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DISSERTATION SUMMARY

Characterization of the nodulation receptor kinase (NORK) protein in *Medicago truncatula*

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Legumes are able to establish root endosymbioses with rhizobial bacteria and arbuscular mycorrhizal fungi. These relationships enable these plants to fix atmospheric nitrogen and acquire phosphorus from the soil, respectively. The legume-rhizobia relationship begins with a molecular dialog in the rhizosphere. Plant exudates (flavonoids) induce the production of the so-called Nod factor (NF) in the bacteria (Dénarié et al. 1996). NFs induce nodule formation on roots and they are the major determinant of host specificity of the interaction. Since responses are induced in the plant by NF concentrations in a nano- to picomolar range, it has been postulated that NFs are recognized by specific receptor(s). Recently, several genes have been identified and characterized that are involved in the perception and early signal transduction of the NF thereby in the nodulation process.

The nodulation receptor kinase (*NORK*) gene was the first symbiotic legume gene isolated via map-based cloning from *Medicago* (Endre et al. 2002). It is predicted to play a key role in the NF signal transduction pathway in the plant. The gene encodes for a receptor-like kinase that contains a unique (NORK extacellular-sequence-like - NSL) motif and three leucine-rich repeats (LRRs) in the extracellular domain, a transmembrane domain (TM) and an intracellular serine/threonin kinase domain.

In order to elucidate the role of the NORK protein in nodulation we designed different chimaeric and truncated *NORK* constructs to test their activity in transgenic roots with mutant background. The transformation experiments were carried out by the *Agrobacterium rhizogenes* system. The plant material used for transformation is a NORK mutant, non-nodulating *Medicago truncatula (Mt)* line TR25. In order to assess the effects of transgenes the transgenic roots were observed by light microscopy after 3-9, 14, 21 days post inoculation (dpi) with *Sinorhizobium meliloti (Sm)* carrying a plasmid containing the *lacZ* gene. For infection studies the roots were fixed and stained for β-galactosidase activity (Oldroyd and Long 2003).

In the case of the chimaeric constructs, the extracellular domain (NSL + LRR) of the *Mt NORK* gene was replaced with the extracellular domains of other legume species, *Melilotus albus (Ma)* or *Vicia hirsuta (Vh)*, respectively. Both constructs complemented the *Mt* NORK mutant plants, pink, nitrogen fixing nodules appeared on the transformed roots 21 dpi with *Sm*. We could observe the different early plant responses (root hair deformations, infection thread and nodule primordia formation) normally induced by the bacteria. The nodulation process did not show any delay compared to the control transformed roots. The truncated constructs did not contain the NSL domain or the NSL and the LRR domains from the extracellular part. These constructs did not complement the non-nodulating phenotype of the mutant plants, no nodules on the transformed roots were detected.

Vh is a more distant relative of *Mt* than *Ma*, and it has another symbiotic partner, *Rhizobium leguminosarum* bv. *viciae*, which produces NF with a structure different from that of *Sm*. From these experiments we concluded that the NORK protein does not take part in the NF binding directly, but the whole protein is necessary for the activation of the signal transduction pathway.

References

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