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Commentary

Fungal signals and calcium-mediated transduction pathways along the plant defence–symbiosis continuum

Plant roots reside in a complex subterranean world in contact with diverse soil microorganisms that trigger intricate transcriptional and developmental responses. Focusing on fungal interactions, plants use the perception of specific chitin-based molecules to discriminate between symbiotic partners, such as arbuscular mycorrhizal (AM) fungi, which plants host in their root tissues, and fungal pathogens, which they actively oppose. Calcium is central to the transduction, acting as a common mediator of downstream signalling (Zipfel & Oldroyd, 2017).

Over 20 years of research has led to a model in which chitin-derived signals and their respective induced Ca^{2+} signatures are part of either symbiotic- or immunity-related intracellular signalling cascades. Short chitin oligomers (COs) based on a tetrameric/pentameric backbone (CO4/CO5) as well as their derivatives harbouring a lipid tail (mycLCOs) have been characterized as powerful symbiotic signals and have been shown to trigger repeated transient elevations in nuclear and perinuclear Ca^{2+} levels. This Ca^{2+} spiking is a commonly accepted hallmark of plant symbiotic signalling, shared by other beneficial interactions, such as symbiotic nitrogen fixation in legumes and actinorhizal plants (Maillet *et al.*, 2011; Genre *et al.*, 2013; Sun *et al.*, 2015; Barker *et al.*, 2017). Instead, longer COs such as octamers (CO8) are acknowledged elicitors of plant immunity (Bjornson *et al.*, 2021) and are known to trigger a transduction pathway based on a rapid Ca^{2+} influx in the plant cell (Ranf *et al.*, 2011). However, emerging evidence suggests that this view may be overly simplistic. Indeed, CO8 was shown to activate nuclear Ca^{2+} spiking in *Medicago truncatula* roots (Feng *et al.*, 2019; Zhang *et al.*, 2021), and CO4 is known to trigger mild plant immunity-related responses, such as reactive oxygen species burst and activation of the MAPK cascade (Bozsoki *et al.*, 2017).

Similarly, a recent study investigating compartment-specific Ca^{2+} signatures activated by different fungal signals demonstrated that CO8, CO4, and mycLCOs induce a rapid cytosolic Ca^{2+} influx, followed by a longer-lasting nuclear Ca^{2+} spiking in *Lotus japonicus* roots (Fig. 1). Combining pharmacological and genetic approaches, the first Ca^{2+} influx was shown to be related to plant immunity and functionally uncoupled from symbiotic Ca^{2+} spiking. Moreover, the amplitude of the Ca^{2+} influx, as well as the level of induction of immunity marker genes, was critically dependent on elicitor concentration (Binci *et al.*, 2024). These data

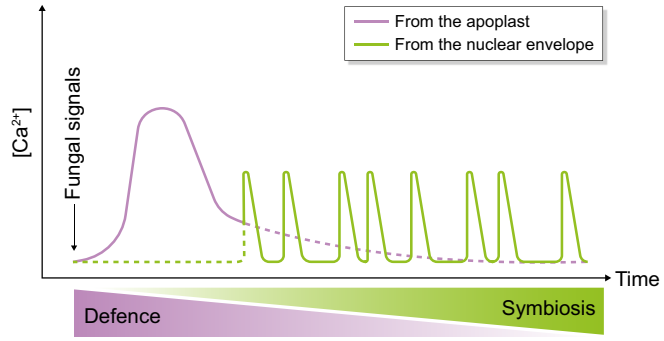

Fig. 1 Biphasic nature of plant Ca^{2+} signalling in response to fungal signals. The nonbinary nature of chitin-based fungal elicitors is mirrored by the partial overlap in plant defence- and symbiosis-related signalling (magenta and green triangles). In both cases, this is mediated by Ca^{2+}, but distinct kinetics of evoked Ca^{2+} changes are emerging. Upon fungal signal perception (black arrow), a rapid and steep Ca^{2+} influx from the apoplast (magenta) is associated with the activation of defence responses. By contrast, the triggering of symbiotic signalling is delayed in time (green) and involves the activation of repetitive nuclear-centred Ca^{2+} oscillations (Ca^{2+} spiking) generated from the nuclear envelope.

corroborate previous findings (Feng *et al.*, 2019; Zhang *et al.*, 2021) and outline a complex picture in which both immunity- and symbiosis-related responses are elicited by different fungal molecules through distinct intracellular Ca^{2+} changes.

In our recent Viewpoint (Giovannetti *et al.*, 2024), which was intended to be included in the present special issue, but was published in an earlier issue of the journal in error, such intertwined fungus–plant communication circuits are discussed to spotlight the continuum of both fungal elicitors and Ca^{2+}-mediated signals in the transduction pathways that mediate plant defence and symbiosis. Based on the cumulative knowledge gathered over the last two decades of research and recent advances in the field, we outline three main challenges for future studies.

First, the classical binary separation of chitin-derived fungal molecules into symbiotic and pathogenic signals according to their length and decorations does not reflect the complexity of plant–fungus chemical communication in light of recent findings. It is noteworthy that short-chain COs promote AM formation (Volpe *et al.*, 2020, 2023), whereas long-chain oligomers decrease it (Zhang *et al.*, 2021) when administered exogenously. However, when applied as purified molecules to axenic seedlings, COs of either size can trigger both symbiosis- and immunity-related responses (Genre *et al.*, 2013; Sun *et al.*, 2015; Bozsoki *et al.*, 2017; Feng *et al.*, 2019; Zhang *et al.*, 2021; Binci *et al.*, 2024), suggesting that chitin-based signals are only one part of a broader communication system (Fig. 2), where the concentration, relative abundance, and solubility of each class of COs within fungal exudates, along with more subtle chemical features (such as lateral

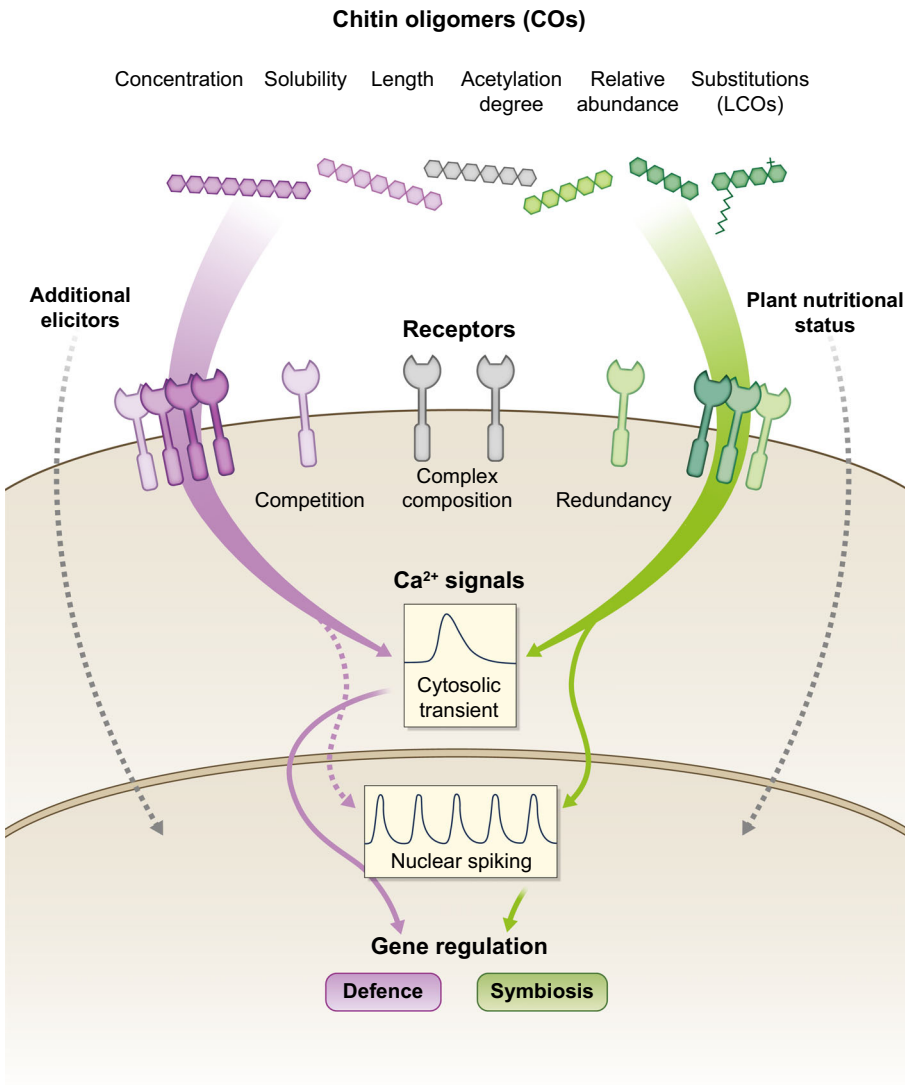

Fig. 2 Chitin-based fungal signals and Ca^{2+}-mediated plant signalling in defence and symbiosis. Plant-interacting fungi release a mix of short- and long-chain chitin oligomers (COs) that elicit symbiosis or defence in their host plants. Although a clear distinction between defence-associated long-chain COs and symbiosis-related short-chain molecules has been proposed, recent research has indicated that this distinction may not be as obvious as previously thought. Additional factors such as CO concentration, relative abundance, solubility, and degree of acetylation may be major determinants of downstream responses. An increasing number of studies on plant CO receptors is revealing a second level of complexity: distinct plant species use distinct receptors, and the same receptors may participate in different complexes based on the availability of individual COs, functional redundancy, and competition between receptors for complex formation. Finally, both pathogenic and symbiotic fungi cause changes in intracellular Ca^{2+} concentration, which is a key node in several plant signal transduction pathways. A transient cytosolic Ca^{2+} elevation is associated with the perception of both fungal elicitors. By contrast, nuclear-centred Ca^{2+} spiking is an acknowledged feature of symbiotic signalling. Nevertheless, when applied as purified molecules, long-chain COs have also been shown to induce nuclear Ca^{2+} spiking (dashed magenta arrow). Disentangling this complicated signalling scenario, which underpins the effective and clear regulation of gene expression for defence or symbiotic responses, is now a major challenge in the biology of plant–microbe interactions. In this context, the simultaneous perception of additional (nonchitinous) signals that are more specific to symbionts or pathogens and the physiological conditions of the plant, such as its nutritional status, are starting to emerge as important contributing factors (dashed grey arrows). Modified from Giovannetti *et al*. (2024).

substitutions or acetylation degree), or the simultaneous perception of additional fungal signals, corroborate the activation of one pathway or the other.

Second, other factors, such as the nutritional status of the plant (Li *et al*., 2022) and the complexity of the root microbiota, have been suggested to play a relevant role in directing plant decisions towards welcoming or opposing an approaching fungus. Such physiology-based processes may overlap and dominate direct signal exchanges between fungi and plants.

Finally, Ca^{2+} dynamics in beneficial and detrimental plant–microbe interactions have often been investigated using distinct tools, largely because of historical circumstances. Indeed, the field of plant

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immunity has mainly relied on aequorin-based Ca^{2+} assays in entire seedlings, plant organs, and suspension-cultured cells, providing accurate and sensitive Ca^{2+} measurements (Knight *et al.*, 1991; Chandra *et al.*, 1997; Mithöfer *et al.*, 1999; Lecourieux *et al.*, 2002; Zuppini *et al.*, 2004; Ranf *et al.*, 2011). In addition to an aequorin-based approach in plant cell populations that first demonstrated the involvement of Ca^{2+} in AM signalling (Navazio *et al.*, 2007), the endosymbiotic field has primarily adopted fluorescent Ca^{2+}-dependent dyes and genetically encoded Ca^{2+} indicators (GECIs) for imaging Ca^{2+} dynamics at the single-cell level (Ehrhardt *et al.*, 1996; Shaw & Long, 2003; Kosuta *et al.*, 2008; Chabaud *et al.*, 2011; Sieberer *et al.*, 2012; Genre *et al.*, 2013; Sun *et al.*, 2015). More recently, the use of fluorescent GECIs to investigate immunity Ca^{2+} signalling (Thor & Peiter, 2014; Keinath *et al.*, 2015; Kelner *et al.*, 2018) and bioluminescent GECIs in symbiotic interactions (Binci *et al.*, 2024; Teyssier *et al.*, 2024) clarified that the use of complementary tools may provide more robust findings. Furthermore, extending investigations into plant organellar Ca^{2+} signalling (Stael *et al.*, 2012; Costa *et al.*, 2018; Resentini *et al.*, 2021) may be essential to fully understand the complexity of the whole cellular Ca^{2+} network in beneficial plant–fungus interactions. Indeed, Ca^{2+} released from intracellular stores seems to play a crucial role not only in symbiosis (Charpentier *et al.*, 2016; Del Cerro *et al.*, 2022) but also in immunity signalling (Wang *et al.*, 2024). In short, an integrative approach combining different GECIs, merging distinct advantages, that is, qualitative and quantitative analyses of Ca^{2+} signals, appears to be critical for fully dissecting and reconstructing Ca^{2+} signalling in plant–microbe interactions.

Notably, most of our understanding of plant–fungus beneficial interactions has relied on a small number of plant species, mainly belonging to angiosperms. Nevertheless, recent advancements have been facilitated by comparative phylogenomics and the use of diverse model plant species that represent different evolutionary trajectories. These approaches have elucidated the conservation of a set of plant genes implicated in symbiosis across a spectrum of terrestrial plants (Vernié *et al.*, 2024). For example, studies on the AM host liverwort *Marchantia paleacea*, whose compact genome provides a simpler system for investigating membrane receptors, have shown that short and long COs induce fast and intense cytosolic Ca^{2+} elevations under the control of LysM receptor-like kinases (Teyssier *et al.*, 2024), tracing the nonbinary nature of symbiosis- and immunity-related signalling back to the most ancient common ancestors of land plants.

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