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How to maintain embryogenic capacity of embryogenic lines initiated from Douglas-fir immature embryos

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Douglas-fir (*Pseudotsuga menziesii* (Mirb) Franco) is a native conifer from the Pacific North-West of the US and Canada, and is one of the most important timber species used in the world with both high productivity in a range of climatic conditions and highly valuable wood properties (quality) as well as strong tolerance to diseases and insects. In Europe, Douglas-fir is a major species for reforestation with increasing demand for its wood. Therefore, adaptation of new varieties to climate change and associated stresses is one challenging question for ongoing breeding programs. Efficient selection and vegetative propagation of improved varieties appeared to be key issues to maintain productivity in plantation forestry (Lelu-Walter *et al.* 2013). However, as in many other conifers, early maturation is preventing clonal forestry through conventional multiplication methods in Douglas-fir (Bastien *et al.* 2013).

Somatic embryogenesis from immature seeds, coupled with cryopreservation, is a promising retroactive clonal propagation system of selected trees, currently developed for an increasing number of conifer species (Klimaszewska *et al.* 2016). Excluding patents, there are only a few published studies on Douglas-fir somatic embryogenesis. One recurrent problem is the sustainable multiplication of initiated embryogenic material, i.e. embryonal masses (EMs). Currently, EMs obtained after initiation are subcultured every two weeks in clumps on proliferation medium. Yellowish, non-embryogenic callus (NEC), wich is interspersed with EM, is frequently observed during this process.

In this work we used high-resolution optical microscopy for cytological observation of three embryogenic lines initiated in 2012 (from different genotypes: D1, D2, D3) that were previously classified according to their macro-morphology as either "pure", white-translucent EM as typically described in conifer species (D1, D2) or as a mixture of EMs and NEC (D3). Using confocal microscopy with FDA/PI staining it was shown that EMs from a "pure" line (D2) are apparently interspersed with viable, persistent non-embryogenic cells of unknown origin. In addition, dead cells were observed in embryo suspensors from embryogenic lines suggesting actively occurring apoptotic and autophagic programmed cell death (PCD) that is required for normal embryo development. To tentatively reduce the occurrence of non-embryogenic cells, it is necessary to frequently subculture the EMs (each week), to vigorously dissociate them in liquid medium before transferring onto filter paper as a thin cell layer (Reeves *et al.* submitted).

Key words: Douglas-fir, somatic embryogenesis, multiplication, embryonal masses, morphology, cytology.

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