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STUDY OF *MtN5* TRANSCRIPTIONAL CONTROL AND OF ITS INVOLVEMENT IN *MEDICAGO TRUNCATULA* NODULATION PATHWAY

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The symbiosis between legumes and rhizobia starts with an exchange of molecular signals between the two partners. In response to the plant-derived flavonoids, bacteria synthesize Nod Factors (NFs), which are able to induce a series of events, such as ion fluxes, root hair deformation and the expression of the early nodulin genes, that eventually lead to the formation of root nodules. We previously demonstrated that *MtN5* is an early nodulin, required for the establishment of the symbiosis and also present in mature nodules. In order to investigate the role of *MtN5* in root nodules induction pathway, its expression profile during the early stages of infection was studied. *MtN5promoter::GUS* fusion showed that the promoter was active in epidermis and root hairs a few hours after inoculation, whilst in mature nodules, GUS was observed in the distal zone. The *in silico* analysis of the promoter sequence revealed the presence of two consensus regions (AAAGAT and CTCTT), also found in the promoter region of genes activated in rhizobia-colonized cells within nodules. Other motifs identified are putatively responsible for the hormonal control of gene expression. On the basis of these observation, the responsiveness of *MtN5* gene toward bacteria-derived molecules (*i.e.* NFs, EPS, Chitin Oligomers) and plant hormones (*i.e.* NAA, BAP, Ethylen precursor ACC) was tested. In a time course nodulation experiment, *MtN5* showed to be co-expressed with early markers of rhizobia infection, such as *RIP1, NIN* and *ENOD11*, and resulted to be more precocious than *ENOD20* and *MtN6*. In transgenic adventitious root silenced for *MtN5* expression (*MtN5hp* roots), we observed that upon rhizobia infection the nodulin *MtNIN* was not induced, whilst *ENOD11* was strongly upregulated with respect to control roots. Furthermore, in *MtN5hp* roots the expression of *FLOT4*, a nodulin gene known to be involved in the infection thread growth, was unaffected by the inoculation with symbiotic bacteria, in contrast with what observed in control roots. With the aim of gaining a further insight on the role of *MtN5* in the establishment of symbiosis, we carried out microscopic observation of infected *MtN5hp* roots by means of rhizobia carrying a reporter gene. *MtN5hp* roots displayed a reduced colonization of nodule primordia as compared to control. All these observations suggest that the activity of *MtN5* is required for the penetration of bacteria within the nodules.