Knowledge of the organization of plant meristems has increased over the last years by studies on model plant species like *Arabidopsis thaliana*. In the root of this model plant, stem cells are surrounding the quiescent center (QC) cells, which function to prevent stem cell differentiation. Stem cells and QC form the stem cell niche. The position of this niche is marked by the highest concentration of PLETHORA (PLT) in a gradient of PLT activity. PLTs are transcription factors and are part of the small AINTEGUMENTA-like (AIL) AP2 gene clade of transcriptional regulators within the large AP2/ERF family. Among this clade, PLT1–4 are essential for root formation as their higher order mutants show increasingly severe root phenotypes.

On roots of legumes new organs, so-called root nodules, are formed as a result of the interaction between these plants and soil-borne bacteria collectively known as rhizobia. In the model legume *Medicago truncatula*, that forms nodules with a persistent meristem at its apex, nodule formation is initiated by dedifferentiation of cortical cells which divide and form the nodule primordium. Specific secreted lipochito-oligosaccharides (Nod factors) allows the rhizobia, through the Nod factor signaling cascade, to control infection and the formation of a nodule meristem at the apex of the primordium. As nodules are formed on roots it has been hypothesized that the nodule developmental program is derived from the lateral root developmental program.

Recently, we showed that 4 *Medicago* PLT (MtPLT1 to 4) genes redundantly control nodule formation and nodule meristem maintenance. This is reminiscent of the redundant function of their orthologous PLT genes in Arabidopsis root development and lends support to the hypothesis that nodule formation is derived from root developmental programs. Based on our results and that of others it was suggested that rhizobia recruited major regulators of root development. Recruitment may evolve through specialization of protein function or through specific cis-regulatory elements. In the latter case, it is expected that the Arabidopsis PLT1 to 4 promoters are not active in the nodule or active at places different from the othologous Medicago promoters. To determine this, we studied the *pAtPLT1*, 2, 3 and 4 promoter mediated expression patterns in Medicago roots and nodules. The length of the promoter regions for *AtPLT* genes used in this study are indicated in Table 1, with a comparison to the *MtPLT* promoters used in Franssen et al., 2015. We constructed transcriptional fusions of the *pAtPLT* promoters. To co-localize with the stem cell niche in Medicago roots (Fig. 1, panels A–D: orange arrow), similar to the patterns observed and described in Arabidopsis roots. As nodules are formed on roots it has been hypothesized that the nodule developmental program is derived from the lateral root developmental program.

The highest activation of the *pAtPLT*:GUS expression co-localizes with the stem cell niche in Medicago roots (Fig. 1, panels A–D: orange arrow), similar to the patterns observed and described in Arabidopsis roots. The activation patterns of *pAtPLT3*:GUS and *pAtPLT4*:GUS, however, do not extend in the vasculature as far as the *pMtPLT3*:GUS and *pMtPLT4*:GUS do. Nevertheless, promoter activation patterns are very similar and therefore the *pAtPLT* promoters must contain conserved cis-regulatory elements that are sufficient to drive gene expression in the Medicago root meristem.

We then studied whether *pAtPLT*:GUS fusions are expressed in the nodule and nodule meristem (Fig. 1E–J). This meristem is composed of a central part, the nodule central meristem (NCM) and of nodule vascular meristems (NVM) that
are located at the periphery of the NCM.\textsuperscript{16,18} Whereas \textit{MtPLT1} and \textit{MtPLT2} are expressed highest in the NVM, \textit{MtPLT3} and \textit{MtPLT4} are expressed at equal levels in the NCM and NVM.\textsuperscript{16} Similarly, \textit{pAtPLT1::GUS} and \textit{pAtPLT2::GUS} are highly activated in the NVM (Fig. 1E,F) and the activation patterns of \textit{pAtPLT3::GUS} and \textit{pAtPLT4::GUS} includes both NCM and NVM (Fig. 1G,H). In addition to activation in the NCM and the NVM, expression of \textit{pMtPLT3::GUS} and \textit{pMtPLT4::GUS} was also observed in cells of the infection zone.\textsuperscript{16} Serial sections of \textit{pAtPLT3::GUS} and \textit{pAtPLT4::GUS} nodules were analyzed and these show that also the \textit{pAtPLT3} and \textit{pAtPLT4} promoters are active in the infection zone (Fig. 1I,J). The level of activation of the \textit{pAtPLT3::GUS} and \textit{pAtPLT4::GUS} is lower in the infection zone than in the NM, like for \textit{pMtPL3::GUS} and \textit{pMtPLT4::GUS}.\textsuperscript{16} Thus, in the Medicago nodule the spatial activation of \textit{pAtPLT1} to 4 is similar to that of \textit{pMtPLT1} to 4. Therefore, all \textit{cis}-regulatory elements for spatial activation in root and specific areas of the nodule must be present in the orthologous Arabidopsis and Medicago \textit{PLT1} to 4 promoters, suggesting that in Medicago no nodule specific \textit{cis}-regulatory

### Table 1. Length of Arabidopsis PLT promoters used in this study compared with Medicago PLT promoters.

| Arabidopsis ID | Medicago ID      | Length (Kb)\textsuperscript{1} | Length (Kb)\textsuperscript{2} |
|----------------|------------------|---------------------------------|---------------------------------|
| PLT1           | At3g20840        | 4.5                             | 1.5                             |
| PLT2           | At1g51190        | 1.3                             | 1.3                             |
| PLT3           | At5g10510        | 4.6                             | 2.7                             |
| PLT4/BBM       | At5g17430        | 4.2                             | 1.1                             |

\textsuperscript{1}As described in\textsuperscript{7}.

\textsuperscript{2}As described in\textsuperscript{16}.

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**Figure 1.** \textit{pAtPLT::GUS} activity in Medicago root and nodule meristem. (A-D) The \textit{pAtPLT1::GUS} (A), \textit{pAtPLT2::GUS} (B), \textit{pAtPLT3::GUS} (C) and \textit{pAtPLT4::GUS} (D) expression patterns overlap in the Medicago root, with the highest activity in the stem cell niche of the root meristem (arrow). (E-F) Top views of \textit{pAtPLT1::GUS} (E) and \textit{pAtPLT2::GUS} (F) nodules show highest GUS activity in discrete regions in the nodule meristem periphery corresponding to the NVM (arrows), and lower GUS activity throughout the nodule meristem. (G-H) \textit{pAtPLT3::GUS} (G) and \textit{pAtPLT4::GUS} (H) display expression throughout the nodule meristem (arrowheads). (I-J) Nodule sections showing highest \textit{pAtPLT3::GUS} (I) and \textit{pAtPLT4::GUS} (J) activity in the central part of the nodule meristem (arrowhead) and lower activity in the infection zone (bracket). Nodules were sampled 16 d after inoculation.
elements have been acquired to enable the expression of PLT genes in nodules.

We show that promoters of AtPLT1, 2, 3 and 4 genes are activated in Medicago roots in regions previously shown to display the orthologous MtPLT1 to 4 promoter activity. This indicates that cis-elements and the regulatory machinery conferring spatial expression of PLT1 to 4 in the root meristem are conserved among Arabidopsis and Medicago. Delimiting the activation pattern of pAtPLT3::GUS and pAtPLT4::GUS in the differentiation zone compared with the more extended vascular expression of pMtPLT3::GUS and pMtPLT4::GUS, maybe due to differences in promoter sizes tested (Table 1).

Also in the nodule, an organ that cannot be formed on Arabidopsis roots, pAtPLT1 to 4:GUS reporters are activated in the same locations as their Medicago orthologues. This indicates that for the spatial activation in nodules all cis-elements that are present in pAtPLT1 to 4 promoter regions tested here. Based on the similar activation patterns of At- and MtPLT1 to 4 in roots as well as in nodules, it is conceivable that the regulatory mechanisms directing PLT expression in these organs share several components.

A next and interesting question is whether the conservation of PLT promoter or even PLT protein function between Arabidopsis and Medicago is sufficient for cross complementation. However, single mutants (Arabidopsis) or knock downs (Medicago) display only mild phenotypes.7,16,22 Therefore, this should ideally be tested and Medicago is sufficient for cross complementation. However, our studies indicate that Rhizobium, and by inference the Nod factor signaling cascade, is able to activate pAtPLT1 to 4 during nodule development. The Arabidopsis genome, however, appears to lack the genetic information for a critical component of this cascade.23,24 This raises the question which inputs are essential or sufficient for PLT activation during nodulation. Nod factor application and testing expression in nodulation mutants can be instructive to answer this in Medicago. In parallel, the mechanisms underlying PLT expression in Arabidopsis can be useful to further dissect how Rhizobium co-opted genes involved in root development for nodule formation.

Similar to the role of PLT genes in Arabidopsis, the Rhizobium-mediated induction of PLT gene expression during nodule formation correlates with developing tissues and organs.6,16 It is likely that regulated genes/targets of MtPLT proteins will show an overlap with those regulated by AtPLT proteins.25 In addition, comparing the MtPLT targets regulated in roots versus nodules may reveal specific PLT-mediated developmental programs required for either organ.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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