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Antioxidant activity in tomato plants cv. Micro-Tom induced to temperature and flooding stress

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Workshop Information

I Workshop of Plant Biology (I Workshop de Biologia Vegetal) was held in the Bioscience Institute – UNESP, campus of Rio Claro, Brazil, during August 20 and 21, 2012. Workshop was a scientific event organized by Post-graduate students from that Institute aiming to integrate Post-graduate and Graduate students from different areas related to Plant Biology (Anatomy, Ecology, Evolution, Morphology, Physiology, and transitional areas) from different Universities. Workshop Organization offered a large number of speaking activities, scientific discussions, and extra short-courses to improve the knowledge and formation of students in Plant Biology.

Scientific Committee

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INTRODUCTION

Micro-Tom is a miniature (dwarf) tomato cultivar, first designed for home gardening purposes, being used nowadays as genetic model in studies, representing the Solanaceae family, besides being a model system for fleshy fruit development, in the defense of plants and stress tolerance (Aoki et al. 2010).

The stress caused by flooding, in which plants are subjected to a hypoxic state, can cause changes in physiological functions, among them an immediate reduction in gas exchange, reduction in oxygen availability in the soil, which leads to decrease in respiration of roots, inhibiting ATP synthesis and contributing to the reduction of the energy available for the growth and development of the vegetable (Pezeshki 1994).

With respect to thermal stress by increasing the temperature, Wahid et al. (2007) address that high temperatures, in a transitory or constant way, cause a series of morpho-anatomical, physiological and biochemical changes in the plant, that affect its growth and development. Heat and flooding stress responses are reasonably well described in the literature, however, there is less information on the combined effects or impacts on biochemical processes that occur in the plant. In ideal conditions, cellular homeostasis is achieved by a coordinated action of several metabolic pathways, which may differ in their biophysical and molecular properties. Thus, stress factors can affect these pathways and the coordinated behavior differently, changing the metabolic flow of these pathways, mainly generating reactive oxygen species (ROS) (Kochhar and Kochhar 2005).

Although ROS are toxic, they are usually produced and metabolized by the cellular system. However, under stress conditions, they can be produced in excess, and must be rapidly metabolized, which prevents the formation of destructive radicals, thus protecting against uncontrolled oxidation Gratão et al. (2008).

To neutralize and/or mitigate the adverse effects of these radicals, plants have developed a series of enzymatic and nonenzymatic detoxification systems through the production of antioxidant enzymes, seeking to protect cells from oxidative damage. The enzymes that stand out in the removal and/or elimination of ROS are superoxide dismutase (SOD),

catalase (CAT) and ascorbate peroxidase (APX) (Vasconcelos et al. 2009).

Therefore, the objective of this study was to analyze the effect of flooding, of high temperature and of the combination of both on the activity of the antioxidant system, in order to better understand the complex process of plant response to environment.

MATERIAL AND METHODS

Wild tomato (*Solanum lycopersicum* Mill. cv *Micro-Tom*) seeds were put to germinate in Gerbox ® in the dark at 24° C. Eight days after germination they were planted in pots with a capacity of 0.5 kg containing sieved and washed sand as substrate in a 1:1 proportion and were kept in a growth chamber under controlled conditions: temperature at 21 ± 2ºC, 12h photoperiod , photosynthetically active photons flux of 200 µmol m^{-2} s^{-1}. It was placed in Hoagland complete nutrient solution with pH adjusted to 6.0-6.5 three times a week and irrigation performed daily.

When the plants were in full vegetative stage (65 days after transplantation) they were subjected to different treatments: 1 - flooding of the roots by seven days; 2 - high temperature (37 °C) for two days; 3 - flooding for seven days followed by high temperature for two days; 4 - high temperature followed by flooding for seven days. The control treatment consisted of plants that remained in normal conditions of temperature and irrigation. Four repetitions were used per treatment, with the repetition consisted of a pot containing a plant.

To enzyme activity, two hundred milligrams of root and leaf tissue were macerated in liquid nitrogen for the enzyme activity assays following the method of Bradford (1976). SOD activity was assessed by the ability of the enzyme to inhibit the blue of nitrotetrazolium (NBT) photoreduction (Giannopolitis and Ries 1977) in a reaction medium composed of 100 mM potassium phosphate (pH 7.8), 14 mM methionine, 0.1 µM EDTA, 75 mM NBT and 2 mM riboflavin. The control was the reaction medium without the sample. The tubes were illuminated for seven minutes in a box with a 20 W fluorescent lamp. A tube kept in the dark was used as the blank. The readings were performed at 560 nm. One unit of SOD is the amount of enzyme capable to inhibit the photoreduction of NBT by 50% under the tested conditions.

The CAT activity was determined as described by Azevedo et al. (1998) with minor modifications. The activity was monitored by the decrease in absorbance at 240 nm for two minutes in a reaction medium incubated at 28 ºC, containing 100 mM potassium phosphate buffer (pH 7.0) and 12.5 mM H_2O_2. The activity of APX was determined as described by Nakano and Asada (1981), tracking the oxidation rate of ascorbate at 290 nm. The reaction medium that was incubated at 28 ºC was composed of 100 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, and 0.1 mM H_2O_2. The decrease in absorbance was monitored for two minutes following the initiation of the reaction. The activity of DHAR was performed according to Nakano and Asada (1981). The incubation buffer, which consisted of 50 mM potassium phosphate (pH 7.0), 2.5 mM reduced glutathione, 0.2 mM dehydroascorbate (DHA) and 0.1 mM EDTA, was placed in a water bath at 28 ºC. The readings were performed by observing the increase in absorbance at 265 nm, which indicated the formation of ascorbate (Nakano and Asada 1981). Finally, the activity of GR was based on the method of Cakmak et al. (1993), tracking the rate of oxidation of NADPH by the decrease in absorbance at 340 nm for two minutes. The reaction medium, incubated at 28ºC, consisted of 50 mM potassium phosphate buffer (pH 7.8), oxidized 1 mM glutathione and 0.075 mM NADPH.

RESULTS AND DISCUSSION

Among the various enzymes involved in elimination of ROS, SOD is a key enzyme, being the first line of defense to oxidative stress. SOD performs the dismutation of the superoxide radical ($O_2^{\bullet-}$) to hydrogen peroxide (H_2O_2). In this study, it was found that, both in the roots and leaves of tomato plants, the activity of this enzyme differed from control in all treatments (Figure 1). This shows that the plants effectively triggered, at a first moment, the antioxidant enzymatic system in order to prevent oxidative damage. Although SOD is part of the first plant tolerance adjust to oxidative stress, its product, H_2O_2, is also a free radical as harmful as its substrate. Thus, a greater degree of protection against oxidative damage must require rapid removal of H_2O_2 by the subsequent enzymes of the antioxidant system, such as CAT and APX. Thus, it was found that the CAT and APX activity in tomato roots and leaves was much higher than its control in all treatments.

The increase in enzyme activity of the antioxidant system found in plants flooded and subjected to high temperature (37° C) shows that in both stresses there is a production increasing of reactive species of O_2 with subsequent removal by the enzymatic system. Thus, the antioxidant enzymatic system of this species appears to be critical for conferring tolerance to flooding and high temperature.

According to Zhang and Kirkham (1996), the capacity to maintain, at high levels, SOD, CAT and APX activity, under environmental stress conditions, is essential for maintaining the balance between

formation and removal of H_2O_2 from the intracellular environment. According to Cakmak and Horst (1991), the reduction in CAT activity and the increase in the activities of peroxidases indicate that, in plants maintained under conditions of stress, the generated H_2O_2 is most consumed in oxidative processes, such as lipid peroxidation, than eliminated from the metabolism.

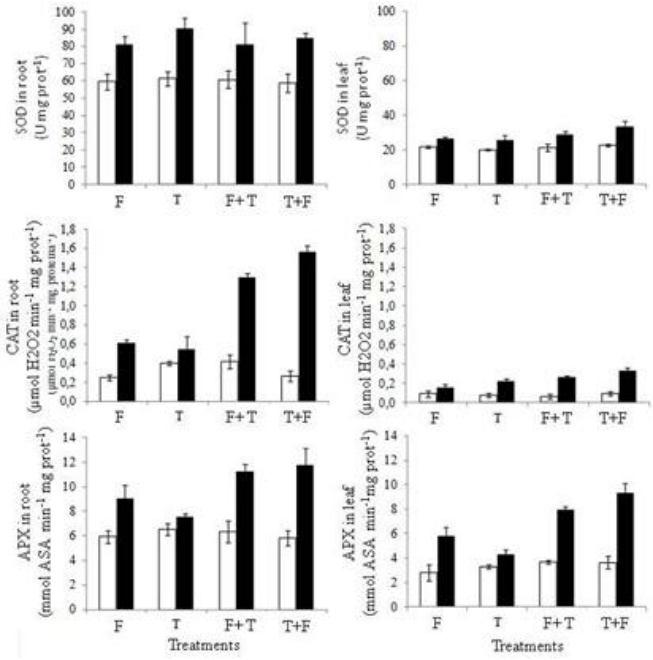

Figure 1. Specific activity of SOD, CAT and APX on roots and leaf in Micro-Tom Tomato plants subjected to different treatments: F - flooding of the roots by seven days; T - high temperature (37 °C) for two days; F + T - flooding for seven days followed by high temperature for two days; T + F - high temperature followed by flooding for seven days. Control (□), treatment (■). Bars represent the standard error of the mean, n=3.

CONCLUSIONS

The results show that the enzyme activity of the antioxidant system of the plants subjected to combined stresses of temperature and flooding was much larger than that of plants subjected to stress by flooding of the root or high temperature.

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Review process and quality of English writing is responsibility of Workshop Scientific Committee.