

Poster Abstract - E.21

NEW METHODS FOR IN VITRO REGENERATION AND GENETIC MODIFICATION OF ELITE POPULUS EURAMERICANA CLONES

P. CALLIGARI*, G. DELIA*, S. ZELASCO*, M. CONFALONIERI**, S. BOTTI***, S. PIETRA*, T. COLLOT*, G. NERVO*

*) CRA - Istituto di Sperimentazione per la Pioppicoltura, Casale Monferrato (AL)

**) CRA - Istituto Sperimentale per le Colture Foraggere, Lodi

***) Dipartimento di Genetica e di Microbiologia, Università degli Studi di Pavia

P. × euramericana, poplar clones, in vitro regeneration, genetic transformation

Poplars represent an important wood resource in the temperate regions in the world. In Italy, poplar plantation are the major sources of domestic roundwood and have also significant potential for paper, biomass energy productions, cleaning up environmental pollution, phytoremediation and enhanced amenity and landscape restoration value. Interspecific *P.euramericana* hybrids (*P.deltoides* × *P.nigra*) still nowadays represent the bulk of European intensive poplar cultivation and most of them have been selected at the Poplar Research Institute of Casale Monferrato. The introduction of new desirable traits in these hybrids by classical breeding has been delayed because of the large size of plants, the long sexual generation cycles and the prolonged period required in order to evaluate adult traits. The use of genetic engineering could allow the solution of these problems and shorten the length of breeding programmes. Applications of genetic modifications include improved resistance to pests and diseases, altered wood properties and composition, herbicide tolerance and growth rate. Plant transformation vectors and methodologies have been improved to increase the efficiency of plant transformation protocols for *P. × euramericana* clones, generally considered recalcitrant. Elimination of markers is advocated since the antibiotic resistance genes may be transferred to pathogenic bacteria. To achieve an efficient and reproducible *in vitro* shoot regeneration protocol, we have preliminarily tested two different protocols (Leplè et al. 1995; Confalonieri et al. 1997) by using stem internodes and leaf explants, excised from *in vitro* plantlets of different clones (I-214, Neva, Arno). All experiments failed to obtain a good shoot regeneration efficiency. Subsequently, the same explants were cultured on different basic media (MS, B5) with different combinations of growth regulators; by using stem internodes a regeneration efficiency of 70% was obtained. β-glucuronidase assays for transient expression were performed on poplar stem internodes following co-cultivation with *A. tumefaciens* strains. Significant progress were observed also in developing transgenic plants devoid of antibiotic marker genes. Experiment of stable transformation are currently under way even if differences were observed among the tested poplar clones.