

## CALCIUM SPIKING IN ARBUSCULAR MYCORRHIZAS: THE WHO AND WHERE OF PRESYMBIOTIC SIGNALING

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Arbuscular mycorrhizas (AM) are symbiotic associations between 90% of land plants and obligate fungal symbionts belonging to Glomeromycota. AM fungi improve plant nutrient uptake and resistance against pathogens by colonizing the root through intra/intercellular hyphal development and the formation of arbuscules, the highly branched structures that mediate nutrient exchange. Early recognition of the symbiont by the host plant is a crucial step in the interaction, required for setting up a range of local and systemic responses. Certain of these plant responses depend on the so-called 'common SYM' signal transduction pathway which, in legumes is partly shared with the nitrogen-fixing symbiosis involving rhizobia. In this case, calcium is known to play a key role as a second messenger. Since the bacterial symbiosis evolved more recently than the AM association, it is thought that rhizobia have exploited the ancient AM signaling pathway by mimicking symbiotic fungal signals. Therefore, by analogy with bacterial Nod factors, it has been proposed that AM fungi release signal molecules termed Myc factors, which should activate the SYM pathway and its calcium signals to induce AM-specific responses. Furthermore, due to the wide host range of AM fungi, such signals should not be limited to legumes, but extend to all AM host plants.

The aim of this study was to investigate Ca<sup>2+</sup> responses to AM fungi in the host root epidermis following fungal contact or diffusible signal perception in both legumes and nonlegumes.

We report that sustained nuclear Ca<sup>2+</sup> spiking can be detected in hyphopodium-contacted epidermal cells of both *M. truncatula* and carrot roots, thus demonstrating that fungal-activated Ca<sup>2+</sup> spiking occurs in the cell type targeted by AM hyphopodia, and is most probably part of an ancient plant signalling pathway predating the divergence between the rosid and asteroid clades. In addition, we have been able to show that exudates of germinated AM spores (but not purified rhizobial NFs) are able to trigger nuclear Ca<sup>2+</sup> spiking in the outer root tissues of ROCs, and that this response is limited to the AM-responsive root zone. Ca<sup>2+</sup> spiking responses to both hyphopodia formation and AM exudate application are dependent on genes of the common SYM pathway in *M. truncatula*, but independent of the NFP gene encoding the LysM receptor-like kinase which mediates NF perception.

Together, these findings provide additional evidence that Ca<sup>2+</sup> spiking is a key component of a highly conserved AM-activated signalling pathway required for intracellular fungal infection, and furthermore suggest that cameleon-expressing ROCs can provide valuable AM-specific bio-tests for the future characterization of fungal symbiotic signals.

AM specificity in the SYM pathway-mediated response is a point of major interest in legumes, which must discriminate between symbiotic fungi and bacteria. This point will also be discussed in the light of our recent studies on the specificity of calcium signatures in either case.