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Poster Abstract – C.37

*PLEOROTUS OSTREATUS pox*A1b GENE EXPRESSION IN TOBACCO PLANTS

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Phenols are very abundant compounds in the nature, due to their natural occurrence in the environment such as lignin and as pollutants from industrial activities. Among them, olive oil mills are the major environmental hazardous for releasing effluents (olive oil mill wastewaters-OMW), which are characterised by low pH and heavy presence of mono and polyaromatic compounds such as polyphenols. Those compounds are refractory to biodegradation and have high level of phytoxoxic and microbial inhibitory compounds (El Hajjouji *et al.* 2008). It has been estimated that over 2.5 million metric tons per year of olive oil are produced worldwide, 98% of those in Mediterranean countries (FAOSTAT, 2007) and in most of them, OMW are used to be discharged in fresh waters. However, some European countries, including Italy (Law No. 574/1996), allow their spreading on agriculture soils between 50 to 80 m^3 per year. As results, it has been reported several effects on crops such as higher rate of mortality and low percentage of seed germination. In nature, some ligninolytic fungi are able to reduce the content of aromatic compounds and among them *Pleurotus ostreatus* (P.o.) has been intensively studied for its ability to degrade phenolic compounds into less toxic ones thanks to the production of several extracellular enzymes including laccases (Giardina *et al.* 1999) Therefore, our aim is to overexpress laccases in plant roots in order to evaluate their ability for reducing phenol content in OMW. In a previous SIGA communication (Galante *et al.*, 2005) we have reported that *Nicotiana tabacum* plants expressing the gene *pox*C from P.o. were able to release the enzyme in the root exudates into the *in vitro* culture medium; moreover, the laccase was fully active as resulted by an ABTS assay. Giardina *et al.* (1999) have also demonstrated that the laccase POXA1b is more stable than the other P.o. isoenzymes. Hence, the P.o. *pox*A1b cDNA was cloned into the pGreen binary vector under the CaMV35S promoter and electroporated in *Agrobacterium tumefaciens* strain LBA4404, which has been used as carrier to transfer the *pox*A1b cassette into the genome of *N. tabacum* cv Samsun NN. The differentiated rooted shoots, selected in the presence of kanamycine, were screened by PCR analysis using specific primers for the *pox*A1b gene. The evaluation of laccase activity was performed by detection of a green band on native polyacrilamide gel electrophoresis using ABTS as crhomogenic substrate. Quantification of phenol-oxidase activity was firstly performed by enzymatic assays in leaves protein extracts. In addition, transgenic plants were grown *in vitro* to measure the enzymatic activity of root exudates. In order to verify the synergic effects on phenols degradation of both POXA1b and POXC laccases, transgenic tobacco lines expressing each of those enzymes will be crossed and their progenies will be evaluated.