Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 2A.45

MOLECULAR ANALYSIS OF GENE EXPRESSION LEVELS IN *POPULUS* SPP. TRANSGENIC LINES

DONNARUMMA F.*, PAFFETTI D.**, FLADUNG M.***, BIRICOLT S.****, ERNST D.*****, ALTOSAAR I.******, VETTORI C.*

*) Plant Genetics Institute, CNR, Via Madonna del Piano 10, 50019 Sesto Fiorentino (Italy) - francescadonnarumma@gmail.com; cristina.vettori@cnr.it
**) Department of Agricultural and Forest Economics, Engineering, Sciences and Technologies, University of Florence, Via San Bonaventura 13, 50145 Florence (Italy) - donatella.paffetti@unifi.it
***) Johann Heinrich von Thuenen Institute (vTI), Forestry and Fisheries Institute for Forest Genetics, Sieker Landstr. 2, D-22927 Grosshansdorf (Germany) - matthias.fladung@vti.bund.de
****) Dipartimento di Ortoflorofrutticoltura, Università degli Studi di Firenze, Viale delle Idee 30, 50019 Sesto Fiorentino (Italy) - stefano.biricolti@unifi.it
*****) Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Neuherberg (Germany) - ernst@helmholtz-muenchen.de
******) Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Ontario (Canada) - altosaar@uottawa.ca

Transgenic poplars, cry gene, transgene copy number, real time PCR

Insect-tolerant poplars have been obtained using several types of insecticidal genes coding for *Bacillus thuringiensis*-toxins. In transgenic plants, transgene copy number can greatly affect the expression level and genetic stability of the target gene, making estimation of transgene copy numbers an important area of genetically modified plant research.

In this study, *Populus alba* and *P. tremula* x *P. tremuloides* transgenic lines, obtained via *Agrobacterium*-mediated transformation, carrying *cry1Ab* and *nptII* genes in the T-DNA region, were investigated by PCR and Real Time PCR (RT-PCR) analysis to estimate the transgene copy number as well as expression of the inserted gene in transgenic poplar, respectively. The plants were vegetatively propagated in growth chambers over 2 years. Ten individuals from each clone were planted in containers with "forest soil", and grown in a climate chamber.

All lines contained one copy of *cry* gene and two of them showed that the copy number was different for the *cry1Ab* and *nptII* genes, suggesting rearrangements or multiple but incomplete copies of the transferred DNA. The copy number was concordant among the 3 individuals of each lines analysed and with those determined from the same transgenic lines kept in micropropagation for 2 years. The transcript levels from both genes were determined in 3 individuals for each line growing in climatic chambers. High levels of mRNA expression were detected with respect to the stable endogenous *actin* gene for both transgenic lines. Comparing the transcript level of inserted genes among lines, a significant low level of *nptII* gene ($p = 0.005$) in the line carrying 3 copies was observed.

The evaluation of the copy number of the inserted genes has indicated their stability after 2 years of micropropagation. The lower expression level of the *nptII* inserted gene in one line could suggest that factors like position effects or DNA rearrangements lead to differential expression.

The screening of the transcriptomic variations in transgenic plants carrying the *cry* gene and the comparison with position effects or DNA rearrangements is in course. The final aim is to unravel possible pleiotropic transcriptomic effects following *cry* gene expression in *P. alba* and *P. tremula* x *P. tremuloides* transgenic lines.