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Investigation of the elements of Rho (Rop) GTPase-dependent signalling in *Medicago* sp.: Identification of Rop guanine nucleotide exchange factors (ROPGEFs) in *Medicago truncatula*

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ABSTRACT Rop GTPase-dependent signalling plays a significant role in plant development as well as during the responses of plants to environmental signals. The elements of these diverse signalling pathways just started to be revealed during the last couple of years, especially in *Arabidopsis thaliana*. In our laboratory we aim to identify these elements from the model species *Medicago truncatula* in order to allow investigations of Rop GTPase-mediated signalling during *Medicago-Sinorhizobium* symbiosis and somatic embryogenesis. Here we report on the in silico identification of seven Rop guanine nucleotide exchange factor (RopGEF) sequences and their primary characterization including gene expression profiling in various plant organs.

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KEY WORDS

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gene expression
real-time PCR
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Rop GTPases are members of the Ras superfamily of small GTP-binding proteins and represents 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 exchangers (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.arabidop->

[sis.org/abrc/](http://www.arabidop-)and The European Medicago Genome Database (UrMeLDB) 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>.

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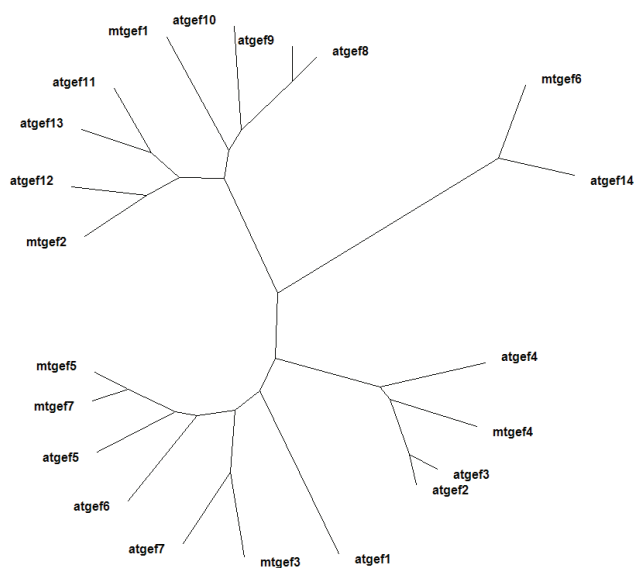


Figure 1. Unrooted phylogenetic tree of 14 *Arabidopsis thaliana* and 7 *Medicago truncatula* RopGEF proteins deduced from cDNA or genomic nucleotide sequences.

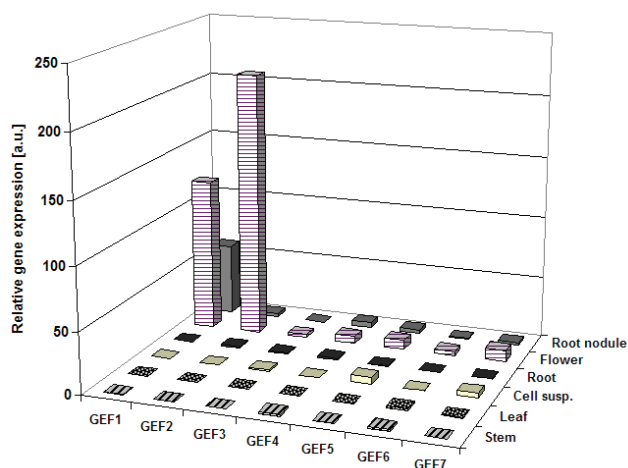


Figure 2. Relative gene expression of *Medicago truncatula* RopGEF genes in various plant organs. Expression is normalized to the constitutively expressed SecA (Secret Agent, O-linked N-acetyl glucosamine transferase) gene (Lohar et al., 2006). The relative expression is shown in arbitrary units where the average of the normalized expression values in all investigated samples represents 1 a.u.

com. Real-time quantitative RT-PCR was assayed on an ABI Prism 7700 sequence detection system using according to the manufacturer’s (Applied Biosystems) instruction. The Secret Agent (O-linked N-acetyl glucosamine transferase), a constitutively expressed gene (Lohar et al. 2006), was used as an endogenous control for data normalization.

Results and Discussion

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 par-

tial 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

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.

Name	Seq. Accession	Forward	Reverse	UPL #
Secret Agent	gbAC146559.29	cgtttctctctccaccgttt	aacctccctctgcttgatt	68
MtGEF1	gbAC134823.41	ctcgcaagaagcgatct	tcaaggagcattgcatcaag	11
MtGEF2	gbAC173288.3	cggtgacaatgcaagacaa	tgctggaaccttcggtgta	151
MtGEF3	gbAC170582.2	cccttcaaaggaggaaaat	cccctacacctctgagga	157
MtGEF4	gbAC150440.3	gcaggccaaggtcagatatt	gcattgtgtcaagctctgc	145
MtGEF5	embCT963133.4	cccaggacagatattgcatc	caagtagcatgttatcgatttgc	14
MtGEF6	embCU302336.1	gctcgtgcagacattcatc	gcctcgaagcatcacagtcca	11
MtGEF7	MtD06210	tgacttcagaccaagatcag	tgacttcagaccaagatcag	11

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

- Berken A (2006) ROPs in the spotlight of plant signal transduction. *Cell Mol Life Sci* 63:2446-2459.
- Berken A, Thomas C, Wittinghofer A (2005) A new family of RhoGEFs activates the Rop molecular switch in plants. *Nature* 436:1176-1180.
- Gu Y, Li S, Lord EM, Yang Z (2006) Members of a Novel Class of *Arabidopsis* Rho Guanine Nucleotide Exchange Factors Control Rho GTPase-Dependent Polar Growth. *Plant Cell* 18:366-381.
- Lohar DP, Sharopova N, Endre G, Peñuela S, Samac D, Town C, Silverstein KA, VandenBosch KA (2006) Transcript Analysis of Early Nodulation Events in *Medicago truncatula*. *Plant Physiol* 140:221-234.
- Nibau C, Wu H, Cheung AY (2006) RAC/ROP GTPases: 'hubs' for signal integration and diversification in plants. *Trends Plant Sci* 11:309-315.
- Shichrur K, Yalovsky S (2006) Turning ON the switch--RhoGEFs in plants. *Trends Plant Sci* 11:7-59.
- Yang Z, Fu Y (2007) ROP/RAC GTPase signaling. *Curr Opin Plant Biol* 10:490-494.
- Zhang Y, McCormick S (2007) A distinct mechanism regulating a pollen-specific guanine nucleotide exchange factor for the small GTPase Rop in *Arabidopsis thaliana*. *Proc Natl Acad Sci U S A* 104:18830-18835.