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ORIGINAL ARTICLE

Drought Tolerance Induced by Foliar Application of Abscisic Acid and Sulfonamide Compounds in Tomato

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The effects of exogenous abscisic acid and two sulfonamide compounds: Sulfacetamide and Sulfasalazine were studied on tolerance of tomato (*Lycopersicon esculentum* Mill. cv. Super chief) under drought stress and compared with abscisic acid effects. Eight-week old plants were treated with ABA (10 and 25 mg/L), Sulfacetamide (25, 50 and 100 mg/L) and Sulfasalazine (25, 50 and 100 mg/L). Solutions were sprayed daily and sampling was done at 0 h, 48 h, 96 h, 144 h and 48 h after re-watering (recovery phase or 192 h). Treated plants showed relatively greater drought tolerance. This indicates that, Sulfacetamide and Sulfasalazine may improve resistance in tomato, like ABA, increasing levels of proline, glycine betaine and malondialdehyde and the activity of ascorbate peroxidase. Daily application of Sulfasalazine and Sulfacetamide during drought stress period was effective in increasing tomato plants tolerance to drought as was ABA.

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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). Plant cells are protected against the detrimental effects of reactive oxygen species (ROS) by a complex antioxidant system comprising of the non-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. 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). ABA is an important phytohormone, which inhibits growth under severe environmental conditions and protects plants against stresses, such as drought, salinity, and 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. 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. Osmoprotectants such as carbohydrates, glycinebetaine and proline are some of these accumulated compounds that involves in drought tolerance in plants. 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 osmoprotectants level in tomato plants.

MATERIALS AND METHODS

Plant material and treatments

Chemicals, ABA, sulfacetamide and sulfasalazine were commercially purchased (Sigma-Aldrich Chemie GmbH, Fluka). 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 (wa ered regularly) and stressed (without receivi g solution). Sampling was done every 48 hours from ay 0 to day 6 and final sampling was done 2 days fter irrigation (recovery phase).

Glycine betaine measurement

Dried leaf materials (0.5 g) were finely grounded and mechanically shakened with 20 mL deionized water for 24 h at 25 ˚C. Then samples filtered and filterates diluted with 2N H_2SO_4 (1:1). Aliquots were cooled in ice water for 1h. Cold KI-I_2 reagent (0.20 mL), 15 g of iodine and 20.0 g of KI dissolved in 100 mL water (Grieve *et al*. 1983) was added and gently stirred with a vortex mixture. Samples stored at 4 ˚C for 16 h and then centrifuged at 10000 ×g (model 57.462) for 15 min at 0 ˚C. The supernatant was separated carefully with a fine glass tube. Formed periodide crystals, dissoulved in 9 mL of 1.2-dichloroethane. After 2 h, the absorbance was measured at 365 nm using glycinebetaine as standard. Glycine betaine content was measured in leaves sprayed with ABA (10 and 25 mg/L), SS (25, 50 and 100 mg/L) and Sa (25, 50 and 100 mg/L) on 0, 48, 96 and 144 h of drought period and 48 h after re-watering

Proline content

Free proline content of leaves was estimated using the acid ninhydrin method of Bates *et al*. 1973. One hundred fifty milligram of leaf tissues was grounded in a mortar and pestle with 6 mL of 3 % (w/v) sulfosalicylic acid aqueous solution and the homogenate was filtered through Whatman No. 1 filter paper, then 2 mL of the filtered extract was taken for the analysis to which 2 mL acid ninhydrin and 2 mL of glacial acetic acid were added. The reaction mixture was incubated in a boiling water bath. Four milliliter of toluene was added to the reaction mixture and the organic phase was

extracted, in which a toluene soluble reddish chromophore was obtained, which was read at 520 nm using toluene as blank by UV-visible spectrophotometer (WPA model S2100). Proline content was measured in leaves that sprayed with ABA (10 and 25 mg/L), SS (25, 50 and 100 mg/L) and Sa (25, 50 and 100 mg/L) on 0, 48, 96 and 144 h of drought period and 48 h after re-watering.

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 nM 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.

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 μmol MDA per g fresh weight.

Statistics

We subjected all data to SPSS (version 15.0 for windows statistical software package). Means ±SE were calculated from three replicates. The statistical differences were expressed at $P \leq 0.05$.

RESULTS

During drought stress treatment, levels of glycine betaine (GB) in leaves that sprayed with ABA 10 and 25 mg/L, increased continuously peaking on 144 h, then reduced on 192 h (re-watering). Glycine betaine content in plants that were sprayed with SS 20, 50 and 100 mg/L and Sa 100 mg/L increased on 48 h and 96 h, gradually. In leaves that received Sa 25 and 50 mg/L the levels of GB decreased continuously during drought period and after re-watering (192 h or recovery phase). Glycine betaine content of leaves that were sprayed with SS 100 mg/L, reduced on 144 h but increased after re-watering (192 h). In all plants, except for the SS 100 mg/L treatment, glycine betainelevel decreased at recovery phase (re-watering) (Fig 1). All treatments showed the same trend for levels of proline, increasing continuously to a peak on 144 h before declining after the plants were re-watered. Spraying with Sa 100 mg/L also led to increased level of proline at 48 h and 144 h decreased on 96h and 192 h. The increased level of proline in sprayed leaves with SS 50 mg/L was obvious to a peak on 144 h. In all treatments the level of proline was higher than the proline content of drought-stressed leaves without spraying (Fig 2). MDA content showed changes between 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 3). 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 4).

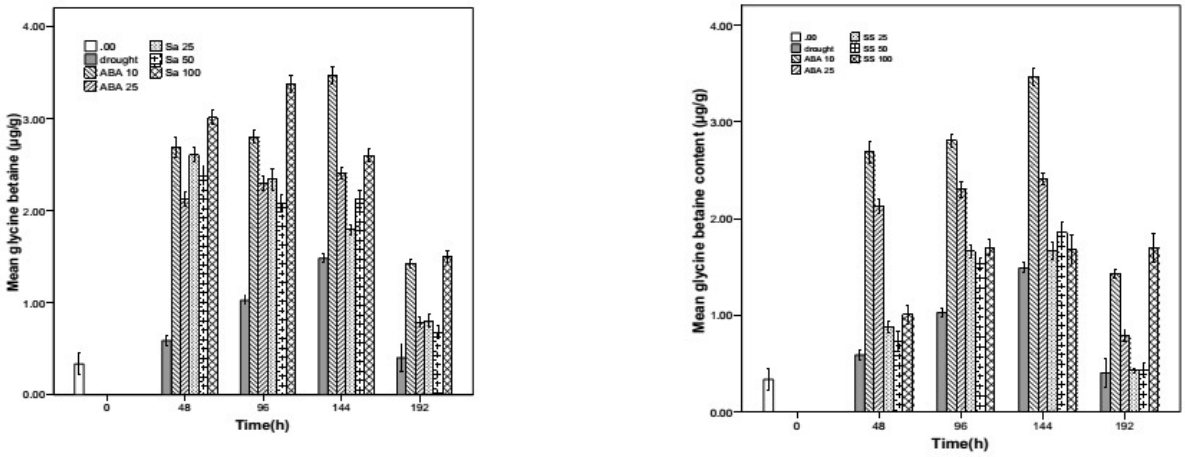

Figure 1 : Effects of ABA and SA (left) and SS (right) on glycine betaine content in tomato plants

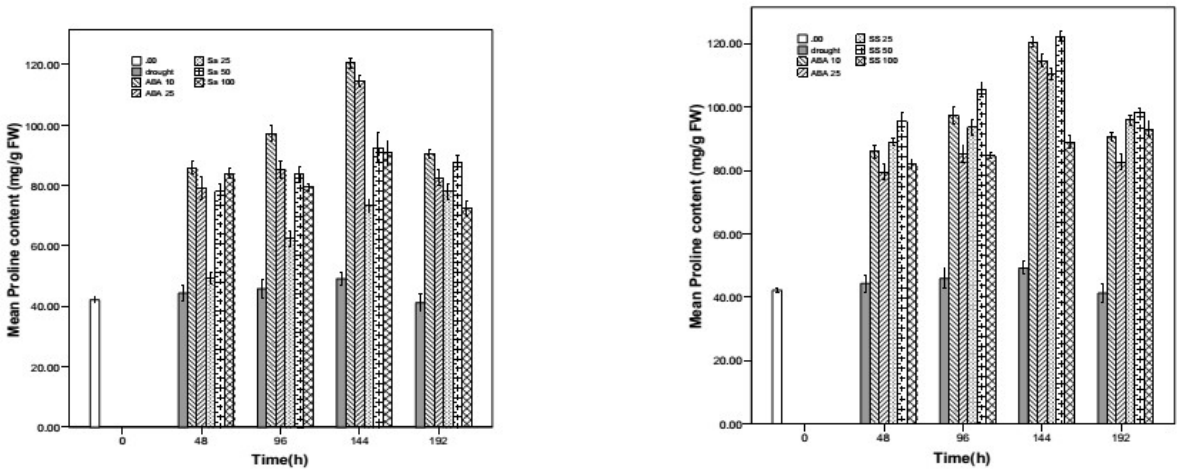

Figure 2. Effects of ABA and SA (left) and SS (right) on proline content in tomato plants

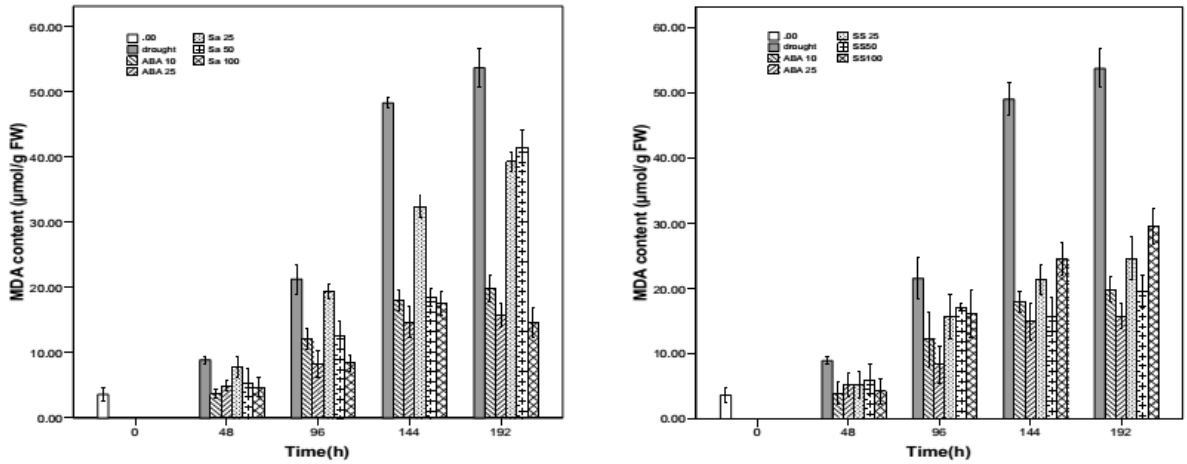

Figure 3. Effects of ABA and SA (left) and SS (right) on MDA content in tomato plants

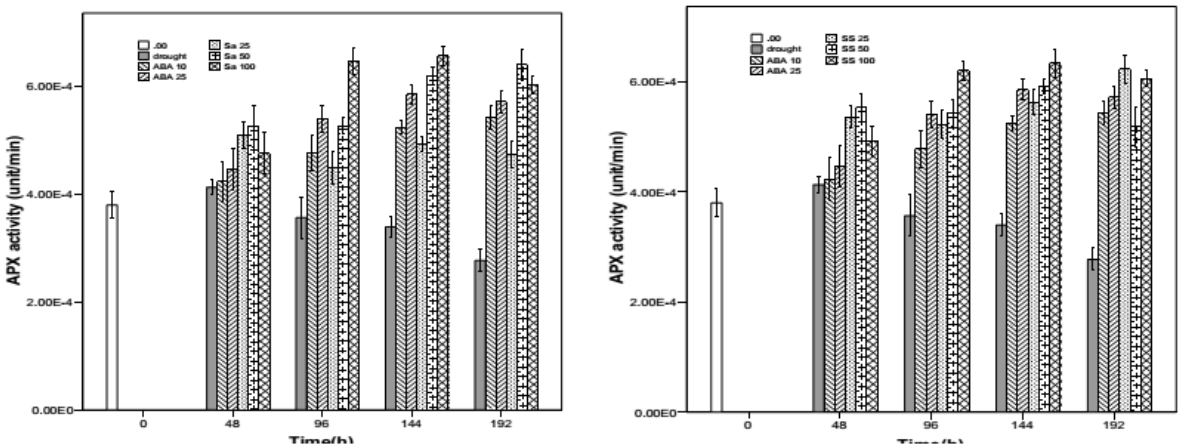

Figure 4. Effects of ABA and SA (left) and SS (right) on APX activity in tomato plants

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 indicate 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, 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). 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 increasin of the activity of antioxidant enzymes, such as APX, CAT, SOD, and POD which in turn protects plants from oxidative stress (Ma *et al*., 2008). The role of antioxidant defense system and more specially the role of APX was examined. Although there were variations in observed results, in general, APX activity 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 H_2O_2 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 that assayed in this work (APX). Under the imposed-drought conditions, proline and glycine betaine (GB) were increased in leaves. We have compared effects of two sulfonamide compounds on stress tolerance of tomato in drought conditions. Increased proline and glycine betaine accumulation were reported in water-stressed plants (Hamada 2000). Accumulation of glycine betaine under stress condition suggests its involvement in stress tolerance as it has been proposed that tolerant species normally accumulate more GB than sensitive species in response to stress but, glycine betaine accumulation and stress tolerance is species specific (Ashraf *et al*. 2007). Glycine betaine might be able to stabilize macromolecular activity as well as membrane integrity (Sakamoto *et al*. 2002). In present study, the amount of glycine betaine increased in plants that sprayed with ABA, SS and Sa under water deficit but decreased in plants recipient SS 100 mg/L on 144 h after water withholding, and then decreased on recovery phase (192 h). Proline accumulates in many plant species under a broad range of stress conditions such as water deficit, salinity, high temperatures and high light intensity (Mansour 2000). Claussen (2004) has reported that the highest amount of proline had been observed in young leaves of tomato under osmotic stress condition. An increase in proline content of tomato leaves was observed in plants that were sprayed with ABA, Sa and SS. In this investigation, the levels of proline increased generally in all treatments from hour 0 to hour 144 of drought period, and then decreased after re-watering (192 h). This shows that plants were sprayed with SS and Sa could tolerate water deficit like plants that were received ABA compared with drought-stressed group. Increases in concentration of proline were due to water loss and increased synthesis; all other drought-induced increases in organic-acid concentrations were a consequence of water loss only. Water stress caused a linear increase in the proline content of leaves coinciding with the increase in water stress period. Maximum increase in proline content was observed in leaves recipient SS 50 mg/L and (10 and 25 mg/L) on 144 h of drought period.

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