Generation of transgenic maritime pine somatic embryos with altered expression of genes involved in Nitrogen metabolism and Wood formation

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This study was initiated in the frame of the Sustainpine project (2009 PLANT-KBBE, (http://www.scbi.uma.es/sustainpine/) and aimed at connecting expression of candidate genes involved in nitrogen metabolism and wood formation with phenotype. The targeted genes were *asparagine synthase* (*ASN*1); *arginase* (*ARS20*); *ornithine d-aminotransferase* (*dOAT*); *PII* (a nitrogen sensor protein) and *LIM* (a transcription factor involved in wood formation).

The five candidate genes were overexpressed and/or knockout (RNAi strategy) in a reference embryogenic line (PN519) using an *Agrobacterium tumefaciens* coculture method initially developed in both France (FCBA, INRA) and Portugal (IBET) (see Trontin et al. 2013, Proceedings of the IUFRO Working Party 2.09.02, Brno conference, pp 184-187) with the following small modifications: increased bacterial density (up to an $O.D_{600}$ 1.2-1.4), acetosynringone concentration (200 μM) during infection and coculture; and the use of 0.5 mg/L phosphinothricin during eight weeks for selection of putative transgenic embryogenic tissue. Transgenic embryogenic tissues were tested for the presence of the *bar* gene (PCR), and amplified for production of cotyledonary somatic embryos. Aliquots of the PCR-positive lines were cryopreserved or used for expression analysis of each targeted candidate gene by RT-qPCR compared to a PN519 non-transformed control.

Under these conditions we were able to obtain between 4-20 PCR-positive lines depending on the gene. For most of the genes, at least three of the lines were significantly up- or down-regulated compared to the non-transgenic control.

The number of mature cotyledonary embryos obtained in maturation experiments was lower in transgenic lines than in the controls, and most SEs were arrested at the precotyledonary stage. Plants obtained had no well-developed roots.

To facilitate further SE development we assayed the effect of cellulose and dialysis membranes as cell physical support but none of then favored further maturation. Ongoing experiments included addition of active charcoal (AC) and pulses of ABA. Results of these experiments will be presented and the possible relation between gene expression and maturation process will be discussed.

In order to induce root development in the germination period, mature somatic embryos were transferred to 1/4 DCR medium with AC as described by Tereso et al. (2006, Plant Growth Regul. 50:57–68). This treatment allowed the recovery of plantlets that will be acclimatized and used for metabolic assays.

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