Scientia Horticulturae 159 (2013) 172–177

Contents lists available at SciVerse ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Effects of endogenous abscisic acid, jasmonic acid, polyamines, and polyamine oxidase activity in tomato seedlings under drought stress

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ARTICLE INFO

Article history:
Received 13 February 2013
Received in revised form 25 April 2013
Accepted 16 May 2013

Keywords:
Drought stress
Abscisic acid
Jasmonic acid
Polyamines
Polyamine oxidase activity
Tomato

ABSTRACT

Drought stress causes various physiologic and biochemical effects in plants. The phytohormones in plant systems are closely involved in responses against drought stress. However, information on the regulatory effect of abscisic acid (ABA), and free polyamine (PA) metabolism on the accumulation of JA is limited. Elucidating the endogenous mechanisms that confer stress resistance is essential to providing insights into the potential of plants to adapt to environmental change. This study aims to determine the relationship between the concentrations of abscisic acid (ABA) and jasmonic acid (JA) and the accumulation of free PAs (putrescine, spermine, and spermidine), as well as with polyamine oxidase (PAO) activity, in tomato (*Lycopersicon esculentum* M.) seedlings grown hydroponically under polyethylene glycol – induced drought stress. The results indicate that the concentrations of endogenous ABA, JA, and free polyamines, and the PAO activity in the roots and leaves of tomato seedlings were generally higher in the treatment groups than in the untreated controls. A significantly positive correlation was observed between the concentrations of endogenous polyamines and PAO activity ($R = 0.708^{**}$) in roots and leaves of tomato seedlings. The time course in the present experiment demonstrated that the ABA concentrations increase in the roots prior to that in the leaves. Therefore, under drought stress, the higher concentrations of endogenous spermine and spermidine in the roots and leaves stimulate the simultaneous accumulation of endogenous ABA and JA with increasing PAO activity.

1. Introduction

Plants encounter various stressful environmental conditions in their life cycle. About one-third of the global potentially viable land suffers from an inadequate water supply. Drought stress causes various physiologic and biochemical effects on plant populations (Farooq et al., 2009). Plants have systems for tolerating environmental stresses. Some phytohormones are closely associated with these systems. For instance, abscisic acid (ABA), as one of the major phytohormones linking plant responses to stress (Hirayama and Shinozaki, 2007), is ubiquitous in higher plants, and interacts with membrane phospholipids to stabilize plant cell membranes against stress conditions, and to enhance tolerance as a messenger in stress perception response pathways, such as in drought stress (Guschina et al., 2002). Vegetative development is frequently influenced by environmental stress. Many studies have shown that ABA acclimatization occurs in plants exposed to drought, which indicates that ABA plays an important role in desiccation tolerance (Hirayama and Shinozaki, 2007). On the other hand, polyamines (PAs) are ubiquitous nitrogen-containing polycationic compounds found in all eukaryotic cells. The most abundant PAs in plants are putrescine (Put), spermidine (Spd) and spermine (Spm). As plant hormones, PAs are susceptible to environmental changes as indicated by the massive increase in PAs in plants for regulating plant growth and development under stress conditions, and for preventing stress-induced damage (Yamaguchi et al., 2007). PAs are closely associated with plant resistance to water stress (Groppa and Benavides, 2008). Environmental stress induces considerable increases or decreases in cellular PA concentrations, depending on the type of stress, plant species, and duration of stress application (Kasinathan and Wingler, 2004), which may mediate intracellular hormonal effects or act as a secondary hormonal messenger. Different stresses may influence PA metabolism in different manners and specific functions when exposed to stress conditions (Sharma and Dietz, 2006). PA oxidase (PAO) is one of the key enzymes that regulate the PA metabolic pathway in different model plant systems (Groppa and Benavides, 2008). Aside from PAs and ABA, jasmonic acid (JA) is also a key

Abbreviations: ABA, Abscisic acid; JA, Jasmonic acid; PA, Polyamine; Pas, Putrescine/spermine and spermidine; PAO, Polyamine oxidase; PEG, Polyethylene glycol; Put, Putrescine; PVP, Poly vinyl pyrrolidone; Spd, Spermidine; Spm, Spermine.

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http://dx.doi.org/10.1016/j.scienta.2013.05.013

mediator in the signaling pathways in plant defense systems (Overmyer et al., 2003). Several studies provide ample evidence that JA concentrations increase rapidly and transiently in response to various types of stress, such as salt stress (Pedranzani et al., 2003), and desiccation stress (Shan and Liang, 2010), which both show that JA contributes to stress tolerance. PAs enhance the desiccation tolerance of plants by regulating endogenous ABA concentrations under water stress conditions. Under chill stress, the rice seedlings showed a remarkable ABA-increase and simultaneous enhanced PA synthesis (Gill and Tuteja, 2010). ABA treatment increases the PA concentrations in sugarcane as a response to desiccation, whereas JA treatment is ineffective under these processes (Nieves et al., 2001). ABA and JA generally increased in response to salinity, these phytohormones may have separate and interactive effects on how plants respond and adapt to stress in natural environments (Wang et al., 2001). There was also a remarkable JA-increase in two populations of *Pinus pinaster* Ait. under water stress, thus the JA-response was much more prominent under water stress than under cold stress(Pedranzani et al., 2007). Different patterns of JAs have been found in different varieties of the same species (e.g., tomato) related to the stress tolerance (Pedranzani et al., 2003). All these studies suggest that these phytohormones contribute to drought tolerance.

Until now, the relationship between ABA, JA, and free PA metabolism in plant populations is unclear aside from the respective roles of each under drought stress. Information on the regulatory effect of ABA and free PA on JA accumulation in plants is limited. The mechanism by which endogenous substances modify stress tolerance in plants has to be further elucidated. To our knowledge, this is the first investigation to link responses to polyethylene glycol (PEG)-induced drought stress and the phytohormones ABA, JA, and PA in the same plant population. The findings would have important implications in revealing the mechanisms that confer stress resistance and provide insights into the potential of plants to adapt to environmental change.

The objectives of this study are as follows: (1) to determine whether ABA and endogenous JA interact with PAs and to elucidate the underlying mechanisms in tomato seedlings (*Lycopersicon esculentum* M.) under PEG-induced drought stress using two cultivars with different degrees of drought resistance; (2) to find the relationship among endogenous ABA, JA, and PAs in plants under drought stress; (3) to identify differences in desiccation tolerance between the two tomato cultivars; and (4) to verify the roles of these hormones.

2. Materials and methods

2.1. Plant materials and treatments

The study was conducted from October to December of 2009 and repeated in May to July of 2010 in an environmentally controlled greenhouse at the Horticultural College in Northwest A & F University, Yangling, Shanxi Province, China (34° 20′ N, 108° 7′ E). The two tomato cultivars HuangGuan (drought-susceptible), which originated from the southwestern climatic region (Guangzhou, 23° 8′ N, 113° 17′ E), and MaoFen802 (drought-resistant), which originated from northwestern climatic region (Yangling), were selected for this study. Both tomato cultivars were selected among eight cultivars that originated from different climatic regions of China. The seeds of the two tomato cultivars were surface-sterilized with 50% NaClO (8% active Cl_2) for 10 min, thoroughly rinsed in distilled water, and germinated at 25 °C in moistened vermiculite for 3–4 days. The germinated seeds were then sown individually in 10 cm-diameter plastic pots filled with homogenized soil that contained 4 g of a slow-release fertilizer (Osmocote 17-6-12 with micronutrients), and were placed in a naturally lit greenhouse under semicontrolled environmental condition. The temperatures in the greenhouse ranged from 20 °C to 30 °C in the day and from 15 °C to 18 °C at night, with a 12 h photoperiod under a photosynthetic photon flux density of 130 $\mu mol\,m^{-2}\,s^{-1}$, and relative aerial humidity fluctuating between 60% and 75%. The plants were watered with half strength Hoagland's solution.

After the 4th leaf of the seedlings was fully expanded, seedlings were selected for uniformity and transplanted to troughs containing 7 L of full-strength Hoagland's solution (pH 6.5 ± 0.1; EC from 2.2 $mS\,cm^{-1}$ to 2.5 $mS\,cm^{-1}$). The nutrient solution was aerated using an air pump to supply oxygen intermittently (40 $min\,h^{-1}$) to maintain the dissolved oxygen at 7.8 $mg\,L^{-1}$ ± 0.2 $mg\,L^{-1}$. The solutions were renewed every 5 d and pre-culturing was conducted at the sixth leaf stage.

Experimental treatments were started 7 days after preculturing. The treatments were as follows: (a) control, full-strength Hoagland solution; and (b) polyethylene glycol 6000 (PEG6000) treatment, 10% PEG, which is equivalent to an osmotic potential of −0.40 MPa. The troughs were arranged in a completely randomized block design with three replicates, providing 12 troughs with 54 plants per treatment. At the beginning (0 h), and after 6, 12, 24, 36, and 48 h of treatment, roots and leaves were sampled from each treatment, rinsed in distilled water, flash frozen in liquid nitrogen, and stored at −72 °C for further use. The content of ABA and JA, as well as the three free PAs (Put, Spm, and Spd) were simultaneously measured and recorded with three replicates.

2.2. Determination of the ABA content

ABA was extracted from plant tissues using the method by Kondo et al. (2001) with minor modifications, depending on the plant material. During extraction for each cultivar and treatment, ABA content was determined for each sample. Then, 50 mg of sample plant material were kept on a shaker at 4 °C in the dark to prevent ABA isomerization. A series of organic solvent mixtures were used to remove interfering substances. After the filtrate was dried, it was re-dissolved in 10 mL of 0.1 M phosphate buffer at pH 8, and the pH adjusted to 2.5. ABA was extracted with ethyl acetate. The extracts were vacuum-dried, dissolved in 1 mL of absolute methanol, and passed through 0.45 μm filters. ABA was quantified using high-performance liquid chromatography (HPLC; Waters 600E system, Waters Corporation, USA) equipped with two pumps and a diode array UV2487 detector. The separation was carried out on a 4.5 mm × 250 mm C–18 column (Spherisorb ODS 2–3 mm) using an isocratic system of solvent (water/methanol/acetic acid, 40:60:1). The mobile phase solutions were filtered through at 0.45 μm membrane filter and degassed before use. The high-performance liquid chromatography was operated at a flow rate of 0.8 $mL\,min^{-1}$ and was adjusted to pH 2.8; detection was performed at 254 nm. Identification and quantification were performed using pure ABA (cis–trans isomer, Sigma) as the external standard. The ABA concentration was calculated as $ng\,g^{-1}$ fresh weight.

2.3. Determination of the JA content

The JA content was determined via enzyme-linked immunosorbent assay (ELISA), using polyclonal anti-JA antibodies prepared using the method by Albrechet et al. (Albrecht et al., 1993) with slight modifications. Then, 0.5 g of samples were ground in liquid nitrogen with 3 mL of 80% methanol (1% PVP), allowed to stand overnight at 4 °C, ultrasonicated, and centrifuged at 10,000 × g for 20 min at 4 °C. The supernates were collected, and the precipitates were extracted once more with 80% methanol. The supernatant liquid was pooled, dried with N^2, and redissolved in 1.5 mL of the extraction solution before the ELISA. According to ELISA process,