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EVALUATION OF ALTERNATIVE SPLICING IN THE *CITRUS SINENSIS* GLUTATHIONE S TRANSFERASE GENE FAMILY

LICCIARDELLO C.*, D'AGOSTINO N.**, REFORGIATO RECUPERO G.*,
CHIUSANO M.L.**

*) C.R.A.-Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee, Corso Savoia 190, 95024 Acireale (Italy)
**) Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples "Federico II", Via Università 100, 80055 Portici (Italy)

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The glutathione S transferase (GST) gene family was characterized in *Citrus sinensis* L. Osbeck by screening a large collection of Expressed Sequence Tags (ESTs). Unique GST encoding transcripts were identified and the longest Open Reading Frame (ORF) detection was performed on each of them in order to find out full-length sequences. In addition, an *Arabidopsis*-based GST class assignment was undertaken and finally Semi-Quantitative Reverse Transcription Polymerase Chain Reaction (SemiQ RT-PCR) analyses were performed to assess the expression levels of the putative GST genes in the albedo, flavedo, flesh, young and adult leaf tissues and ovary.

Considering the computational analysis, two sequences of the *phi* class and one sequence of the *tau* class were assumed to be intron-retaining sequences (IR).

Since, exon-intron structure of GST genes have been already described (three exons/two introns for *phi* genes and two exons/one intron for *tau* genes), we concluded that the *phi* class sequences retain both the introns and the *tau* sequence retains the only one intron it owns.

In maize, a class *tau* GST gene, *bronze2* (*bz2),* whose transcript variants are known to live both as spliced and unspliced (i.e. IR) *bz2* forms, is predicted to encode 26 kDa protein and a trucated protein products of 14 kDa, respectively (Marrs and Walbot, 1997, Plant Physiology 113: 93-102). We assume that the *tau* class Citrus GST we analysed, might maintain the same behaviour of the maize counterpart.

On the other hand, no spliced and unspliced forms have been previously described in literature for *phi* class GST transcripts.

RT-PCR performed on a *phi* class GST transcript, we assembled *in silico* and which is identical to the sequence DQ207360 deposited at GenBank, generates one band of the expected size in fruit tissues (albedo, flavedo and flesh), while two amplicons of different size are observed in leaves and ovary. These two amplicons were extracted, sequenced and the sequences obtained were BLAST-searched against the GenBank non-redundant nucleotide database.

As a consequence, the upper band resulted to be the unspliced transcript form (both the introns are retained), while the lower band resulted to be the spliced transcript form which generates the same protein product described in the DQ207360 GenBank record. In conclusion, we demonstrated that both the transcript forms are detectable in the cells analysed, though we are still investigating on the functional role of the intron retention for these transcript variants.

We also underwent a wider screening on possible splicing variants within the *phi* class to better investigate on possible amplification mechanisms of gene products.