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Research article

In-field study on traditional Italian tomato landraces: The constitutive activation of the ROS scavenging machinery reduces effects of drought stress

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ABSTRACT

The involvement and the efficiency of the antioxidants scavenging system upon drought were examined by comparing traditional tomato landraces with respect to an industrial commercial genotype (Red Setter); for the first time, comprehensive analyses of physiological, biochemical and molecular parameters were investigated directly under real field conditions, in a typical agricultural environment of Southern Italy.

The characterization of the responses upon drought evidenced peculiar changes in stomatal conductance, ascorbate peroxidase and catalase activities and expression in drought tolerant tomato landraces, with respect to the industrial genotype.

An *in silico* analysis (promoter and co-expression study) coupled to a phylogenetic investigation of selected enzymes was performed, reinforcing the hypothesis of a basal activation of ROS scavenging machinery in the Mediterranean landraces.

Thus our data suggest a constitutive increase in the expression and activities of specific enzymes involved in ROS detoxification that can play a pivotal role in the drought response shown by tomato landraces.

Therefore, traditional landraces could represent an important source of useful genetic variability for the improvement of commercial varieties; their ROS detoxifying capabilities denote peculiar aspects worth being explored to better describe their specific stress tolerance.

1. Introduction

Water scarcity represents a major limiting factor for plant cultivation and crop productivity (Reynolds and Tuberosa, 2008); this stressful condition afflicts numerous countries worldwide impacting in the economy of the agriculture sector with huge losses in crop yields (Boyer, 2010). It is well known that at plant level drought cause inhibition of leaf growth, stomata closure with reduction in CO_2 uptake, a decrease of the photosynthetic activity, and a consequent massive decrease in biomass production (Avramova et al., 2015).

Drought stress effects in plants are counteracted by the activation of several different biological processes, such as cellular rehydration by osmoprotectants synthesis (Sharma et al., 2011); physiological and biochemical adjustments (Krannich et al., 2015); trascriptomic re-organization by expression of genes encoding proteins involved in signal transduction or regulation of transcription (Iovieno et al., 2016) and many others.

One of the main mechanisms conferring acclimation and tolerance of plants to drought stress is the regulation of the levels of reactive oxygen species (ROS), which are induced by different abiotic stresses, such drought, salinity or heavy metals accumulation (Gill and Tuteja, 2010).

ROS accumulation is mainly induced by gas exchange reduction, resulting in O_2 accumulation, CO_2 limitation and the concomitant over-reduction of the photosynthetic electron transport chain (ETC) during photorespiration (Miller et al., 2010). Furthermore, ROS cause protein oxidation, thus inhibiting many enzymatic activities, and inducing active proteolysis; a widespread damage to DNA is observed upon oxidative stress (Gill and Tuteja, 2010).

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Therefore, the tolerance to drought cannot escape an accurate detoxification of the ROS excess by different ROS scavenging systems, composed by both scavenging enzymes, and non-enzymatic antioxidants compounds (Mittler et al., 2004).

A crucial enzyme involved in ROS detoxification is catalase (CAT - EC 1.11.1.6), present in higher plants as three different isoforms: CAT1 and CAT2 localized in both the cytosol and peroxisomes; CAT3 confined into mitochondria (Willekens et al., 1994). CAT is induced in response to drought and salt stresses, and specifically in leaves allowed the removal of H_2O_2 excess from photorespiration during stress (Sofo et al., 2015). This action appears required to avoid the over-reduction of the photosynthetic ETC and photoinhibition (De Pinto et al., 2013).

An essential process in ROS detoxification is represented by the ascorbate-glutathione cycle (Sofo et al., 2015); this cycle is sustained by several enzymatic reactions, using glutathione as the original electron donor (Carfagna et al., 2011), and it ensures adequate photosynthetic rates even under stress (De Pinto et al., 2013).

In higher plants, ascorbate peroxidase isoforms (APX, EC 1.11.1.11) play a pivotal role in the ascorbate-glutathione cycle, and are localized in distinct sub-cellular districts: cytosol, glyoxysome, stroma and thylakoids (Noctor and Foyer, 1998). Increase of APX expression (Iovieno et al., 2016; Landi et al., 2016), occurrence, and activities (Kausar et al., 2012; Sofo et al., 2015) were reported upon drought, flooding and salinity, thus highlighting the crucial role of APX as ROS scavenger.

More recently an emerging role in abiotic stress response and ROS detoxification has been suggested for Glucose 6 phosphate dehydrogenase (G6PDH – EC 1.1.1.49 – Nemoto and Sasakuma, 2000; Cardi et al., 2011, 2015; Landi et al., 2016), the first and rate-limiting enzyme of the Oxidative Pentose Phosphate Pathway (OPPP); G6PDH is not a specific antioxidant enzyme, but its activity (together with the activity of the following - and strictly related - 6-phosphogluconate dehydrogenase EC 1.1.1.44) is able to produce moieties of reductants, as NADPH, required for the ROS scavenging systems (Esposito, 2016). The presence in higher plants of different G6PDH isoforms has been demonstrated (Kruger and von Schaewen, 2003; Meyer et al., 2011; Esposito, 2016). The cytosolic isoform contributes for the major (60–80%) part of the G6PDH total activity in plant cells (Esposito et al., 2001; Castiglia et al., 2015).

Water deprived plants show metabolic and growth perturbations at various levels, depending on tolerance capability and stress intensity and duration (Patanè et al., 2016). Among crops, tomato (*Solanum lycopersicum*) represents an important fleshy fruit model, and its completely sequenced genome represents a precious resource for scientists and breeders (The tomato genome consortium, 2012). Tomato - the sixth most important value food crop worldwide (FAOSTAT, 2013) - is drought-sensitive, presenting serious yield reduction upon water scarcity (Kissoudis et al., 2015). In the past decades, tomato breeding has been very successful, greatly ameliorating fruit and yield characteristics (Aflitos et al., 2014); on the other hand, no major improvements have been reached in the development of new genotypes adapted to stress, possibly due to the high complexity of the traits involved.

The exacerbation of drought expected in the near future in the Mediterranean area (Galmes et al., 2013) rapidly requires new drought tolerant and high yielding tomato varieties, expected to satisfy the growing demand for this crop (Iovieno et al., 2016).

To this aim, the traditional tomato landraces of the Mediterranean area present genetic traits useful for molecular breeding improvement. In the last centuries the semi-arid meteorological conditions of this region, together with to the widespread cultivation on the behalf of small communities and single farmers, generated thousands of different local landraces, showing different interesting features at the plant and fruit level, including drought tolerance and fruit long shelf-life (Conesa et al., 2014; Patanè et al., 2016; Van Oosten et al., 2016).

To our knowledge, neither genomic nor enzymatic analyses are yet available for ROS enzymatic antioxidant system in traditional tomato Mediterranean landraces. The goal of this research is the evaluation - during a field experimentation - of the efficiency of the enzymatic scavenging to counteract the drought-induced oxidative stress.

To this purpose, enzymes involved in ROS detoxification were analyzed and compared to a common commercial tomato cultivar used for agro-industrial processing; moreover a bioinformatic characterization of these enzymatic families in tomato is proposed in order to identify the phylogenetic origin of these peculiar properties.

2. Materials and methods

2.1. Plant material and stress treatments

Plants of *Solanum lycopersicum*, L. 1753 of landraces *Crovarese* (CRO), *Lucariello* (LUC), *Giallo Beneventano* (GB), *Landrace Siciliana* (LS) and cultivar Red Setter (RED) (control) were used in this study (Table 1). Tomato seeds of CRO and LUC were provided by two Companies, “Arca 2010”, and “Semiorto Sementi” respectively. Seeds of GB were provided by the Department of Agriculture (IPA Sector) of Regione Campania; LS and RED seeds were from the CNR-IBBR UOS Portici germoplasm collection.

Plants were grown from seedlings to two-leaves stage plantlets in a nursery, and then planted in an open field at Acerra, NA (40°57′6″12 N; 14°22′37″56 E) during May–August 2015. The deep soil is characterized by high fertility. The fields used in this study developed on volcanic material and showed a loam texture formed by 40.9% sand, 32.8% silt and 26.3% clay (Guida et al., 2017).

Plants were grown under full irrigation regime for 30 days and then, and then divided into two groups (each genotype arranged in a row) organized in separate and randomized, spaced blocks: i) control group with irrigated plants (15 plants for each genotypes); ii) drought group totally deprived of irrigation (15 plants for each genotypes). Leaf samples from control and drought groups (5 replicate for treatment) were collected after 45 days from the start of water withholding (Supplemental Table S1). Leaves samples were randomly collected from control and stressed plants by selecting the apical mature leaves from the second/third intermeddle node (too young, and senescent leaves were not selected).

The experimental time length was 45 days on the basis of previous studies (Landi et al., 2016), and experimental data available (Landi and Grillo, personal communication), in order to ensure that all the landraces and genotypes reached a severe drought stress status under field conditions, mimicking the standard growth condition of the landraces. Watering of plants followed an experimental pattern described in Supplementary Table 1. During the experimental time length no rainfall occurred, thus no further water exceeding the experimental design was received by the plants.

Subsequently, control plants were irrigated while drought stressed plants were irrigated with 50% of control water for the rest of the cultivation cycle.

2.2. Stomatal conductance

Stomatal conductance was measured using the AP4 Porometer (Eijkelkamp Soil and Water, Giesbeek, The Netherlands), according to Manufacturer's instructions. In brief, stomatal conductance (gs, mmol H_2O m^{-2} s^{-1}) was determined during the daylight at 15, 30