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Towards functional genomics of transcription factor genes associated to growth and wood formation in maritime pine

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Maritime pine is a major forest tree in southern Europe for sustainable delivery of bio-based products through advanced plantation forestry of improved varieties. Early selection is needed to reduce breeding cycle and accelerate variety deployment in the context of climate change. Both gene(s) and genome-wide information should provide opportunities for predictive, marker-assisted selection. Reverse genetics, defined as ectopic expression or silencing of candidate genes can be useful for functional dissection of traits of interest. Functional genomics of 9 transcription factor (TF) genes associated to growth and wood formation (*MYB1,8,14,20,23, DOF5, MADBOX4, NACx, NACataf*) was initiated in maritime pine through genetic transformation of embryogenic tissue. Twelve TF constructs designed for constitutive overexpression (OE) or silencing (RNAi) were studied: 6 constructs (batch 1) from previous projects (*MYB1*-RNAi, *MYB8*-OE/RNAi, *MYB14*-RNAi, *DOF5*-OE/RNAi) as well as 3 constructs for putative gene targets of *MYB* (*CAD*-RNAi) and *DOF5* (*GS1a*-OE, *GS2*-OE); and 6 constructs (batch 2) obtained during ProCoGen (*MYB23*-OE, *MYB20*-OE, *MADBOX4*-OE, *NACx*-OE/RNAi, *NACataf*-OE).
Phosphinothricin-resistant lines could be cryopreserved for all but one (*MYB20-OE*) constructs. Transformation rate was estimated in the range 9.6-22.0 (OE) or 0.4-18.0 (RNAi) transgenic lines per gram embryogenic tissue (batch 1). Somatic embryos were obtained from 1-3 lines per construct (25 lines in total) and successfully converted to plants (batch 1). Acclimatization rates were similar to controls and transgenic plants were confirmed for most lines. Putative adverse effect of *MYB14*-RNAi, *DOF5*-RNAi, and *MYB8*-OE on transformation rate was observed. *DOF5*-RNAi apparently stimulated germination rate. Plant growth data and morphology are available for constructs from batch 1 after up to 12 (*MYB1*-RNAi, *MYB8*-OE/RNAi, *DOF5*-OE/RNAi, *GS2*-OE, *GS1a*-OE) or 42 months (*MYB14*-RNAi, *CAD*-RNAi) growth. Both transgene copy number and targeted gene expression data are available from transgenic plants obtained from *MYB14*-RNAi and *CAD*-RNAi lines. Transgenics and controls from batch 1 were sampled at age 16 (*MYB1*-RNAi, *MYB8*-OE/RNAi, *DOF5*-OE/RNAi) or 70 months (*MYB14*-RNAi, *CAD*-RNAi). Molecular characterization of *MYB1,8,14* and *DOF5* plant material has been performed by qPCR and transcriptomic analysis using microarray is underway. Wood analyses have been initiated for *MYB14*- and *CAD-RNAi* plants (in progress).