REVIEW ARTICLE

Biology of callose (β-1,3-glucan) turnover at plasmodesmata

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Abstract The turnover of callose (β-1,3-glucan) within cell walls is an essential process affecting many developmental, physiological and stress related processes in plants. The deposition and degradation of callose at the neck region of plasmodesmata (Pd) is one of the cellular control mechanisms regulating Pd permeability during both abiotic and biotic stresses. Callose accumulation at Pd is controlled by callose synthases (CalS; EC 2.4.1.34), endogenous enzymes mediating callose synthesis, and by β-1,3-glucanases (BG; EC 3.2.1.39), hydrolytic enzymes which specifically degrade callose. Transcriptional and post-translational regulation of some CalSs and BGs are strongly controlled by stress signaling, such as that resulting from pathogen invasion. We review the role of Pd-associated callose in the regulation of intercellular communication during developmental, physiological, and stress response processes. Special emphasis is placed on the involvement of Pd-callose in viral pathogenicity. Callose accumulation at Pd restricts virus movement in both compatible and incompatible interactions, while its degradation promotes pathogen spread. Hence, studies on mechanisms of callose turnover at Pd during viral cell-to-cell spread are of importance for our understanding of host mechanisms exploited by viruses in order to successfully spread within the infected plant.

Abbreviations

BG	β-1,3-glucanase; β-1,3-glucan hydrolase
CalS	Callose synthase
ER	Endoplasmic reticulum
GSL	Glucan synthase-like
MP	Movement protein
Pd	Plasmodesmata
PR	Pathogenesis related
SAR	Systemic acquired resistance
SEL	Size exclusion limit

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Introduction

Plasmodesmata (Pd) are trans-wall membranous channels that interconnect the cytoplasm, plasma membrane and endoplasmic reticulum (ER) of contiguous plant cells. Cell-to-cell transport via Pd is involved in such diverse processes as plant development, spread of RNA silencing, and host reactions to pathogen infection. Although the regulation of Pd permeability to different macromolecules involved in these processes is presumed to be essential, our understanding of the controlling mechanisms is rudimentary. One of the factors involved in regulating Pd permeability is callose, a β-1,3-linked homopolymer of glucose that contains some β-1,6-branches. In this article, we review current knowledge about callose regulation mechanisms.

We also discuss in detail about the regulation of callose accumulation by both endogenous and pathogen-derived factors which are involved in regulating viral movement.

Callose as a regulator of Pd permeability

Callose is known to be involved in various biological processes in plants (reviewed by Chen and Kim 2009; Kauss 1996; Ostergaard et al. 2002; Verma and Hong 2001). For example, callose is temporarily deposited at cell plates during cytokinesis and then is hydrolyzed as the new wall matures (Hong et al. 2001a,b; Samuels et al. 1995; Thiele et al. 2009); is involved in pollen development (Stone and Clarke 1992); and its temporal accumulation at Pd promotes cotton fiber elongation (Ruan et al. 2004). Besides these developmental processes, callose deposition is induced by various abiotic and biotic stresses, including metal exposure (Sivaguru et al. 2000; Ueki and Citovsky 2002), pathogen attack (Hofmann et al. 2010; Jacobs et al. 2003; Nishimura et al. 2003), and wounding (Jacobs et al. 2003).

In addition, the reversible accumulation of callose at the neck region of Pd (Northcote et al. 1989) is involved in regulating Pd permeability to macromolecules. It is one of the few host factors clearly demonstrated to control Pd-mediated cell-to-cell transport. Callose accumulation at Pd restricts the channel aperture to inhibit cell-to-cell transport of macromolecules, whereas down-regulation of callose accumulation relaxes it to allow more macromolecular trafficking (Beffa and Meins 1996; Beffa et al. 1996; Botha and Cross 2000; Bucher et al. 2001; Epel 2009; Iglesias and Meins 2000; Northcote et al. 1989). The level of callose accumulation is primarily controlled by a balance between activities of callose synthase (CalS) and β-1,3-glucanase (BG) that degrades callose (Kauss 1985; Levy et al. 2007b). Also, a recently identified Pd associated callose binding protein (PDCB, see below) may participate in regulating the accumulation level of this polysaccharide, possibly by stabilizing it (Simpson et al. 2009). These factors together participate in regulation of Pd-callose levels and thus Pd permeability.

In Arabidopsis (*Arabidopsis thaliana*), 12 genes were identified as callose synthases, designated as *GLUCAN SYNTHASE-LIKE* (*AtGSL1 to AtGSL12*), or as *CALLOSE SYNTHASE* (*AtCalS1 to AtCalS12*) according to other nomenclature (see Table 1 for correlation) (reviewed by Chen and Kim 2009; Verma and Hong 2001). In this review, we use "CalS" for callose synthase in general, and "GSL" for specific Arabidopsis callose synthases. The expression of *AtGSLs* is differentially regulated by both developmental and physiological signals (Chen and Kim 2009; Dong et al. 2005, 2008; Hong et al. 2001a,b). CalSs

Table 1 Correlation of two nomenclatures for Arabidopsis callose synthase gene family: AtCalS and AtGSL nomenclatures

GSL nomenclature	Gene ID	CalS nomenclature[a]
GSL1	AT4G04970	CalS11
GSL2	AT2G13680	CalS5
GSL3	AT2G31960.1	CalS2
GSL4	AT3G14570	CalS8
GSL5	AT4G03550	CalS12
GSL6	AT1G05570	CalS1
GSL7	AT1G06490	CalS7
GSL8	AT2G36850	CalS10
GSL9	AT5G36870	CalS4
GSL10	AT3G07160	CalS9
GSL11	AT3G59100	CalS6
GSL12	AT5G13000	CalS3

[a] CalS nomenclature is based on Verma and Hong (2001)

are large proteins (1,770–1,950 amino acids in Arabidopsis), located in the plasma membrane; they possess many transmembrane helices and large cytoplasmic region (Brownfield et al. 2009). CalSs exhibit high substrate specificity for nucleotide sugar uridine diphosphate glucose (UDP-glucose). However, studies to date failed to assign a catalytic center containing the consensus UDP-glucose binding site in CalS peptides (Brownfield et al. 2009). It was suggested that some of these enzymes function as part of a multiple subunit complex consisting of other polypeptides that do not possess catalytic domains (Verma and Hong 2001). Among the 12 *At*GSLs, some appeared to be involved in non-Pd related processes: for example, *At*GSL1, *At*GSL2, *At*GSL5, *At*GSL8, and *At*GSL10 are demonstrated to be involved in callose synthesis essential for pollen development, fertility, and/or viability (Dong et al. 2005; Enns et al. 2005; Huang et al. 2009; Nishikawa et al. 2005; Toller et al. 2008). *At*GSL8 is also involved in callose formation at cell plate during both shoot apical and root meristem development (Chen et al. 2009; Thiele et al. 2009).

Regulation of non-cell autonomous pathway by Pd-callose

Intercellular signal transduction may play a central role in providing positional information, in many cases, for plant development (reviewed by Heinlein and Epel 2004; Lehesranta et al. 2010; Lucas et al. 2009; Ruiz-Medrano et al. 2004). Intercellular signal transduction may be mediated through apoplastic pathways or by direct cell-to-cell transport of cell nonautonomous proteins by symplastic exchanges via Pd. Since callose is presumed to be involved in Pd trafficking, it is reasonable to speculate that

this polysaccharide plays an active role during the regulation of non-cell autonomous protein trafficking. A recent study revealed that callose and *At*GSL8 may play a crucial role during stomatal patterning (Guseman et al. 2010). An Arabidopsis line that has double mutations in receptor-like kinases, ERECTA (*ER*) and ERECTA-LIKE1 (*ERL1*), *er erl1*, shows weak phenotype in stomatal patterning, i.e., higher stomatal density. Genetic screening of EMS-mutagenized pool of this "sensitized line," *er erl1*, identified a mutant termed *chor*, that shows clustered stomata and incomplete development of precursor cells. *CHOR* was identified as At*GLS8*, and the mutant line showed incomplete cytokinesis and was defective in callose formation at cell plate and Pd in leaf epidermal cells. Consistently, *chor* shows much higher symplastic permeability in epidermal cells, with increased size exclusion limit (SEL, the size of the largest molecule capable of moving from cell to cell) compared to wild-type plants. Moreover, SPEACHLESS (SPCH), one of the bHLH proteins that specify the initiation of stomatal lineage, shows ectopic distribution in the *chor* epidermal cells, possibly due to the aberrant cell-to-cell leakage of the protein due to enlarged SEL. These observations suggest that GLS8 is crucially involved in regulating the stomatal differentiation by restricting distribution of stomatal development regulator SPCH (Guseman et al. 2010).

Pd permeability and β-1,3-glucanases

Studies of BGs from various plant species revealed that these enzymes localize to extracellular spaces/cell walls (Bol et al. 1990; Delp and Palva 1999) and vacuoles (Castresana et al. 1990). Earlier studies showed that there are three distinct classes of tobacco (*Nicotiana tabacum*) BGs. Class I tobacco BGs consists of vacuole isoforms (Castresana et al. 1990; Shinshi et al. 1988), while class III comprises a pathogen-induced BG, pathogenesis-related (PR)-Q′, an acidic extracellular protein (Payne et al. 1990). Class II consists of three subgroups, termed PR2a, 2b, and 2c (Ori et al. 1990; Ward et al. 1991). PR2a proteins are acidic, extracellular type, while PR2b are closely related to 2a, but with basic/neutral isoelectric point and potential C-terminal vacuole-targeting sequence (Ward et al. 1991). PR2c, also termed SP41, is an extracellular protein specifically expressed in the stylar matrix (Ori et al. 1990).

Bioinformatic analyses identified about 50 BG-related genes in Arabidopsis (Doxey et al. 2007). Phylogenetic studies and microarray-based expression analyses showed that these genes can be classified into several clusters based on tissue-specific expression, response to physical stresses, hormones, and pathogens (Doxey et al. 2007). Sequence analysis of these Arabidopsis BGs showed that there are five protein domain architectural classes, all of which contain a N-terminal secretion signal and a core glucosyl hydrolase family 17 domain, and some contain one or two repeats of a cellulose binding module, and hydrophobic C-terminal sequence (for details, see Doxey et al. 2007). Also, some Arabidopsis BGs are predicted to be glycosyl phosphatidyl inositol (GPI) anchored, which links the protein to the extracellular face of the plasma membrane (Borner et al. 2002, 2003; Elortza et al. 2003; Levy et al. 2007a). For example, *At*BG_pap (*A. thaliana* Pd-associated protein), a GPI anchored protein that associates with Pd, is involved in Pd regulation (Levy et al. 2007a). Its loss leads to high constitutive accumulation of callose at Pd due to reduced degradation, which results in limited cell-to-cell diffusion of cytoplasmic GFP (Levy et al. 2007a).

Several studies using different plant systems demonstrated that these enzymes are induced during different developmental stages. For example, induction of a class I BG is observed in the process of tobacco seed germination and dormancy break (Leubner-Metzger 2001, 2002, 2005; Leubner-Metzger and Meins 2000, 2001). Also, some BGs belonging to PR2 group, are induced by pathogen infection and are active in digesting fungal cell wall β-1,3-glucans (Dong et al. 1991; Leubner-Metzger and Meins 1999). Some BGs are identified in flowers, including anther, stylar, ovary, stamen, and pollen grain (Bucciaglia et al. 2003; Delp and Palva 1999; Hird et al. 1993).

Reversible callose accumulation is known to be involved in regulating symplastic connectivity at different stages of plant development. For example, in the shoot apex of birch (*Betula pubescens*), callose deposition is induced by short photoperiod, and as a result, symplastic connectivity is shut down (Rinne et al. 2001). In this system, the recovery of symplastic connectivity in spring is likely mediated by a BG whose expression level remains unchanged, but whose localization is altered by a temperature shift (Rinne et al. 2001). During the rapid phase of cotton fiber elongation, Pd are sealed by callose deposition, and symplastic connectivity between cotton fiber cell and underlying seed coat epidermis is lost. This loss in symplastic connectivity allows for the development of high osmotic and turgor pressures required for rapid cell wall expansion (Ruan et al. 2001, 2004, 2005). After this rapid elongation phase callose deposits at those Pd disappear. Disappearance of callose and reopening of Pd synchronize with transcription activation of a fiber-specific BG gene, suggesting that the enzyme plays a crucial role in controlling the symplastic connection between cotton fiber cells and adjacent epidermal cells at different developmental stages (Ruan et al. 2004).

Other callose associated Pd regulators

Besides these enzymes, other noncatalytic proteins associated with Pd or cell wall are apparently involved in the regulation of callose levels. A group of Pd-targeted proteins, termed Pd callose binding proteins (AtPDCBs), was recently identified from Arabidopsis (Simpson et al. 2009). AtPDCBs possess a signal sequence for GPI-anchoring and an X8 domain which is responsible for carbohydrate binding. It was shown that AtPDCBs have specific callose binding activity in vitro, and that their overexpression leads to increased callose deposition at Pd and inhibits symplastic GFP cell-to-cell diffusion (Simpson et al. 2009), thus demonstrating their role in controlling Pd permeability based on callose regulation.

The class 1 reversibly glycosylated polypeptides (^{C1}RGPs) are another class of Pd-targeted proteins that are delivered to the plasma membrane of Pd through ER-Golgi pathway (Sagi et al. 2005). In At^{C1}RGP2 overexpressing tobacco, callose accumulation is increased in source leaves compared to wild-type plants, while viral spread and development are impaired (Zavaliev et al. 2010). These observations implicate ^{C1}RGPs in callose regulation, and in turn, Pd mediated molecular trafficking. The mechanism of callose up-regulation mediated by this protein is as yet unknown.

Callose deposition is also known to be induced by oxidative conditions, presumably by activating CalSs (Bolwell et al. 2002; Verma and Hong 2001). Recent studies revealed that the cellular factors involved in metabolism of reactive oxygen species (ROS) may participate in callose regulation and, in turn, Pd-mediated macromolecular trafficking (Benitez-Alfonso et al. 2009; Benitez-Alfonso and Jackson 2009; Stonebloom et al. 2009). During a screen for genes regulating Pd permeability at phloem/nonvasculature interfaces, an Arabidopsis mutant "gfp arrested trafficking 1" (gat1) defective in the thioredoxin-m3 (TRX-m3) gene was identified (Benitez-Alfonso et al. 2009). The diffusion of GFP out of the sieve element was shown to be reduced in gat1 mutants compared to wild-type plants. The GAT1/TRX-m3 is expressed in the meristem and mostly localized to nongreen plastids. The gene is likely involved in the regulation of redox status homeostasis, since gat1 mutants accumulate high levels of ROS and, importantly, higher levels of callose in the root tips, though its accumulation level at Pd is not defined. Conversely, when GAT1/TRX-m3 was constitutively overexpressed, the cell-to-cell trafficking in mature epidermal cells was increased compared to wild-type plants, demonstrating that GAT1/TRX-m3 regulates Pd-mediated macromolecular trafficking into both positive and negative directions. In addition, root meristemless 1 (rml1), an Arabidopsis mutant which is defective in the production

of the antioxidant glutathione (Cairns et al. 2006), or wild-type plants treated with oxidants, also showed decreased macromolecular trafficking at the phloem/nonvasculature interface (Benitez-Alfonso et al. 2009). Collectively, these observations suggest that the cellular redox status is involved in callose regulation, and thus in regulation of Pd permeability. The mechanism for this regulation is unclear and is of major interest.

Interestingly, Pd-mediated trafficking can be differentially regulated by redox balance, depending on the intracellular ROS levels. Screening of embryos of EMS-mutagenized Arabidopsis lines for altered SEL by a dye loading assay using 10-kDa F-dextran (Kim et al. 2002) identified a mutant termed increased size exclusion limit 1 (ise1) (Stonebloom et al. 2009). The mutant shows larger SEL during its early embryonic stage, and is defective in the expression of ISE1, which encodes DEAD-Box RNA helicase localized to mitochondria (Stonebloom et al. 2009). In ise1 plants or in Nicotiana benthamiana whose ISE1 was transiently silenced, like in gat1, ROS accumulate at higher levels than in wild-type plants. However, in contrast to gat1, in ISE1 knockout/knockdown backgrounds the cell-to-cell macromolecular trafficking is significantly increased (Stonebloom et al. 2009). Callose deposition levels in these mutants were not characterized, and the mechanistic reasons for the opposing effects of ROS on Pd-mediated trafficking in ise1 and gat1 backgrounds need to be examined. Nonetheless, these studies revealed a possible mode of Pd regulation by cellular redox status change.

A phenomenon of callose in plant–virus interactions

Callose, apart from being a developmental regulator of symplastic connectivity, is also deposited at Pd in response to various stresses, such as wounding (Fig. 1a), plasmolysis, high temperature, chemical treatment, metal toxicity, and pathogen invasion (reviewed by Levy and Epel 2009; Stass and Horst 2009; Voigt and Somerville 2009). Callose deposition at cell wall appears to be a nonspecific defense response to infection by many pathogens, and it is presumably aimed at restricting pathogen invasion and/or spread. There are different patterns of stress-induced callose deposition at cell walls determined both by pathogen and host factors. In response to fungal and bacterial infections callose-rich papillae are formed at pathogen penetration sites between the plasma membrane and the cell wall (Aist 1976). Accumulation of callose has been detected at pit fields and walls in plants infected with wide range of viruses. In Nicotiana glutinosa infected with Tobacco mosaic virus (TMV), callose accumulation was detected in walls of initially infected leaf epidermal cells after 20 h post inoculation (hpi), prior to local lesion formation
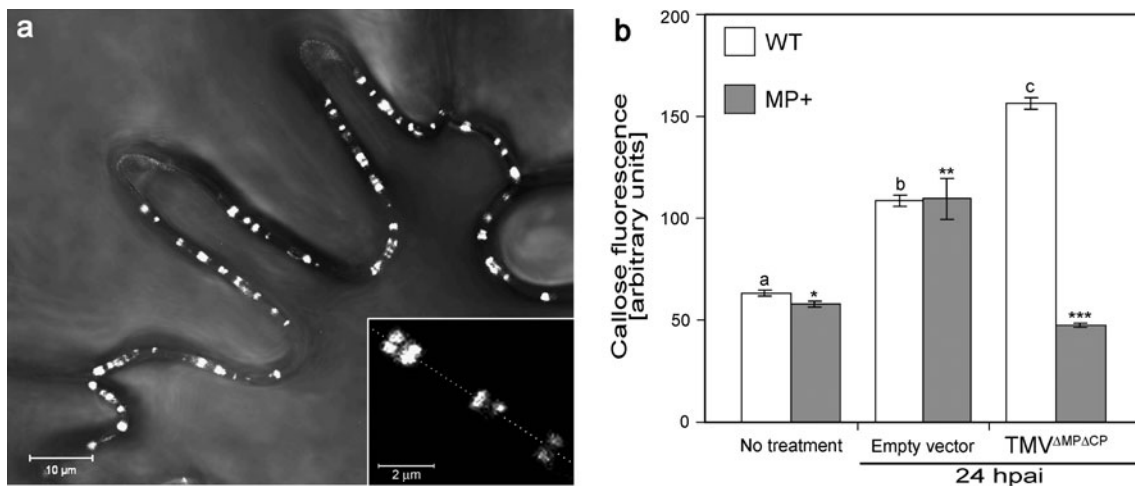
Fig. 1 Stress-induced callose accumulation at plasmodesmata in leaf epidermal cells of *N. benthamiana*. **a** Confocal image showing the pattern of wound-induced callose accumulation at pit-fields in aniline blue stained tissue. *Insert*: double fluorescent spots of callose at individual pit-fields (*dotted line* marks middle lamella). Callose was stained by incubating a strip of leaf tissue in 0.1% aniline blue for 1–2 min before observation. Intact cells adjacent to cut were observed. **b** Callose accumulation at pit-fields quantified after transient transformation with mutant TMV. Callose was quantified 24 h post agro-infiltration (24 hpai) with either a mutant TMV $(TMV^{\Delta MP\Delta CP})$ which replicates, but is unable to move cell-to-cell, or an empty vector as control for agroinfection. Both are compared to control with no treatment. Virus replication alone induces high accumulation of callose in wild-type (WT) plants to levels above both controls. Virus replication coupled with cell-to-cell movement in transgenic plants expressing viral movement protein (MP+) triggers callose degradation at Pd to levels similar to control without treatment. Panel **b** is reproduced from Guenoune-Gelbart et al. (2008)

(Shimomura and Dijkstra 1975). Callose accumulation was observed also in living cells of leaf tissues around TMV necrotic lesions, suggesting the formation of a physical barrier to slow virus spread (Beffa et al. 1996; Krasavina et al. 2002; Schuster and Flemming 1976; Shimomura 1979; Shimomura and Dijkstra 1975; Stobbs and Manocha 1977; Wu and Dimitman 1970). Similarly, callose was observed to accumulate around local lesions in *Sorghum bicolor* infected with *Maize dwarf mosaic virus* (Choi 1999); in *Gomphrena globosa* infected with *Potato virus X* (PVX) (Allison and Shalla 1974; Pennazio et al. 1981); and in potato plants infected with *Potato virus Y* (PVY) (Hinrichs-Berger et al. 1999). Callose also accumulates as a systemic response to viral infection in resistant hosts. The development of systemic acquired resistance (SAR) to TMV in resistant tobacco correlated with high callose accumulation in systemic tissues (Shimomura 1979). It should be noted that in most of these studies no Pd-callose of virus-infected plants was directly observed or measured, but rather the aniline blue-stained callose fluorescence of entire cell wall of leaf tissues was observed. It is known that the level of defense response (including callose deposition) in incompatible interactions is much stronger than in compatible interactions (Malamy et al. 1990). Therefore it is suggested that callose depositions observed in cell walls around viral-induced necrotic lesions include in addition to Pd also non-Pd associated callose due to stronger defense reaction in these plants.

In susceptible hosts, the levels of callose accumulation are very low or similar to uninfected plants (Krasavina et al. 2002; Shimomura and Dijkstra 1975). Yet, in these compatible plant/virus interactions callose was also implicated in limiting viral spread. Arabidopsis is a systemic host for seed transmissible *Turnip vein clearing virus* (TVCV). Yet, the virus is unable to penetrate the host gametes and is not transmitted through seeds of systemically infected plants (Lartey et al. 2008). This reproductive resistance was attributed to high callose accumulation observed at Pd between ovule and funiculus as well as in pollen exine in TVCV-infected Arabidopsis compared to healthy plants (Lartey et al. 2008). It was shown recently that infection of *nn* cultivar of tobacco with TMV results in inherited changes in resistance of progeny of infected plants (Kathiria et al. 2010). The F1 progeny of TMV infected plants exhibited significant delay in systemic symptoms development upon challenge with TMV, as well as increased resistance to fungal and bacterial infections. Compared to control plants, the resistant progeny showed higher frequency of homologous recombination along with high levels of callose accumulation at Pd and of PR1 expression (Kathiria et al. 2010).

Certain nonpathogen-associated stimuli, which lead to callose accumulation at Pd, may also inhibit virus spread. Stimulation of callose synthesis at Pd of vascular tissues by cadmium treatment inhibits the systemic spread of TVCV in tobacco (Ueki and Citovsky 2002). Cadmium treatment

induces high levels of cdiGRP (cadmium induced glycine-rich protein) which localizes to cell walls of vasculature and triggers high callose accumulation in this tissue. Deficiency in cdiGRP results in enhanced TVCV spread even in the presence of cadmium, while its overexpression blocks the virus (Ueki and Citovsky 2002). External application of elicitors of plant defense responses, like chitosan and β-1,3-glucan sulfate, induces high callose accumulation in treated tissues and subsequent inhibition of viral spread (Iriti and Faoro 2008; Lu et al. 2010; Menard et al. 2004). Although callose accumulation is an immediate cellular response to these elicitors, they are suggested to limit only an early phase of viral spread, since elicitors further activate broader resistance mechanisms including programmed cell death (PCD) and SAR, which restrict viral spread.

The above observations demonstrate that, regardless of the signal that induces callose deposition, there is always a strong negative correlation between callose accumulation in cell wall and virus spread. It should be noted that callose accumulation during biotic stress is only one of many other defence reactions which may also inhibit viral spread.

Regulation of callose synthesis during stress

Given that callose is a barrier to viral spread, then for efficient movement between cells and throughout the plant, viruses must somehow overcome the deposition of callose at Pd. Callose deposition at Pd is mediated by CalS. The degree, rate and reversibility of stress induced callose deposition are highly dependent on the species, tissue type, and type of stimuli (reviewed by Roberts 2005; Stass and Horst 2009; Voigt and Somerville 2009). The level of callose at Pd at any time point is a result of two parallel processes: synthesis by CalS and hydrolysis by BG. Control mechanisms differentially regulating the activity, level, and targeting of these enzymes to Pd, as well as substrate availability for both enzymes, will determine the net level of callose at Pd. In some tissues (e.g., epidermis and phloem), stress-induced callose synthesis is a rapid process occurring within minutes after stimulation (Fig. 1a) (Furch et al. 2010; Radford et al. 1998). Such rapid and localized synthesis of callose is most likely regulated at the protein level, by the activation of CalS complexes at the plasma membrane. This activation was shown to be mediated by high local concentrations of Ca^{2+} (Kauss 1985) and proteases (Nakashima et al. 2003). Burning the leaf tip in bean and tomato (Solanum lycopersicum) caused electropotential waves and led to protein plugging of the sieve plate pores within 15–45 s at a distance of 3 cm away from the stimulation point (Furch et al. 2007). This reaction

was followed by callose deposition at the pores and pore Pd units (PPUs) which reached its maximum after 20 min, with subsequent slower degradation after 1–2 h. Similar results were shown for pumpkin (Cucurbita maxima), where callose deposition reached a maximum at 10 min followed by slower degradation within 50 min (Furch et al. 2010). It was suggested that electropotential waves trigger Ca^{2+} influx into the sieve element lumen causing rapid callose deposition, and that callose degradation occurs when Ca^{2+} levels decrease (Furch et al. 2008).

In some cases, CalSs may also be regulated at the transcriptional level during stress. This was demonstrated in Arabidopsis, in which of the 12 CalS genes assayed, only two, AtGSL5 and AtGSL6, were strongly up-regulated by salicylic acid (SA) and fungal infection (Dong et al. 2008; Jacobs et al. 2003; Ostergaard et al. 2002). Normally, AtGSL5 is expressed in floral organs and is suggested to play a role in pollen maturation and fertility. However its expression is also highly induced in leaves subjected to wounding, pathogen infection, and SA treatment (Dong et al. 2008; Ostergaard et al. 2002). Loss of AtGSL5 in Arabidopsis (pmr4 and gsl5 mutations) results in low or lack of callose accumulation upon infection with compatible fungi (Jacobs et al. 2003; Meyer et al. 2009; Nishimura et al. 2003; Shimada et al. 2006; Ton and Mauch-Mani 2004; Vogel and Somerville 2000; Wawrzynska et al. 2010) and bacteria (Flors et al. 2008; Kim et al. 2005). However, unexpectedly, the mutants were resistant to these pathogens compared to susceptible wild-type plants. The resistance to fungi was suggested to result from the constitutive up-regulation of SA dependent SAR pathway in pmr4/gsl5 mutants, which includes induction of PR genes with antifungal activity (Jacobs et al. 2003; Nishimura et al. 2003). In fact, PR associated BG (AtBG2) was up-regulated in healthy pmr4 mutants, and was further highly induced upon fungal infection compared to infected wild-type plants (Nishimura et al. 2003). These observations show that, under stress conditions, both callose synthesis and degradation are controlled by SA dependent signaling. Nishimura et al. (2003) hypothesized that during biotic stress, SA-induced accumulation of either callose or AtGSL5 triggers negative feedback mechanism which represses further SA-dependent defense responses that might be potentially damaging. Therefore the loss of AtGSL5 results in up-regulated defense mechanisms. Infection of pmr4 mutants with cyst-forming nematodes similarly resulted in low callose accumulation around syncytium, a group of host cells with dissolved walls formed at site of nematode feeding. This again had no significant effect on nematode development (Hofmann et al. 2010). However, in an Arabidopsis AtBG_pap mutant, which has high and constitutive accumulation of callose at Pd, nematode development was arrested and syncytia formation was

impaired (Hofmann et al. 2010). *AtBG_pap* encodes a constitutive Pd-associated BG (Levy et al. 2007a), which is not transcriptionally regulated by pathogens or wounding (Levy and Epel, unpublished results). These data suggest that though callose accumulation may not be detrimental to nematode invasion, yet it is an important factor in blocking its spread. In fact, the dissolution of cell wall during formation of syncytium was observed to initiate along the Pd of cells at the nematode feeding site (Golinowski et al. 1996). Interestingly, *pmr4/gsl5* mutants apparently have normal punctate pattern of stress-induced callose deposition in epidermal cells (Jacobs et al. 2003; Nishimura et al. 2003), suggesting that the mechanism of callose accumulation at Pd might be distinct from that of papillae. It is likely that some other constitutive CalSs (e.g., *At*GSL8; see above) activated at the protein level are responsible for the rapid callose accumulation at Pd during stress. However, the effect of *At*GSL5 on Pd-callose levels has yet to be demonstrated.

β-1,3-Glucanases in virus infection

Induction and control of expression

Callose degradation at Pd is mediated by BG (Levy et al. 2007a). Unlike most CalSs which are posttranslationally regulated, most BGs are regulated at the transcriptional level during stress. Thus the onset of degradation of rapidly deposited callose, as opposed to synthesis, is rather a slower cellular process (Furch et al. 2007; Simmons 1994). Among the 50 Arabidopsis BG genes, only a few are induced upon pathogen infection (Doxey et al. 2007). The transcription of *AtBG1*, *AtBG2*, *AtBG3*, and *At4g16260* was shown to be highly induced in Arabidopsis upon fungal and bacterial infection (Dong et al. 1991; Doxey et al. 2007; Nawrath and Metraux 1999). The increased expression of these PR-BGs was also observed in global gene expression analysis of virus infected Arabidopsis plants (Agudelo-Romero et al. 2008; Ascencio-Ibanez et al. 2008; Babu et al. 2008; Huang et al. 2005; Whitham et al. 2003). In *Cauliflower mosaic virus* (CaMV) infected Arabidopsis, *At*BG2 (PR-2) expression was highly induced in systemic leaves along with PR-1 and PR-5 (Love et al. 2005). Yang et al. (2007) analyzed the gene expression profile at four successive zones (0 to 3) of a radial infection site in *Turnip mosaic virus* (TuMV) infected Arabidopsis leaves. A subset of stress related genes was detected, among them *At*BG2 showed highest induction within and adjacent to the infection site (zones 0–2) and no induction in zone 3 (far from infection site). Interestingly, other cell wall modifying genes like xyloglucan hydrolase 6 (XTH6), pectin methyl-esterase 3 (PME3), and expansin 10 (EXP10) showed opposite expression profiles, being suppressed in zones 0 and 1, but expressed at normal levels outside of infection site, zones 2 and 3 (Yang et al. 2007). The latter genes are responsible for cell wall extensibility, and their suppression in infected tissue is probably aimed at reducing cell wall permeability. These observations suggest functional relevance of localized induction/suppression of stress-related genes to virus spread. But what would be the effect of induced callose degradation on virus spread? Assuming that the induction of PR-BGs in the infection front is coupled with their targeting to callose deposits at Pd, it would have positive effect on virus spread. In tobacco and *N. glutinosa* the levels of BG activity were highly and locally induced during local lesion formation upon infection with TMV and *Tomato spotted wilt virus* (TSWV), and systemically induced upon infection with *Broad bean wilt virus* (BBWV) (Kauffmann et al. 1987; Moore and Stone 1972b; Sanada et al. 1986; Ye et al. 1990). Likewise, high local and systemic BG activity was measured in cucumber (*Cucumis sativus*) plants infected with TNV (Ji and Kuc 1995).

PR proteins are part of the nonspecific host defense reaction, and are involved in the phenomenon of local and systemic acquired resistance (Hull 2002). Tobacco PR-BGs have been most extensively studied with respect to plant pathogen interactions. The transcript and protein levels of a class I BG from tobacco were highly and locally induced in TMV infected plants (Vogeli-Lange et al. 1994; Vogeli-Lange et al. 1988). The expression appeared in a localized pattern, concentrating in a ring of cells around the local lesion. A similar localized gene expression pattern was observed for class II acidic isoform of BG (PR-2) in tobacco (Hennig et al. 1993), and for an acidic BG (*gluB*) in potato (Mac et al. 2004) infected with TMV.

Effect on virus spread

The localized induction and expression of PR-BGs during virus infection suggest that these proteins may have important biological functions in viral pathogenicity. The constitutive expression of PR-*BG*s in many species of transgenic plants results in increased resistance to pathogenic fungi (reviewed by Grover and Gowthaman 2003; see also Lusso and Kuc 1996; Melchers et al. 1993; Sela-Buurlage et al. 1993; Wrobel-Kwiatkowska et al. 2004), as well as in delay of root colonization by mycorrhizal fungus (Vierheilig et al. 1995). In these transgenic plants, higher resistance was suggested to result either directly from hydrolytic activity of BG on fungal cell wall β-1,3-glucans, and/or indirectly from the activation of defenses by elicitors generated by excess BG activity. In contrast to the case of fungal invasion, in the case of virus infection the direct

activity of PR-BGs cannot be explained as part of host resistance mechanism to viral spread. On the contrary, intensive callose degradation at Pd walls of infected cells would result in more opened Pd which in turn would promote viral spread. This hypothesis was proposed by Moore and Stone (1972b) who measured high BG activity in cells around TMV-induced lesions and suggested that "BG could have a role in facilitating the spread of virus through removal of callose deposits." Little information is available on the effect of PR-BG overexpression on viral infection. Yet, it could be predicted that the spread of viruses in *BG*-overexpressing plants will be enhanced due to expected net reduction in Pd-callose levels. Indeed, tobacco plants constitutively expressing a modified *BG* from thermophylic bacterium *Thermotoga neapolitana* (Movsesian et al. 2001) had significantly larger local lesions upon TMV infection compared to control plants (Serova et al. 2006). The extent of viral spread was estimated after incubation of inoculated leaves at 31°C for 24 h (virus spread) and subsequent shift to 23°C (lesion formation). The strongest effect was observed in transgenic plants with BG targeted to the apoplast compared to plants expressing cytoplasmic BG, which had no- or very small effect on virus spread. The callose levels in transgenic plants expressing extracellular BG were significantly lower than in control plants with cytoplasmic BG (Serova et al. 2006). In another report, using transgenic tobacco constitutively expressing class II acidic BG (PR-N), there was no detectable change in the local spread of TMV and no difference in systemic spread of *Tobacco etch virus* (TEV) and *Tobacco vein mottling virus* (TVMV) between transgenic and control plants (Lusso and Kuc 1996). Yet, these plants showed increased resistance to fungal infection. Although PR-N transgenic tobacco had higher BG activity, the level of callose in these plants at Pd was not measured.

As opposed to BG overexpression, the deficiency in BG results in reduced susceptibility to viral infection due to net increase in Pd callose levels (Beffa et al. 1996; Iglesias and Meins 2000). In mutant plants, in which a class I basic PR-BG, *Nt*GLA, was silenced by antisense transformation, there was a delay in local spread of TMV in mutant tobacco and delayed mosaic disease symptoms of TNV in mutant *N. silvestris* plants (Beffa et al. 1996; Iglesias and Meins 2000). The local and systemic spread of PVX (Iglesias and Meins 2000), and the systemic spread of TVCV (Ueki and Citovsky 2002), were similarly reduced in *Nt*GLA deficient tobacco. Stress induced callose accumulation in response to wounding, high temperature, and xylanase treatment was markedly increased in *Nt*GLA-deficient tobacco (Iglesias and Meins 2000; Ueki and Citovsky 2002), and this was correlated with reduced BG activity (Beffa et al. 1996; Iglesias and Meins 2000). The mutant plants also had reduced Pd SEL, as determined by an inhibition of cell-to-

cell movement of the *Cucumber mosaic virus* 3a MP-GFP fusion protein and FITC-dextrans (Iglesias and Meins 2000). When the *Nt*GLA coding sequence was cloned into the TMV genome and the recombinant virus was inoculated to wild-type tobacco plants, the virus spread faster than without the gene (Bucher et al. 2001). *Nt*GLA is a basic isoform of tobacco PR-BGs, which is localized to vacuole, but it was shown to be secreted to apoplast upon proteolitic processing (Kunze et al. 1998; Melchers et al. 1993). Therefore, it is probable that by this mechanism the vacuolar BGs are targeted to their substrate, Pd-callose, during virus infection. Acidic BG isoforms were also shown to be induced and secreted upon TMV infection in tobacco (Kauffmann et al. 1987; Payne et al. 1990; Ward et al. 1991) and TNV infection in cucumber plants (Ji and Kuc 1995).

These findings support the view that BGs do not protect the host against viral infection, but rather they are exploited by viruses, having positive effect on their spread mediated by secretion of BG to the apoplast and thus leading to callose hydrolysis at Pd.
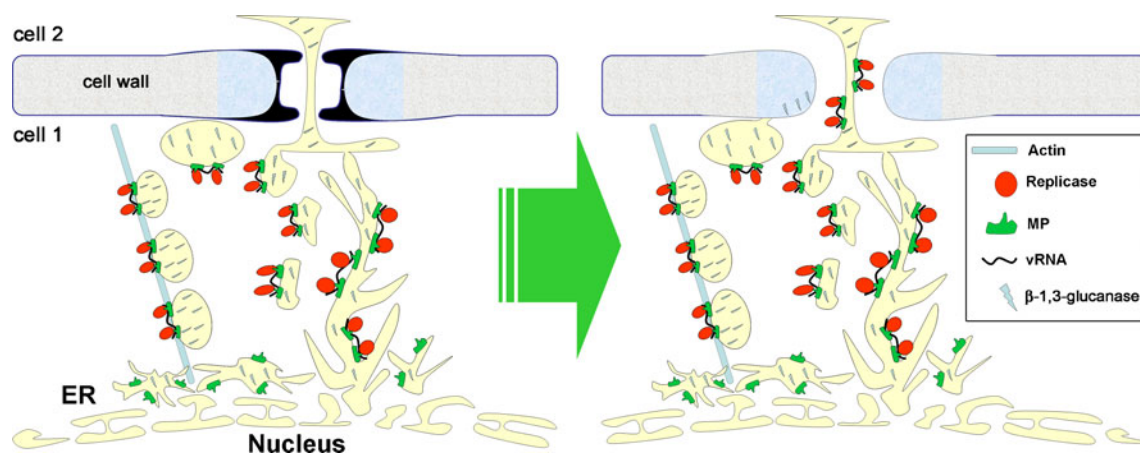
Mechanism of Pd gating by β-1,3-glucanases

Secreted proteins are inserted or translocated into the ER lumen for subsequent trafficking by ER vesicles, which fuse with plasma membrane (Jurgens and Geldner 2002). A common feature of PR proteins, including BGs, is that they are targeted to ER lumen either for subsequent secretion to apoplast (acidic proteins), or accumulation in the vacuole (basic proteins) (Bol et al. 1990). It was shown that elicitor treatment of tobacco rapidly induced local and systemic expression of PR-*BG* which followed the earlier induction of ER lumenal chaperons: lumenal binding protein (*BiP*), protein disulfide isomerase (*PDI*), endoplasmin, and calreticulin (Jelitto-Van Dooren et al. 1999). Interestingly, the expression *BiP* could be detected as early as 2 h after treatment, followed by induction of *BG* at 4 h. Early induced chaperons are suggested to prepare the ER system for intensive synthesis and secretion of PR proteins during pathogen infection (Jelitto-Van Dooren et al. 1999; Wang et al. 2005). The endomembrane system apparently plays an important role in virus replication and trafficking. The cell-to-cell spread of many plant viruses is dependent on one or more viral movement proteins (MPs) (Epel 2009; Lucas 2006). The TMV genome encodes a 30-kDa MP (MP^{TMV}), which targets and gates Pd to facilitate movement of viral complexes (Wolf et al. 1989). Upon synthesis, MP^{TMV} targets to the ER membrane and coordinates virus replication and movement through Pd, and at later stages it is associated with ER-derived membranous inclusion bodies (Heinlein et al. 1998; Mas and Beachy 1999; Reichel and

Beachy 1998). The precise mechanism of Pd gating mediated by MP^{TMV} is not well understood. Some recent studies suggest that the Pd targeting of MP^{TMV} occurs via actin-ER network (Wright et al. 2007), and its cell-to-cell movement is dependent on endocytic recycling machinery (Lewis and Lazarowitz 2010). Other studies, in contrast, show no involvement of microfilaments in viral spread and suggest the involvement of nonconventional targeting mechanisms (Avisar et al. 2008; Prokhnevsky et al. 2005). Moreover, it was shown that the gating effect of MP^{TMV} is a result of its ability to promote callose degradation at Pd accumulated as a stress response to viral infection (Guenoune-Gelbart et al. 2008; Liarzi and Epel 2005). Using TMV and *N. benthamiana* as its susceptible host, it was shown that TMV replication in the absence of MP^{TMV} results in callose accumulation at Pd, while callose levels are reduced when replication occurs in the presence of MP^{TMV} (Fig. 1b) (Guenoune-Gelbart et al. 2008). Pd-callose levels are unaffected in MP^{TMV} transgenic plants with no virus replication (Fig. 1b), suggesting that both replication and MP^{TMV} activity are necessary to promote callose hydrolysis at Pd. It was proposed that TMV must somehow recruit and/or activate BGs at Pd, or, alternatively, inactivate CalS (Epel 2009).

Taken together, it could be proposed that the secretion pathway of PR-BGs to cell wall might be up-regulated by a virus during infection to enhance Pd-callose degradation and subsequent promotion of viral spread. The possible mechanism of Pd "gating" is illustrated in Fig. 2, which shows that the cell-to-cell spread of TMV is facilitated by callose degradation at Pd mediated by stress induced BG.

Conclusions and future directions

Though callose is clearly playing a vital role in defining symplastic connection between cells, it is important to note that there are other factors that regulate Pd without altering callose deposition during apical shoot meristem development (Bayer et al. 2008; Rinne and van der Schoot 1998). Future studies may reveal the involvement of these pathways and their possible crosstalk with Pd-callose turnover in regulation of Pd permeability to coordinate various tissue differentiations.

Since plants do not have an adaptive immune system their survival under pathogen attack depends on the speed with which they activate defense mechanisms against pathogen invasion and spread. Callose deposition at cell wall, including Pd, in both compatible and incompatible host–pathogen interactions is an important part of plant's defense response. The level of callose at Pd, and thus Pd permeability, is a result of balance between its deposition and degradation, both of which are under control of stress signaling. During virus infection there is apparently a shift in this balance towards more callose degradation than deposition thus allowing rapid viral spread to as many cells as possible before onset of host resistance mechanisms. Such a shift in callose levels could result by either suppressing the stress-induced callose synthesis or enhancing its hydrolysis, or both. There are experimental evidences in support of the latter mechanism, in which viruses deploy PR-BGs to gate Pd. However, virus-induced inhibition of CalS activity during infection cannot be excluded.

Fig. 2 A schematic model illustrating the gating of Pd during virus infection, exemplified by TMV cell-to-cell spread. Callose (*black*) is deposited in the cell wall at the neck region of Pd in response to viral infection, resulting in constricted Pd aperture. Viral RNA (*vRNA*) initiates synthesis of replicase, movement protein (*MP*), and coat protein (not shown). MP associates with the endoplasmic reticulum (*ER*). Plant defense response to virus replication leads to synthesis of pathogenesis related β-1,3-glucanase which accumulates in the ER lumen. ER associated bodies containing replicase, MP and β-1,3-glucanase are formed. The bodies traffic to plasma membrane and deliver their lumenal cargo containing β-1,3-glucanase to the cell wall. When at cell wall, β-1,3-glucanase hydrolyzes callose, allowing Pd to dilate. MP:vRNA:replicase complex diffuses in the ER-desmotubule continuum to the next cell through dilated Pd. Reproduced from Levy et al. (2007b) with modifications

Further research is needed to reveal the mechanisms of Pd regulation by callose turnover during stress. The identity of possible Pd-specific CalS complex and mode of its localized regulation during stress conditions are yet unclear. Although, the involvement of Pd-callose in virus spread is clearly shown, yet the underlying cellular mechanism controlling the targeting of PR-BGs to Pd, the kinetics of callose turnover at Pd during infection and its spatiotemporal correlation with viral cell-to-cell spread, need to be determined. If Pd-callose is a barrier to slow viral spread, then its deposition has to precede the virus both in space and time. Loss-of-function mutants of PR-*BG*s, as well as the use of specific inhibitors of BG activity *in situ* will help to understand the kinetics of Pd gating during virus infection mediated by callose turnover. Several specific inhibitors of BG hydrolytic activity *in vitro* have been described previously. Among them, (2,3)-epoxypropyl-β-D-laminaribiose (Hoj et al. 1989), carbodiimide, *N*-acetylimidazole, and 2-hydroxy-5-nitrobenzylbromide (Moore and Stone 1972a), had highest inhibition at low concentrations. However, the effectiveness and specificity of these inhibitors *in planta* have not been tested and need to be determined.

Conflict of interest The authors declare that they have no conflict of interest.

References

Agudelo-Romero P, Carbonell P, de la Iglesia F, Carrera J, Rodrigo G, Jaramillo A, Perez-Amador MA, Elena SF (2008) Changes in the gene expression profile of *Arabidopsis thaliana* after infection with *Tobacco etch virus*. Virol J 5:92

Aist JR (1976) Papillae and related wound plugs of plant cells. Annu Rev Phytopathol 14:145–163

Allison AV, Shalla TA (1974) The ultrastructure of local lesions induced by *Potato virus X*: a sequence of cytological events in the course of infection. Phytopathology 64:784–793

Ascencio-Ibanez JT, Sozzani R, Lee TJ, Chu TM, Wolfinger RD, Cella R, Hanley-Bowdoin L (2008) Global analysis of Arabidopsis gene expression uncovers a complex array of changes impacting pathogen response and cell cycle during geminivirus infection. Plant Physiol 148(1):436–454

Avisar D, Prokhnevsky AI, Dolja VV (2008) Class VIII myosins are required for plasmodesmatal localization of a closterovirus Hsp70 homolog. J Virol 82(6):2836–2843

Babu M, Griffiths JS, Huang TS, Wang A (2008) Altered gene expression changes in Arabidopsis leaf tissues and protoplasts in response to *Plum pox virus* infection. BMC Genomics 9:325

Bayer E, Thomas C, Maule A (2008) Symplastic domains in the Arabidopsis shoot apical meristem correlate with PDLP1 expression patterns. Plant Signal Behav 3(10):853–855

Beffa R, Meins F (1996) Pathogenesis-related functions of plant beta-1, 3-glucanases investigated by antisense transformation—a review. Gene 179(1):97–103

Beffa RS, Hofer RM, Thomas M, Meins F Jr (1996) Decreased susceptibility to viral disease of beta-1, 3-glucanase-deficient plants generated by antisense transformation. Plant Cell 8 (6):1001–1011

Benitez-Alfonso Y, Jackson D (2009) Redox homeostasis regulates plasmodesmal communication in Arabidopsis meristems. Plant Signal Behav 4(7):655–659

Benitez-Alfonso Y, Cilia M, San Roman A, Thomas C, Maule A, Hearn S, Jackson D (2009) Control of Arabidopsis meristem development by thioredoxin-dependent regulation of intercellular transport. Proc Natl Acad Sci USA 106(9):3615–3620

Bol JF, Linthorst HJM, Cornelissen BJC (1990) Plant pathogenesis-related proteins induced by virus infection. Annu Rev Phytopathol 28:113–138

Bolwell GP, Bindschedler LV, Blee KA, Butt VS, Davies DR, Gardner SL, Gerrish C, Minibayeva F (2002) The apoplastic oxidative burst in response to biotic stress in plants: a three-component system. J Exp Bot 53(372):1367–1376

Borner GH, Sherrier DJ, Stevens TJ, Arkin IT, Dupree P (2002) Prediction of glycosylphosphatidylinositol-anchored proteins in Arabidopsis. A genomic analysis. Plant Physiol 129(2):486–499

Borner GH, Lilley KS, Stevens TJ, Dupree P (2003) Identification of glycosylphosphatidylinositol-anchored proteins in Arabidopsis. A proteomic and genomic analysis. Plant Physiol 132(2):568–577

Botha CEJ, Cross RHN (2000) Towards reconciliation of structure with function in plasmodesmata—who is the gatekeeper? Micron 31(6):713–721

Brownfield L, Doblin MS, Fincher GB, Bacic A (2009) Biochemical and molecular properties of biosynthetic enzymes for (1,3)-beta-glucans in embryophytes, chlorophytes and rhodophytes. In: Bacic A, Fincher GB, Stone BA (eds) Chemistry, biochemistry and biology of (1-3)-beta-glucans and related polysaccharides. London Academic Press, pp 283–326

Bucciaglia PA, Zimmermann E, Smith AG (2003) Functional analysis of a beta-1, 3-glucanase gene (Tag1) with anther-specific RNA and protein accumulation using antisense RNA inhibition. J Plant Physiol 160(11):1367–1373

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

Cairns NG, Pasternak M, Wachter A, Cobbett CS, Meyer AJ (2006) Maturation of Arabidopsis seeds is dependent on glutathione biosynthesis within the embryo. Plant Physiol 141(2):446–455

Castresana C, de Carvalho F, Gheysen G, Habets M, Inzé D, Van Montague M (1990) Tissue-specific and pathogen-induced regulation of a *Nicotiana plumbaginifolia* beta-1, 3-glucanase gene. Plant Cell 2:1131–1143

Chen XY, Kim JY (2009) Callose synthesis in higher plants. Plant Signal Behav 4(6):489–492

Chen XY, Liu L, Lee E, Han X, Rim Y, Chu H, Kim SW, Sack F, Kim JY (2009) The Arabidopsis callose synthase gene GSL8 is required for cytokinesis and cell patterning. Plant Physiol 150 (1):105–113

Choi CW (1999) Modified plasmodesmata in sorghum (*Sorghum bicolor* L. Moench) leaf tissues infected by *Maize dwarf mosaic virus*. J Plant Biol 42(1):63–70

Delp G, Palva ET (1999) A novel flower-specific Arabidopsis gene related to both pathogen-induced and developmentally regulated plant beta-1, 3-glucanase genes. Plant Mol Biol 39(3):565–575

Dong X, Mindrinos M, Davis KR, Ausubel FM (1991) Induction of Arabidopsis defense genes by virulent and avirulent *Pseudomo-*

nas syringae strains and by a cloned avirulence gene. Plant Cell 3 (1):61–72

Dong X, Hong Z, Sivaramakrishnan M, Mahfouz M, Verma DP (2005) Callose synthase (CalS5) is required for exine formation during microgametogenesis and for pollen viability in Arabidopsis. Plant J 42(3):315–328

Dong X, Hong Z, Chatterjee J, Kim S, Verma DP (2008) Expression of callose synthase genes and its connection with Npr1 signaling pathway during pathogen infection. Planta 229(1):87–98

Doxey AC, Yaish MW, Moffatt BA, Griffith M, McConkey BJ (2007) Functional divergence in the Arabidopsis beta-1, 3-glucanase gene family inferred by phylogenetic reconstruction of expression states. Mol Biol Evol 24(4):1045–1055

Elortza F, Nuhse TS, Foster LJ, Stensballe A, Peck SC, Jensen ON (2003) Proteomic analysis of glycosylphosphatidylinositol-anchored membrane proteins. Mol Cell Proteomics 2(12):1261–1270

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(3):333–349

Epel BL (2009) Plant viruses spread by diffusion on ER-associated movement-protein-rafts through plasmodesmata gated by viral induced host beta-1, 3-glucanases. Semin Cell Dev Biol 20 (9):1074–1081

Flors V, Ton J, van Doorn R, Jakab G, Garcia-Agustin P, Mauch-Mani B (2008) Interplay between JA, SA and ABA signalling during basal and induced resistance against *Pseudomonas syringae* and *Alternaria brassicicola*. Plant J 54(1):81–92

Furch AC, Hafke JB, Schulz A, van Bel AJ (2007) Ca^{2+}-mediated remote control of reversible sieve tube occlusion in *Vicia faba*. J Exp Bot 58(11):2827–2838

Furch AC, Hafke JB, van Bel AJ (2008) Plant- and stimulus-specific variations in remote-controlled sieve-tube occlusion. Plant Signal Behav 3(10):858–861

Furch AC, Zimmermann MR, Will T, Hafke JB, van Bel AJ (2010) Remote-controlled stop of phloem mass flow by biphasic occlusion in *Cucurbita maxima*. J Exp Bot 61 (13):3697–3708

Golinowski W, Grundler FMW, Sobczak M (1996) Changes in the structure of *Arabidopsis thaliana* during female development of the plant parasitic nematode *Heterodera schachtii*. Protoplasma 194:103–116

Grover A, Gowthaman R (2003) Strategies for development of fungus-resistant transgenic plants. Curr Sci 84(3):330–340

Guenoune-Gelbart D, Elbaum M, Sagi G, Levy A, Epel BL (2008) *Tobacco mosaic virus* (TMV) replicase and movement protein function synergistically in facilitating TMV spread by lateral diffusion in the plasmodesmal desmotubule of *Nicotiana benthamiana*. Mol Plant Microb Interact 21(3):335–345

Guseman JM, Lee JS, Bogenschutz NL, Peterson KM, Virata RE, Xie B, Kanaoka MM, Hong Z, Torii KU (2010) Dysregulation of cell-to-cell connectivity and stomatal patterning by loss-of-function mutation in Arabidopsis CHORUS (GLUCAN SYNTHASE-LIKE 8). Development 137(10):1731–1741

Heinlein M, Epel BL (2004) Macromolecular transport and signaling through plasmodesmata. Int Rev Cytol 235:93–164

Heinlein M, Padgett HS, Gens JS, Pickard BG, Casper SJ, Epel BL, Beachy RN (1998) Changing patterns of localization of the *Tobacco mosaic virus* movement protein and replicase to the endoplasmic reticulum and microtubules during infection. Plant Cell 10(7):1107–1120

Hennig J, Dewey RE, Cutt JR, Klessig DF (1993) Pathogen, salicylic acid and developmental dependent expression of a beta-1, 3-glucanase/GUS gene fusion in transgenic tobacco plants. Plant J 4(3):481–493

Hinrichs-Berger J, Harfold M, Breger S, Buchenauer H (1999) Cytological responses of susceptible and extremely resistant potato plants to inoculation with *Potato virus Y*. Physiol Mol Plant Pathol 55:143–150

Hird DL, Worrall D, Hodge R, Smartt S, Paul W, Scott R (1993) The anther-specific protein encoded by the *Brassica napus* and *Arabidopsis thaliana* A6 gene displays similarity to beta-1, 3-glucanases. Plant J 4(6):1023–1033

Hofmann J, Youssef-Banora M, de Almeida-Engler J, Grundler FM (2010) The role of callose deposition along plasmodesmata in nematode feeding sites. Mol Plant Microb Interact 23(5):549–557

Hoj PB, Rodriguez EB, Stick RV, Stone BA (1989) Differences in active site structure in a family of beta-glucan endohydrolases deduced from the kinetics of inactivation by epoxyalkyl beta-oligoglucosides. J Biol Chem 264(9):4939–4947

Hong ZL, Delauney AJ, Verma DPS (2001a) A cell plate specific callose synthase and its interaction with phragmoplastin. Plant Cell 13(4):755–768

Hong ZL, Zhang Z, Olson JM, Verma DPS (2001b) A novel UDP-glucose transferase is part of the callose synthase complex and interacts with phragmoplastin at the forming cell plate. Plant Cell 13(4):769–779

Huang Z, Yeakley JM, Garcia EW, Holdridge JD, Fan JB, Whitham SA (2005) Salicylic acid-dependent expression of host genes in compatible Arabidopsis-virus interactions. Plant Physiol 137 (3):1147–1159

Huang L, Chen XY, Rim Y, Han X, Cho WK, Kim SW, Kim JY (2009) Arabidopsis glucan synthase-like 10 functions in male gametogenesis. J Plant Physiol 166(4):344–352

Hull R (2002) Matthews' plant virology, 4th edn. London Academic Press

Iglesias VA, Meins F Jr (2000) Movement of plant viruses is delayed in a beta-1, 3-glucanase-deficient mutant showing a reduced plasmodesmatal size exclusion limit and enhanced callose deposition. Plant J 21(2):157–166

Iriti M, Faoro F (2008) Abscisic acid is involved in chitosan-induced resistance to *Tobacco necrosis virus* (TNV). Plant Physiol Biochem 46(12):1106–1111

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(11):2503–2513

Jelitto-Van Dooren EP, Vidal S, Denecke J (1999) Anticipating endoplasmic reticulum stress. A novel early response before pathogenesis-related gene induction. Plant Cell 11(10):1935–1944

Ji C, Kuc J (1995) Purification and characterization of an acidic beta-1, 3-glucanase from cucumber and its relationship to systemic disease resistance induced by *Colletotrichum lagenarium* and *Tobacco necrosis virus*. Mol Plant Microb Interact 8(6):899–905

Jurgens G, Geldner N (2002) Protein secretion in plants: from the trans-Golgi network to the outer space. Traffic 3(9):605–613

Kathiria P, Sidler C, Golubov A, Kalischuk M, Kawchuk LM, Kovalchuk I (2010) *Tobacco mosaic virus* infection results in an increase in recombination frequency and resistance to viral, bacterial and fungal pathogens in the progeny of infected tobacco plants. Plant Physiol 153(4):1859–1870

Kauffmann S, Legrand M, Geoffroy P, Fritig B (1987) Biological function of pathogenesis-related proteins: four PR proteins of tobacco have 1, 3-beta-glucanase activity. EMBO J 6(11):3209–3212

Kauss H (1985) Callose biosynthesis as a Ca^{2+}-regulated process and possible relations to the induction of other metabolic changes. J Cell Sci Suppl 2:89–103

Kauss H (1996) Callose synthesis. In: Smallwood M, Knox JP, Bowles DJ (eds) Membranes: specialized functions in plants. BIOS Scientific, Oxford, pp 77–92

Kim I, Hempel FD, Sha K, Pfluger J, Zambryski PC (2002) Identification of a developmental transition in plasmodesmatal function during embryogenesis in *Arabidopsis thaliana*. Development 129:1261–1272

Kim MG, da Cunha L, McFall AJ, Belkhadir Y, DebRoy S, Dangl JL, Mackey D (2005) Two *Pseudomonas syringae* type III effectors inhibit RIN4-regulated basal defense in Arabidopsis. Cell 121 (5):749–759

Krasavina MS, Malyshenko SI, Raldugina GN, Burmistrova NA, Nosov AV (2002) Can salicylic acid affect the intercellular transport of the *Tobacco mosaic virus* by changing plasmodesmal permeability? Russ J Plant Physiol 49(1):71–77

Kunze I, Kunze G, Broker M, Manteuffel R, Meins F Jr, Muntz K (1998) Evidence for secretion of vacuolar alpha-mannosidase, class I chitinase, and class I beta-1, 3-glucanase in suspension cultures of tobacco cells. Planta 205(1):92–99

Lartey RT, Ghoshroy K, Ghoshroy S (2008) Association of selective deposition of (1-3)-beta-glucan in floral tissues with restricted movement of *Turnip vein-clearing virus* in Arabidopsis: a possible mechanism for non-seed transmission. Plant Pathol J 7(2):120–130

Lehesranta SJ, Lichtenberger R, Helariutta Y (2010) Cell-to-cell communication in vascular morphogenesis. Curr Opin Plant Biol 13(1):59–65

Leubner-Metzger G (2001) Brassinosteroids and gibberellins promote tobacco seed germination by distinct pathways. Planta 213 (5):758–763

Leubner-Metzger G (2002) Seed after-ripening and over-expression of class I beta-1, 3-glucanase confer maternal effects on tobacco testa rupture and dormancy release. Planta 215(6):959–968

Leubner-Metzger G (2005) beta-1, 3-Glucanase gene expression in low-hydrated seeds as a mechanism for dormancy release during tobacco after-ripening. Plant J 41(1):133–145

Leubner-Metzger G, Meins F (1999) Functions and regulation of plant beta-1, 3-glucanases (PR-2). In: Datta SK, Muthukrishnan S (eds) Pathogenesis-related proteins in plants. CRC, Boca Raton, pp 49–76

Leubner-Metzger G, Meins F Jr (2000) Sense transformation reveals a novel role for class I beta-1, 3-glucanase in tobacco seed germination. Plant J 23(2):215–221

Leubner-Metzger G, Meins F Jr (2001) Antisense-transformation reveals novel roles for class I beta-1, 3-glucanase in tobacco seed after-ripening and photodormancy. J Exp Bot 52 (362):1753–1759

Levy A, Epel BL (2009) Cytology of the (1-3)-beta-glucan (callose) in plasmodesmata and sieve plate pores. In: Bacic A, Fincher GB, Stone BA (eds) Chemistry, biochemistry and biology of (1-3)-beta-glucans and related polysaccharides. London Academic Press, pp 439–463

Levy A, Erlanger M, Rosenthal M, Epel BL (2007a) A plasmodesmata-associated beta-1, 3-glucanase in Arabidopsis. Plant J 49(4):669–682

Levy A, Guenoune-Gelbart D, Epel BL (2007b) beta-1, 3-Glucanases: plasmodesmal gate keepers for intracellular communication. Plant Signal Behav 2(5):404–407

Lewis JD, Lazarowitz SG (2010) Arabidopsis synaptotagmin SYTA regulates endocytosis and virus movement protein cell-to-cell transport. Proc Natl Acad Sci USA 107(6):2491–2496

Liarzi O, Epel BL (2005) Development of a quantitative tool for measuring changes in the coefficient of conductivity of plasmodesmata induced by developmental, biotic, and abiotic signals. Protoplasma 225(1–2):67–76

Love AJ, Yun BW, Laval V, Loake GJ, Milner JJ (2005) *Cauliflower mosaic virus*, a compatible pathogen of Arabidopsis, engages three distinct defense-signaling pathways and activates rapid systemic generation of reactive oxygen species. Plant Physiol 139(2):935–948

Lu H, Zhao X, Wang W, Yin H, Xu J, Bai X, Du Y (2010) Inhibition effect of *Tobacco mosaic virus* and regulation effect on calreticulin of oligochitosan in tobacco by induced Ca^{2+} influx. Carbohydr Polym 82(1):136–142

Lucas WJ (2006) Plant viral movement proteins: agents for cell-to-cell trafficking of viral genomes. Virology 344(1):169–184

Lucas WJ, Ham BK, Kim JY (2009) Plasmodesmata—bridging the gap between neighboring plant cells. Trends Cell Biol 19 (10):495–503

Lusso M, Kuc J (1996) The effect of sense and antisense expression of the PR-N gene for β-1, 3-glucanase on disease resistance of tobacco to fungi and viruses. Physiol Mol Plant Pathol 49:267–283

Mac A, Krzymowska M, Barabasz A, Hennig J (2004) Transcriptional regulation of the gluB promoter during plant response to infection. Cell Mol Biol Lett 9(4B):843–853

Malamy J, Carr JP, Klessig DF, Raskin I (1990) Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection. Science 250(4983):1002–1004

Mas P, Beachy RN (1999) Replication of *Tobacco mosaic virus* on endoplasmic reticulum and role of the cytoskeleton and virus movement protein in intracellular distribution of viral RNA. J Cell Biol 147(5):945–958

Melchers LS, Sela-Buurlage MB, Vloemans SA, Woloshuk CP, Van Roekel JS, Pen J, van den Elzen PJ, Cornelissen BJ (1993) Extracellular targeting of the vacuolar tobacco proteins AP24, chitinase and beta-1, 3-glucanase in transgenic plants. Plant Mol Biol 21(4):583–593

Menard R, Alban S, de Ruffray P, Jamois F, Franz G, Fritig B, Yvin JC, Kauffmann S (2004) Beta-1, 3-glucan sulfate, but not beta-1, 3-glucan, induces the salicylic acid signaling pathway in tobacco and Arabidopsis. Plant Cell 16(11):3020–3032

Meyer D, Pajonk S, Micali C, O'Connell R, Schulze-Lefert P (2009) Extracellular transport and integration of plant secretory proteins into pathogen-induced cell wall compartments. Plant J 57 (6):986–999

Moore AE, Stone BA (1972a) A beta-1, 3-glucan hydrolase from Nicotiana glutinosa: II. Specificity, action pattern and inhibitor studies. Biochim Biophys Acta 258(1):248–264

Moore AE, Stone BA (1972b) Effect of infection with TMV and other viruses on the level of a beta-1, 3-glucan hydrolase in leaves of *Nicotiana glutinosa*. Virology 50(3):791–798

Movsesian NR, Alizade X, Musiichuk KA, Popov Iu G, Piruzian ES (2001) Transgenic tobacco plants expressing bacterial genes encoded the thermostable glucanases. Genetika 37(6):745–753

Nakashima J, Laosinchai W, Cui X, Brown RMJ (2003) New insight into the mechanism of cellulose and callose biosynthesis: proteases may regulate callose biosynthesis upon wounding. Cellulose 10:369–389

Nawrath C, Metraux JP (1999) Salicylic acid induction-deficient mutants of Arabidopsis express PR-2 and PR-5 and accumulate high levels of camalexin after pathogen inoculation. Plant Cell 11 (8):1393–1404

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

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

Northcote DH, Davey R, Lay J (1989) Use of antisera to localize callose, xylan and arabinogalactan in the cell-plate, primary and secondary cell walls of plant cells. Planta 178:353–366

Ori N, Sessa G, Lotan T, Himmelhoch S, Fluhr R (1990) A major stylar matrix polypeptide (Sp41) is a member of the pathogenesis-related proteins superclass. EMBO J 9(11):3429–3436

Ostergaard L, Petersen M, Mattsson O, Mundy J (2002) An Arabidopsis callose synthase. Plant Mol Biol 49(6):559–566

Payne G, Ward E, Gaffney T, Goy PA, Moyer M, Harper A, Meins F, Ryals J (1990) Evidence for a 3rd structural class of beta-1, 3-glucanase in tobacco. Plant Mol Biol 15(6):797–808

Pennazio S, Redolfi P, Sapetti C (1981) Callose formation and permeability changes during the partly localized reaction of *Gomphrena globosa* to *Potato virus X*. Phytopathol Z 100:172–181

Prokhnevsky AI, Peremyslov VV, Dolja VV (2005) Actin cytoskeleton is involved in targeting of a viral Hsp70 homolog to the cell periphery. J Virol 79(22):14421–14428

Radford JE, Vesk M, Overall RL (1998) Callose deposition at plasmodesmata. Protoplasma 201(1–2):30–37

Reichel C, Beachy RN (1998) *Tobacco mosaic virus* infection induces severe morphological changes of the endoplasmic reticulum. Proc Natl Acad Sci USA 95(19):11169–11174

Rinne PL, van der Schoot C (1998) Symplasmic fields in the tunica of the shoot apical meristem coordinate morphogenetic events. Development 125(8):1477–1485

Rinne PL, Kaikuranta PM, van der Schoot C (2001) The shoot apical meristem restores its symplasmic organization during chilling-induced release from dormancy. Plant J 26(3):249–264

Roberts AG (2005) Plasmodesmal structure and development. In: Oparka KJ (ed) Plasmodesmata. Annu Plant Rev, Vol. 18, Oxford Blackwell Publishing, pp 1–32

Ruan YL, 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(1):47–60

Ruan YL, Xu SM, 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(4):4104–4113

Ruan YL, Llewellyn DJ, Furbank RT, Chourey PS (2005) The delayed initiation and slow elongation of fuzz-like short fibre cells in relation to altered patterns of sucrose synthase expression and plasmodesmata gating in a lintless mutant of cotton. J Exp Bot 56 (413):977–984

Ruiz-Medrano R, Xoconostle-Cazares B, Kragler F (2004) The plasmodesmatal transport pathway for homeotic proteins, silencing signals and viruses. Curr Opin Plant Biol 7(6):641–650

Sagi G, Katz A, Guenoune-Gelbart D, Epel BL (2005) Class 1 reversibly glycosylated polypeptides are plasmodesmal-associated proteins delivered to plasmodesmata via the Golgi apparatus. Plant Cell 17 (6):1788–1800

Samuels AL, Giddings TH Jr, 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(6):1345–1357

Sanada M, Matsushita K, Shimokawa H, Itoh R (1986) Comparison of beta-1, 3-glucan hydrolase activity in some tobacco plants inoculated with *Tobacco mosaic virus*. Ann Phytopathol Soc Jpn 52:320–329

Schuster G, Flemming M (1976) Studies on the formation of diffusion barriers in hypersensitive hosts of *Tobacco mosaic virus* and the role of necrotization in the formation of diffusion barriers as well as in the localization of virus infections. Phytopathol Z 87:345–352

Sela-Buurlage MB, Ponstein AS, Bres-Vloemans SA, Melchers LS, Van Den Elzen P, Cornelissen B (1993) Only specific tobacco (*Nicotiana tabacum*) chitinases and beta-1, 3-glucanases exhibit antifungal activity. Plant Physiol 101(3):857–863

Serova VV, Raldugina GN, Krasavina MS (2006) Inhibition of callose hydrolysis by salicylic acid interferes with *Tobacco mosaic virus* transport. Dokl Biochem Biophys 406:36–39

Shimada C, Lipka V, O'Connell R, Okuno T, Schulze-Lefert P, Takano Y (2006) Nonhost resistance in Arabidopsis–*Colletotrichum* interactions acts at the cell periphery and requires actin filament function. Mol Plant Microb Interact 19 (3):270–279

Shimomura T (1979) Stimulation of callose synthesis in the leaves of Samsun *NN* tobacco showing systemic acquired resistance to *Tobacco mosaic virus*. Ann Phytopathol Soc Japan 45:299–304

Shimomura T, Dijkstra J (1975) The occurrence of callose during the process of local lesion formation. Neth J Plant Pathol 81:107–121

Shinshi H, Wenzler H, Neuhaus JM, Felix G, Hofsteenge J, Meins F (1988) Evidence for N-terminal and C-terminal processing of a plant defense-related enzyme—primary structure of tobacco prepro-beta-1, 3-glucanase. Proc Natl Acad Sci USA 85 (15):5541–5545

Simmons CR (1994) The physiology and molecular biology of plant 1, 3-beta-d-glucanases and 1, 3;1, 4-beta-d-glucanases. Crit Rev Plant Sci 13(4):325–387

Simpson C, Thomas C, Findlay K, Bayer E, Maule AJ (2009) An Arabidopsis GPI-anchor plasmodesmal neck protein with callose binding activity and potential to regulate cell-to-cell trafficking. Plant Cell 21(2):581–594

Sivaguru M, Fujiwara T, Samaj J, Baluska F, Yang Z, Osawa H, Maeda T, Mori T, Volkmann D, Matsumoto H (2000) Aluminum-induced 1→3-beta-d-glucan inhibits cell-to-cell trafficking of molecules through plasmodesmata. A new mechanism of aluminum toxicity in plants. Plant Physiol 124:991–1006

Stass A, Horst WJ (2009) Callose in abiotic stress. In: Bacic A, Fincher GB, Stone BA (eds) Chemistry, biochemistry and biology of (1-3)-beta-glucans and related polysaccharides. London Academic Press, pp 499–524

Stobbs LW, Manocha MS (1977) Histological changes associated with virus localization in TMV-infected Pinto bean leaves. Physiol Plant Pathol 11:87–94

Stone BA, Clarke AE (1992) Chemistry and biology of 1→3-beta-glucans. La Trobe University Press, Victoria

Stonebloom S, Burch-Smith T, Kim I, Meinke D, Mindrinos M, Zambryski P (2009) Loss of the plant DEAD-box protein ISE1 leads to defective mitochondria and increased cell-to-cell transport via plasmodesmata. Proc Natl Acad Sci USA 106 (40):17229–17234

Thiele K, Wanner G, Kindzierski V, Jurgens G, Mayer U, Pachl F, Assaad FF (2009) The timely deposition of callose is essential for cytokinesis in Arabidopsis. Plant J 58:13–26

Toller 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(5):911–923

Ton J, Mauch-Mani B (2004) Beta-amino-butyric acid-induced resistance against necrotrophic pathogens is based on ABA-dependent priming for callose. Plant 38(1):119–130

Ueki S, Citovsky V (2002) The systemic movement of a tobamovirus is inhibited by a cadmium-ion-induced glycine-rich protein. Nat Cell Biol 4(7):478–486

Verma DP, Hong Z (2001) Plant callose synthase complexes. Plant Mol Biol 47:693–701

Vierheilig H, Alt M, Lange J, Gut-Rella M, Wiemken A, Boller T (1995) Colonization of transgenic tobacco constitutively expressing pathogenesis-related proteins by the vesicular–arbuscular mycorrhizal fungus *Glomus mosseae*. Appl Environ Microbiol 61 (8):3031–3034

Vogel J, Somerville S (2000) Isolation and characterization of powdery mildew-resistant Arabidopsis mutants. Proc Natl Acad Sci USA 97(4):1897–1902

Vogeli-Lange R, Hansengehri A, Boller T, Meins F (1988) Induction of the defense-related glucanohydrolases, beta-1, 3-glucanase and chitinase, by *Tobacco mosaic virus* infection of tobacco leaves. Plant Sci 54(3):171–176

Vogeli-Lange R, Frundt C, Hart CM, Nagy F, Meins F Jr (1994) Developmental, hormonal, and pathogenesis-related regulation of the tobacco class I beta-1, 3-glucanase B promoter. Plant Mol Biol 25(2):299–311

Voigt CA, Somerville SC (2009) Callose in biotic stress (pathogenesis). In: Bacic A, Fincher GB, Stone BA (eds) Chemistry, biochemistry and biology of (1-3)-beta-glucans and related polysaccharides. London Academic Press, pp 525–562

Wang D, Weaver ND, Kesarwani M, Dong X (2005) Induction of protein secretory pathway is required for systemic acquired resistance. Science 308(5724):1036–1040

Ward ER, Payne GB, Moyer MB, Williams SC, Dincher SS, Sharkey KC, Beck JJ, Taylor HT, Ahl-Goy P, Meins F, Ryals JA (1991) Differential regulation of beta-1, 3-glucanase messenger RNAs in response to pathogen infection. Plant Physiol 96(2):390–397

Wawrzynska A, Rodibaugh NL, Innes RW (2010) Synergistic activation of defense responses in Arabidopsis by simultaneous loss of the GSL5 callose synthase and the EDR1 protein kinase. Mol Plant Microb Interact 23(5):578–584

Whitham SA, Quan S, Chang HS, Cooper B, Estes B, Zhu T, Wang X, Hou YM (2003) Diverse RNA viruses elicit the expression of common sets of genes in susceptible *Arabidopsis thaliana* plants. Plant J 33(2):271–283

Wolf S, Deom CM, Beachy RN, Lucas WJ (1989) Movement protein of *Tobacco mosaic virus* modifies plasmodesmatal size exclusion limit. Science 246(4928):377–379

Wright KM, Wood NT, Roberts AG, Chapman S, Boevink P, Mackenzie KM, Oparka KJ (2007) Targeting of TMV movement protein to plasmodesmata requires the actin/ER network: evidence from FRAP. Traffic 8(1):21–31

Wrobel-Kwiatkowska M, Lorenc-Kukula K, Starzycki M, Oszmianski J, Kepczynska E, Szopa J (2004) Expression of β-1, 3-glucanase in flax causes increased resistance to fungi. Physiol Mol Plant Pathol 65(5):245–256

Wu JH, Dimitman JE (1970) Leaf structure and callose formation as determinants of TMV movement in bean leaves as revealed by UV irradiation studies. Virology 40(4):820–827

Yang C, Guo R, Jie F, Nettleton D, Peng J, Carr T, Yeakley JM, Fan JB, Whitham SA (2007) Spatial analysis of *Arabidopsis thaliana* gene expression in response to *Turnip mosaic virus* infection. Mol Plant Microb Interact 20(4):358–370

Ye XS, Pan SQ, Kuc J (1990) Association of pathogenesis-related proteins and activities of peroxidase, beta-1, 3-glucanase and chitinase with systemic induced resistance to blue mould of tobacco but not to systemic *Tobacco mosaic virus*. Physiol Mol Plant Pathol 36:523–531

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):131–142