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## Interaction between salicylic acid and polyamines and their possible roles in tomato hardening processes

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**ABSTRACT** Long-term pre-treatment of tomato plants with low concentration of salicylic acid can induce abiotic stress tolerance by activating enzymatic and non-enzymatic antioxidant defense system. Changes of antioxidant defense system and catabolism of polyamines in tomato (*Solanum lycopersicum*) plants were investigated. Our results suggest that by affecting the polyamine catabolism salicylic acid can contribute to plant abiotic stress tolerance.  
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**KEY WORDS**

*Solanum lycopersicum*  
proline  
polyamines  
salicylic acid

Salicylic acid (SA) is considered as a hormone-like substance that has important role in the regulation of growth and development. There were several efforts to apply SA as pre-treatment or chemical priming in order to improve abiotic stress tolerance of plants. SA can increase H<sub>2</sub>O<sub>2</sub> contents in tissues which can induce the expression of antioxidant enzymes and increase plant tolerance to abiotic stressors. Our previous studies proved that long-term pre-treatment with salicylic acid (SA) at low concentrations (10<sup>-7</sup> and 10<sup>-4</sup> M SA) can induce the synthesis of polyamines in tomato plants (Szepesi et al. 2009). Polyamines (PAs) are small, organic polycations found in all eukaryotic cells. Putrescine (Put), spermidine (Spd) and spermine (Spm) are the major free PAs in plants and they are involved in various processes such as cell proliferation, growth, morphogenesis, differentiation and programmed cell death (Kumar et al. 1997). Polyamine oxidation is the main catabolic process of polyamines. The main catabolic enzymes are the diamine oxidase(s) (DAO, EC 1.4.3.6) and polyamine oxidases (PAO, EC 1.5.3.3). They can regulate the endogenous content of polyamines and generate H<sub>2</sub>O<sub>2</sub>, which can act as a signaling molecule. H<sub>2</sub>O<sub>2</sub> produced by polyamine catabolism participates in important metabolic pathways involved in stress responses (Papadakis and Roubelakis-Angelakis 2005). The levels of PAs and proline (Pro) could be interrelated because they share certain common intermediates in their biosynthetic and catabolic pathways (Aziz et al. 1998). Interactions between stress-induced Pro and polyamine accumulations may reflect the fact that they share ornithine as a common precursor (Mohapatra et al. 2009). In these experiments we investigated how SA can influence the polyamine catabolism and antioxidant defense system at the

end of the pre-treatment and we evaluated whether elevated polyamine levels were a result of stress-induced injury or a protective response to salicylic acid treatment.

### Materials and Methods

Tomato (*Solanum lycopersicum* cvar Rio Fuego) plants were grown hydroponically in a greenhouse under 180 μmol m<sup>-2</sup> s<sup>-1</sup> light intensity and at 12/12 day/night photoperiod. The temperature was maintained at 25°C and the relative humidity was 55-60 %. Plants were treated for 3 weeks with 10<sup>-7</sup> M and 10<sup>-4</sup> M SA added to the nutrient solution. Samples were prepared from the second, fully expanded leaves and roots in 3 replicates. Experiments were repeated 3-5 times. Data of time-course analysis presented are means±SE for 3 replicates. Free polyamines were determined as described by Flores and Galston (1982). Enzyme activities of diamine oxidase (DAO, EC 1.4.3.6) and polyamine oxidase (PAO, EC 1.5.3.3) were estimated by method of Federico et al. (1985) with some modifications (Moschou et al. 2008). Superoxide dismutase (SOD) activity was determined by measuring the ability of the enzyme to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) in the presence of riboflavin in light (Dhindsa et al. 1981). Catalase (CAT) activity was determined by the decomposition of H<sub>2</sub>O<sub>2</sub> and was measured spectrophotometrically by following the decrease in absorbance at 240 nm (Upadhyaya et al. 1985). APX, MDHAR, DHAR, GR activities were measured by spectrophotometer. Free proline contents were determined as described by Bates et al. (1973). Potassium ion contents were determined by AAS (Hitachi-Z8200).

### Results and Discussion

In order to maintain the integrity of membranes, effective

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enzymatic and non-enzymatic mechanisms for scavenging ROS, among them  $H_2O_2$ , were developed in plants. At the end of SA pre-treatments the activity of the  $H_2O_2$ -generating enzyme, SOD increased in the SA pre-treated leaves while the activity decreased in the roots in the presence of  $10^{-4}$  M SA.  $H_2O_2$  eliminating enzymes, APX and CAT activities increased in the  $10^{-7}$  M SA pre-treated leaves and roots while activities of these enzymes declined in the  $10^{-4}$  M SA pre-treated plants. Free polyamines may function as a substrate for the DAO and PAO and act directly or indirectly as free-radical scavengers. Accumulation of polyamines can stimulate DAO and PAO activities and thus can balance polyamine metabolism in plants. DAO and/or PAO catalyze the oxidative deamination of amino groups of Put or Spd and Spm, respectively, producing the corresponding aldehyde,  $H_2O_2$  and 1,3-diaminopropane in case of Spd and Spm. These enzymes have also been proposed to be involved in lignification and cell wall stiffening in plants. In leaves,  $H_2O_2$  content increased at both SA concentrations compared to the control but this change did not correlate with the activities of enzymes in PA catabolism. Three weeks after treatment with  $10^{-4}$  M SA, Put content increased in the leaves, which resulted in a decreased (Spd+Spm)/Put ratio.

In roots exposed to  $10^{-4}$  M SA Put contents decreased compared to the control, while Spd and Spm contents increased because of elevated DAO and unchanged PAO activities. Root  $H_2O_2$  contents declined in the case of both SA concentrations and free-radical scavenging properties of free polyamines can contribute to the control of  $H_2O_2$  level in the root tissues.

Proline accumulation was slightly enhanced in the leaf tissues after SA pre-treatment and decreased in the root tissues at both SA concentrations. Pro can be metabolized to glutamate, which can also serve as a substrate for PA synthesis, through a number of biochemical steps (Alcázar et al. 2010). It has been suggested that transgenic plants with elevated levels of Pro and PAs exhibit enhanced stress tolerance. Zhao et al. (2007) showed that polyamines improve root  $K^+/Na^+$  homeostasis in barley by regulating ion channel activities. Stimulation of DAO and PAO activities with concomitant accumulation of Pro was observed in salt stressed oat seedlings and tomato leaf discs (Aziz et al. 1998; Alcázar et al. 2010). In these studies inhibitors of DAO was shown to inhibit the accumulation of Pro.

Decarboxylation of arginine was the main pathway of Put accumulation under  $K^+$ -deficiency (Smith and Richards 1964). In our study the total polyamine levels and potassium ion contents changed in parallel in the leaves while in the roots the tendency was the opposite. By investigating

the enzyme activities and PA contents, it can be concluded that SA-induced polyamine synthesis and changes in PA catabolism can contribute to the stress acclimation processes in tomato plants.

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