

Commentary

Walking on a tightrope: cell wall-associated kinases act as sensors and regulators of immunity and symbiosis

Choosing between symbiosis- and immunity-related responses is a decision that plants have to make continuously. As they grow in the soil and the atmosphere, their epidermal cells are probed by a multitude of microbes, attracted by the wealth of organic molecules that plants accumulate in their tissues through photosynthesis. Among this crowd of plant-interacting microorganisms, fungi are an important and particularly challenging group. While symbiotic fungi will gently enter the root tissues offering to trade organic molecules for mineral nutrients, several pathogens will attempt a destructive attack, digesting cell walls and heading straight towards the starch-stuffed parenchymal tissues. The sooner a plant recognises who is who, the better its chances to survive. Indeed, the study of plant perception of fungal signals (Oldroyd, 2013) and downstream activation of signalling pathways (Giovannetti *et al.*, 2024) and appropriate responses has driven several decades of investigation, depicting a complex scenario that has been more exhaustively clarified in the case of pathogenic interactions (Dodds *et al.*, 2024). Our understanding of plant–fungus signalling in mycorrhizal symbioses is undoubtedly less complete (Genre *et al.*, 2020). However, a number of studies have shed light on a few key elicitors, their receptors and the signal transduction pathways (Crosino & Genre, 2022) that mediate fungal accommodation in arbuscular mycorrhizas (AM) through the regulation of plant gene expression and cell reorganisation (MacLean *et al.*, 2017).

‘Zhang and coworkers challenged cotton plants with both an AM fungus (Rhizophagus irregularis) and a fungal pathogen (Verticillium dahliae). This approach led them to the identification of a cell wall-associated Ser/Thr kinase, GhWAK13, as a key player in both interactions.’

The emerging scenario suggests that the mechanisms controlling AM development are largely ruled by the host plant. For example, epidermal cell penetration by the fungal hyphae is blocked in plant mutants (Radhakrishnan *et al.*, 2020) and fungal entry and exit sites

from each cell appear to be defined by the orientation of plant exocytic processes (Genre *et al.*, 2012).

During more advanced stages of AM development, genetic, cellular and transcriptomic studies have revealed that accommodation and senescence of individual arbuscules depend on how efficiently the fungus can provide the plant with mineral nutrients (Javot *et al.*, 2007; Lanfranco *et al.*, 2018). Indeed, the relatively short lifespan of arbuscules – estimated to be only a few days – manifests the delicate equilibrium each plant cell is constantly challenged with. The need to simultaneously facilitate the entry and growth of the beneficial fungus while managing its defence/immunity responses to control arbuscule over-proliferation by activating senescence processes and, likely, preventing simultaneous pathogen infection.

Nevertheless, the characterisation of signals and transduction pathways mediating fungal colonisation of inner root tissues, arbuscule development, functioning and senescence remain elusive. This is largely due to the technical difficulties in targeting inner cortical cells at the precise site and time when such signalling processes are deployed.

The gap in our understanding is now starting to be filled, and the study by Zhang *et al.* (2024; 2180–2194), published in this issue of *New Phytologist*, is taking a major step forward. It provides original and enlightening research, elucidating the intricate molecular mechanisms that govern the nuanced trade-off between facilitating fungal growth and controlling it. Taking a step beyond the systematic comparison of plant responses to either pathogens or symbionts (Carella & Schornack, 2018; Delaux & Schornack, 2021), Zhang and co-workers challenged cotton plants with both an AM fungus (*Rhizophagus irregularis*) and a fungal pathogen (*Verticillium dahliae*). This approach led them to the identification of a cell wall-associated Ser/Thr kinase, GhWAK13, as a key player in both interactions.

While we refer you to the full paper for the complete picture, it is relevant to highlight here a few crucial conclusions emerging from this study. The first hint of an overlap between symbiotic and immune signalling in this tripartite interaction was that *Verticillium* wilt infection was associated with an inhibition of AM development. Second, transcriptomic analyses highlighted that *GhWAK13* expression was upregulated by both the symbiotic and the pathogenic interaction, and in both cases, the protein accumulated in colonised areas. Third, *GhWAK13* silencing inhibited arbuscule development and *Verticillium* wilt infection by stimulating the expression of defence-related genes and the reinforcement of cell walls by promoting cellulose deposition. Together, the present results strongly indicate this novel cell wall-associated kinase as a regulator of immune responses playing a central role in both interactions.

The emerging model (Fig. 1) indicates that during cell wall remodelling, either by fungal (in pathogenic interactions) or by plant enzymes (during AM colonisation), oligogalacturonides

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(OGs; fragments derived from the partial degradation of homogalacturonan in the plant cell wall) are released from cell wall pectins and act as strong immunity elicitors. In this context, *WAK13* activation by such cell wall-derived molecules suppresses immunity. Strong *WAK13* upregulation is critical to prevent deleterious defence responses during AM colonisation and arbuscule development. By contrast, a weaker upregulation is associated with *Verticillium* wilt infection, granting a stronger immunity response, at the cost of limiting symbiosis development.

Two aspects of this model are particularly intriguing. On the one hand, this signalling process appears to be conserved both during interactions with a pathogen, such as *V. dahliae*, and a symbiont, such as *R. irregularis*. This observation suggests the fascinating possibility that root pathogens have partially co-opted root colonisation mechanisms that evolved in symbiotic interactions. On the other hand, with reference to AM symbiosis, the signalling mechanism is strictly localised to the inner root tissues, where signals are generated from the plant cell walls and transduced within cortical cells. These are distinctive features compared with known signalling processes mediating plant–fungus recognition in AM, where fungal wall-generated signals are perceived by root epidermal cells (Crosino & Genre, 2022). Together, this convincingly indicates that we are dealing with two structurally (and most likely temporally) unrelated signalling pathways, whose functional relationship remains to be clarified.

GhWAK13 belongs to the gene family of wall-associated kinases, which plays a crucial role in several plant development processes that involve cell wall remodelling (Stephens *et al.*, 2022). In Arabidopsis, *AtWAK1*, a member of this protein family, can sense cell wall-derived OGs *in vivo*, which triggers downstream immune responses (Decreux *et al.*, 2006; Brutus *et al.*, 2010). This ability has led to *AtWAK1* being

described as a cell wall integrity or pectin sensor (Kohorn, 2016). During the interaction between plants and microorganisms that can invade plant cells, the presence of OGs, which can derive from cell wall pectin, is revealed upon cell wall loosening, expansion or the action of pathogen-derived cell wall-degrading enzymes (CWDEs). Due to their involvement in multiple processes and possible pleiotropic effects triggered in respective knock-out mutants, our precise understanding of WAK proteins at the cellular level has been slowed down (Wagner & Kohorn, 2001). Nevertheless, it has been observed that the disruption of numerous *WAK* genes is usually associated with increased susceptibility to pathogens (Deltiel *et al.*, 2016; Huerta *et al.*, 2023); conversely, overexpression of *WAKs* has been linked to improved resistance to disease (Harkenrider *et al.*, 2016; Feng *et al.*, 2021). Interestingly, Zhang and colleagues have identified *GhWAK13* as a negative regulator of plant immunity activated by the presence of both beneficial and pathogenic fungi. The action of the protein seems to compete with another member of the same family, *GhWAK7A*, which was assigned a crucial role in the chitin-induced immune response (Wang *et al.*, 2020).

Although our understanding of the molecular mechanisms underlying the mutual recognition between AM fungi and plants during the arbuscular phase is limited, several kinases that appear to mediate this process have recently been identified in different plants. In rice, the ARBUSCULAR RECEPTOR-LIKE KINASE 1 and 2, belonging to an undefined receptor-like kinase (RLK) subfamily, govern postarbuscule development and regulation of the AM association (Roth *et al.*, 2018; Montero *et al.*, 2021). In *Medicago truncatula*, KIN3, an active kinase, accumulates in the endoplasmic reticulum of arbusculated cells, exerting a partial suppression of the host immune response (Irving *et al.*, 2022) and a similar phenotype has been shown for its orthologous protein in *Lotus japonicus* (Leng

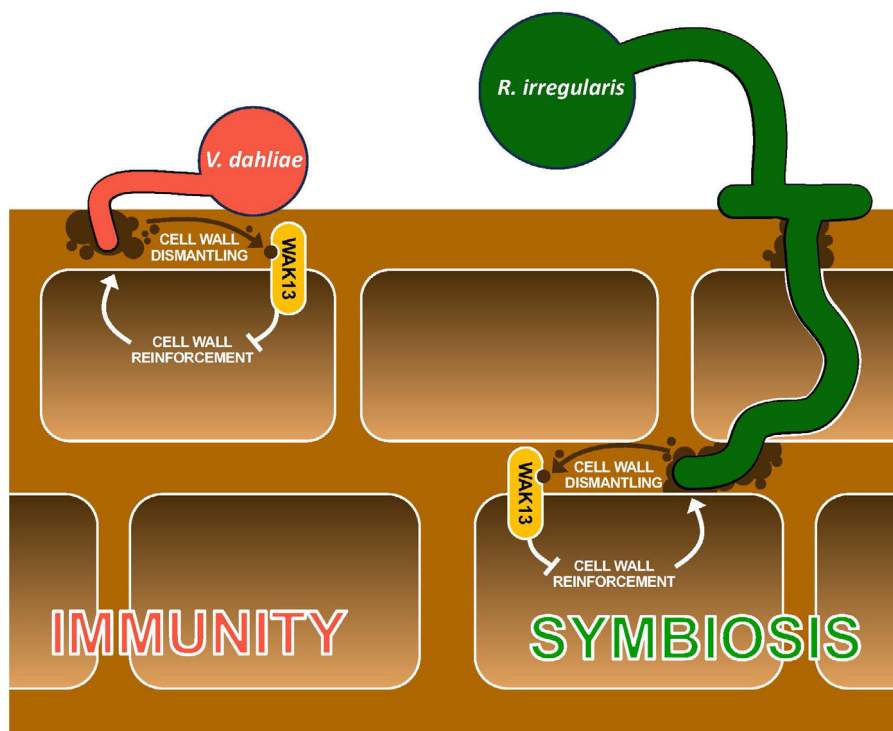


Fig. 1 Schematic representation of the dual role of *GhWAK13* in immunity and symbiosis. The model depicted by Zhang *et al.* (2024; in this issue of *New Phytologist*, pp. 2180–2194) indicates the role of this wall-associated kinase in perceiving cell wall degradation by-products and suppressing defence responses that coordinate cell wall reinforcement during both pathogenic and symbiotic interactions.

et al., 2023). Additionally, the rice kinase gene *OsADK1*, specifically induced in arbusculated cells, proves indispensable for arbuscular development during AM symbiosis, and the protein accumulates in the nucleus (Guo *et al.*, 2022; Shi *et al.*, 2022). Further contributing to the intricate network, *Epr3a*, upregulated in *L. japonicus* roots colonised by AM fungi, exhibits the ability to bind fungal glucans. *Epr3a* promoter activation is recorded in arbuscule-containing root cortical cells, strongly suggesting its involvement in the symbiotic dialogue (Kelly *et al.*, 2023). These results indicate that multiple kinases play a key role in regulating not only AM development, but also lipid trafficking in arbuscules (Ivanov & Harrison, 2024). Damage-associated molecular patterns (DAMPs), such as OGs, may enable plants to sense and mediate continuous checkpoints, providing further evidence of their involvement in this process. The functional connections between WAKs, other kinases and the signal transduction pathways they belong to remain to be clarified.

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