GENETIC TRANSFORMATION OF ELITE P. X CANADENSIS CLONES WITH A MORPHOLOGICAL MARKER GENE.


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Interspecific hybrid poplars (P. deltoides x P. nigra) usually known as P.x canadensis are intensively cultivated worldwide for very interesting commercial wood properties. Despite their importance, relatively few research works have reported successful results in vitro culture and genetic engineering to P x canadensis hybrid clones (Confalonieri et al., 2003). Actually it is very difficult to establish and manipulate in vitro culture tissues of most of these clones for their high recalcitrance (Busov et al., 2005). In this work we show the results in vitro manipulation of clones P x canadensis, selected by C.R.A. - Poplar Research Institute of Casale Monferrato and largely used in intensive poplar cultivations around the world. We tested a number of regeneration protocols using different media and phytormone combinations until we obtained a single regeneration protocol with an efficiency close to 100%. Afterwards we have conducted some genetic transformation experiments employing disarmed vectors carrying either reporter or selective marker genes (uidA, bar, nptII). We also employed a monitoring vector (MAT-type) carrying a morphological marker gene (ipt) in our transformation experiments. PCR analysis to assess the presence of the nptII gene were performed. We obtained positive results only with MAT-vector system. Moreover we selected putative transgenic lines through observation of anomalous phenotypes (ipt-type) and through GUS histochemical assays. We observed 6,7 % and 8,9 % of ipt-phenotypes in Neva and I-214 clones, respectively. Molecular analysis and genetic transformation experiments with other P. x canadensis clones are currently under way to confirm our preliminary results. Considering the commercial importance of hybrid P. x canadensis, these results are very interesting to obtain transgenic plants without antibiotic resistance genes, for a possible use in the open field cultivation, in compliance to the current law on OGM.