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# Glutamate dehydrogenase is differentially regulated in seeded and parthenocarpic tomato fruits during crop development and postharvest storage

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ABSTRACT

Cherry tomatoes are more susceptible than larger fruit varieties to producing auxin-induced parthenocarpic fruits indistinguishable from seeded fruits in most organoleptic characteristics. In this study, the effects of auxin-induced parthenocarpy and of the short-term cold storage on the expression of *GDH* genes, one of the main regulatory enzymes of nitrogen metabolism, were examined. Seeded and parthenocarpic fruits exhibited differences in the pattern of expression of the genes coding for α- and β-subunits of *GDH* during fruit development. Short-term cold storage at 5 °C significantly increased transcript accumulation of *GDH* genes, while storage at 10 °C for five days mostly caused a reduction in the expression of the genes in both fruit types. Finally, no significant differences between the two fruit types were detected in the localization of GDH protein and GDH enzyme activity.



## 1. Introduction

Farmers produce parthenocarpic tomatoes by artificial fruit setting using auxins in order to overcome poor fertilization when the environmental conditions are unfavourable to a satisfactory fruit set (de Jong et al., 2009). Cherry tomatoes, in contrast to many larger fruiting varieties, produce well-formed fruits with no considerable differences in shape and colour compared to seeded tomatoes (Cuartero et al., 1987). Interestingly, while seeded and parthenocarpic tomatoes show insignificant disparities in some of the major quality indexes, they exhibit considerable variations in ascorbic acid and sugar metabolism (Tsaniklidis et al., 2012, 2014; Rounis et al., 2014). These findings suggest that further differences between the two fruit types could exist in other major metabolic routes, such as in nutrient assimilation and turnover.

Nitrogen is a critical macronutrient that must be in reduced state in order to be used by plants. Ammonium is a reduced nitrogen form, easily accessible to plans for assimilation into amino acids and proteins (Masclaux-Daubresse et al., 2006). Ammonium, however, is noxious to plants, causing proton extrusion, pH turbulence, uncoupling of photophosphorylation, etc. (Kronzucker et al., 2001). Glutamate dehydrogenase (GDH-EC 1.4.1.2) regulates to a great extent glutamic acid (Glu), one of the proteinogenic amino acids, which serves as an intermediate molecule during the synthesis of complex amino acids and, thus, plays a pivotal role in ammonium assimilation and amino acid metabolism (Dubois et al., 2003; Masclaux-Daubresse et al., 2006; Bernard and Habash, 2009). Glutamate dehydrogenase is involved in ammonium ion transfer by catalyzing the reversible deamination of Glu to α-ketoglutarate (Ferraro et al., 2012). Hence, this enzyme provides a link between carbohydrate and amino acid metabolism. In ripe tomato fruits, glutamic acid is found at high concentrations, giving them the distinguished 'umami' taste (Sorrequieta et al., 2010). In plants, the higher Km for ammonium in GDH, compared to glutamine synthase (GS), is considered to be involved almost exclusively in Glu breakdown. This biochemical pathway produces α-ketoglutarate and consequently provides carbon skeletons for the tricarboxylic acid cycle (Dubois et al., 2003; Mungur et al., 2005). Moreover,

*Abbreviations:* GDH, glutamate dehydrogenase; Img, immature green; MG, mature green; Br, breaker; RR, red ripe.

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under several conditions where the TCA cycle is inhibited this pathway could be important as an alternative route to 2-oxoglutarate (Sweetlove et al., 2010). GDH enzyme has a hexameric structure consisting of two polypeptides (α- and β-subunit) that fluctuate slightly in mass and charge (Skopelitis et al., 2006). GDH enzyme can be separated by PAGE electrophoresis into seven different anodal-migrating isoenzymes. *GDH* gene families coding for α- and β-subunits of the enzyme have been characterized in *Arabidopsis thaliana* (Turano et al., 1997) and *Nicotiana plumbaginifolia* (Ficarelli et al., 1999). Only recently, a third GDH polypeptide with a minor participation in the hexamere structure of the GDH complex protein was characterized in *Arabidopsis* (Fontaine et al., 2013). In addition, GDH is located in mitochondria, chloroplasts and cytosol (Dubois et al., 2003). GDH is often associated to senescence, stress tolerance and balancing the N/C status of plants (Skopelitis et al., 2006; Labboun et al., 2009; Fontaine et al., 2012). Although GDH function is well characterized, little is known regarding its regulation at the transcriptional level during fruit development. Furthermore, responses to abiotic stress such as cold induction (under post-harvest storage) are vastly uncharted and have not yet been fully investigated. This study attempts to investigate the localization and expression of *GDH* genes, during the development and post harvest storage of cherry tomato fruits, since it is a key enzyme in amino acid composition. In order to investigate further the effects of parthenocarpy on tomato fruit metabolism, the localization of GDH protein and enzyme activity, and transcription of the genes coding for the α- and β-subunits of *GDH* were studied in seeded and parthenocarpic fruits, during their development and following post harvest storage.

## 2. Materials and methods

### 2.1. Plant material and growth conditions

Plants of cherry tomato *Solanum lycopersicum* L. var. *cerasiforme* cv. Conchita F1 (de Ruiter Seeds, Melbourne, Australia), a productive hybrid with long shelf life, were cultivated in a glasshouse of the Agricultural University of Athens, Greece between December and May. Mean minimum and maximum temperatures in the greenhouse were 15.7 ± 2.0 °C and 26.6 ± 4.3 °C, respectively (Spring, [March–May]) and 12.9 ± 1.9 °C and 23.9 ± 4.4 °C (Winter, [October–February]). The average solar radiation was 15.3 MJ/m$^2$ per day. Seedless fruits were produced by the emasculation of closed flowers and subsequent spraying with Ortomone a pre-fixed commercial synthetic auxin preparation (Spirou, Athens, Greece) containing 50 ppm 2-naphthoxyacetic acid (Karapanos et al., 2013). The auxin preparation was applied according to the manufacturer's instructions. Individual flowers were tagged after treatment. Fruits were harvested systematically at the following stages: immature green (ImG-25 DAF (days after flowering) with diameter 15 mm), mature green (MG-38 DAF), breaker (Br-44 DAF with >10% red colour) and red ripe (RR-52 DAF). Post harvest treatments of fruits harvested at breaker stage included storage for 120 h at 5 °C (5d5°C) and storage at 10 °C for 120 h (5d10°C). Storage at 5 °C is typical for storage of fruits at home, while storage at 10 °C was chosen in order to simulate the temperature during the transportation in refrigerator trucks. Each harvest was carried out at 11 am and replicated three times creating three lots. The fruits indented for post harvest treatments were harvested at Br stage. Samples were immediately frozen in liquid nitrogen, homogenized using a pestle and mortar and then stored at −80 °C (Green and Sambrook, 2012).

For postharvest storage, the fruits placed in clear PET cases appropriate for food storage that allowed gas exchange. Their dimensions were 13.1 cm × 11.3 cm × 7 cm. The cases were filled with fruits at the breaker stage. Inside the storage units the relative humidity was 75% and the temperature was adjusted accordingly.

Because of the participation of GDH in nitrogen metabolism, the fertilization plan of the plants was carefully designed in order to maximize production, thus simulating a commercial crop. The fertilization plan was based on the findings of Sainju et al. (2003) with some modifications. The fertilizers used for this experiment were high performance soluble complex NPK fertilizers containing micronutrients.

**Table 1**
Primer sequences of genes used for qPCR.

| Gene | Encoded enzyme/protein | Primer sequence | Accession no. |
|---|---|---|---|
| *GDH α* | α-Subunit of tomato glutamate dehydrogenase | Sense: CAAGCCGCTC-GAATTCTTGG Antisence: CCTCC-CAAAGCACAAGGGAT | XM_004235152 highly similar to AY366369 from *N. tabacum* (common primers were designed for all putative tomato α-GDH subunits) |
| *GDH β* | β-Subunit of tomato glutamate dehydrogenase | Sense: TTCCATGATGCACATCC AAT Antisence: CCAACTGATCCAGAAGC TGA | NM_001246921 |
| *UBQ* | Ubiquitin | Sense: GCAGACTATAA-CATCCAGAAAGAG Antisence: AACAA-CAAAGCACACAGCCATC | X73156.1 |

### 2.2. qPCR analysis

Samples for qPCR analysis were prepared mixing 1 g from each lot (described above). Total RNA was isolated from each sample using RNeasy extraction Kit (Qiagen, Hilden Germany). Quantity and quality of total RNA were assessed by spectrophotometric and electrophoretic analysis, measuring the absorbance at 260 nm and the absorbance ratio of 260/280 nm in Nanodrop (Thermo, Wilmington, USA) and by 1.5% (w/v) agarose-gel electrophoresis. To eliminate total DNA, samples were treated with RNAse free DNaseI (Takara, Otsu Shiga Japan) for 60 min at 37 °C (Delis et al., 2011). The complete DNA removal was tested with primers designed against the ubiquitous expressed gene of *UBIQUITIN* (*UBQ*), while *S. lycopersicum* genomic DNA was used as a positive control.

First strand cDNA was reverse transcribed by the Affinity Script$^{TM}$ Multi Temperature reverse transcriptase using oligo primers according to the manufacturer's instructions (Stratagene, Santa Clara, USA). The resulting first-strand cDNA was then normalized for the expression of the housekeeping gene of *UBQ*. Gene specific primers for the tomato *GDH* (α-subunit) (Purnell et al., 2005), *GDH* (β-subunit) (Ferraro et al., 2012), and *UBQ* genes were designed with Beacon designer v 7.01 software (Table 1). Quantitative real time PCRs were performed on a MX-3005P system (Stratagene, Santa Clara, USA) with Kapa Fast Universal 2× qPCR Master Mix (Kapa, Woburn, USA) and gene-specific primers. The cycling profile consisted of an initial denaturation step of 3 min, followed by 40 cycles of 30 s at 95 °C, 45 s at 60 °C, and 11 s at 72 °C and a final elongation step of 5 min at 72 °C. The primer specificity and the formation of primer–dimers were monitored by dissociation curve analysis and agarose gel electrophoresis on a 2% (w/v) gel. In all samples examined, a single amplicon was detected.

The expression levels of *S. lycopersicum UBQ* gene were used as internal standards to normalize concentrations of the cDNA templates. For the relative quantification of gene expression, a