Transformation of somatic embryos in maritime pine (*Pinus pinaster*) mediated by *Agrobacterium*: current state and challenges

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Genetic transformation using *Agrobacterium tumefaciens* provides an efficient tool for unraveling gene function in conifers, including maritime pine (e.g. Sustainpine project, http://www.scbi.uma.es/sustainpine). For this species, a reference FCBA/INRA protocol (2002, 2007) using as model the somatic embryo line PN519 (initiated at FCBA in 1999) and modified pCambia1301 binary vector providing both hygromycin (HYG) and phospinothricin (PPT) selection (pCbar vector obtained from FCBA) is producing high and reliable transformation yield and efficiency (low escape rate). However recent experiments with alternative binary vectors and selection procedures were weakened by very low selection efficiency (i.e. associated with high escape rates). In this work, we optimized our "droplet" method for different vectors to specifically improve transformation efficiency.

Somatic embryos were transformed with the binary vectors pCAMBIA1305.2 (*35S::GUSPlus*, *35S::HPT*), pCbar (*35S::GUSA*, *35S::HPT* and *UBI-1::BAR*) and mt-gb CD3-988 (*35S::GFP*, *35S::BAR*) encoding *β-GLUCURONIDASE* (*GUS*) or *GREEN FLUORESCENT PROTEIN* (GFP) as reporter genes and *HYGROMYCIN PHOSPHOTRANSFERASE* (*HPT*) and/or *PHOSPHINOTRICIN-ACETYL-TRANSFERASE (PPT*) as selectable marker genes. T-DNA integration was confirmed by PCR and reporter gene activity was tested by either GUS (pCambia1305.2, pCbar) or GFP (mt-gb CD3-988) screening assays. Transgenic, PCR-positive lines with resistance to PPT (pCbar, mt-gb CD3-988) were further confirmed by chlorophenol red assay. The frequency of *Agrobacterium* regrowth following transformation was significantly reduced by intensifying the decontamination procedure (use of antibiotics) of cocultivated embryogenic tissue. Furthermore, with a progressive selection system the transformation efficiency has improved the success rate by up to 48% (pCAMBIA1305.2), 77% (pCbar) or 80% (mt-gb CD3-988).