Βασιλική Σκιαδά – Διδακτορική διατριβή

Colonization of legumes by an endophytic *Fusarium solani* strain K. Early-stage molecular signaling and sub-cellular responses.

Πρότυπα αποικισμού ψυχανθών από τον ενδοφυτικό μύκητα *Fusarium solani* στέλεχος Κ. Μοριακή σηματοδότηση και υπο-κυτταρικές αποκρίσεις στα πρώιμα στάδια της αλληλεπίδρασης.

Abstract

Legumes interact with a plethora of microbes in their root system ranging from beneficial symbionts to pathogens. Symbiont recognition initiates at the pre-contact level, with typical symbiotic rhizobial Nod- and the putative glomeromycetes Myc-Factors (LCOs, and mix of COs/LCOs, respectively) identified via LysM-receptor like kinases at the plant cell Plasma Membrane (PM). This triggers a so-called common symbiotic signaling pathway (CSSP), including the induction of nuclear calcium spiking in legume root epidermis. At the post-contact level, legumes colonization by symbionts is well described, with similarities to infestation by pathogenic fungi. On the other hand, plant cellular responses to endophytic fungi are relatively underreported.

The aim of the present work, was to investigate the early stages of interaction of an endophytic fungal isolate, *Fusarium solani* strain K (FsK), with the model legume *Lotus japonicus*, at the molecular and at the cellular level.

Commonalities arose with symbiont recognition at the molecular level, as FsK induced the expression of *LysM* receptors for chitin-based molecules, CSSP members and CSSP-dependent genes in *L. japonicus*. Activation of defense genes also occurred. In addition, FsK exudates, comprising heat labile, and/or chitinase sensitive molecules, induced nuclear calcium spiking at the epidermis of *M. truncatula* Root Organ Cultures (ROCs) harboring cameleon reporters, and this response was proven to be CSSP-dependent. This response was, furthermore, recorded in response to other fungal exudates, derived from mutualistic or pathogenic fungi. Results were complemented by mutant analysis: FsK intraradical progression was stimulated in *LysM* mutants examined, whereas in CSSP mutants it was dependent on genes acting downstream the Ca^{2+} spiking response (*LjCCaMK*, *LjCYCLOPS*). It was therefore shown through this work that the CSSP is a more common pathway than previously envisaged.

To study the interaction at the cellular level, either *L. japonicus* whole plants or *M. truncatula* ROCs (labelled for specific cellular compartments, thus allowing a more detailed investigation), were employed. Optical, confocal, and TEM microscopy revealed a polarized reorganization of the legume root cell: endoplasmic reticulum and cytoplasm accumulation and nuclear placement at contact sites, occasional development of papillae underneath hyphopodia and membranous material rearrangements towards penetrating hyphae. Cell death was recorded during colonization process, and fungal hyphae were observed within the vascular bundle of the plant. FsK proceeds in the aerial part of the plant, where it

grows epiphytically and endophytically. Differentiated round structures, of unknown function, were also recorded in the stem in epidermal/upper cortical cells. It was therefore pointed through this work that the establishment of FsK within legume tissues requires fungal growth adaptations and plant cell-autonomous responses, known to occur during both symbiotic and pathogenic plant-fungal interactions. Unique responses were also observed, worth of additional investigation.

To further gain insight on FsK-*Lotus* interaction, the lifecycle of inoculated plants was followed, and no apparent phenotypic defects or growth promotion traits were recorded in comparison to controls, under the normal conditions examined. Furthermore, the ability of the endophyte to complete its lifecycle in association with its host was examined by measuring conidiation events in the substrate. Propagule formation recorded was independent of the plant, at least up to the last time point examined.

Results at the molecular level, were furthermore complemented with transcriptome profiling of FsK-inoculated *L. japonicus* plants, at early stages of the interaction. A large repertoire of the legume host transcripts is differentially expressed: genes coding for proteins involved in membrane transport, hormonal regulation, lipid metabolism, cell modifications, defense are activated, indicating the molecular and cellular alterations necessary for recognition and accommodation of another eukaryotic organism within the plant cell.