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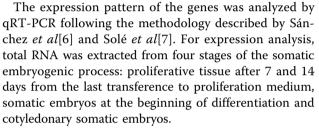
Expression pattern of the GRAS gene family during somatic embryogenesis in pine

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The GRAS protein family of putative transcription factors, which includes SHORT-ROOT (SHR), SCARE-CROW (SCR) and SCARECROW-LIKE (SCL) proteins, is involved in root development in Arabidopsisthaliana and other plant species [1]. In forest species, genes with homology to the A. thalianaSCR 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. thalianaSHR 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 sativaSCARECROW-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.



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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