Advances in the Maturation of Somatic Embryos of *Pinus taeda* and *Pinus palustris*

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Somatic embryogenesis (SE) is a clonal propagation technique with the potential to capture the benefits of traditional breeding by realizing large-scale, clonal propagation of selected genotypes. Furthermore, it is a suitable tissue for genetic transformation. However, for most pine species, the percentage of SE initiation, subsequent conversion of immature embryos to mature embryos, and the production of viable seedlings, is still low, thereby reducing the applicability of this technique. In SE, maturation is described as the initiation of the development of somatic embryos prior to desiccation and subsequent germination During *in vitro* maturation of somatic embryos, different osmotic agents are provided in the medium to increase the osmotic potential mimicking normal seed development. Mature somatic embryos have accumulated storage substances (including carbohydrates, protein and lipids) that will be used for germination. Each of these stages is critical for optimal development of the future plantlets *ex vitro*. The aim of the current project was to test different osmotic agents (ABA, maltose and phytagel) for the maturation and germination of somatic embryos of *Pinus taeda* line J8189 (CellFor). First, a combination of two maltose concentrations (3% and 6%,) and four concentrations of ABA (0, 40, 80 and 120 µM) was tested. Maturation was achieved in all treatments except at 0 µM ABA on both maltose concentrations. The number of mature embryos did not differ significantly between the two maltose concentrations ($p=0.625$). However, the number of somatic embryos was statistically different amongst ABA concentrations ($p=0.003$).The highest number of mature embryos was obtained using 40 µM ABA, with more vigorous embryos observed at 6% maltose. Subsequently, complete germination of mature embryos was achieved on ½ MS medium containing 3% sucrose, with and without desiccation pre-treatment. Secondly, in order to increase the number of mature embryo per mg of fresh culture, the best maturation treatment from above was tested in combination with four Phytagel concentrations (0.3, 0.6, 0.9 and 1.2% w/v). The number of mature embryos was increased with the use of 0.6% Phytagel ($p >0.0001$). After that, germination of mature embryos was achieved using the same germination medium above but with a desiccation pretreatment of only 10 min. The same ABA and maltose concentrations were tested on cell lines of *P. taeda* and *P. palustris* of interest to the Virginia Department of Forestry. Two of the four lines showed embryo head formation, but did not progress further. Currently, maturation of these cell lines are being tested in two independent experiments combining 1) ABA with auxin, and 2) ABA with anti-auxin (PCIB), as both treatments have improved maturation in other conifers studies.

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