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Research article

Glucose-6-phosphate dehydrogenase plays a central role in the response of tomato (*Solanum lycopersicum*) plants to short and long-term drought

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ABSTRACT

The present study was undertaken to investigate the expression, occurrence and activity of glucose 6 phosphate dehydrogenase (G6PDH – EC 1.1.1.49), the key-enzyme of the Oxidative Pentose Phosphate Pathway (OPPP), in tomato plants (*Solanum lycopersicum cv. Red Setter*) exposed to short- and long-term drought stress.

For the first time, drought effects have been evaluated in plants under different growth conditions: in hydroponic laboratory system, and in greenhouse pots under controlled conditions; and in open field, in order to evaluate drought response in a representative agricultural environment.

Interestingly, changes observed appear strictly associated to the induction of well known stress response mechanisms, such as the increase of proline synthesis, accumulation of chaperone Hsp70, and ascorbate peroxidase.

Results show significant increase in total activity of G6PDH, and specifically in expression and occurrence of cytosolic isoform (cy-G6PDH) in plants grown in any cultivation system upon drought.

Intriguingly, the results clearly suggest that abscissic acid (ABA) pathway and signaling cascade (protein phosphatase 2C – *PP2C*) could be strictly related to increased G6PDH expression, occurrence and activities.

We hypothesized for G6PDH a specific role as one of the main reductants' suppliers to counteract the effects of drought stress, in the light of converging evidences given by young and adult tomato plants under stress of different duration and intensity.

1. Introduction

Glucose 6 phosphate dehydrogenase (G6PDH – EC 1.1.1.49) is well known as the first and rate-limiting enzyme of the Oxidative Pentose Phosphate Pathway (OPPP), catalyzing the oxidation of glucose-6-phosphate (G6P) to 6-phospho- δ -glucono-1,5-lactone, spontaneously converted – or by the action of lactonase (EC 3.1.1.31) - to 6 phospho-gluconic acid; together with following 6-phosphogluconic acid dehydrogenase (6PGDH - EC 1.1.1.44) to ribulose-5-phosphate, these reactions produce moieties of reducing equivalents as NADPH (Kletzien et al., 1994; Castiglia et al., 2015).

It is widely recognized that different intermediates of the OPPP are used for biosynthetic pathways (e.g. ribose-5P, erythrose 4P for nucleotides biosynthesis), while a considerable part of the reducing power produced in the OPPP is utilized for nitrogen assimilation in plants (Bowsher et al., 1992; Esposito et al., 2003, 2005) and algae (Huppe and Turpin, 1996; Esposito et al., 2006; Ferrara et al., 2013).

A primary role in the regulation of G6PDH (and therefore of the whole OPPP) is played by NADPH/$NADP^+$ ratio. In photosynthetic organisms, this ratio is low during active biosynthetic processes (Huppe and Turpin, 1996) and it is modulated by stress conditions (Nemoto and Sasakuma, 2000); when the ratio is high the G6PDH activity decreases (Esposito et al., 2005).

It has been previously demonstrated the presence in higher

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plants of at least three different G6PDH isoforms, playing different roles in plant metabolism (Kruger and von Schaewen, 2003). Two compartmented enzymes are found in the plastids: P1-G6PDH seems exclusively found in green tissues (Esposito et al., 2005) and it is similar to algal isoform (Esposito et al., 2006), while P2-G6PDH is predominant in roots and heterotrophic tissues (Esposito et al., 2001). It is however known that the major part of the activity can be ascribed to the cytosolic isoform (Cy-G6PDH).

The cytosolic isoforms support the major part of the G6PDH total activity in plant cells, contributing for 60–80% of total rate measured (Esposito et al., 2005). The expression of these isoforms is induced at transcriptional level by abscissic acid (ABA – Hou et al., 2006) and/or by a sugar-sensing mechanism (Lejay et al., 2008). Cy-G6PDH is tightly regulated at post-transcriptional level by various mechanisms such nitrogen levels (Esposito et al., 2001, 2003). Moreover, cy-G6PDH is generally insensitive to light effects (Fickenscher and Scheibe, 1986), which exert the main control on the activity of P1-G6PDH (Wenderoth et al., 1997).

In plants, in the last years several studies described the key functions of G6PDH in stress-response mechanisms. G6PDH plays an important role in maintaining the redox state of plant cell under nutrient deficiency (Esposito et al., 2003); upon salt stress the oxidative burst is counteracted, at least in part, by G6PDH, both by expression and activities of cytosolic and plastidial isoforms (Nemoto and Sasakuma, 2000; Cardi et al., 2015; Valderrama et al., 2006) utilizing possibly different regulation patterns.

As a major example, under salt stress condition cy-G6PDH in *Arabidopsis thaliana* undergoes to a specific regulatory mechanism induced by the phosphorylation of Thr_{467} by Glycogen Synthase Kinase 3 (ASKα) (Dal Santo et al., 2012); and this mechanism is possibly linked to a sugar-sensing signal (Lejay et al., 2008).

Although a major involvement of G6PDH activity during the plant general response to abiotic stress has been widely proven, little is known about possible, specific relationships between this reaction and the response and tolerance to drought.

Drought stress represents a constant menace for the world agricultural system, because it poses one of the most important constraints to plant growth, and consequently to crop productivity, in many regions all over the world (Fita et al., 2015).

In response to drought conditions, plants activate different mechanisms to reduce injuries and limit effects on growth and development, resulting in the induction of the expression of many genes involved in different biological processes, such osmolyte synthesis and accumulation (Xing and Rajashekar, 2001; Burg and Ferraris, 2008), abscisic acid (ABA) synthesis and signaling (Mehrotra et al., 2014), protection from Reactive Oxygen Species (ROS) (Gill and Tuteja, 2010), aquaporins activation (Maurel and Chrispeels, 2001), transcription factors regulation (Janiak et al., 2015), maintenance of leaf greenness (Rolando et al., 2015) and many others.

G6PDH could play a primary role during stress response being responsive to ABA signaling pathway and favoring ROS scavenging functions. In fact, G6PDH promoter presents different ABA Responsive Elements (ABRE elements); thus, its expression is in part modulated by this phytohormone (Cardi et al., 2011). Moreover, during drought plant cells increase their request for reducing power in order to sustain the antioxidant defense system and counteract ROS accumulation and consequent damages (Gill and Tuteja, 2010). Therefore, the enhanced G6PDH activity would be able to provide NADPH for the antioxidant system(s) in order to remove ROS excess (Dal Santo et al., 2012).

Curiously, G6PDH has been characterized in many model organisms such *Arabidopsis* (Wakao and Benning, 2005), barley (Cardi et al., 2013; Castiglia et al., 2015), tobacco (Scharte et al., 2009), wheat (Nemoto and Sasakuma, 2000), potato (Wendt et al., 2000) and others, but few information are known about tomato (*Solanum lycopersicum*), which represent the tenth horticultural crops cultivated worldwide, and the fourth vegetable in Italy (FAOSTAT, 2013). Most of the tomato varieties are sensitive to drought that halt the plant development, reduce fruit size and affect fruit quality properties (Nuruddin et al., 2003; Rai et al., 2013). Therefore, tomato is cultivated in Mediterranean environments using a consolidated irrigation schedule lasting for the whole growth season, to guarantee quality standard as well as sufficient yields. Tomato breeding objectives is actually focused on the development of drought-tolerant varieties, which could be able to grow under limited water supply. This is particularly urgent, considering the pressing need to cope with water scarcity, and the randomness of rains, as predicted by global climatic changes (Eckardt et al., 2009; Ripoll et al., 2014).

The aim of this paper is to elucidate the role(s) of G6PDH in response to drought stress in tomato plants. For the purpose, tomato plants were grown in different environments, from controlled laboratory hydroponics, to greenhouse pots, and finally in open field under common cultivation practices. Gene expression and enzymatic activity of G6PDH were examined to determine the involvement of this enzyme in drought stress response.

We hypothesized that up-regulation of G6PDH gene(s), and the activation of cytosolic G6PDH rate are required to respond to the oxidative stress condition induced by water deprivation.

This possible role(s) of G6PDH in the mechanisms of drought response in tomato is discussed.

2. Materials and methods

2.1. Plant materials, growth conditions and stress treatments

Plants of tomato, *Solanum lycopersicum*, L. 1753, cultivar Red Setter, were used in this study. Seeds were germinated in soil in a greenhouse.

For experiments in hydroponics, seedlings at two-leaves stage (25 days after sowing) were transferred in a hydroponic system, and grown in a 5 L solution containing $Mg(NO_3)_26H_2O$ (384 mg/L), $Ca(NO_3)_24H_2O$ (812.9 mg/L), KNO_3 (101.5 mg/L), K_2SO_4 (319.3 mg/L), KH_2PO_4 (204.8 mg/L), Hydromix (14.0 mg/L) for 3 weeks. Then plants were divided in three groups: “control” plants were kept in the same nutritive solution; “drought” plants grown in 15% PEG 8000 MW, (Sigma-Aldrich), added to the hydroponic solution; “salt “ stressed plants grown in the hydroponic solution supplemented with 150 mM NaCl. Leaves of tomato were collected from each group after 3 h, 6 h, 24 h, 48 h from stress imposition.

Plants in greenhouse were grown from seedlings at two-leaves stage transferred in 30 cm diameter soil-filled plastic pots, and irrigated regularly for 30 days. Then plants were divided in two groups: i) control group was kept in full irrigated regime; ii) drought group was deprived of water for 16 days; then leaves from control and drought groups were collected for further analyses.

Open field plants were grown starting from seedlings at two-leaves stage planted in a field at Acerra, NA (40°57′6″12 N; 14°22′37″56 E) during May–July 2015, and grown under full irrigation regime for 60 days. Then, plants were divided in two groups: i) control group with irrigated plants; ii) drought group totally deprived of water. Leaves from control and drought groups were collected after 30 days (48% less water than control); and 45 days (58% less water than control) from the start of water withholding.

2.2. Stomatal conductance measurements

Stomatal conductance was measured using the AP4 Porometer (Eijkelkamp – Giesbeek, The Netherlands), according to