

Somatic embryogenesis - a tool for expression analysis in conifers

Rupps, A., Raschke, J., Zoglauer, K.

Humboldt-University of Berlin, Institute of Biology, AG Botany & Arboretum,
Invalidenstrasse 42, 10115 Berlin

There is an increasing demand for applied sciences and in conifer breeding for molecular analysis of conifers' ontogenesis, in particular concerning growth and development. Even though the spruce genome was recently sequenced, molecular mechanisms underlying the control of embryogenesis in conifers are scarcely known - not least because of missing expression data and functional studies but also due to the difficulties in accessing starting material.

We used somatic embryos of *Larix decidua* Mill. as a system to facilitate experimental procedures which guaranteed availability of starting material and precisely controllable culture conditions.

As a prerequisite, the substitutability of the somatic for the zygotic system was verified by comparing expression levels using semi-quantitative RT-PCR. Transcript levels of embryogenesis-related genes have additionally been confirmed by the means of quantitative real-time-PCR. Furthermore several protocols for tissue and cell specific expression analysis (*in-situ*-hybridization) have been tested for different embryogenic stages: The integrity of the tissue was shown by a pre-staining series, hybridization of tissue sections was compared to the whole mount procedure and the influence of the respective probe type (DNA or RNA probe) and labeling (DIG, rhodamine) was analyzed. Thus, we collected important clues for successful hybridization and present first results for the detection of localization sites for genes of interest.

Somatic embryos offer the possibility of detailed molecular studies in niche organisms like conifers. Despite the above listed advantages of the somatic system, protocols to gain expression data cannot merely be transferred from other distantly related plant taxa but need to be independently developed. We could show similarities in expression behavior concerning the genes of our interest, which leads to the assumption that the studied embryogenesis-related genes in conifers and angiosperms have a conserved role. Yet, the information on the expression pattern of homologous genes is essential and changes in regulatory gene functions have to be taken into account, when comparing model plant data of Magnoliopsida to those of Coniferopsida, which separated 300 million years ago.