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THE SILENCING OF A GST GENE INCREASES THE CONTENT OF HEALTH-PROMOTING DICAFFEOYLQUINIC ACIDS IN TOMATO

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There is considerable interest in preventive medicine and food industry in the development of strategies to increase the content of natural antioxidants in edible plants.

Tomato, in addition to lycopene, contains a number of flavonoids and phenolic acids which, synergistically or additively, provide protection against the damage induced by free radicals during oxidative stress, and reduce the risk of certain chronic diseases in human beings.

Among phenolic acids, the mono-caffeoylquinic acids (e.g. chlorogenic acid, CGA) and to an even greater extent the dicaffeoylquinic acids (diCQAs) have been found to possess marked antioxidative properties. However, the development of strategies to increase diCQAs content in plants is hampered by the lack of information on genes involved in their biosynthetic pathways.

Incubation of CGA in crude extracts of tomato fruits led to the formation of two new products, absent in the control reactions (boiled enzyme), which were identified by LC-MS as isomers of diCQAs. We thus hypothesized the presence of a transferase catalysing the synthesis of diCQAs using CGA as acyl donor.

The enzymatic activity increased with advancing fruit ripening, reached the highest value in fully ripe tomato fruits and was accompanied by accumulation of diCQAs.

The enzyme was purified from fully ripe fruits using a combination of ammonium sulphate precipitation and anion exchange chromatography. The final protein fraction resulted in 387 fold enrichment of enzymatic activity, and was subjected to trypsin digestion and mass spectrometric sequencing: the *Tau* Glutathione S-transferase (GST) was selected as a potential candidate gene.

To assess GST functional role, a Virus-induced gene silencing strategy was applied in purple tomato lines which, following expression of the transcription factors *Delia* and *Rosea* from snapdragon, accumulate high level of anthocyanins. These lines make it possible a visual monitoring of VIGS experiments by combining silencing of a candidate gene with that of *Del-Ros* module, which leads to easily recognizable red-coloured sectors within the purple background. As evidenced by LC-MS analyses, red-coloured *Del*/*Ros*/GST-silenced sectors contained 2.1 fold more diCQAs than red coloured *Del*/*Ros* silenced portions. Our results show the involvement of the newly isolated gene in diCQAs metabolism, whose silencing may significantly enhance the content of diCQAs in tomato.