Rop GTPases are members of the Ras superfamily of small GTP-binding proteins and represent the only “signalling type” small GTPase family in plants (Berken 2006). They are involved in many cellular processes including the establishment of cell polarity and tip growth, cell elongation, signalling during stress, hormonal and pathogen responses (Nibau et al. 2006). These small (21 kD) proteins serve in these processes as molecular switches as their signal transduction activity depends on their GDP- or GTP-bound conformation. Therefore the regulation of their nucleotide binding and GTPase activities have to be tightly regulated in order to ensure proper functioning. This regulation is exerted on Rop GTPases by three protein families, namely by guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs) and guanine nucleotide dissociation inhibitors (GDIs) (Yang and Fu 2007). Rop GEFs are GTPase activators as they promote the exchange of Rop-bound GDP to GTP. Therefore they potentially serve as the link between receptors and Rop GTPases and play a significant role in the activation of Rop-dependent signalling cascades (Shichrur and Yalovsky 2006).

In our laboratory we investigate the elements of Rop-GTPase-dependent signalling cascades in Medicago species and here we report on the identification and primary characterization of the members of the Medicago RopGEF protein family.

**Materials and Methods**

DNA sequences have been downloaded from the Arabidopsis Biological Resource Center (ABRC) at http://www.arabidopsis.org/abrc/and The European Medicago Genome Database (UrMeLD) at http://mips.gsf.de/proj/plant/jsf/medi/. The accession numbers and the used nomenclature are shown in Table 1. Sequence alignment has been made using the ClustalW algorithm. Phylogenetic analysis was carried out by the Phylip 3.67 program package(http://evolution.genetics.washington.edu/phylip.html).

To analyze the gene expression in different organs, roots, root nodules, leaves, stems, flowers were harvested from mature greenhouse plants. Additionally, cells from an exponentially growing cell suspension culture were also collected. All plant material was frozen in liquid nitrogen immediately after harvesting. Total RNA has been extracted from the frozen plant material using the RNazol reagent (Sigma, St-Louis, USA). To avoid genomic DNA contamination, each RNA preparation was treated with RNAse free DNase according to the manufacturer’s instructions (Sigma). RNA was then quantified measuring absorbance at 260 nm using a spectrophotometer (NanoDrop Technologies, Wilmington, USA) and loaded on a denaturing agarose gel to check concentration and integrity.

2.5 µg total RNA was reverse transcribed using oligo dT primers and reverse transcriptase (RevertAid M-MuLV, Fermentas, Vilnius, Lithuania) according to the manufacturer’s instructions. The cDNAs were diluted to 200 µl with sterile H2O.

Appropriate primer pairs and an appropriate sequence specific TaqMan probe allowing the differential identification of the Medicago RopGEF genes (Table 1) have been designed by the Universal Probe Library Assay Design Center (Roche Applied Science) at https://www.roche-applied-science.
The RopGEF protein family of *Arabidopsis thaliana* has only been recently identified (Berken et al. 2005; Gu et al. 2006). This family consists of 14 members carrying a plant specific protein domain with RopGEF activity called PRONE (plant Rop GTPase-dependent nucleotide exchanger). Based on this conserved sequence a database screening approach was carried out in order to identify related protein in *Medicago truncatula*. Until now six full length and one additional partial sequences could be classified into the Medicago RopGEF family (Table 1., Fig. 1). Phylogenetic comparison of the 14 *Arabidopsis* and the 7 *Medicago* RopGEF protein sequences revealed that the family can be divided into four groups and all group is represented among the identified *Medicago* sequences (Fig. 1).

As a first step in their characterization, the relative expression level of the seven *Medicago* RopGEF genes was determined in various plant organs and in exponentially growing cultured cells (Fig. 2). As it can be seen on Figure 2, two of the investigated Medicago RopGEF genes (GEF1 and 2) have a very high relative expression in the flower. In *Arabidopsis thaliana* a group of pollen specific RopGEF proteins have also been identified (AtRopGEF8-12; Zhang and McCormick, 2007). These proteins have a characteristic C-terminal inhibitory region that has to be phosphorylated in

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**Table 1.** Accession numbers of the nucleotide sequences, the PCR primers and the numbers of corresponding Universal Probe Library probes used for the real-time PCR experiments.

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<th>Name</th>
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order to allow GEF activity (Zhang and McCormick 2007). The flower-expressed Medicago proteins have a similar C-terminal region (data not shown). It is interesting to note that the MtGEF1 gene is also expressed at a relatively high level in root nodules in addition to the flower (Fig. 2). The other five Medicago RopGEF genes are expressed in all investigated organs at a relatively low level (Fig. 2). The regulation of their activity is rather at the post-transcriptional level.

Further studies will clarify the biochemical and developmental roles of Medicago RopGEF genes especially during the establishment of Medicago-Sinorhizobium symbiosis, root nodule development and somatic embryogenesis.

Acknowledgements

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References