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Accumulation of free polyamines enhances the antioxidant response in fruits of grafted tomato plants under water stress

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ABSTRACT

Polyamines, small aliphatic polycations, have been suggested to play key roles in a number of biological processes. In this paper, attempts were made to investigate the possibility of improving antioxidant response of tomato fruits in relation with endogenous free polyamines content. We studied the reactive oxygen species and polyamines content, and antioxidant and polyamine-biosynthesis enzyme activities in fruits of ungrafted and grafted tomato plants under moderate water stress. We used a drought-tolerant cultivar (Zarina) and drought-sensitive cultivar (Josefina) to obtain reciprocal graft, selfgraft and ungraft plants. Fruits contained higher endogenous polyamine content during the course of the experiment relative to the control, coupled with higher arginine decarboxylase and spermine synthase activities in Zarina ungrafted and *ZarxJos*. In these cultivars, tomato fruits showed a lower reactive oxygen species generation and higher catalase and superoxide dismutase activities, suggesting that a higher content in polyamines (especially spermine) exerted a positive effect on antioxidant systems. All of these data suggest that spermine leads to more effective reactive oxygen species scavenging (less tissue damage) in tomato fruits, which may function collectively to enhance dehydration tolerance.

1. Introduction

Tomato crop worldwide is always threatened by adverse environmental stresses, among which drought is one of the most devastating factors. Drought retards plant growth, reduces fruit size and yield and promotes leaf abscission (García-Sánchez et al., 2007). Accordingly, it is important to develop appropriate strategies that can be taken to tackle the drought stress. In this sense, grafting is a horticultural technique, practiced for many years and in many parts of the world, used to overcome many abiotic stresses (Estañ et al., 2005; Venema et al., 2008). For example, grafted plants have been used recently to induce resistance to low and high temperatures (Rivero et al., 2003; Zhou et al., 2009), enhance nutrient uptake (Ahmed et al., 2007), improvement of fruit quality (Giorgi et al., 2005) and increase synthesis of endogenous hormones (Dong et al., 2008). So, the application of grafting in greenhouse tomato production has increased in recent years (Dorais et al., 2008), especially in the Mediterranean area where weather conditions may be adverse (Rosales et al., 2006). Grafting could be used to contribute to food security increasing the efficiency of use water (Albacete et al., 2015). Nevertheless, the number of studies related to grafting as a means of improving the water-stress resistance of tomato is limited and further studies are required before grafting can be recommended as a useful tool for growers to cope with drought.

Drought results in water deficit and loss of cell turgor. In addition, it evokes overproduction of highly reactive oxygen species (ROS) like superoxide (O_2^-) and hydrogen peroxide (H_2O_2), leading to oxidative stress. It has been well documented that plants have developed an array of mechanisms to cope with these abnormal physiological disorders. Accumulating evidence has been acquired to show that under stressful conditions plants may undergo physiological, biochemical, cellular and molecular alterations (Yamaguchi-Shinozaki and Shinozaki, 2005). One approach for the plants to respond and adapt to adverse milieus is the accumulation of compatible solutes, also known as osmoprotectants, for osmotic adjustment and maintenance of cell turgor. Moreover, the plants posses a complex antioxidant system to detoxify ROS, including low-molecular mass antioxidants as well as antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Arbona and Gómez-Cadenas, 2008). These two mechanisms might work independently or in synergy to mitigate stress-induced cell death and consequently enhance stress tolerance. It is thus conceivable that a given compound that can function both as osmoprotectant and ROS scavenger will serve as a robust effector to

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counteract the drought stress. In this regard, polyamines (PAs) can be regarded as a satisfactory candidate to meet the two requirements.

PAs are low molecular and aliphatic nitrogen organic cation and play an important role in the control of cell metabolism. Putrescine (Put), spermidine (Spd) and spermine (Spm) are the main PAs (Groppa and Benavides, 2008). Many researchers have shown the relationships of PAs metabolism (including changes in forms, contents, biosynthetic and catabolic enzymes of PAs) with plant responses to various abiotic stress conditions (Radyukina et al., 2009; Zhang et al., 2009). Being positively charged at physiological pH, they can interact with various cellular macromolecules like nucleic acids, protein and membrane phospholipids and regulate relevant processes (Martin-Tanguy, 2001). So, PAs may stabilize the membranes, scavenge free-radical, modulate the activities of certain ion channels and control many aspects of DNA, RNA and protein turnover under drought stress (Groppa and Benavides, 2008; Alcázar et al., 2010). PAs can bind to antioxidant enzymes, such as SOD and CAT, and allow them to permeate to the sites of oxidant stress within cells (Tang et al., 2005). Moreover, intensive work has revealed that PAs play important roles in stress response, although the definitive modes of action remain a matter of speculation (Kusano et al., 2008).

Described above suggests that modulation of endogenous PAs content in can be regarded as a convenient and effective strategy to enhance stress tolerance. However little information is available about the role of endogenous PAs in combating water stress improving antioxidant response in fruits. To address this issue, this work attempts investigate whether exist relation between PAs content and antioxidant enzymes activities in fruits of grafting tomato plants under moderate water stress. In a previous work, we selected the most drought tolerant (cv. Zarina) and sensitive (cv. Josefina) among 5 commercial tomato cultivars, and analyses certain biochemical indicators in these plants (Sánchez-Rodríguez et al., 2010). Therefore, in the present work our aim was evaluate the usefulness of grafting as a tool for increasing the water stress resistance in tomato fruits. We study the response to moderate water stress with different combinations. Finally, this work is to offer new evidence supporting PAs protective roles against oxidative stress caused by water deficit in fruit.

2. Material and methods

2.1. Plant material and treatments

Two tomato (*Lycopersicon esculentum* Mill.) cultivars, Zarina and Josefina, were used as scion and rootstock. The seeds of these cultivars were germinated and grown for 30 days in a tray with wells (each well $3 \times 3 \times 10$ cm) in the nursery Semillero Saliplant S.L. (Carchuna, Granada). Grafting was performed when seedlings has developed 3–4 true leaves. In the vermiculite trays used for germination, the seedlings were cut over the cotyledons, using the shoot as scion and the remaining plant part as rootstock. Grafts were made immediately after cutting the plants and grafting clips were used to adhere the graft union. Self-grafted plants were included as controls. After grafting, seedlings were covered with a transparent plastic lid to maintain a high humidity level and to facilitate graft formation and were left in the shade for 24 h. The plastic was opened slightly every day to allow reduction in relative humidity and it was removed 6 days after grafting. Afterwards, ungrafted and grafted plants were transferred to a cultivation chamber at the Plant Physiology Department of the University of Granada under controlled conditions with relative humidity of $50 \pm 10\%$, at 25 °C/15 °C (day/night), and a 16 h/8 h photoperiod with a PPFD (photosynthetic photon-flux density) of 350 $\mu mol\, m^{-2}\, s^{-1}$ (measured with an SB quantum 190 sensor, LI-COR Inc., Lincoln, NE, USA). Under these conditions, the plants grew in individual pots (25 cm upper diameter, 17 cm lower diameter, and 25 cm high) of 8 L in volume and filled with a 1:1 perlite:vermiculite mixture. Throughout the experiment, the plants were grown in a complete nutrient solution (Sánchez-Rodríguez et al., 2010). The water-stress treatments began 45 days after germination when the plants had started flowering period and was maintained during 65 days (terminal drought). The control treatment received 100% field capacity (FC) irrigation, whereas moderate water stress corresponded to 50% field capacity. Flowers were mechanically pollinated every 2 days. Uniformly ripe healthy fruits, at the red-ripe stage, were harvested. The experimental design was a randomized complete block with 12 treatments (Zarina ungrafted, Josefina ungrafted, Zarina self-grafted, Josefina self-grafted, *JosxZar* and *ZarxJos* well-watered 100% FC and water stress 50%) (Fig. 1) arranged in individual pots with 6 plants per treatment (one plant per pot) and 3 replications each.

2.2. Malondialdehide and H_2O_2 concentration

For the MDA assay, fruits were homogenized with 5 mL of 50 mM solution containing 0.07% $NaH_2PO{\cdot}2H_2O$ and 1.6% $Na_2HPO_4{\cdot}12H_2O$ and centrifuged at $20{,}000 \times g$ for 25 min in a refrigerated centrifuge. For measurement of MDA concentration, method of Heath and Packer (1968) was used. Results were expressed as nmol g^{-1} DW.

The H_2O_2 concentration in fruits extract was colorimetrically measured as described by Mukherje and Choudhuri (1983). Leaf samples were extracted with cold acetone to determine the H_2O_2 levels. The intensity of yellow color of the supernatant was measured at 415 nm. The result of H_2O_2 concentration was expressed as $\mu mol\, g^{-1}$ DW.

2.3. Antioxidant enzymatic activities

SOD (EC 1.15.1.1) activity was assayed by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT), according to the methods of Giannopolitis and Ries (1977) with some modifications (Yu et al., 1998). Fruits material were homogenized in liquid N_2 with buffer Heppes-HCl 50 mM pH 7.6 and centrifuged at 4 °C for 10 min. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm.

CAT (EC 1.11.1.6) activity was determined by following the consumption of H_2O_2 at 240 nm for 5 min (Nakano and Asada, 1981). Fruits material were homogenized in liquid N_2 with buffer Heppes-HCl 25 mM pH 7.8 and centrifuged at 4 °C for 15 min. The reaction mixture contained 25 mM Tris-acetate buffer (pH 7.0), 0.8 mM Na-EDTA and 20 mM H_2O_2, and enzyme assay was performed at 25 °C.

2.4. Polyamine forms content

Fruit material (0.7 g) was homogenized in 1 mL of 6% (v/v) cold perchloric acid (PCA), kept on ice for 1 h, and then centrifuged at $21{,}000 \times g$ for 30 min. The pellet was extracted twice with 1 mL of 5% PCA and recentrifuged. The three supernatants were pooled and used to determine the levels of free and PS conjugated PAs, whereas the pellet was used to determine the levels of bound PAs. The pellet was resuspended in 5% PCA and hydrolyzed for 24 h at 110 °C after being mixed with 12 N HCl (1:1, v/v). The hydrolyzates were filtered, dried at 70 °C, and then resuspended in 1 mL of 5% PCA for analysis of bound PAs. For conjugated PAs, 1 mL of the supernatant were mixed with 1 mL of 12 N HCl and hydrolyzed under the conditions described above. The supernatant, hydrolyzed supernatant, and the pellet were benzoylated in accordance with the method of Aziz and Larher (1995). The benzoyl derivatives were separated