

POSTER PRESENTATION

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Expression pattern of the GRAS gene family during somatic embryogenesis in pine

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The GRAS protein family of putative transcription factors, which includes SHORT-ROOT (SHR), SCARECROW (SCR) and SCARECROW-LIKE (SCL) proteins, is involved in root development in *Arabidopsis thaliana* and other plant species [1]. In forest species, genes with homology to the *A. thaliana* SCR gene have been involved in the induction of somatic embryogenesis in *Picea glauca* (Moench) Voss [2] and *Pinus taeda* L. [3] as well as in the development of radial patterning of roots in *Pinus sylvestris* L. [4]. Schrader *et al*[5] also reported the expression of genes with homology to the *A. thaliana* SHR gene in cambial region of *Populus tremula x tremuloides*. Increased levels of mRNA of *Pinus radiata* SHR (*PrSHR*), *Pinus radiata* SCARECROW-LIKE1 (*PrSCL1*) and *Castanea sativa* SCARECROW-LIKE1 (*CsSCL1*) have been associated with the early stages of adventitious root induction in *Pinus radiata* D. Don and *Castanea sativa* Mill., respectively [6-9].

In addition to *PrSHR* and *PrSCL1*, we have identified 13 new GRAS genes belonging to the different GRAS clades in the pine genome. The objective of this work is the analysis of the spatiotemporal expression patterns of the pine GRAS gene family during somatic embryogenesis in *Pinus radiata* D. Don. Somatic embryogenesis has become the first biotechnology showing great potential for mass propagation of conifers for application in forestry, allowing the implementation of multivarietal forestry (MVF) [10,11]. Despite major advances in clonal regeneration by somatic embryogenesis or organogenesis, many forestry species are recalcitrant [12]. More knowledge of the regeneration process regulation is necessary to improve the capacity of vegetative regeneration.

The expression pattern of the genes was analyzed by qRT-PCR following the methodology described by Sánchez *et al*[6] and Solé *et al*[7]. For expression analysis, total RNA was extracted from four stages of the somatic embryogenic process: proliferative tissue after 7 and 14 days from the last transference to proliferation medium, somatic embryos at the beginning of differentiation and cotyledonary somatic embryos.

In general, the transcripts of the pine GRAS genes accumulated at the highest levels in cotyledonary somatic embryos. In addition, the transcript levels of *PrSCR*, *PrSHR*, *PrSCL1*, *PrSCL6*, *PrSCL8*, *PrSCL11* and *PrSCL12* showed an increase in somatic embryos at the beginning of differentiation. No differences in *PrSCL10* transcript levels were found between the four stages analyzed. Transcript levels of *PrSCL16* were undetectable at all stages. *In situ* hybridization for spatial expression analysis will confirm differential expression domains.

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