



Titolo del programma:

Metabolic engineering of flavonoids pathway to improve the nutritional value of tomato

Results

The main objective of this collaborative project is to evaluate the healthy potential of some new tomato transgenic lines enriched in specific classes of polyphenols, already developed during the course of this collaborative project.

During my stage at the John Innes Centre, I carried out a (bio)chemical characterisation of main polyphenols fractions detected in the fruit of some newly developed tomato lines. In particular, we were able to obtain, by traditional crossing, two new lines accumulating high levels of resveratrol in a normal cv. Moneymaker and in a purple tomato background, respectively. The genotype of these tomato lines was preliminarily confirmed by PCR using specific primers. Fruits from positive tomato lines were lyophilised and solvent extracted. Hydrophilic and lipophilic polyphenols fractions were separated by RP-HPLC and main polyphenol classes identified by MS and UV spectra analyses using synthetic standards. Polyphenols classes were also quantified, using five-point regression calibration graphs of the UV absorption of solutions of commercially available standards. The antioxidant capabilities of tomato fruit extracts was carried out by TEAC (Trolox equivalent antioxidant capacity) assay. The method is based on the ability of antioxidant molecules to quench the long-lived $ABTS^{\cdot+}$, a blue-green chromophore with characteristic absorption at 734 nm, compared with that of Trolox, a water-soluble vitamin E analog. The addition of antioxidants to the preformed radical cation reduces it to ABTS, determining a decolorization. Fruits from different tomato lines were washed, deprived of seeds and homogenized under nitrogen in a mortar. The lyophilized sample was extracted twice with water/ethyl acetate solution (1:1 vol/vol) under agitation for 60 min at room temperature, centrifuged and the fractions collected. Tomato extracts were immediately analyzed for their antioxidant capacity.

Results indicated that the fruit from the new tomato lines accumulated significantly high amount of resveratrol and related glycosylated forms. In particular, we were able to identify three different glycosylated forms of resveratrol, together with its piceid. In particular the line in the cv Moneymaker background showed the presence of about 1.2 mg of total resveratrol (comprising resveratrol, its piceid and three other glycosylated form) per gram of fresh weight. In the line obtained in the purple background, the synthesis levels were about 50 % lower than those recorded from the previous line, even though the concomitant accumulation of anthocyanins renders this line a very useful tool to investigate the health beneficial effects of both these classes of polyphenols.

These results were mirrored by the data relative to the anti-oxidant capabilities of these lines. Indeed, the high resveratrol line in the cv Moneymaker background showed a significant higher (about four times) antioxidant power in the lipophilic fraction in comparison with control tomatoes. Conversely, the purple line showed a slight increase in the lipophilic fraction but a significant increase (about three times higher) in



the hydrophilic fraction. Notably, the total antioxidant capability of this line was about three time higher than that recorded from control tomato.

Further work is now in progress to evaluate the health beneficial effects of these new tomato lines in a number of in vitro and in vivo human disease models. Preliminary results indicated that an extract obtained from the resveratrol accumulating purple line was able to significantly reduce the vitality of a number of cancer cell lines including breast, ovarian and gut cancer models.

In fede
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