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Commentary

Title: Cellulose/callose glucan network: the key to powdery mildew resistance in plants?

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“Since the presence of both callose and cellulose material has been indicated in papillae induced by [...] fungi in a variety of hosts [...] this might represent a rather general phenomenon.”

(Hächler & Hohl, 1982)

The plant cell wall represents the first barrier against intruding pathogens. Especially in response to fungi and oomycetes, localized cell wall modification, so-called papillae, at sites of attempted pathogen penetration is one of the earliest plant defense responses that has been analyzed on a cellular level, starting over 150 years ago (deBary, 1863). Papillae are generally thought to function as a physical barrier to slow or even stop pathogen invasion (Stone & Clarke, 1992) and appear to be induced in essentially all plants following pathogen challenge. Hence, localized papillae formation can be regarded as a ubiquitous plant defense response, which is not specific to a phylum or even a species compared with many other defense responses. Papillae are complex structures that contain diverse chemical components with a clear antimicrobial function, like phenolic compounds and thionins (McLusky *et al.*, 1999), or hydrogen peroxide as a reactive oxygen species, used by peroxidases to promote cross-linking of proteins and phenolics for cell wall reinforcement (Thordal-Christensen *et al.*, 1997). In contrast, the precise function of the carbohydrate polymers has not been elucidated in detail yet. This is surprising as the most prominent cell wall polymer of papillae, the (1,3)- β -glucan callose, was already described by Mangin (1895) over 120 years ago. The existence of the (1,4)- β -glucan cellulose in pathogen-induced papillae was indicated in cellular and biochemical analyses starting over 100 years ago and finally proven and visualized in potato tuber infected by the oomycete *Phytophthora infestans* (Hächler & Hohl, 1982). Recently, a detailed histochemical study revealed a similar distribution of the two glucan polymers in papillae of the Poaceae and important crop barley (*Hordeum vulgare*) induced by the adapted powdery mildew *Blumeria graminis* f.sp. *hordei* (*Bgh*). The inner core of effective papillae, where *Bgh* invasion was unsuccessful, was composed of callose (and in the case of barley also considerable amounts of arabinoxylan) surrounded by cellulose (Chowdhury *et al.*, 2014). In this issue of *New Phytologist* (pp. X-Y and X-Y), Douchkov *et al.* (2016) and Chowdhury *et al.* (2016) make the latest contribution to the importance and function of glucan polymers in pathogen-induced papillae in barley. They could not only show that both, callose and cellulose are required to form and establish effective papillae against *Bgh*, but also provide the precise information about the genes that encode the respective cellulose and callose synthase, respectively. This would open new opportunities in studying regulatory mechanisms of papillae formation in an important crop and support new molecular breeding approaches for increased penetration resistance.

But what do we really know about the function of the two glucan polymers callose and cellulose in papillae? In general, it has long been observed that prevention of pathogen invasion is associated with relatively high amounts of callose and cellulose deposited at sites of attempted penetration, ideally forming effective papillae. However, the effectiveness of callose deposition in pathogen-induced papillae was challenged by the observation that callose-rich papillae were also consistently found at sites of successful penetration (Aist, 1976). Moreover, disruption of the pathogen-induced callose synthase PMR4 (POWDERY MILDEW RESISTANT 4; also known as GSL5 [GLUCAN SYNTHASE-LIKE 5]) in the model plant *Arabidopsis* (*Arabidopsis thaliana*) prevented callose deposition at sites of attempted fungal penetration, but unexpectedly increased resistance to powdery mildew (Nishimura *et al.*, 2003). Because observed powdery mildew resistance was caused by hyperactivation of the salicylic acid pathway in the absence of callose deposition and/or the callose synthase PMR4, an additional function of callose synthases in regulating plant-defense related pathways other than callose biosynthesis can be anticipated. A function of pathogen-induced callose synthase in regulating defense pathways is not restricted to *Arabidopsis* but can also be

concluded from results in tomato (*Solanum lycopersicum*). Silencing of the callose synthase gene *SIPMR4*, the ortholog of *PMR4*, resulted in increased resistance to the adapted powdery mildew *Oidium neolycopersici* (Huibers *et al.*, 2013). In contrast, silencing of *HvGsl6*, encoding the callose synthase from barley that falls into the *PMR4* clade (Chowdhury *et al.*, 2016), also decreased callose accumulation in powdery mildew-induced papillae; however, resulting in an increased penetration rate of *Bgh*. Hence, degree of callose deposition in papillae seems to correlate with penetration resistance in barley; and reveals an active function of callose in providing resistance to powdery mildew. In conclusion, the pathogen-induced callose synthase in barley might not or to a lesser degree be involved in regulating other plant defense pathways compared to the dicotyledonous plants *Arabidopsis* and tomato. As a consequence, barley, and putatively other Poaceae, would represent a more suitable target for successfully modifying pathogen-induced callose biosynthesis for increased powdery mildew resistance. Compared to dicots, less, unexpected alteration of other defense pathways and unwanted pleiotropic effects would be expected. Regarding increased powdery mildew resistance through callose modification, overexpression of *PMR4* in *Arabidopsis* induced elevated, early callose deposition at infection sites and complete penetration resistance to the adapted powdery mildew *Golovinomyces cichoracearum* (Ellinger *et al.*, 2013). Transient expression of the *Arabidopsis PMR4* gene in barley leaves increased *Bgh* penetration resistance (Blümke *et al.*, 2013), indicating that elevated callose deposition in powdery mildew-induced papillae would improve their effectiveness in stopping fungal invasion also in barley. Combining this result with the recently gained knowledge from the study of Chowdhury *et al.* (2016), *HvGsl6* overexpression in barley would constitute a promising approach in increasing powdery mildew resistance. Interestingly, penetration resistance to powdery mildew in *Arabidopsis PMR4* overexpression lines was not only a consequence of elevated callose deposition but also an extensive migration of callose fibrils into the pre-existing cellulosic cell wall, which was revealed by super-resolution microscopy (Eggert *et al.*, 2014). The formation of an enlarged cellulose/callose glucan polymer network provided resistance to cell wall-hydrolyzing enzymes, which can directly be associated with penetration resistance.

This brings us directly to the role of cellulose in establishing effective papillae against intruding powdery mildews. Even though a cellulose layer that surrounds the callosic core of pathogen-induced papillae, as reported for potato (Hächler & Hohl, 1982) and recently in great detail for barley (Chowdhury *et al.*, 2014), has not been reported for *Arabidopsis* yet, the detection of the cellulose/callose network showed the importance of cellulose in forming effective papillae also in *Arabidopsis*. However, to pin down the function of cellulose to a specific callose synthase would be rather problematic in *Arabidopsis* because the cellulose fibrils involved in cellulose/callose network formation are not pathogen-induced but part of the regular cellulosic cell wall. Therefore, disruption of any of the known cell wall cellulose synthase is accompanied with severe pleiotropic effects. The situation in barley is different. Here, papillae-associated cellulose deposition could be associated with one specific cellulose-modifying enzyme encoded by the cellulose synthase-like D2 gene (*HvCslD2*) (Douchkov *et al.*, 2016). Silencing of *HvCslD2* significantly reduced cellulose deposition at pathogen-induced papillae, resulting in increased susceptibility to powdery mildew. This provided direct evidence for an active role of cellulose in forming effective papillae. Hence, barley-powdery mildew interaction currently represents the most suitable pathosystem to study the role of cellulose in effective papillae formation. Similar to the approach in modifying callose biosynthesis, overexpression of *HvCslD2* could provide new insight into the function of cellulose. One main question would be whether elevated cellulose deposition at forming, powdery mildew-induced papillae might correlate with increased penetration resistance. In this regard, a further structural analysis of the cell wall polymer architecture by using super-resolution microscopy would reveal the extent to which cellulose/callose networks also occur in barley papillae and whether also arabinoxylan would interact. Here, it is likely that these glucan networks may not only be formed in association with the pre-

existing, cellulosic epidermal cell wall but also the pathogen-induced, papillae-associated cellulose layers.

Considering the current knowledge of the occurrence and function of glucan polymers in pathogen-induced cell wall depositions, it can be hypothesized that the interaction of cellulose and callose might be a key mechanism in forming effective papillae that successfully prevent powdery mildew infection. Cellulose/callose polymer networks would be supportive in generating cell wall regions that could be more resistant to cell wall hydrolyzing enzymes (Eggert *et al.*, 2014). In addition, these glucan networks might impede the ingress of so-called effectors that are required for successful invasion and colonization of the host by biotrophic pathogens like powdery mildews. In this regard, it is noteworthy to mention that cellulose/callose polymer networks were not only reported from plant-pathogen interaction sites, but were also formed in unchallenged epidermal leaf cells of the C4 Poaceae maize (*Zea mays*) and *Miscanthus x giganteus* (Falter *et al.*, 2015). Interestingly, powdery mildews do not infect C4 grasses. Summing up, there is growing indication that the statement of Hächler & Hohl (1982) about the general presence of both cellulose and callose in pathogen-induced, host papillae could be correct; and might be complemented with extension of “effective” papillae and the occurrence of “cellulose/callose polymer networks” in these papillae.

- Aist JR 1976.** Papillae and Related Wound Plugs of Plant Cells. In: Baker KF, Zentmeyer GA, Cowling EB eds. *Annual Review of Phytopathology*. Palo Alto: Annual Reviews mInc., 145-163.
- Blümke A, Somerville SC, Voigt CA. 2013.** Transient expression of the *Arabidopsis thaliana* callose synthase PMR4 increases penetration resistance to powdery mildew in barley. *Advances in Bioscience and Biotechnology* 4: 810-813.
- Chowdhury J, Henderson M, Schweizer P, Burton RA, Fincher GB, Little A. 2014.** Differential accumulation of callose, arabinoxylan and cellulose in nonpenetrated versus penetrated papillae on leaves of barley infected with *Blumeria graminis* f. sp. hordei. *New Phytol* 204(3): 650-660.
- Chowdhury J, Schober MS, Shirley NJ, Singh RR, Jacobs AK, Douchkov D, Schweizer P, Fincher GB, Burton RA, Little A. 2016.** Down-regulation of the *glucan synthase-like 6 gene* (*HvGsl6*) in barley leads to decreased callose accumulation and increased cell wall penetration by *Blumeria graminis* f. sp. hordei. *New Phytol*.
- deBary A. 1863.** Recherches sur le développement de quelques champignons parasites. *Annales des Sciences Naturelles. Botanique et Biologie végétale* 20: 5-148.
- Douchkov D, Lueck S, Hensel G, Kumlehn J, Rajaraman J, Johrde A, Doblin MS, Beahan CT, Kopischke M, Fuchs R, et al. 2016.** The barley (*Hordeum vulgare*) cellulose synthase-like D2 gene (*HvCslD2*) mediates penetration resistance to host-adapted and nonhost isolates of the powdery mildew fungus. *New Phytologist*.
- Eggert D, Naumann M, Reimer R, Voigt CA. 2014.** Nanoscale glucan polymer network causes pathogen resistance. *Sci Rep* 4: 4159.
- Ellinger D, Naumann M, Falter C, Zwikowics C, Jamrow T, Manisseri C, Somerville SC, Voigt CA. 2013.** Elevated early callose deposition results in complete penetration resistance to powdery mildew in Arabidopsis. *Plant Physiol* 161: 1433-1444.
- Falter C, Zwikowics C, Eggert D, Blümke A, Naumann M, Wolff K, Ellinger D, Reimer R, Voigt CA. 2015.** Glucanocellulosic ethanol: the undiscovered biofuel potential in energy crops and marine biomass. *Scientific Reports* 5: 13722.
- Hächler H, Hohl HR. 1982.** Histochemistry of papillae in potato-tuber tissue infected with *Phytophthora infestans*. *Botanica Helvetica* 92(1): 23-31.
- Huibers RP, Loonen AE, Gao D, Van den Ackerveken G, Visser RG, Bai Y. 2013.** Powdery mildew resistance in tomato by impairment of *SIPMR4* and *SIDMR1*. *PLoS One* 8(6): e67467.

- Mangin L. 1895.** Recherches sur les Péronosporées. *Bulletin de la Société d'Histoire Naturelle d'Autun* **8**: 55-108.
- McLusky SR, Bennett MH, Beale MH, Lewis MJ, Gaskin P, Mansfield JW. 1999.** Cell wall alterations and localized accumulation of feruloyl-3'-methoxytyramine in onion epidermis at sites of attempted penetration by *Botrytis allii* are associated with actin polarisation, peroxidase activity and suppression of flavonoid biosynthesis. *Plant Journal* **17**(5): 523-534.
- 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**(5635): 969-972.
- Stone BA, Clarke AE. 1992.** *Chemistry and Biology of (1→3)-β-glucans*. Bundoora: La Trobe University Press.
- Thordal-Christensen H, Zhang Z, Wei Y, Collinge DB. 1997.** Subcellular localization of H₂O₂ in plants. H₂O₂ accumulation in papillae and hypersensitive response during the barley-powdery mildew interaction. *Plant Journal* **11**(6): 1187-1194.