cellulose formation.

Recently, Zavaliev *et al*. (2010) have shown that *Arabidopsis* class 1 reversibly glycosylated polypeptides (C1RGPs) are plasmodesmal-associated proteins. According to them, the transgenic tobacco *(Nicotiana tabacum)* plants constitutively expressing GFP tagged AtRGP2 under the control of the CaMV 35S promoter are stunted, have a rosette-like growth pattern, and in source leaves exhibit strong chlorosis, increased photoassimilate retention and starch accumulation that results in elevated leaf specific fresh and dry weights. Basal callose levels around plasmodesmata (PD) of leaf epidermal cells in transgenic plants are higher than in WT. Such a phenotype is characteristic of virus-infected plants and some transgenic plants expressing Pd-associated viral movement proteins (MP). The local spread of Tobacco mosaic virus (TMV) is inhibited in AtRGP2: GFP transgenics compared to WT. Taken together these observations suggest that overexpression of the AtRGP2: GFP leads to a reduction in PD permeability to photoassimilate, thus lowering the normal rate of translocation from source leaves to sink organs. Such a reduction may also inhibit the local cell-to-cell spread of viruses in transgenic plants. The observed reduction in PD permeability could be due to a partial PD occlusion caused either by the accumulation of AtRGP2: GFP fusion in PD, and/or by constriction of PD by the excessive callose accumulation.

Future prospective: Callose is involved in various biological processes associated with plant growth, development and stress responses. Although remarkable progress has been made over the last decade, a number of questions remain unanswered: 1. What is the precise biochemical mechanism of callose synthesis? 2. What are the functional components of the callose synthase complex? 3. What is the function of callose during functional megaspore selection? 4. Do GSL proteins interact with each other in homo-oligomers or in hetero-oligomers? 5. Is there any cross-talk between callose-associated regulation mechanism(s) and other signal pathways? Answering these questions is essential for a deeper understanding of callose function in plants. In the near future, based on previous progress, multiple approaches such as biochemistry, cell biology, genetics and system biology will need to be employed to unravel these mysteries.

REFERENCES

Aidemark M, Andersson CJ, Rasmusson AG & Widell S 2009 Regulation of callose synthase activity in situ in alamethicin-permeabilized Arabidopsis and tobacco suspension cells *BMC Plant Biology* **9** 27-31.

Ahokas H 1980 Cytoplasmic male sterility in barley. II. Physiological characterization of the msm 1-Rfm 1a system. *Physiol. Plant* **48** 321-238.

Alche JD & Rodriguez-Garcia MI 1997 Fluorochromes for detection of callose in meiocytes of olive (Olea europaea L.) Biotechnic & Histochemistry **72** (6) 285-290.

Anger EM & Weber M 2006 Pollen-wall formation in Arum alpinum. *Ann. Bot.* **97**(2) 239-244.

Anvari SF & Stosser R 1978 Ein neue fluorezenzmikroscopische Methode zur Beurteilung der Befruchtungs-fahigkeit der Samenanlagen bei Prunus. *Z. PflZucht.* **81** 333-336.

Anvari SF & Stosser R 1981 Uber das Pollenschauch-wachstum beim Apfel. Mitt. *Klosterneuberg* **31** 24-30.

Bansal S & Chauhan SVS 2012 Abnormal behaviour of callose, pollen sterility and depression of exine formation in the anthers of tomato (Lycopersicon esculentum Mill.). *Internat. J. Plant Reprod. Biol.* **3**(1) In Press.

Bhandari NN, Bhargava M & Geier T 1981 A persisting cellulosic wall of microspore mother cells during microsporogenesis in Allium tuberosum Rottl. and Cyclamen persicum Mill. *Ann. Bot.* **48** 425-431.

Binh NTH & Hendrychová-Tomková J 1982. A case of early dissolution of the microsporocyte callose wall in male-sterile (CMS) sweet pepper *(Capsicum annuum L.) Biologia Plantarum* **24**(4) 260-265.

Bino RJ 1985a Ultrastructural aspects of male sterility in *Petunia hybrida. Protoplasma* **127** 130-140.

Bino RJ 1985b Histological aspects of microsporogenesis in fertile, male sterile and restored fertile Petunia hybrida. *Thero. Appl. Genet.* **69** 423-428.

Brownfield L, Wilson S, Newbigi E, Bacic A & Read S 2008 Molecular control of the glucan synthase-like protein NaGSL1 and callose synthesis during growth of Nicotiana alata pollen tubes. *Biochem* J. **41** 4 43–52.

Brownfield L, Ford K, Doblin MS, Newbigin E, Read S & Bacic A. 2007 Proteomic and biochemical evidence links the callose synthase in Nicotiana alata pollen tubes to the product of the NaGSL1 gene. *Plant* J. **52** 147–156.

Bucciaglia PA & Smith AG 1994 Cloning and characterization of Tag1, a tobacco anther ß-1,3-glucanase expressed during tetrad dissolution. *Plant Mol. Biol.* **24** 903-914.

Bucher GL, Tarina C, Heinlein M, Di Serio F, Meins F, Jr & Iglesias VA 2001 Local expression of enzymatically active class I β-1,3-glucanase enhances symptoms of TMV infection in tobacco. *Plant J.* **28** 361–369.

Chasan R. 1992 Breaching the Callose Wall. *Plant Cell* **4** 745-746.

Chauhan SVS, Chaudhary Meenu & Chauhan Seema 2009. Premature dissolution of microsporocyte callose wall causes male sterility in ethaphone treated cotton *(Gossypium arboretum L.). J. Plant Reprod. Biol.* **1**(1)49-52.

Chen Xiong-Yan & Kim Jae-Yean 2009 Callose synthesis in plants. *Plant Signal Behav* **4**(6) 489-492.

Clarke AE & Stone BA 1984 The Biology and Chemistry of β-1,3-glucans. London: MacMillan .

Crafts AS 1951 Movement of assimilates viruses, growth regulators, and chemical indicators in plants. *Bot Rev.***17** 203-284.

Currier HB 1957 Callose substance in plant cells. *Am. J. Bot*. **44**(6) 478-488.

Currier HB & Strugger S 1956 Aniline blue and fluorescence microscopy of callose in bulb scales of *Allium cepa L. Prtoplasma* **45** 552-559.

DeSloover JL 1961 Etudes sur les cycadales. 1. Meiose et megasporogenese chez Encephalartos poggei Asch. *Cellule* **62** 103-116.

Dickinson HG & Heslop-Harrison J 1970 The ribosome cycle, nucleoli, and cytoplasmic nucleoloids in the meiocytes of Lilium. *Protoplasma* **69** 187-200.

Dickinson HG & Lewis D 1975 Interaction between the pollen grain coating and the stigmatic surface during compatible and incompatible intraspecific pollinations in Raphanus. In: *The Biology of the Male Gamete* (eds. Duckett JG & Racey PA), Pp. 165-175. New York, London: Academic Press.

Donofrio NM & Delaney TP 2001 Abnormal Callose Response Phenotype and Hypersusceptibility to Peronospora parasitica in Defense-Compromised Arabidopsis nim1-1 and Salicylate Hydroxylase-Expressing Plants. Molecular *Plant-Microbe Interaction* **14** (4) 439-450.

Dong X. 2005. Functional investigation of Arabidopsis callose synthase and the signal transduction pathway. Ohio State University . The PhD thesis.

Dong X, Hong Z, Chatterjee J, Kim S & Verma DPS. 2008 Expression of callose synthase genes and its connection with Npr1 signaling pathway during pathogen infection. *Planta.* **229** 87–98.

Dong X, Hong Z, Sivaramakrishnan M, Mahfouz M & Verma DPS 2005 Callose synthase (CalS5) is required for exine formation during microgametogenesis and for pollen viability in Arabidopsis. *Plant J*. **42** 315–328.

Dumas C & Knox RB 1983 Callose and determination of pistil viability and incompatibility. *Theor. appl. Genet.* **67** 1-10.

Echlin P 1972 The ultrastructure and ontogeny of pollen in Helleborus foetidus L. IV. Pollen grain maturation. *J. Cell Sci.* **11** 111-129.

Enns LC, Kanaoka MM, Torii KU, Comai L, Okada K & Cleland RE 2005 Two callose synthases, GSL1 and GSL5, play an essential and redundant role in plant and pollen development and in fertility. *Plant Mol Biol.* **58** 333–349.

Esau K 1939 Development and structure of the phloem tissue. *Bot. Rev*. **5** 373-432.

Esau K 1950 Development and structure of the phloem tissue. *Bot. Rev.* **16** 67-114.

Frost DJ, Read SM, Drake RR, Haley BE, Wasserman BP, Tenga N, Chen T, Jin B, Wu Z, Huanga Z, Li X, Wanga Y, Mu X, & Lin J 2006 Abnormalities in pistil development result in low seed set in *Leymus chinensis* (Poaceae) *Flora* **201** 658-667.

Flors V, Ton J, Jakab G & Mauch-Mani B 2005 Abscisic Acid and Callose: Team Players in Defense against Pathogens? *J. Phytopathology* **153** 377–383.

Franco F & Iriti M 2007 Callose synthesis as a tool to screen chitosan efficacy in inducing plant resistance to pathogens *Caryologia* **60**(1-2) 121-124.

Feder N & O'brien TP 1968 Plant microtechnique: some principles and new methods. *Am. J. Bot*. **55** 123-142.

Frankel R, Izhar S & Nitsan J 1969 Timing of callase activity and cytoplasmic male sterility in *Petunia. Biochem Genet* **3** 451-455.

Gardiner W & Ito T 1887 On the structure of mucilage-secreting cells of Blechnum occidentale and Osmunda regalis. *Ann. Bot*. **1** 33-35.

Gorska-Brylass A 1967 Transitory callose envelope surrounding the generative cell in pollen grains. *Acta Soc. Bot. Pol.* **36** 419-422.

Gorska-Brylass A 1970 The "callose stage" of the generative cells in pollen grians. *Grana* **10** 21-30.

Hamissou M, Haygood M & Patton C 2003 Induction of callose biosynthesis in Arabidopsis, an activation of the--plant defense mechanism. Free Library.

Heslop-Harsison J 1964 Cell walls, cell membranes and protoplasmic connections during meiosis and pollen development. In: *Pollen Physiology and Fertilization* (ed. Linskens HF), pp. 39-47. Amsterdam: North Holland.

Heslop-Harrison J 1966a Cytoplasmic connexions between angiosperm meiocytes. *Ann. Bot*. **30** 221-230.

Heslop-Harrison J. 1966b Cytoplasmic continuities during spore formation in flowering plants. *Endeavour* **25** 65-72.

Heslop-Harrison J 1968 Synchronous pollen mitosis and the formation of the generative cell in massulate orchids. *J. Cell Set.* **3** 457-466.

Heslop-Harrison J 1971 Pollen: Development and Physiology. Butterworths, London

Heslop-Harrison J & Mackenzie A 1967 Autoradiography of soluble (2-l4C) thymidine derivatives during meiosis and microsporogenesis in Lilium anthers. *Cell Sci.* **2** 387-400.

Hirano Y & Brunner I 2006 Quantitative determination of callose in tree roots *J Plant Physiol.* **163**(12) 1333-1336.

Izhar S & Frankel R 1971 Mechanism of male sterility in Petunia: The relationship between pH, callase activity in anthers and the breakdown of the microsporogenesis. *The. Appl. Genet.* **41** 104-108.

Jacobs AK, Lipka V, Burton RA, Panstruga R, Strizhov N, Schulze-Lefert P, & Fincher GB 2003 An arabidopsis callose synthase, GSL5, is required for wound and papillary callose formation. *Plant Cell.* **15** 2503–2513.

Jalouzot MF 1970 Mise en evidence de parois callosiques au cours de la megasporogenese et de l'oogenese d'Oenothera biennis. *C.r. hebd. Seanc. Acad. Set., Paris, D,* **270** 317-319.

Jensen WA 1962 Botanical Histochemistry. San Francisco: Freeman.

Jensen WA 1968 Cotton embryogenesis: The zygote. *Planta* **79** 346-366.

Jensen, WA 1974 Reproduction in flowering plants. In: Dynamic Aspects of Plant Ultrastructure (ed. Robards AW), pp. 481-503. London: McGraw-Hill.

Jos JS, Nair RB &Sreekumari MT 1990 Diverse types of male sterility in cassava. *The Indian J. Genet.* **50**(1) 23-25.

Kapil RN & Tiwari SC 1978 Plant embryological investigations and fluorescence microscopy: An assessment of integration. *Int. Rev. Cytol.* **53** 291-331.
Zygote special callose wall in Ericaceae 135

Knox RB & Heslop-Harrison J 1970 Direct demonstration of the low permeability of theangiosperm meiotic tetrad using a fluorogenic ester. *Z. PflPhysiol.* **62**, 451—459.

*Kuster E 1903 Pathologische Pflananatomie. Gustav Fischer, Jena

Lucas WJ & Lee J 2004 Plasmodesmata as a supracellular control network in plants. *Nat Rev Mol Cell Biol.* **5** 712–726.

Mackenzie A, Heslop-Harrison J & Dickinson HG 1967 Elimination of ribosomes during meiotic prophase. *Nature*, Lond. **215** 997-999.

Mamun EA, Alferd S, Cantrill LC, Overall RL & Suttan BG 2006 Effect of chilling on male gametophyte development in rice. *Cell Biol. International* **30** 583-591.

*Mangin L 1889 Observations sur la membrane du grain de pollen mur. *Bull. Soc. Bot. France* **36** 274-284.

*Mangin L. 1890 Sur la callsoe, nouvelle substance fondamentale existant dans la membrane. Compt. Rend. *Acad. Sci. Paris* **110** 644-647.

*Mangin L 1892 Observations sur la presence de la callose chez les Phanergames. *Bull. Soc. Bot. France* **39** 260-267.

Martinez-Tellez J & Crossa-Raynaud P 1982 Contribution a l'etude du processus de la fecondation chez trois especes dt*Prunus: P.persica* (L.) Batsch., *P. cerasifera* Ehrh., *P. mahaleb* L. grace a l'utilisation de couples de varietes male-steriles et male-fertiles.*j4^ronowje* **2** 333-340.

McCormick S 1993 Male gametophyte development. *Plant Cell* **5**(10) 1265-1275.

*McConchie CA 1983 The reproductive biology of

some Australian aquatic monocotyledons. Ph.D. thesis, University of Melbourne, Australia.

Mepham RH & Lane GR 1970 Observations on the fine structure of developing microspores of *Tradescantia bracteata. Protoplasm* **70** 1-20.

Myklestad S, Djurhuus R & Mohus A 1981 Demonstration of exo-(β-1, 3)-d-glucanase activity in some planktonic diatoms. *J.Exp. Marine Biol. Ecol.* **56**(2-3) 205-211.

Nanda K & Gupta SC 1974 Malfuctioning tapetum and callose wall behavior in Allium cepa microsporangia. *Beitr. Biol. Pflanz*. **50** 465-472.

Nishimura MT, Stein M, Hou B, Vogel JP, Edwards H & Somerville SC 2003 Loss of a callose synthase results in salicylic acid-dependent disease resistance. *Science.* **301** 969–972.

Nishikawa S, Zinkl GM, Swanson RJ, Maruyama D & Preuss D 2005 Callose (β-1,3 glucan) is essential for *Arabidopsis* pollen wall patterning, but not tube growth. *BMC Plant Biology* **5** 22-31.

Nguyen Thi Hoa Binh NTH & Hendrychová-Tomková J 1982. A case of early dissolution of the microsporocyte callose wall in male-sterile (CMS) sweet pepper *(Capsicum annuum L.) Biologia Plantarum* **24**(4) 260-265.

Nguyen Thi Hoa Binh and Jarmila Hendrychová-Tomková 1982 A case of early dissolution of the microsporocyte callose wall in male-sterile (CMS) sweet pepper *(Capsicum annuum L.) Biologia Plantarum* **24**(4) 260-265.

Oliver FW 1887 On the obliteration of the sieve-tubes in Laminariaceae. *Ann. Bot.* **1** 95-117.

Olson AR & Cass DD 1981 Changes in megagametophyte structure in Papaver nudicaule L. (Papaveraceae) following in vitro placental pollination. *Am. J. Bot.* **68** 1333-1341.

Østergaard L, Petersen M, Mattsson O & Mundy J 2002 An Arabidopsis callose synthase *Plant Molecular Biology*, **49** (6) 559-566.

Pearce RB 1986 Chlorantine fast green Bll as a stain for callose in oak phloem *Biotechnic and Histochemistry* **61**(1) 47 – 50.

Periasamy K & Amalathas J 1991 Absence of Callose and Tetrad in the Microsporogenesis of Pandanus odoratissimus with Well-formed Pollen Exine *Annals of Botany* **67** 29-33.

Plegt L & Bino RJ 1989 β-glucuronidase activity during development of the male gametophyte from transgenic and non-transgenic plants. *Mol. Ge. Genet.* **216** 321-327.

Popova AF, Ivanenko GF, Ustinova A Yu & Zaslavsky VA 2008 Localization of callose in microspores and pollen grains in Sium latifolium L. plants in different water regimes. *Cytology and Genetics* **42**(6) 363-368.

Rawlins TE & Takahashi WN 1952 Technics of plant histochemistry and virology. The National Press. Millbrae, California.

Rendle AB 1889 On the vesicular vessels of the onion. *Ann. Bot.* **3** 169-177.

Richmond TA 2000 Somerville CR The cellulose synthase superfamily. *Plant Physiol*. **124** 495–498.

Ridgway CS 1913 The occurrence of callose in root hairs. *Plant World* **16** 116-122.

Rinne PLH & Van der Schoot C 1998 Symplasmic fields in the tunica of the shoot apical meristem coordinate morphogenetic events. *Development.* **125** 1477–1485.

Rinne PLH, Kaikuranta PM & Van Schoot CD 2001 The shoot apical meristem restores its symplasmic organization during chilling-induced release from dormancy. *Plant* J. **26** 249–264.

Rodkiewicz B 1967 Walls with callose in the megaspore and hypostase of ovules of Antirrhinum majus observed in a fluorescence microscope. *Bull. Acad. pol. Sci. Cl. IlSerSd. biol*. **15** 493-495.

Rodkiewicz B 1970 Callose in cell walls during megasporogenesis in angiosperms. *Planta* **93** 39-47.

Rodkiewicz B & Gorska-Brylass A 1968 Callose in the walls of the developing megasporocyte and megaspores in the orchid ovule. *Ada Soc. Bot. Pol.* **37** 19-28.

Ruan Y, Llewellyn DJ & Furbank RT 2001 The control of single-celled cotton fiber elongation by developmentally reversible gating of plasmodesmata and coordinated expression of sucrose and K^+ transporters and expansin. *Plant Cell*.**13** 47–60.

Ruan Y, Xu S, White R, Furbank RT 2004. Genotypic and developmental evidence for the role of plasmodesmatal regulation in cotton fiber elongation mediated by callose turnover. *Plant Physiol*. **136** 4104–4113.

Russell SD 1979 Fine structure of megagametophyte development in Zea mays. Can. J. Bot. 57 1093-1110.

Samuels AL, Giddings TH, Staehelin LA 1995 Cytokinesis in tobacco BY-2 and root tip cells: A new model of cell plate formation in higher plants. *J Cell Biol*. **130** 1345–1357.

Schildknecht PHPA, Marília de M Castro M de M & Vidal BC 2004 Histochemical analysis of the root epidermal mucilage in maize and wheat *Can. J. Bot.* **82**(10) 1419–1428.

Scott R, Hodge R, Paul W & Draper J 1991The molecular biology of anther differentiation. *Plant Sci* **80** 167-191.

Sedgley M 1977 Reduced pollen tube growth and the presence of callose in the pistil of the male floral stage of

the avocado. *Scientia Horticulturae* **7**(1) 27-36.
Shivanna KR 2003 Pollen Biology and Biotechnology. Oxford & IBH Publishing Co., Pvt. Ltd. New Delhi
Shivanna KR & Johri BM 1985 The angiosperm pollen: structure & function. Wiley Eastern Ltd., New Delhi.
Smith MM & Mccully M 1978 A critical evaluation of the specificity of aniline blue induced fluorescence. *Protoplasma* **95** 229-254.
Spencer HJ 1939 On the nature of the blocking of laticiferous system at the leaf base of Hevea brasiliensis. *Ann. Bot*. N.S. **3** 231-235.
Southworth D 1971 Incorporation of radioactive precursors into developing pollen walls. In *Pollen Development and Physiology* (ed. Heslop-Harrison J), Pp. 115-120. London: Butterworths.
Staehelin LA & Hepler PK 1996Cytokinesis in higher plants *Cell* **84** 821–824.
Steiglitz H 1977 Role of ß-1,3-glucanase in postmeiotic microspore release. *Dev. Biol*. **57** 87-97.
Stieglitz H & Stern H 1973 Regulation of β-1, 3-glucanase activity in developing anthers of *Lilium. Dev. Biol.* **34**(1) 169-173.
Stone BA & Clarke AE 1992 Chemistry and Biology of (1-3)-β-D-Glucans. Victoria, Australia: La Trobe University Press
Stosser R & Anvari S F 1982 Pollen tube growth and fruit set as influenced by senescence of stigma, style and ovules. *Ada hort*. **139** 13-22.
Street HE & Withers LA 1974 The anatomy of embryogenesis in culture. In *Tissue Culture and Plant Science* (ed. Street HE), Pp. 71-100. New York, London: Academic Press.
Thiele K, Wanner G, Kindzierski V, Jürgens G, Mayer U, Pachl F & Assaad FF 2008 The timely deposition of callose is essential for cytokinesis in *Arabidopsis. Plant J.*
Thoday MG 1911 On the biological relationship between Cuscuta and its host. *Ann. Bot.* **2**5 655-681.
Thomas RC 1928 Composition of fungus hyphae. I. The Fusaria. *Am. J. Bot.* **17** 779-788.
Töller A, Brownfield L, Neu C, Twell D & Schulze-Lefert P 2008 Dual function of Arabidopsis glucan synthase-like genes GSL8 and GSL10 in male gametophyte development and plant growth. *Plant J.* **54** 911–923.
Valluri JV & Soltes EdJ 1990 Callose formation during wound-inoculated reaction of *Pinus elliottii to Fusarium subglutinans Phytochemistry* **29**(1) 71-72.
Verma DPS & Hong Z 2001 Plant callose synthase complexes. *Plant Mol Biol.* **47** 693–701.
Vijayaraghavan MR & Shukla AK 1977 Absence of Callose Around the Microspore Tetrad and Poorly Developed Exine in Pergularia daemia. *Ann. Bot.* **41**
Williams EG, Knox RB, Kaul V & Rouse JL 1984 Post-pollination callose development in ovules of Rhododendron and Ledum (Ericaceae): zygote special wall. *J. Cell Sci.* **69** 127-135.
Warmke HE & Overman MA 1972 Cytoplasmic male sterility in sorghum. 1. Callose behavior in fertile and sterile anthers. *J Hered* **63** 103-108.
W J, Horner HT & Palmer RG 1997 Genetics and cytology of a new genic male sterile soybean [*Glycine max* (L.) Merr]. *Sex Plant Reprod*.**10** (1) 13-21.
Wang X & Hong Z 2010 Precocious pollen germination in Arabidopsis plants with altered callose deposition during microsporogenesis. Planta (In Press).
Waterkeyn L 1961 Etude des depots de callose au niveau des parois sporocytaires, au moyen de la microscopie de fluorescence. *C.r. hebd. Seanc. Acad. Sci., Paris* **252** 4025—4027.
Waterkeyn L 1962 Les parois microsporocytaires de nature callosique chez Helleborus et Tradescantia. *Cellule* **62** 223-255.
Waterkeyn L 1964 Callose microsporocytaire et callose pollinique. In *Pollen Physiology and Fertilization* (ed. H. F. Linskens), Pp. 52-58. Amsterdam: North Holland.
Whelan EDP 1974 Discontinuties in the callose wall, intermeiocyte connections, and cytomixis in angiosperm meiocytes. *Can. J. Bot.* **52** 1219-1224.
Williams EG, Knox RB, Kaul V & Rouse JL 1984 Post-pollination callose development in ovules of Rhododendron and Ledum (Ericaceae): Zygote special wall. *J. Cell Sci.* **69** 127-135.
Williams E, Heslop-Harrison J & Dickinson HG 1973 The activity of the nucleolus organising region and the origin of cytoplasmic nucleoloids in meiocytes of Lilium. *Protoplasma* **11** 79-93.
Williams EG, Knox RB & Rouse JL 1982 Pollination subsystems distinguished by pollen tube arrest after incompatible interspecific crosses in Rhododendron (Ericaceae). *Cell Sci.* **53** 255-277.
Wilms H J, Went VJL, Cresti M & Ciampolini F 1983 Adventive embryogenesis in *Citrus. Caryologia* **36** 65-78.
Xie B, Wang X & Hong Z 2009 Precocious pollen germination in Arabidopsis plants with altered callose deposition during microsporogenesis. *Planta* **231** 809-823.
Zavaliev R, Sagi G, Gera A & Epel BL 2010 The constitutive expression of Arabidopsis plasmodesmal-associated class 1 reversibly glycosylated polypeptide impairs plant development and virus spread. *J. Exp. Bot.* **61**(1) 31-42.