Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - L.04

PHENOL-OXIDASE ACTIVITY IN TRANSGENIC TOBACCO PLANTS FOR ORGANIC POLLUTANTS DEGRADATION

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Nicotiana tabacum, laccase, poxC, phenols, phytoremediation

Under the term “phytoremediation” are listed several approaches based on the use of plants to clean up the environment (air, water, and soil) from organic and inorganic pollutants. Our Plant Biotechnology Research Group is interested in phytoremediation of soils from polycyclic aromatic hydrocarbons (PAHs), since the presence in Campania Region of several areas polluted by wastewaters from olive-oil mills and tanning plants. However, although some plant species were found to be able to metabolize these xenobiotics to non-toxic compounds, the efficiency of this treatment is still too low. Our aim is to obtain plants expressing heterologous enzymes that might make phytoremediation procedures more effective. Our research approaches was to express *in planta* fungal laccase (E.C1.10.3.2; p-benzenediol:oxygenoxidoreductase) genes from *Pleurotus ostreatus*, targeting transgenic enzymes to the apoplast, in order to have them available into the soils by means of plants root exudates.

In a previous communication (http://www.siga.unina.it/SIGA2003/3_16.htm) we reported the expression of the *poxC* gene under the CaMV35S[2] constitutive promote into several *Nicotiana tabacum* cv *Samsun* NN transgenic plants; we shown also the integration of the transgene from single to multiple copies into the tobacco genome and its expression. However, some transgenic clones behaved differently in comparison with the untransformed control, showing from moderate to strong reduced growth, modified leaf shape, and a floral abortion. At that time, we suggested the interference of transgenic laccase activity with the correct lignin synthesis and/or degradation in these plants, even if not all transgenic plants behaved in this different manner in respect to the control.

Further results are presented in this communication on the analysis of some transgenic clones. Using ABTS as chromogenic laccase substrate, we ascertained that the enzyme is released from transgenic plants into solid and liquid medium *via* root exudates and it is still active. Hence, laccase activity was tested again with ABTS assay on total, extra cellular, and cytoplasmic protein fractions and on root exudates. All transgenic clones have shown a total laccase activity many times more efficient than the untransformed control; the extracellular activity ranged from 8 to 100 times the control one. The latter did not show any activity in root exudates, whereas transgenic clones showed a large range of activity. Interestingly, the clone showing the most aberrant phenotype was also the one showing the highest enzyme activity in root essudates. Total phenols content in control and some transgenic clone leaves was determined by HPLC. All transgenic clones have shown phenols content equal to or lower than the control. Preliminary results on the proteome analysis by 2D protein gel electrophoresis have shown some differencies among control and transgenic clones, to be confirmed in further analysis.

We are now developing: a metabolic profiling approach to better understand which major metabolic pathway was modified by the transgene insertion/expression; *in vivo* assays of phenol-oxidase activity in

transgenic plants on PAHs; the co-expression of the *pox*A1b gene into root-cells of *pox*C transgenic clones.