



Comparative effects of abscisic acid and two Sulfonamide compounds on tomato under drought conditions

Leila Zeinali Yadegari*, Reza Heidari, Jalil Khara

Department of Biology, Faculty of Science, Urmia University, Urmia, Iran

Abstract

The effects of exogenous abscisic acid (ABA) and its two agonists, Sulfacetamide (Sa) and Sulfasalazine (SS) on tolerance of tomato (*Lycopersicon esculentum* Mill. Cv. Super chief) under drought stress were studied. Eight-week plants were treated with ABA (25 and 50 mg/L), Sulfacetamide (Sa) (25, 50 and 100 mg/L) and Sulfasalazine (SS) (25, 50 and 100 mg/L). Solutions were sprayed daily and sampling was done at 48 h, 96 h, 144 h and 48 h after re-watering (recovery phase). Treated plants showed relatively greater drought tolerance. This indicates that, Sulfacetamide and Sulfasalazine improved resistance in tomato, like ABA, increasing activity of antioxidant enzymes, i.e., catalasthise (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX). On the other hand, abscisic acid, Sulfacetamide and Sulfasalazine reduced H₂O₂ and MDA contents in the plants under study. Daily application of these agonists during moisture stress period was effective in increasing tomato plants tolerance to drought as was ABA.

Keywords: abscisic acid; antioxidant; drought; Sulfacetamide; Sulfasalazine

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Introduction

Higher plants are continually being exposed to adverse environmental stresses, such as drought, salinity, cold and extreme temperatures (Choi et al., 2011). Among these abiotic stresses, drought is a major limiting factor for the crop plants growth and development (Choi et al., 2011). Aerobic organisms constantly face the problems due to reactive oxygen species (ROS) that are partially reduced forms of molecular oxygen and are formed by the inevitable leakage of electrons onto molecular oxygen during electron transport activities of

chloroplasts, mitochondria and plasma membranes (Asada, 1994). Production of ROS has been found to be stimulated in plants under a variety of environmental stresses (Sgherri et al., 1996). The ROS include superoxide radical (O₂⁻), hydroxyl free radical (·OH), hydrogen peroxide (H₂O₂) and singlet oxygen and cause peroxidation of lipids, denaturation of proteins, mutation of DNA and various types of cellular oxidative damage (Smirnoff, 1993). Peroxidation of membrane lipids and oxidation of -SH groups of proteins have been regarded as an index of oxidative stress (Smirnoff, 1993; Boominathan et al., 2002). Plant cells are protected against the detrimental effects of ROS by a complex antioxidant system comprising of the non-

*Corresponding author

E-mail address: zeinali_l@yahoo.com

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enzymatic as well as enzymatic antioxidants (Noctor and Foyer, 1998). Peroxidases which are located in cytosol, vacuole as well as in extracellular space scavenge H_2O_2 by oxidation of substrates. GPXs require a phenolic compound guaiacol as electron donor to decompose H_2O_2 (Asada et al., 1994). In higher plants, several distinct isozymes of APX, which convert H_2O_2 to H_2O using ascorbate as an electron donor, are localized in cytosol and various organelles (Madhusudhan et al., 2003). APX plays an important role in protecting cells against damaging effect of H_2O_2 . Since ascorbate is oxidized to monodehydroascorbate in APX catalyzed H_2O_2 decomposition, a system for regeneration of ascorbate is necessary. Catalases eliminate H_2O_2 by breaking it down to H_2O and O_2 and do not require any reducing equivalent (Blokhina et al., 2003; Patterson et al., 1984). To survive in stress conditions, small molecules such as abscisic acid (ABA), regulate plant growth and development (Melcher et al., 2010). ABA is an important phytohormone, which inhibits growth under severe environmental conditions and protects plants against stresses, such as drought, salinity, cold and pathogen exposure. Under these conditions, ABA levels increase by induction of ABA biosynthesis. Exogenous application of ABA, also, increases plant resistance to drought. ABA binds to its selective receptor, PYR/PYL/RCAR in membrane with micromolar affinities. This receptor was discovered with application of a synthetic ABA agonist termed Pyrabactin (Park et al., 2009) that is a member of sulfonamides. Some sulfonamides have no structural similarity with ABA, but mimic its effects on plant resistance to drought via induction of ABA signal transduction pathway. PYR/PYL/RCAR proteins are a new family of proteins that were reported as candidate ABA sensors (Ma et al., 2009) and were found to bind ABA and inhibit the activity of specific protein phosphatase enzyme type 2C (PP2C) (Melcher et al., 2010) thus, allowing the plant to respond stress. Because of high cost in the commercial production and low stability of ABA exposed to light, cheap synthetic agonists of ABA which are active in triggering the drought tolerance and have minimal adverse effects on the environment are needed. The present work is an attempt to

find and introduce the capability of two sulfonamide compounds among sulfonamide components, namely Sulfacetamide and Sulfasalazine, in activation of some antioxidant enzymes and altering MDA level in tomato plants.

Materials and Methods

Plant material and treatments

Sterilized seeds of tomato (*Lycopersicon esculentum* Mill. Cv. Super chief) were soaked in distilled water for 12 h. The seeds were sown in pots (20×30 cm) containing sand and soil in ratio of 5:1. The plants were watered with half-power Hoagland nutrient solution daily for 8 weeks. Drought was imposed by water withholding for a period of 6 days. During this period, ABA (25 and 50 mg/L), Sulfacetamide and Sulfasalazine (25, 50 and 100 mg/L) solutions were sprayed on leaves, daily. Two groups were determined as control (watered regularly) and stressed (without receiving solution). Sampling was done every 48 hours from day 0 to day 6 and final sampling was done 2 days after irrigation (recovery phase). Samples of leaves were collected, washed and used to measure MDA content, APX, CAT and GPX activities.

Enzyme extraction and enzyme assays

Fresh tomato leaf tissues (500 ml) were used to prepare enzyme extract. Plant materials were ground in 3 ml of 50 mM Tris-HCl (pH=7.5) buffer containing 3 mM $MgCl_2$, 1 mM EDTA, using pre-cooled mortar and pestle. Extraction buffer for APX contained 0.2 mM ascorbate. The mixture was then centrifuged at 5000 rpm at 4 °C for 20 min. The supernatant was used for determination of enzyme activity (Kang et al., 2002).

Ascorbate peroxidase (APX) (EC 1.11.1.11)

APX activity was assayed by monitoring the oxidation of ascorbic acid according to the method of Nakano et al. (1981). The reaction mixture included 2.5 ml of 50 mM phosphate buffer (pH=7.0) with 0.1 ml H_2O_2 (1%) and 0.1 ml enzyme extract. APX activity was calculated using

an extinction coefficient of 2.8/ (mM cm) within 1 min at 240 nm.

$$\text{Unit (mM/min)} = \frac{\frac{\text{doD}}{\text{min (slope)}} \times \text{Vol. of assay (.0001)}}{\text{Extinction coefficient (2.8)}}$$

Catalase (CAT) (EC 1.11.1.6)

CAT activity was determined using the method of Aebi (1984). The reaction mixture contained 2.5 ml of 50 mM phosphate buffer (pH= 7.0) with 0.2 ml hydrogen peroxide (1%) and 0.3 ml enzyme extract. CAT activity was measured by monitoring a decrease in absorbance of H₂O₂ using an extinction coefficient 0.0436 (mM cm) at 240 nm within 1 min.

$$\text{Unit (mM/min)} = \frac{\frac{\text{doD}}{\text{min (slope)}} \times \text{Vol. of assay (.0003)}}{\text{Extinction coefficient (0.0436)}}$$

Guaiacol peroxidase (GPX) (EC 1.11.1.7)

GPX activity was measured by the method of Upadhyaya et al. (1985). The reaction mixture including 2.5 ml of 50 mM phosphate buffer (pH= 7.0) contained 1ml guaiacol (1%), 1 ml H₂O₂ (1%) and 0.1 ml enzyme extract. Activity was determined using an extinction coefficient 26.6 (mM cm) at 420 nm within 1 min.

$$\text{Unit (mM/min)} = \frac{\frac{\text{doD}}{\text{min (slope)}} \times \text{Vol. of assay (.0001)}}{\text{Extinction coefficient (26.6)}}$$

MDA content

Lipid peroxidation was measured in terms

of content of malondialdehyde (MDA), a product of lipid peroxidation, following the method of Buege and Aust (1978). Leaf samples (0.1 g) were homogenized in 5 ml 20% (w/v) trichloroacetic acid (TCA) in 0.5% (w/v) thiobarbituric acid (TBA) then centrifuged at 10000 ×g for 15 min. The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10000 g for 15 min, the absorbance of the supernatant was recorded at 532 nm. The value for nonspecific absorption at 600 nm was subtracted. MDA content was expressed as μ mol MDA per g fresh weight.

All data were subjected to analyze using SPSS software (version 15.0 for Windows). Means ±SE were calculated from three replicates. The statistical differences were expressed at P ≤ 0.05.

Results

APX activity increased gradually in ABA (10 and 25 mg/L) treatment in all sampling times but decreased in ABA 25 mg/L treatment at 192 h (recovery). In Sa treatments (25, 50 and 100 mg/L) APX activity increased in general, but in the case of 100 mg/L there was a significant increase at 96 and 144 h. Also, SS treatments (25, 50 and 100 mg/L) showed a gradual increase in ascorbate peroxidase activity except for Sa (25 and 50 mg/L) at 96 h and SS (50 and 100 mg/L) at 192h which showed a decreased activity. In SS concentration 100 mg/L, APX activity increased at 48 h that was higher than its function in ABA treatments 10 and 25 mg/L and SS treatment 50 mg/L, but was lower than SS concentration 25 mg/L (Fig. 1 a, b).

In Sa 25 mg/L , GPX activity was between ABA 10 and 25 mg/L at 48, 96 and 144 h, and

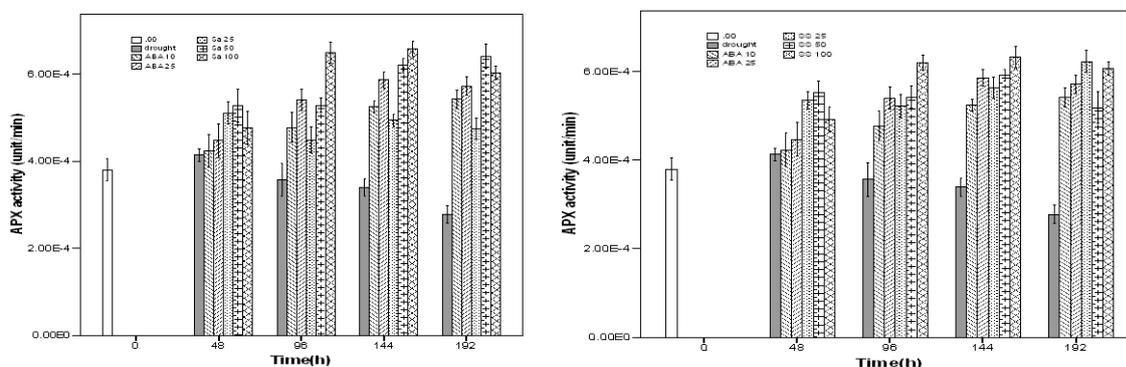


Fig. 1. Effects of ABA and SA (left) and SS (right) on APX activity in tomato plants

again increased at 192 h (recovery). Sa concentrations 50 and 100 mg/L showed a gradual increase but decreased at 192 h. In SS treatments (25, 50 and 100 mg/L) an increased GPX activity was observed at 48 and 96 h. In SS 25 and 100 mg/L concentrations it decreased at 144 h (Fig. II. a, b). CAT activity increased significantly in Sa 25 mg/L treatment, except for those at 96 h which decreased. In Sa 50 and 100

mg/L treatments a gradual increase was observed but in SA 50 mg/L, catalase activity was higher than its activity in ABA 10 and 25 mg/L at 96 and 144 h. Furthermore, in SS concentrations (25, 50 and 100 mg/L), there was a general increase in CAT activity at all sampling times and in SS 100 mg/L it was between ABA 10 and 25 mg/L treatments (Fig. III. a, b).

MDA content showed changes between

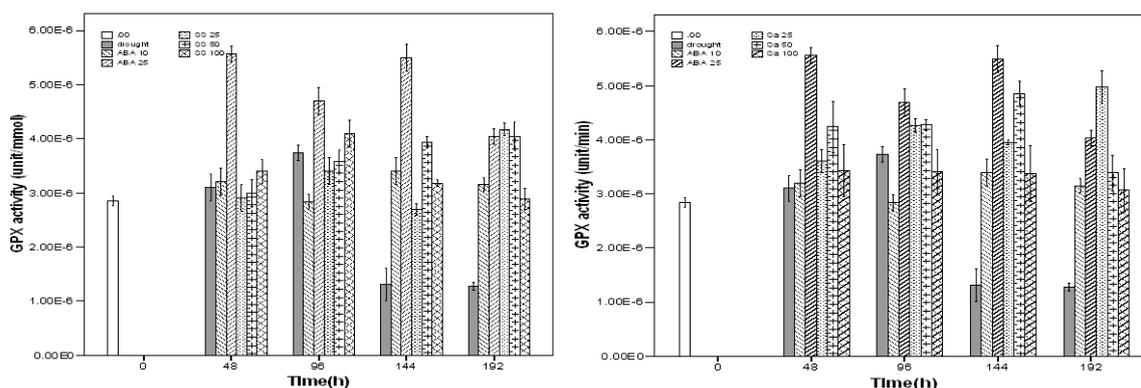


Fig. II. Effects of ABA and SA (left) and SS (right) on GPX activity in tomato plants

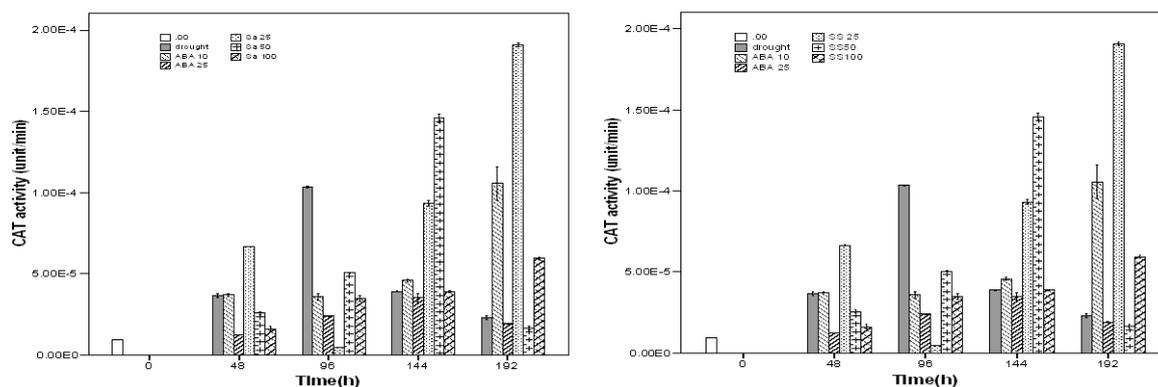


Fig. III. Effects of ABA and SA (left) and SS (right) on CAT activity in tomato plants

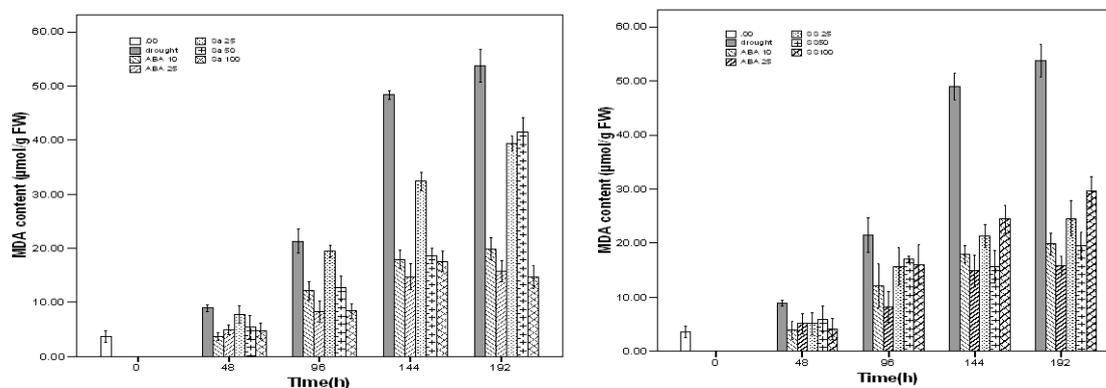


Fig. IV. Effects of ABA and SA (left) and SS (right) on MDA content in tomato plants

treated tomato plants. It increased slowly compared with drought-stressed group and at recovery phase it decreased generally except in Sa 25 and 50 mg/L and all three concentrations of SS which raised compared with the same treatments in drought-stress times (48, 96 and 144 h). In general, Sa and SS could increase tomato plants tolerance to water deficit like ABA (Fig. IV. a, b).

Discussion

Among environmental abiotic stresses, drought is one of the major limiting factors for crop plants growth and is becoming increasingly critical due to changes in the global climate (Hura et al., 2007). Plant drought tolerance can be improved by ABA (Raghavendra et al., 2010). Exogenous ABA reduces the accumulation of dry matter (Buege and Aust, 1978). It has been shown that exogenous application of ABA significantly decreased plant height, total leaf area, total biomass accumulation, while significantly increased root/shoot ratio, ABA concentration, and water use efficiency (Ma et al., 2008).

Results of the present study clearly indicated the enhanced activities of the enzymes of ascorbate-glutathione cycle, signifying a potential role of these enzymes in providing antioxidative defense under drought stress conditions. At higher levels of drought stress, generation of superoxide anion increased lipid peroxidation, more declines in soluble proteins, thiols as well as non-enzymatic antioxidants ascorbate and glutathione were observed compared to mildly stressed rice seedlings (Sharma and Dubey, 2005; Heath and Packer, 1968). Production of reactive oxygen species (ROS) under drought stress conditions induces protective responses and cellular damage (Blokina et al., 2003). MDA content can indicate the extent of oxidative stress in plants. It increased in drought-stressed plants during stress time even at recovery phase. MDA level also increased in ABA, SS and Sa application, but had significant differences with drought-stressed group. MDA level in SS 25 mg/L application was near its content in ABA 25 mg/L sprayed plants. In SS 50 mg/L, MDA content decreased at 144 h and this result was between its level in ABA (10

and 25 mg/L) at 144 and 192 h. In all applied concentrations of SS and Sa, MDA level was lower than drought-stressed plants. Results of Sa concentrations 50 and 100 mg/L were better than SS treatments 25 and 100 mg/L at 144 h of stress period. Plants are able to improve their stress tolerance often by antioxidant system. On the other hand, a higher amount of ROS triggers increasing of the activity of antioxidant enzymes, such as APX, CAT, SOD, POD and GPX, which in turn protects plants from oxidative stress (Davey et al., 2000; Ma et al., 2008). CAT besides the SOD and POD are the most important detoxifying enzymes, working together with APX and GPX to promote the scavenging of ROS (Hernandez et al., 2001). The role of antioxidant defense system and more specially the role of APX, CAT and GPX were examined. Although there were variations in observed results, in general, APX, CAT and GPX activities increased MDA content and showed reduction compared with drought-stressed plants. These results indicate that applied concentrations of SS and Sa can raise tomato plants tolerance to drought compared with ABA (10 and 25 mg/L) and drought-stressed group. Formation of MDA is an oxidative effect of ROSs such as H₂O₂ on membrane lipids; therefore, MDA level increased in drought-stressed plants during stress period, but decreased in sprayed ones with ABA, SS and Sa. This is a result of increased activity of antioxidant enzymes activities especially those assayed in this work (APX, CAT and GPX).

In conclusion, drought significantly affected the antioxidant enzymes activity and MDA content of tomato plants. Exogenous ABA, SS and Sa can help the plants to avoid stress effects resulting from drought to some extent. Our study reveals the SS and Sa agonistic activities with ABA to increase tomato plants resistance in drought condition. These findings are very helpful when determining how some sulfonamide components can act as ABA agonist that possess higher stability against light and cheap production cost rather than ABA.

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