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Unplugging the callose plug from sieve pores

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The presence of callose in sieve plates has been known for a long time, but how this polysaccharide plug is synthesized has remained unsolved. Two independent laboratories have recently reported the identification of callose synthase 7 (CalS7), also known as glucan synthase-like 7 (GSL7), as the enzyme responsible for callose deposition in sieve plates. Mutant plants defective in this enzyme failed to synthesize callose in developing sieve plates during phloem formation and were unable to accumulate callose in sieve pores in response to stress treatments. The mutant plants developed less open pores per sieve plate and the pores were smaller in diameter. As a result, phloem conductivity was reduced significantly and the mutant plants were shorter and set fewer seeds.

Introduction

The term "callose" was first introduced to describe aniline blue-reactive polysaccharide present in plant cells more than 100 years ago.[1] Aniline blue is a triphenylmethane dye containing a benzophenone derivative fluorochrome and binds specifically to β-1,3-glucan and β-1,3-xylan chains.[2] The chemical nature of callose was later determined to be a β-1,3-glucan.[3] The sieve plate of phloems is one of the several anatomical locations of higher plants where the presence of callose was first discovered. It is now known that callose is an essential polysaccharide component of plant cells and can account for up to 80% of dry mass in many specialized cell walls including the callose wall, pollen tubes, and the growing cell plate.[1,4] Its synthesis and accumulation are controlled tightly during cell division, cell growth and differentiation in higher plants. Its accumulation can also be induced by biotic or abiotic stress treatments.[1,4,5] Callose is present in the sieve plate at a basal level under normal growth conditions. When plants are subject to stress, it accumulates rapidly and drastically, plugging the sieve pores. It has been unknown how this callose is synthesized and what enzyme is responsible for it. Two recent papers from independent laboratories have reported the finding of a phloem-specific callose synthase in Arabidopsis.[6,7]

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CalS7 is a Unique Member of the CalS Family

For nearly two decades, callose was thought to be a by-product of the cellulose synthase complex (reviewed in ref. 8). This misconception has finally been put to rest by the cloning of the first *Callose Synthase* (*CalS*) gene and the demonstration of its catalytic activity.[5,9] Arabidopsis contains 12 *CalS* genes, also referred to as *glucan synthase-like* (*GSL*).[10] Multiple *CalS* genes appear to have evolved in plants to meet the needs for callose biosynthesis in different tissues and in response to a range of biotic and abiotic stresses. CalS1 is localized to the cell plate and responsible for the callose biosynthesis at cytokinesis.[5,9] CalS5 plays a major role in the synthesis of the callose wall during pollen formation,[11,12] whereas CalS11 and CalS12 together are required specifically for the synthesis of the interstitial callose wall that separate the four spores in a tetrad.[13] CalS5 is also required for the formation of the callose plug and tube wall during pollen tube growth.[11,12] CalS9 is required for both symmetric and asymmetric mitosis during male gamete development.[14-16] CalS10 plays a pivotal role in the division plane orientation of the asymmetric mitosis during microgametogenesis in flowers[14,17] and stomatal formation in leaves.[18,19] CalS12 can be induced by pathogen infection and wounding.[20-22] In Arabidopsis *cs7* mutants, callose could not be detected in the sieve plate (**Fig. 1B**) and the transport function of the phloem was affected.[6,7] In mature sieve plates of control plants subjected to stress treatments, a thick layer of callose is deposited between the plasma membrane and the primary cell wall surrounding the sieve pores. Mutant plants defective in CalS7 failed to synthesize this stress-induced callose layer in sieve plates. Compared to other CalS isoforms, CalS7 contains two additional putative transmembrane domains at the N-terminal region.[6] It remains unsolved if these unique transmembrane domains serve as a targeting signal for its localization in the sieve plate.

CalS7 is Phloem-Specific

Previous observations using the CalS promoter-GUS reporter system has shown that different *CalS* genes exhibit distinct tissue-specific expression patterns.[21] Although several *CalS* gene promoters appear to be active in the vascular tissues, their function in vascular transport has not been examined. For *CalS7*, its gene expression pattern was determined using in situ RNA hybridization and promoter-reporter analysis (**Fig. 1A**). The two different techniques resulted in consistent conclusion showing the phloem-specific expression of *CalS7* in various plant tissues examined.[6,7] Analysis of callose deposition also suggests that CalS7 is specifically and primarily responsible for callose

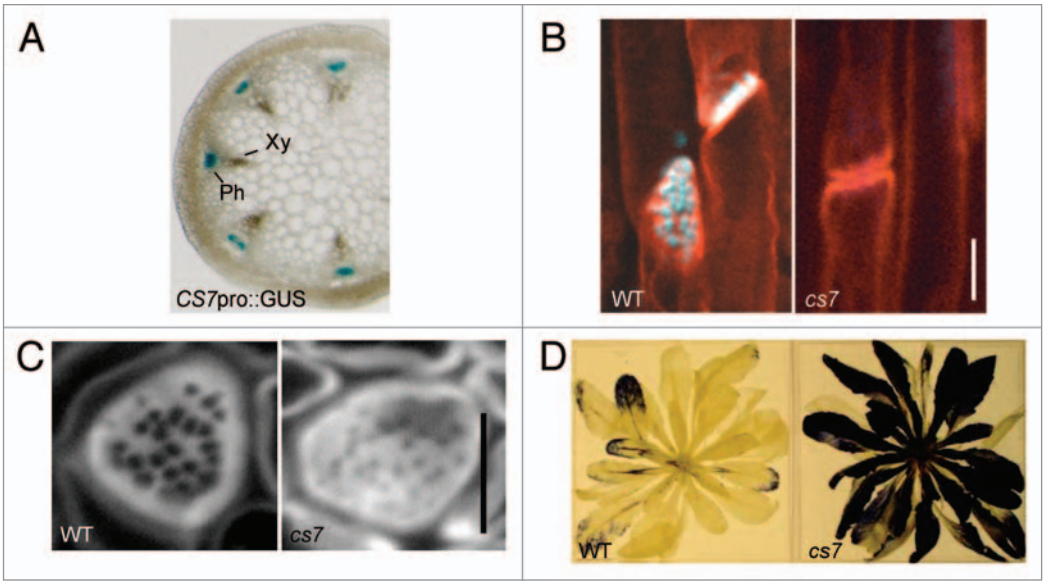

Figure 1. Lack of phloem callose and its effect on carbohydrate transport in *cs7* mutant. (A) Expression of $CalS7_{PRO}$::*GUS* in the stem. GUS activity was specifically detected in phloems (Ph), but not in xylem (Xy). (B) Confocal images of the phloem of stem samples stained with aniline blue and propidium iodine. Callose (light blue) was present in the sieve plate of the wild-type (WT), but absent in *cs7* plants. (C) Confocal images of aberrant sieve pores in *cs7* plant. (D) Accumulated starch in leaves of *cs7* plant, as indicated by iodine staining. Bars, 5 μm in (B and C). (A and B) reproduced in reference 6 and (C and D) from reference 7 ©American Society of Plant Biologists).

deposition in the phloem.[6] Because both protoxylem and protophloem are differentiated from the meristematic procambium cells, it would be interesting to study at what stage of vascular tissue development the *CalS7* gene is turned on.

Callose and Sieve Plate Development

Despite the prominent presence of callose in sieve plates, its role in sieve plate development has been a topic of debate for a long time.[23] The fact that callose is absent in the phloem cells of certain plants and that callose accumulation is highly induced by wounding and chemical treatments has led to the argument that callose might not play a major role in phloem

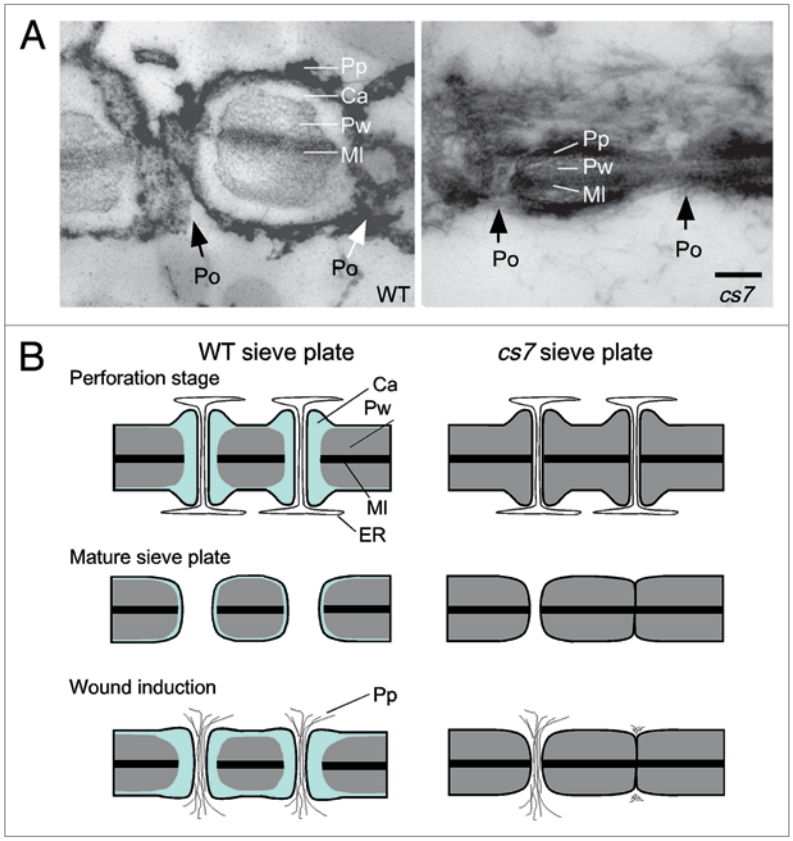

Figure 2. Role of callose in sieve pore formation and wounding response. (A) Electron microscope images of mature sieve pores in the stem of wild-type (WT) and *cs7* plants. Note the lack of callose layer and the aberrant sieve pores in *cs7*. (B) Model of lack of callose in *cs7* plant on sieve pore formation and wounding response. At the beginning of phloem perforation, callose is deposited surrounding the plasmodesmatal pores. During perforation, the callose deposit and a portion of the primary wall and middle lamella surrounding the plasmodesmatal pores are degraded, leading to the formation of widened sieve pores, which contain a residual callose layer under normal growth conditions. Wounding and other stresses can induce callose accumulation and P-protein aggregation in the sieve pores of mature phloems. In *cs7* mutant, no callose is synthesized in the plasmodesmatal pores at the beginning of perforation, which leads to the formation of aberrant sieve pores that are either very narrow or blocked. With no induced callose deposition, the sieve pores of *cs7* plants are controlled only by P proteins. Bar, 250 nm. Pp, phloem P protein; Ca, callose; Pw, primary cell wall; Ml, middle lamella; Po, sieve pore. (A) reproduced from reference 6.

development. In the new studies,[6,7] the use of *cs7* mutants has proved to be critical for the elucidation of the potential roles of callose in sieve plate development. It was observed that the lack of callose in *cs7* phloem cells hampered the perforation of phloem sieve elements, resulting in the formation of sieve plates with less and narrower pores (**Fig. 1C and 2A**).[6] These data argue in favor of the hypothesis that callose acts as a mode that specifies the pore site and size during phloem development (**Fig. 2B**).

Callose and Phloem Transport

The passage of photosynthate through the sieve pores is controlled mechanically by the aggregates of the phloem-specific P proteins and the deposition of callose.[23-25] P protein aggregation in the sieve pores was not affected in *cs7* mutant.[6] One would immediately ask if the phloem transport is affected in the absence of callose in *cs7* mutant plants. To anser this question, two experiments via pulse-chase $^{14}CO_2$ labeling and sugar content measurement were conducted in *cs7* plants (**Fig. 1D**). Data obtained from these experiments suggest that photosynthate is not efficiently transported from the source to the sink, resulting in starch accumulation in leaves and carbon starvation in the inflorescence bud clusters in *cs7* plants.[7] As consequence, the mutant plants were shorter and produced much fewer seeds. Further tests using techniques that can measure direct transport of biologically relevant biomolecules through the phloem should be performed in the characterized *cs7* plants. This will likely to shed new light onto the mechanism of phloem conductivity control in higher plants.

The identification of CalS7 as a phloem-specific callose synthase has answered a long standing question about the enzyme responsible for the callose deposition in sieve pores. It also provides a tool for future research aimed at identifying the enzyme complex and understanding the regulation mechanisms of callose biosynthesis in the phloem.

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