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Callose synthesis during reproductive development in monocotyledonous and dicotyledonous plants

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Callose, a linear β-1,3-glucan molecule, plays important roles in a variety of processes in angiosperms, including development and the response to biotic and abiotic stress. Despite the importance of callose deposition, our understanding of the roles of callose in rice reproductive development and the regulation of callose biosynthesis is limited. *GLUCAN SYNTHASE-LIKE* genes encode callose synthases (GSLs), which function in the production of callose at diverse sites in plants. Studies have shown that callose participated in plant reproductive development, and that the timely deposition and degradation of callose were essential for normal male gametophyte development. In this mini-review, we described conserved sequences found in GSL family proteins from monocotyledonous (*Oryza sativa* and *Zea mays*) and dicotyledonous (*Arabidopsis thaliana* and *Glycine max*) plants. We also describe the latest findings on callose biosynthesis and deposition during reproductive development and discuss future challenges in unraveling the mechanism of callose synthesis and deposition in higher plants.

Introduction

Callose is a β-1, 3-linked homo polymer of glucose that contains some 1,6 branches. It is widespread in multicellular green algae and higher plants, including monocots and dicots, and is an essential component of specialized cell walls or cell wall-associated structures at various stages of plant development.[1] Callose was first detected by aniline blue (a triphenylmethane dye) staining more than 100 years ago, and it was thought to be the by-product of the cellulose synthase complex for nearly 2 decades until the first GLUCAN SYNTHASE-LIKE gene (*GSL*) was cloned.[2-4] Callose is synthesized from UDP-glucose, which binds directly to the catalytic subunit of callose synthase (GSL).[5] All GSLs are encoded by genes belonging to the GSL family and they are conserved across several species. Although many studies of GSL function have been conducted, especially in recent years, the number of recent review articles is limited. Here, we summarized the functions of callose, the regulatory mechanisms governing its biosynthesis, and the deposition of callose during plant reproductive development.

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Callose functions in plant reproductive development

Callose (β-1,3-glucan) is widespread in angiosperms. It is synthesized by GSLs and deposited at various stages of plant development. For example, in higher plants callose is synthesized and deposited to the cell plate, cell wall, and other locations within microsporocytes and megaspores during reproductive development.

Over the last 50 years, multiple key roles of callose in anther development have been proposed. Callose walls, temporary cell walls consisting of callose deposited between the primary cell wall and the plasma membrane, may function in the maintenance of microsporocyte morphology and to shield microsporocytes from the influence of the surrounding environment.[6] Callose walls prevent microsporocytes fusion and cohesion, and the timely degradation of the walls facilitates the release of microspores from tetrads during late meiosis. Temporary callose walls act as a physical barrier to prevent premature swelling and bursting of microspores; moreover, they appear to participate in the formation of the primexine by providing a mold for pollen exine construction during microsporogenesis. Further, the pollen tube, which functions in the transport of sperm to female gametes, contains callose both in their walls and in the plugs.[7] The cell plate is also mainly composed of callose; callose deposition at the cell plate is tightly linked to the depolymerization of microtubules. Thus, callose plays an essential role in meiosis.

Callose deposition is also associated with female reproductive development. Callose synthesis and deposition was first detected in monosporic and bisporic embryo sac development.[8] Normal endosperm development is essential for proper aril formation; defects in meiosis II related to callose deposition during this process have been shown to cause the formation of meiotic triads in the dyads of *Cytisus multiflorus* and *Cytisus striatus*.[9] In the females of oogamous eukaryotes, the megasporocyte develops into a functional megaspore with a callose-free wall in the chalazal position while the other 3 megaspores are surrounded by a callose layer; which of the 4 megaspores will develop into

functional megaspores or supernumerary megaspores seems to be associated with callose deposition around the tetrad.[10] A study of dandelion ovules revealed the deposition of callose in both diplosporous and sexual dandelions; however, callose deposition was noted mostly at the micropylar pole of the megasporocyte in a diplosporous dandelion, and it was suggested to be correlated with abnormal asynaptic meiosis.[11] Despite these findings, knowledge of the role of callose deposition in embryo sac development is limited.

GSLs in monocotyledonous and dicotyledonous plants

GSLs are comprehensive in various organs of higher plants. Phylogenetic analyses of GSL sequences from rice and *Arabidopsis* indicate that GSLs are conserved and that they can be classified into 4 subgroups; moreover, evidence indicates that the functional differentiation of GSLs arose before the divergence of monocots and dicots. The first subgroup of AtGSLs includes AtGSL1, AtGSL5, AtGSL8, and AtGSL10; the second subgroup contains AtGSL2, AtGSL3, AtGSL6, and AtGSL12; the third subgroup consists of AtGSL7 and AtGSL11; and the fourth subgroup consists of AtGSL4.[12] Most of the *GSL*s in *Arabidopsis* have been cloned, and the functions of the corresponding GSLs have been identified. The number of similar studies in rice is limited, only *GSL5* has been reported so far (**Fig. 1**).[13,14]

The GSL complex

In higher plants, callose deposition varies at different developmental stages, and the variation is biotic and abiotic stress-dependent. Callose synthesis requires several steps and multiple peptides. These proteins combine in diverse plant tissues to form a special complex that is responsible for callose synthesis at specific sites;[2,3,14] the complex contains phragmoplastin, UDP-glucose transferase (Ugt1), Rop1, and annexin. Some reports indicate that this complex contains 6 to 9 polypeptides with a molecular mass of 25–92 kDa.[15-18] The molecular mass of the catalytic complex in plants has been further defined as having a molecular mass of 32–57 kDa by affinity labeling.[19-23] CalS combines to form a heteromeric complex with phragmoplastin, Ugt1, Rho1-like protein, and, possibly, annexin.[24-26] it appears that plant Rop1 controls CalS via UGT. The relationship between tubulin and the GSL complex is supported by data obtained using 2-dimensional blue native/SDS-PAGE.[27] Another study indicated that GSL complex activity requires sucrose synthase, annexin, Ugt1, GTPase, and Ca^{2+}.[27] Thus, callose synthesis in higher plants is not a simple process; it requires the combined action of multiple proteins and other cellular components.

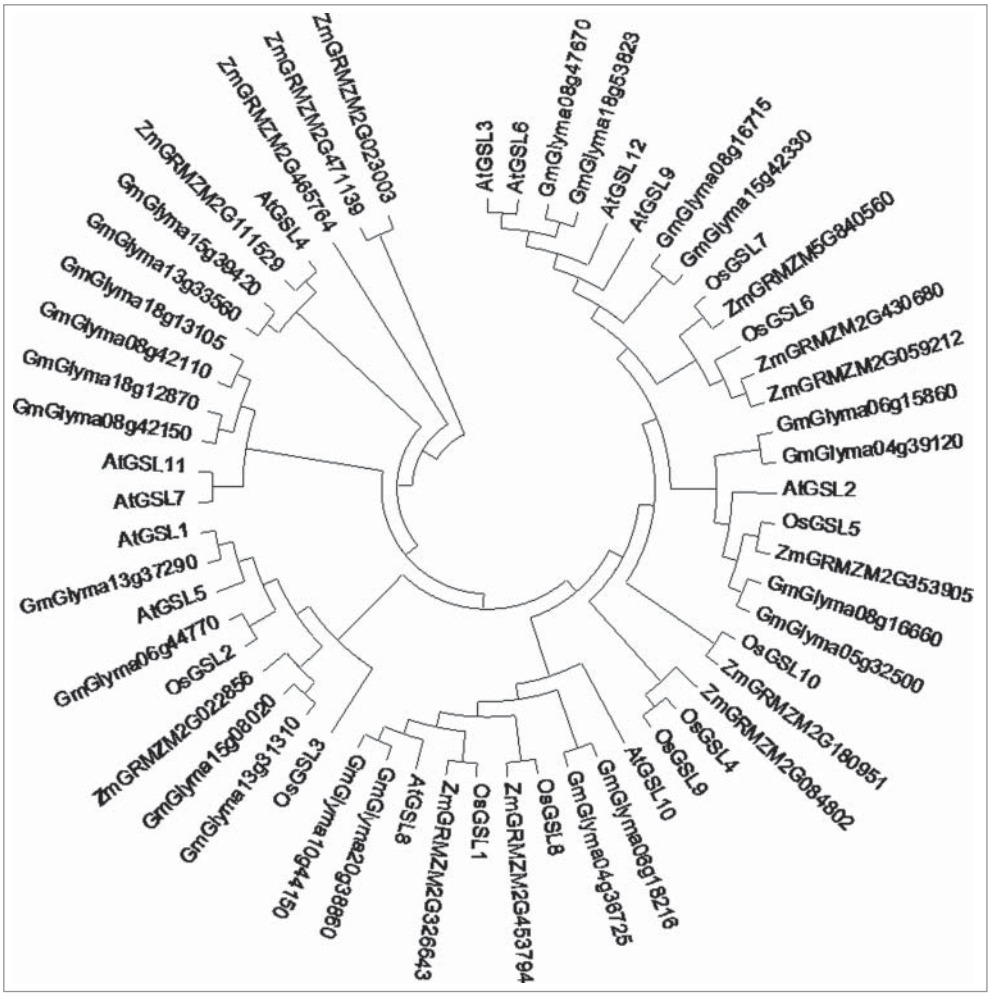

Figure 1. Phylogenetic analysis of glucan synthase-like genes (*GSL*s) in *Oryza sativa*, *Zea mays*, *Arabidopsis thaliana*, and *Glycine max*. The composite tree was produced using Clustalx1.83 and Mega5.0 by aligning deduced GSL amino acid sequences.

Callose biosynthesis during male reproductive development in plants

In higher plants, male reproductive development is a complex biological process in which a special outer wall and a cell plate consisting of callose are deposited in a timely manner. Although the precise molecular mechanisms controlling callose synthesis during microsporogenesis are elusive, the importance of timely callose biosynthesis and deposition during male gametophyte development has been confirmed.[28] The *GSL*s in various higher plant species have been cloned, and the functions of the corresponding proteins have been identified,

especially in *Arabidopsis thaliana*. *Arabidopsis thaliana* possesses 13 putative *GSL*s, and most of them have been analyzed. Two closely related and linked genes, *GSL1* and *GSL5*, may play partly essential and redundant roles while the other *GSL*s may control callose formation at different steps during pollen development.[29] GSL2 (Cals5) is responsible for the deposition of callose at the primary cell wall of the male gametophyte; the *gsl2* mutant has a defective callose wall and cannot form a properly sculpted exine, leading to the production of unviable pollen grains and male sterility.[6,30] Mutants of *AtGSL2* and *OsGSL5*, homologous genes that share 74% identity at the protein level, exhibit defective callose deposition during microsporogenesis, which may limit the development of microspores and cause the formation of an abnormal pollen exine structure.[28] Pollen grains from 2 *Arabidopsis* lines, a *gsl10* (*cals9*) knockout mutant plant and a 35S-*cals5* (*gsl2*) transgenic plant, exhibited precocious germination in the anthers and altered callose deposition; in addition, the progeny of 3 *gsl10* mutant lines exhibited a distorted segregation ratio (1:1:0 instead of 1:2:1). Further research revealed asymmetric meiosis in the microspores of the *gsl10* mutant.[31,32] GSL8 and GSL10 are each essential for microspore development and plant growth; *gsl8* and *gsl10* mutant plants exhibit asymmetric microspore meiosis and dwarfism.[33] A *atgsl8* mutant, unlike other *gsl8* mutants, which are seedling lethal, produces tetraploid meiocytes through random premeiotic endomitosis due to a defect in cell wall formation caused by defective callose deposition.[34-36] ARF17 can bind directly to the *AtGSL2* (*Cals5*) promoter to regulate callose synthesis during male gametophyte development. In the *arf17* mutant, callose deposition was shown to be significantly reduced and the primexine was absent, leading to the formation of a defective mature pollen exine structure.[37]

The regulation of callose synthesis during male reproductive development

Given the critical roles of callose deposition in wide-ranging biological processes, from plant development to environmental stress responses, the regulation of GSL activity in higher plants during male reproductive development has been scrutinized, especially under conditions of stress. In rice, an *in situ* hybridization assay indicated that GSL5 is synthesized in the tapetum and male germ cells in the anthers during reproductive development.[14] During the development of tobacco pollen tubes, it was proposed that GSLs were initially synthesized in the endoplasmic reticulum and then integrated into Golgi bodies for the transport to the subapex of the pollen tube along with actin filaments.[38,39] Other studies indicated that detergents induced GSL activity and that callose deposition increased 3–4 fold in the pollen tubes of *Nicotiana alata*.[40] In rice and barley, the thickness of the callose envelope at the young microspore stage was different under cool conditions. Among the 10 *GSL*s in rice, the expression of *OsGSL5* was dramatically downregulated by cool treatment, it indicated that cool conditions affected GSL activity (especially GSL5) during male reproductive development.[41] Phytosphingosine, an inhibitor of yeast GSLs *in vivo*, was shown to be located at the endoplasmic reticulum.[42]

In *Neurospora crassa* and higher plants, the FKS domain of GSLs binds directly to UDP-glucose.[43] UDP-glucose, as a component of the GSL complex, may bind directly to the catalytic subunit (FKS1 domain) of GSLs. The enzyme is cell plate-specific and interacts with the Rho1-like protein Rop1 and phragmoplastin.[2,3] Alamethicin, Ca^{2+}, and UDP-glucose are required for GSL activity, which may be further stimulated by the addition of Mg^{2+}. Callose deposition ceases in the presence of nucleic acid-binding dyes and marker enzymes, which can remove alamethicin, Ca^{2+}, or UDP-glucose.[27] Yeast GSLs include a catalytic subunit, FKS1, and a regulatory subunit, Rho1p; among them, Rho1p is a Rho-type small GTPase that acts as a molecular switch to activate GSLs by binding GTP.[44,45] In plants and animals, a number of CDKs participate in the regulation of *GSL* RNA splicing.[46] In *Arabidopsis thaliana*, CDKG1 regulates the pre-mRNA splicing of *AtGSL2*. A knockout mutant of *CDKG1* exhibited a low panicle seed setting rate, and further work indicated that the sixth intron of the *CDKG1* pre-mRNA was altered in the *cdkg1* mutant.[47] Consistent with mammalian *CRK7*, a GFP-CDKG1 fusion protein was found to co-localize with spliceosomal components, and it was proposed to couple splicing with transcription (a cyclin-dependent protein kinase, CDKC2, colocalizes with and modulates the distribution of spliceosomal components in *Arabidopsis*). The regulation of GSLs in higher plants includes both transcriptional and post-translational modifications such as phosphorylation and direct translocation.[48,49]

Future Perspectives

Since the first GSL was identified a decade ago, the functions of callose and the regulation of GSL activity during development and in response to biotic and abiotic stress have been studied in bacteria, yeasts, and plants. Although remarkable progress has been made, there are many unanswered questions: How is callose synthesis by GSLs achieved in various plant organs at different developmental stages? What are GSL complexes composed of? What is the precise mechanism of callose biosynthesis? Do GSLs interact with each other as homooligomers or heterooligomers? What is the function of callose in the establishment of a functional megaspore? It has been reported that the pathogen-induced callose defense response is regulated by multiple signals rather than a single conserved pathway, so whether the regulation of callose synthesis is associated with other regulatory mechanisms involved in higher plant reproductive development is unknown. To enhance our understanding of reproduction in higher plants, it will be important to find the answers to these questions through biochemical, cell biological, genetic, and systems biological research.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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