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FLUORODIFEN HERBICIDE TOLERANCE IN TRANSGENIC TOBACCO PLANTS OVEREXPRESSING CsGSTUs

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Abiotic environmental stress conditions, including water deficit, high temperature, salinity, cold, heavy metals, mechanical wounding and exogenous chemicals often impose constraints on plant growth and development under field conditions. Nowadays, the quantity and variety of phytotoxic xenobiotics have been growing as a result of the rapid increase in the number of man made synthetic compounds. Plants encounter them either as a result of industrial pollution or in the form of agrochemicals such as herbicides and pesticides. Such xenobiotics cannot be used for nutrition or as a source of energy, but are nevertheless taken up and accumulated. As plants lack of an excretion pathway like that found in animals, they have developed a detoxification system from toxic compounds that consists of two main processes: chemical transformation and compartmentation. Xenobiotic molecules are firstly activated so that certain functional groups can be exposed to the successive action of several modifying enzymes. Among them, the glutathione transferases (GSTs) catalyze the nucleophilic addition of glutathione (GSH) to the electrophilic groups of a large variety of hydrophobic toxic molecules strongly contributing to the counteracting process. Previously, two gstu genes have been isolated from sweet orange leaves [(*Citrus sinensis*) L. Osbeck)] namely GSTU1 and GSTU2. The encoded proteins differ in three amino acids, all of them included in the C-terminal domain of the enzymes (R89P, E117K, I172V). In order to evaluate the contribution of the mismatched amino acids on the catalytic activity of enzymes, several cross-mutant genes were produced by site-directed mutagenesis followed by the biochemical characterization of the *in vitro* expressed enzymes. In this work, transgenic tobacco plants via *Agrobacterium tumefaciens* mediated tranformation overexpressing both the wild type and mutant *Cs*GSTU genes were generated. Along with the molecular characterization of transformed plants, an *in planta* study to assess their ability in detoxifying herbicides was also performed. Therefore, transgenic plants were subjected to the action of fluorodifen, a diphenyl ether herbicide that cause photooxidative stress by inhibition of the plastid protoporphyrinogen oxidase. The electrolytic leakage assay was carried out to test the damage caused by fluodifen treatment upon transformed and untransformed tobacco plants. The data revealed that the transgenic lines show a sharp reduction of membrane damage compared with the wild type tobacco plants. In fact, tobacco overexpressing both wild type and mutant *Cs*GSTUs show relative electrolyte leakage values ranging between 30% and 33% compared to 62% of wild type plants. These findings suggest that the herbicide-tolerant transgenic tobacco plants can be utilized in phytoremediation programmes against fluorodifen herbicide since the wild type and mutant CsGSTUs might provide an efficient contribution in the detoxification of this xenobiotic in contaminated areas.