MOLECULAR CLONING AND EXPRESSION ANALYSIS IN RESPONSE TO ABIOTIC STRESSES OF TWO TAU-TYPE GSTs FROM ORANGE LEAVES

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Glutathione S-transferases (GSTs) represent a multifunctional family of enzymes grouped into four main classes (tau, phi, theta, and zeta) conjugating endobiotic and xenobiotic compounds to glutathione. In plants, this is considered to be a crucial step in the detoxification process as the S-glutathionylated metabolites are tagged for vacuolar sequestration. In this work, we have isolated two glutathione S-transferases belonging to the tau class GSTs from sweet orange leaves. The cDNA clones contained a complete open reading frame of 651 bp encoding two 216 amino acid proteins. Homology search and sequence alignment showed that the deduced amino acid sequences shared high identity with GSTs from other plant sources, including several strictly conservative motifs and distinctive amino acid residues specific of the tau class GSTs. The genomic clones of both isoforms were also isolated and the analysis of the gene organization confirmed the membership of both enzymes to the tau class GSTs. The encoded proteins differ only for three amino acids: the triplet R89, E117 and I172 found in the isoform named GSTU1 is replaced by the triplet P89, K117 and V172 in the GSTU2 isoform. The successful *in vitro* expression of the proteins led to the functional active form of both enzymes which showed different specific activity against CDNB as substrate, the GSTU1 showing values three fold lower than that observed for the GSTU2 enzyme. The analysis of the gene expressions suggested that the GST isoforms show either different distribution between leaf and flesh, the isoforms being decidedly expressed in the leaf, or cultivar related specificity, the U2 being highly expressed in the leaves of red orange whereas the U1 in the blond orange leaves. Furthermore, we also showed that the expression of U1 gene was remarkably induced in response to cadmium sulphate, CDNB and cyhalothrin treatments as well as to cold stress. On the contrary, the U2 isoform was constitutively expressed probably playing some sort of “default scavenging” activity *in vivo*. Taken together these results suggested that GSTU1 is a stress responsive gene and can be considered as potential target that is genetically modified so as to create novel germoplasm with enhanced stress tolerance.