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FIRST RESULTS ON THE OVEREXPRESSION OF *Cs*GSTU ISOENZYMES IN TRANSGENIC TOBACCO PLANTS

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The glutathione S-transferases (GSTs, EC 2.5.1.18) are members of a multifunctional superfamily of enzymes catalyzing the conjugation of glutathione (GSH) to the electrophilic groups of hydrophobic and usually cytotoxic molecules of either endogenous or exogenous origin. GSTs are widely distributed in nature and are found in several organisms from humans to bacteria. In plants the GSH addition reaction is coupled to the vacuolar compartmentation of the GS-conjugates because of the lack of an effective excretion pathway which is, instead, active in animals. Based on protein sequence similarity, active site residue and gene organization plant GSTs are grouped in four main classes (phi, tau, zeta, theta). The majority of the plant GSTs belongs to the tau (GSTU) and phi (GSTF) classes which are plant specific. Among the plant GST classes, tau is the most numerous and members of this class overlap in their function of enhancing crop stress tolerance. Transgenic plants overexpressing GST subunits active in herbicide detoxification confirmed the GST's role in crop's herbicide selectivity, and, the down-regulation of GST subunit active in the detoxification process can result in reduced tolerance to herbicide of the transformed crop. In a previous study we isolated from sweet orange leaves [(*Citrus sinensis*) L. Osbeck)] two GST genes, namely GSTU1 and GSTU2. The encoded proteins differ only for three amino acids all of them included in the C-terminal domain of the enzymes (R89P, E117K, I172V). In order to understand the significance of the single mismatched residues between U1 and U2 (R89P, E117K and I172V, respectively) site-directed mutagenesis experiments were undertaken to generate several mutate enzymes. Among the mutate enzymes, GST-RKV, obtained by the substitution R89P upon the isoform GSTU2, showed extremely high catalytic efficiency towards GSH and pronounced ability to conjugate GSH to the alkyl halide 4-nitrophenethyl bromide, a molecule of toxicological interest in view of its occurrence as environmental pollutant. Due to these features GST-RKV exhibits great potential for the development of germplasm with novel favourable traits. As a consequence, we report here the first results on generation via *Agrobacterium* transformation of transgenic tobacco plants overexpressing both *Cs*GST isoenzymes and mutants, and *in planta* study of the their role in detoxifying herbicides.