

EVALUATION OF AGRONOMIC PERFORMANCE, MOLECULAR AND BIOCHEMICAL INVESTIGATIONS AND TRANSGENE- SOIL INTERACTIONS OF GM POPLARS

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In vitro grown GM poplar plants (*Populus alba* L) expressing the *bar* and *StSy* transgenes and the *nptII* marker gene (Confalonieri et al., 2000; Giorcelli et al., 2004) were transferred to the greenhouse and cultivated in pots containing soil collected from agricultural land We investigated the tolerance of *bar* GM poplars to a non selective herbicide (BASTA) over three years: neither *bar*-transformed line showed any damage due to the application of the herbicide,

We studied also the stability of *StSy* transgene expression over different seasons by evaluating the susceptibility of the *StSy* GM poplars to different leaf diseases and the amount of resveratrol-like compounds produced by different plant tissues..The trials were performed in 2004 and 2005. The results did not show significant differences between GM and control lines. *In vitro* investigations on the effects of *trans*-piceid, expressed by the tested white poplar lines, against some main fungal parasites of cultivated poplars were performed, to assess how it may affect the growth of fungi without plant mediation. *Venturia populina*, *Discosporium populeum* and *Rosellinia necatrix* were tested on potato-dextrose agar medium containing different amounts of *trans*-piceid. The *trans*-piceid was inhibitory on *V. populina* colonies, especially at concentrations over 100 mg/l of medium, but, on the contrary, its presence was associated with an increased growth of colonies of one *D. populeum* isolate and of two *R. necatrix* isolates. *V. populina*, however, is a quite specific pathogen, so its response to the presence of *trans*-piceid may be triggered by the activation of certain inducible genes. The metabolic pathways of such compound in fungal parasites need to be clarified HPLC-DAD analysis mainly showed the presence of the *trans*-piceid (*trans*-resveratrol 3-glucoside) in all the tested tissues (leaves, stems and roots) in the *StSy* GM poplars.. Seasonal variations of the *trans*-piceid content were found, opposite during time in leaves and roots. Its highest amount (364 mg/kg fresh weight) was detected in leaves during the full vegetative growth whereas in roots just after dormancy (330 mg/kg)

The plants from each transgenic and control line were monitored to evaluate the steady-state level of the *bar* and *StSy* transcripts in apical and basal leaves under conditions of full vegetative growth and dormancy. The evaluation of the *in planta* expression pattern over a three-year period showed significant fluctuations in the steady-state level of the *bar* and *StSy* transcripts. Further

studies were carried out in order to clarify the molecular mechanisms underlying the seasonal fluctuations of the *bar* and *StSy* transcripts.. One possible explanation might involve microRNAs (miRNA). To this purpose, a bioinformatic approach was used to check for the presence of miRNA target sites within the *bar* and *StSy* nucleotide sequence

Different soil samples from *bar* and *StSy* pots were collected to monitor the persistence of recombinant DNA sequences in soil and to assess the possible occurrence of horizontal gene transfer from GM poplars to soil microorganisms. Molecular analyses carried out on both the *bar* and *StSy* trials confirmed the presence of recombinant DNA sequences derived from GM poplar tissues in soil over three year On the same soil samples the total culturable bacterial population and the fraction of kanamycin-resistant bacteria were also analysed. No significant variation was detected in the microbial flora of the soil cultivated with GM poplars in comparison with the soil before GM poplar cultivation. The reported data contribute to a better understanding of the agronomical response and environmental impact for a potential cultivation of GM poplars on a large scale.