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Poster Abstract – G.20

MOLECULAR CLONING AND EXPRESSION ANALYSIS OF GLUTATHIONE S-TRANSFERASE IN BLOOD AND BLOND ORANGE FRUITS

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Glutathione S-transferases (GSTs) are ubiquitary enzymes which have a defined role in xenobiotic detoxification, but a deeper knowledge of their function in endogenous metabolism is still lacking. In this work, we isolated the cDNAs, the genomic clones and the promoter regions of pigmented and non-pigmented orange *gsts*. Having considered gene organization and homology data, we believe that the isolated GST gene is probably involved in the vacuolar import of anthocyanins. In order to confirm this hypothesis we have shown that a strong reduction in GST expression occurs in the non-pigmented orange cultivar [*Citrus sinensis* L. (Osbeck)] (Navel and Ovale) compared to that of the pigmented orange (Tarocco). In accordance with this data, in the crude extracts of both pigmented and non pigmented orange fruit, GST activity was reproducibly detected by providing cyanidin-3-O-glucoside as substrate. However, crude extract of non-pigmented orange showed only 8.5% of GST activity compared to that of pigmented orange suggesting that the GST enzyme involved in cyanidin-3-O-glucoside conjugation to GSH is not largely represented. Moreover, we have shown that cyanidin-3-O-glucoside acted as a powerful competitive inhibitor of 1-chloro-2,4 dinitrobenzene conjugation to GSH in the pigmented orange, whereas it behaves as an in-competitive inhibitor of the enzyme in non-pigmented orange. In addition, we have reported here the successful *in vitro* expression of orange GST cDNAs leading to a GST enzyme which is active against cyanidin3-O-glucoside thus confirming the involvement of the isolated genes in the tagging of anthocyanins for vacuolar import. Finally, after putting together our findings, we have concluded that the low expression level of non pigmented orange *gst* is the result of a likely mutation in a regulatory gene controlling the expression of *gst*.