Comparative study between the physiological role of hydrogen peroxide and salicylic acid in alleviating the harmful effect of low temperature on tomato plants grown under sand-ponic culture.

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Introduction

Tomato (Lycopersicon esculentum L.) is a member of the Solanaceae family and one of the most important vegetables grown in Egypt. It has a particular importance as being a raw material of agricultural industry besides it is used as a fresh vegetable. Tomato is a rich source of lycopene, vitamins, and minerals. Lycopene is responsible for the characteristic deep red color of ripe tomato fruits and tomato products (Helyes et al., 2009). Lycopene is a key intermediate in the biosynthesis of many important carotenoids, such as beta-carotene and xanthophylls and may help in counteract the harmful effects of substances called “free radicals” and different types of cancer (DeStefani et al., 2000).

In nature, plants often face the challenge of severe environmental conditions, which include various biotic and abiotic stresses that exert adverse effects on plant growth and development causing considerable losses in the crop productivity. Hung et al. (2005) stated that the severity of stress depends on numerous intrinsic (e.g. cultivar) and extrinsic (e.g. the fluctuation of temperature, duration of exposure, the water status of soil and the intensity of light) factors. Meanwhile, Miura and Tada (2014) mentioned that the temperature is a major factor of abiotic stresses and it is a key determinant of crop productivity. Since, the amount and rate of the uptake of water and nutrients are decreased by low temperature. Exposure of tomato plants to low temperature caused chilling injury that is caused by exposure to low, but non-freezing temperatures (ca. >10 °C) (Raison and Lyons, 1986). In addition, Sevillano et al. (2009) reported that chilling injury reduced tomato fruit quality. Chilling injury
adversely affect harvested crops in two ways: a primary one that is temperature-dependent and initiated when the temperature falls below a threshold temperature for a specified duration, and causes some metabolic dysfunction. The secondary way is time-dependent and includes a multitude of metabolic processes that can be adversely affected as a consequence of the primary way and lead to the development of measurable symptoms characteristic of chilling injury (Luengwilai et al., 2012). Generally, environmental stress frequently reduced plant growth through over production of reactive oxygen species (ROS) which damage various macromolecules and cellular structures (Apel and Hirt, 2004), cellular membrane, photosynthetic apparatus and enzymes (Lukatkin, 2003) and lead to the death of cells (Upadhyaya et al., 2007; Liu et al., 2010; Goud and Kachole, 2011). These ROS, such as hydrogen peroxide, superoxide and hydroxyl ions, resulting in oxidative damage at the cellular level (Hung et al., 2005; Goud and Kachole, 2011). It is worthy to mention that, ROS may play two very different roles: exacerbating damage or signaling the activation of defense responses (Yi et al., 2014). Since, ROS in low concentration act as signaling molecules mediating a variety of physiological responses, including stomatal movement and gene expression (Suzuki et al., 2012; Yi et al., 2014). Meanwhile, over accumulation of ROS damage almost all cell components including membrane lipids, chloroplasts, pigments, enzymes and nucleic acids (Upadhyaya et al., 2007; Liu et al., 2010; Goud and Kachole, 2011). In this concern, plant species have evolved various mechanisms to cope with environmental stresses. For example, to mitigate stress induced damage, plants may up-regulate various scavenging mechanism like enzymatic antioxidants (superoxide dismutase, peroxidase and catalase) (Noctor and Foyer, 1998) and non-enzymatic metabolites e.g., ascorbic acid (Ahmad et al., 2013) and osmoprotectants (Farooq et al., 2008; Gautam and Singh, 2009; Ahmad et al., 2013). Multiple antioxidant enzymes are involved in the scavenging of ROS. Superoxide dismutases (SOD) react with the superoxide radical to produce hydrogen peroxide ($\text{H}_2\text{O}_2$) that is scavenged by catalases (CAT) and peroxidases (POD). CAT reacts with $\text{H}_2\text{O}_2$ to produce water and oxygen. Among peroxidases, ascorbate peroxidases (APX) and glutathione peroxidase (GPX) which uses ascorbate and glutathione as electron donors, respectively, and leading to $\text{H}_2\text{O}_2$ detoxification in plants.

It is safe to say that ROS have “two-faced”, being “harmful” when produced in excess and “beneficial” at lower concentrations. ROS at these “beneficial” levels play a role in regulating plant development and growth as well as in environmental acclimation (Quan et al., 2008). $\text{H}_2\text{O}_2$ is the most stable form of the ROS and is capable of rapid diffusion across cell membrane (Upadhyaya et al., 2007). $\text{H}_2\text{O}_2$ is a strong oxidizing agent accumulated upon various stress. High level of $\text{H}_2\text{O}_2$ damages photosynthesis and initiates programmed cell death (Dat et al., 2000). On the other hand, when the amount of $\text{H}_2\text{O}_2$ is maintained at normal level by a series of antioxidant enzyme, it acts as a second messenger and coordinates with other important signal molecules to protect plants from stresses and triggering stress tolerance (Noctor and Foyer, 1998; Hung et al., 2005; Quan et al., 2008; Li et al., 2011). $\text{H}_2\text{O}_2$ regulates the expression of numerous genes involved in plant defense and the related pathways such as antioxidant enzymes, defense proteins and transcription factors (Hung et al., 2005; He et al., 2009; Abbass and Mohamed, 2011). $\text{H}_2\text{O}_2$ has been shown to act as a key regulator in a broad range of physiological processes including photosynthesis (Noctor and Foyer, 1998), senescence (Peng et al., 2005), stomatal movement, cell growth and development (Deng et al., 2012). Hung et al. (2005) and Terzi et al. (2014) mentioned that the endogenous $\text{H}_2\text{O}_2$ concentration depended on the balance between its production rates and its utilization by enzymatic and non-enzymatic way. The fluctuation of $\text{H}_2\text{O}_2$ level in plants should spatially and temporally reflect changes in the environment. Exogenous application of $\text{H}_2\text{O}_2$ at low concentrations signals the induction of defense responses in plants against oxidative stresses (Prasad et al., 1994; Morita et al., 1999) and assists in triggering stress resistance mechanism (Hameed et al., 2004; Kumar et al., 2010). Indeed, it has been shown that pretreatment of mung bean seedlings with low concentrations of $\text{H}_2\text{O}_2$ induces chilling tolerance (Yu et al., 2003). $\text{H}_2\text{O}_2$ treatments improved osmotic stress resistance of two cucumber varieties by activating antioxidant system (Yu et al., 2003). In addition, Liu et al. (2011) stated that exogenous $\text{H}_2\text{O}_2$ treatments prevent the increase of oxidative stress and endogenous $\text{H}_2\text{O}_2$ concentration in plants and enhance tolerance of plants to salt stress by enhancing the production of enzymatic and non-enzymatic antioxidants which can quench the ROS and decrease lipid peroxidation. They added that exogenous 100 nM $\text{H}_2\text{O}_2$ treatments decreased the deleterious effect of salt stress on growth of wheat than 50 nM $\text{H}_2\text{O}_2$. Terzi et al. (2014) observed that exogenous applications of $\text{H}_2\text{O}_2$ at low concentration alleviated membrane damages and significantly decreased lipid peroxidation of maize plants under osmotic-stressed conditions by inducing the metabolite levels involved in osmotic adjustment.

Salicylic acid (SA) acts as an endogenic phytohormone from phenolic compounds (among the group of ortho hydroxyl benzoic acid), having the ability of antioxidant defense system and regulates various physiological and biochemical processes in plant such as: stomata conductivity (Hayat et al., 2010), activity of photosynthesis pigments (Hayat et al., 2005), maintenance of tissue water contents and reduced membrane permeability (Farooq et al., 2008), adjustment the activity of antioxidant enzymes (Carvalho et al., 2011), and tolerance to environmental stresses (Kabiri et al., 2012). In addition, Sakhabutdinova et al. (2003) reported that salicylic acid treatments maintain IAA and cytokinin levels in the plant tissues, which enhanced the cell division and dry weight. Miura and Tada (2014) stated that the effects of SA on the physiological processes of plants depend on its concentration, type of plant, the stage of plant growth and environmental conditions. In general, low concentrations of SA may enhance the antioxidant capacity and tolerance to abiotic stresses but high
concentrations of SA may cause cell death or susceptibility to abiotic stresses (Hara et al., 2012). Salicylic acid is an important signaling molecule in plants, improved chilling tolerance and synchronous emergence of maize by activation of antioxidants (Farooq et al., 2008). The application of 0.5 mM SA improved the cold tolerance of maize, cucumber, and rice (Kang and Saltveit, 2002). Potatoes treated with 0.1mM SA exhibited freezing tolerance (Mora-Herrera et al., 2005). The application of 0.5mM SA solution by spraying on the leaves or irrigating the roots of banana seedlings for 1 day improved the chilling tolerance (Kang et al., 2003). SA treatments at low concentrations of (0.1-0.5 mM) promoted tolerance to chilling stress in bean and tomato as reported by Senaratna et al. (2000). Furthermore, Miura and Tada (2014) suggested that cold signaling and SA signaling may be interrelated and that the effect of SA on cold tolerance may be species-specific and dependent on the concentration and period of application.

Regarding the relationship between \( \text{H}_2\text{O}_2 \) and SA, Quan et al. (2008) concluded that \( \text{H}_2\text{O}_2 \) is implicated in SA synthesis where, the conversion of benzoic acid into SA is catalyzed by the \( \text{H}_2\text{O}_2 \) via activating of benzoic-acid-2 hydroxylase. They added that either application of \( \text{H}_2\text{O}_2 \) or SA induces each other.

This work aimed to investigate the physiological role of two signal molecules (\( \text{H}_2\text{O}_2 \) and SA) in enhancement of growth, phytohormones, antioxidant enzymes, yield quantity and quality (TSS and antioxidant activity) of two tomato cultivars grown under low temperature conditions in sand-ponic culture.

Materials and Methods

Experimental procedure

Two pot experiments were conducted during two successive winter seasons (2011-2012) and (2012-2013) at wire house of National Research Centre, Dokki, Giza, Egypt, to study the effect of two levels of \( \text{H}_2\text{O}_2 \) and SA (0.5 mM and 1.0 mM) on growth, fruit yield quantity and quality of two tomato cultivars (Streenb and Floridat) grown under low temperature conditions in sand-ponic culture. Seedlings (one true leaf stage) were transplanted carefully in pots filled with washed sand (2 seedlings/pot) at last week of November during two successive seasons. During this experiment, plants are subjected to low temperature where level of night temperature drops several times below 10°C. The plants were supplied with nutrient solution via irrigation. The nutrient solution contains all necessary elements that required for plant growth. The base solution contained (mg L\(^{-1}\)): 200 potassium, 100 nitrogen, 100 calcium, 54 phosphorus, 64 sulfur, 49 magnesium, 5 iron, 0.5 boron, 0.5 manganese, 0.05 zinc, 0.02 copper, 0.01 molybdenum. Electrical conductivity (EC) and pH were measured and adjusted regularly. Tape water used had: pH of 7.5 and EC of 0.35 dSm\(^{-1}\). The pH of the nutrient solution was measured using a portable pH meter, HI 9023 (Hanna Instruments, Padova, Italy). The experiment was arranged in a complete randomized design with 15 replicates for each treatment. The treatments were two levels of \( \text{H}_2\text{O}_2 \) or SA (0.5 mM and 1.0 mM) which applied exogenously on plants after 15 and 30 days from transplanting.

Data recorded

Random samples of plants were collected at 80 days after transplanting from each treatment to determine some growth parameters (plant height, leaves number/plant, fresh and dry weights of leaves and root/plant) as well as estimate endogenous growth regulators, antioxidant enzymes, malondialdehyde, electrolyte leakage and total chlorophyll (SPAD values). At harvest, tomato fruits were collected weekly and total yield was calculated as g/plant. Fruit quality i.e. total soluble solids (TSS) and antioxidant activity of fruit juice were determined.

Biochemical analysis

Chlorophyll was determined using chlorophyll meter (Model: TYS-A, Zhe Jang Top Instrument Co. LTD., Hangzhou, China). Endogenous growth regulators namely auxins (as indole acetic acid IAA), gibberellic acid (as GA\(_3\)) and abscisic acid (ABA) were extracted and determined by Gas Liquid Chromatography (GLC) according to the method described by Wasfy and Orrin (1975). For enzyme determination: The method used for extracting the enzyme was that of Mukherjee and Choudhuri (1983). Catalase (CAT, EC 1.11.1.6) activity was assayed according to the method of Aebi (1983) by the decrease of absorbance at 240 nm for 1 min as a consequence of \( \text{H}_2\text{O}_2 \) consumption. Peroxidase (POD, EC 1.11.1.7) activity was determined according to the method described by Nakano and Asada (1981) as the increase of absorbance at 470 nm due to oxidation of guaiacol in the presence of \( \text{H}_2\text{O}_2 \) and formation of tetraguaiacol. Polyphenol oxidase (PPO, EC 1.10.3.1) activity was determined according the modified method of Taneya and Sacher (1974). The oxidizing capacity of the enzyme extract was determined spectrophotometrically against pyrogallop. Lipid peroxidation was determined by measuring the malondialdehyde (MDA) content following the method of Dhindsa et al. (1982). Electrolyte leakage (EL) was measured using an electrical conductivity meter (Hanna Instruments, Bedfordshire, England) as described by Goncalves et al. (2007). Total soluble solids (TSS) were determined using a portable refractometer (Brixstix BX 100 Hs; Techniquip Corporation, Livermore, CA). The free radical scavenging activity was determined according to Brand-Williams et al. (1995) using the 1,1-diphenyl-2-picrylhydrazil (DPPH) reagent.
Statistical analysis

Statistically significant differences between means of the two seasons after tested the variances homogeneity according to Snedecor and Cochran (1980) were compared at p ≤ 0.05 using Duncan’s multiple range tests.

Results and Discussion

Growth parameters and SPAD values

Floridat cultivar was characterized by significant increases in growth parameters (plant height, leaves number/plant, fresh and dry weights of leaves and root/plant) and SPAD values than those of Streenb cultivar under all treatments (Table 1). H$_2$O$_2$ treatments (0.5 mM and 1.0 mM) caused the highest significant increase in the growth parameters and SPAD values of two tomato cultivars followed by SA treatments (0.5 mM and 1.0 mM).

H$_2$O$_2$ application might promote cell division (Hameed et al., 2004) and secondary wall formation (Abass and Mohamed 2011). H$_2$O$_2$ takes part in reinforcement of plant cell wall ( lignification, cross-linking of cell wall structural proteins), phytoalexin production and resistance enhancement (Quan et al., 2008). Low doses of H$_2$O$_2$ can increase mass and length of roots (Narimanov and Korystov, 1997). Abass and Mohamed (2011) observed that exogenous application of H$_2$O$_2$ to common bean enhanced the root growth and fresh weight under drought stress. Moreover, Goldani et al. (2012) showed that foliar application of H$_2$O$_2$ can improve oregano shoot and root dry weight and alleviate adverse effects of salinity. Exogenous H$_2$O$_2$ also mediates the growth of primary root, lateral roots, and root hairs (Jiang et al., 2012) and significantly promote the formation and growth of adventitious roots of cucumber (Li et al., 2007).

Salicylic acid is a key signaling molecule in induction of plant defense mechanism and reduces symptoms of environmental stress as well as regulates plant growth and development (Horváth et al., 2007). Khodary (2004) and Stevens et al. (2006) stated that SA treatments ameliorated the negative effect of salt stress on fresh and dry weight of maize and tomatoes plants respectively. The increases in fresh and dry matter of stressed plants in response to SA might be related to the induction of antioxidant response that increased the tolerance of plants to damage (Gunes et al., 2005). SA treatments increased the level of cell division by stimulating the mitotic system of the apical meristem of seedling roots which caused an increase in plant growth (Sakhabutdinova et al., 2003).

SPAD values have a significant association with chlorophyll synthesis and photosynthesis (Khan et al., 2013). He et al. (2009) found that H$_2$O$_2$ pretreatment enhanced the photosynthetic rate in wheat seedlings under drought conditions and attributed this enhancement to the positive role of H$_2$O$_2$ in inducing the expression of antioxidant system and reducing the oxidative damage of cellular membranes. Similarly, Goldani et al. (2012) reported that 5 mM H$_2$O$_2$ increased total chlorophyll and carotenoid content in salt stressed oregano plant by 46.6 and 100.6 % respectively compared to control plant.

Regarding SA effect, Khodary (2004) and Szepesi (2006) found that SA treatment increased the chlorophyll, carotenoid contents, improved the photosynthetic efficiency and enhanced photosynthetic rate, dry weights and maintained membrane integrity, leading to improvement of plant growth in maize and tomato plants respectively under stress conditions. Similarly, exogenous application of SA significantly enhanced net photosynthetic rate which could be due to improving the functional state of the photosynthetic machinery in plants either by the mobilization of internal tissue nitrate or by chlorophyll biosynthesis (Shi et al., 2006). Suitable concentrations of salicylic acid inhibit chlorophyll degradation and increase photosynthesis by inhibition of chlorophyll oxidase enzyme activity (Belkhati et al., 2010).

Table 1. Effect of H$_2$O$_2$ and salicylic acid on some growth parameters and SPAD values of two tomato cultivars grown under low temperature conditions

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Weight of fresh leaves</th>
<th>Weight of dry leaves</th>
<th>Weight of fresh root</th>
<th>Weight of dry root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50.11g</td>
<td>66.70 cde</td>
<td>7.00 e</td>
<td>8.82 d</td>
</tr>
<tr>
<td>H$_2$O$_2$(0.5 mM)</td>
<td>62.14 def</td>
<td>86.12 ab</td>
<td>9.63 cd</td>
<td>12.17 a</td>
</tr>
<tr>
<td>H$_2$O$_2$(1.0 mM)</td>
<td>62.14 def</td>
<td>86.12 ab</td>
<td>9.63 cd</td>
<td>12.17 a</td>
</tr>
<tr>
<td>SA (0.5 mM)</td>
<td>57.35 f</td>
<td>72.17 c</td>
<td>8.62 d</td>
<td>10.70 b</td>
</tr>
<tr>
<td>SA (1.0 mM)</td>
<td>60.91 ef</td>
<td>81.04 b</td>
<td>9.11 d</td>
<td>11.91 a</td>
</tr>
</tbody>
</table>

Data are means of two seasons - Means followed by the same letter for each tested parameter for both cultivars are not significantly different by Duncan’s test (P ≤ 0.05).
Continued Table 1

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant height (Cm)</th>
<th>Number of leaves /plant</th>
<th>SPAD values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>48.5 e</td>
<td>61.0 bcd</td>
<td>20.0 e</td>
</tr>
<tr>
<td>( \text{H}_2\text{O}_2 ) (0.5 mM)</td>
<td>64.0 bc</td>
<td>81.00 a</td>
<td>30.33 c</td>
</tr>
<tr>
<td>( \text{H}_2\text{O}_2 ) (1.0 mM)</td>
<td>66.66 b</td>
<td>83.33 a</td>
<td>32.33 bc</td>
</tr>
<tr>
<td>SA (0.5 mM)</td>
<td>55.0 d</td>
<td>63.0 bc</td>
<td>27.00 d</td>
</tr>
<tr>
<td>SA (1.0 mM)</td>
<td>59.0 cd</td>
<td>65.33 bc</td>
<td>30.66 e</td>
</tr>
</tbody>
</table>

Data are means of two seasons - Means followed by the same letter for each tested parameter for both cultivars are not significantly different by Duncan’s test (\( P \leq 0.05 \)).

Endogenous growth regulators

It was noted that either \( \text{H}_2\text{O}_2 \) or SA treatment (1.0 mM) caused marked increases in endogenous growth regulators (\( \text{GA}_3 \), IAA and ABA) in both tomato cultivars grown under low temperature conditions (Table 2). \( \text{H}_2\text{O}_2 \) treatment had more enhancement effect than SA treatment. In addition, the increases in the amount of ABA under all treatments were more pronounced than IAA and \( \text{GA}_3 \). Since, ABA is involved in stress adaptation. This is principally based on the positive correlation between the accumulation of ABA and chilling tolerance (Orabi, 2004). ABA regulates many important plant developmental processes and induces tolerance to different stresses including drought, salinity and low temperature as reported by Giraudat et al. (1994). ABA production is increased in tissues during these stresses and plays a central role in regulation of stomatal function as a stress signal (Pei et al., 2000; Quan et al., 2008). The increments in ABA under the effect of \( \text{H}_2\text{O}_2 \) treatments was reported by Terzia et al. (2014) who found that ABA content increased in \( \text{H}_2\text{O}_2 \) treated seedlings compared to the control group. Furthermore, the exogenous application of \( \text{H}_2\text{O}_2 \) alone or in combination with drought stress caused significant increase in both IAA and \( \text{GA}_3 \) contents (Abass and Mohamed, 2011). Terzi et al. (2014) mentioned that exogenous \( \text{H}_2\text{O}_2 \) treatment can enhance tolerance of maize seedlings to osmotic stress by increasing some metabolite and phytohormone levels.

Regarding enhancement effect of SA on endogenous growth regulators under low temperature, SA enhanced plant growth and cell division via regulation of other hormones like auxin, cytokinin, gibberellins, ABA (Zarghami et al., 2014) and mitigates abiotic stresses through increasing the growth regulating hormones such as auxins and cytokinins (Shakirova et al., 2003). Moreover, the application of SA on plants increased the amounts of auxin and ABA and prevented the reduction of cytokinin under drought and salinity stress conditions thereby, producing a higher total biomass and seed vigor index (Shakirova et al., 2003 and Kabiri et al., 2012).

Table 2. Effect of \( \text{H}_2\text{O}_2 \) and salicylic acid on some endogenous growth regulators of two tomato cultivars grown under low temperature conditions

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Treatments</th>
<th>( \text{GA}_3 ) \text{mg/100 g fresh leaves}</th>
<th>IAA \text{mg/100 g fresh leaves}</th>
<th>ABA \text{mg/100 g fresh leaves}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streenb</td>
<td>Control</td>
<td>1.042</td>
<td>1.498</td>
<td>0.158</td>
</tr>
<tr>
<td></td>
<td>( \text{H}_2\text{O}_2 ) (1.0 mM)</td>
<td>4.126</td>
<td>2.244</td>
<td>1.755</td>
</tr>
<tr>
<td></td>
<td>SA (1.0 mM)</td>
<td>1.714</td>
<td>3.016</td>
<td>1.499</td>
</tr>
<tr>
<td>Floridat</td>
<td>Control</td>
<td>1.643</td>
<td>1.426</td>
<td>0.219</td>
</tr>
<tr>
<td></td>
<td>( \text{H}_2\text{O}_2 ) (1.0 mM)</td>
<td>3.834</td>
<td>4.110</td>
<td>2.791</td>
</tr>
<tr>
<td></td>
<td>SA (1.0 mM)</td>
<td>2.887</td>
<td>1.351</td>
<td>2.159</td>
</tr>
</tbody>
</table>
Antioxidant enzymes

Floridat cultivar was characterized by higher activity of CAT, POD and PPO than streenb cultivar either in fresh leaf or root tissues (Table 3). H$_2$O$_2$ treatments and SA treatments caused marked decreases in CAT activity accompanied by significant increases in the activities of POD and PPO relative to control plants in fresh leaf and root tissues of both tomato cultivars grown under low temperature conditions. The decreases in CAT activity and increases in POD and PPO activities were more pronounced due to H$_2$O$_2$ treatments than SA treatments. Moreover, H$_2$O$_2$ treatment at 1.0 mM showed the highest significant effect.

H$_2$O$_2$, a stress signal, could trigger the activation of antioxidants in plants to alleviate the oxidative damage and leading to improve physiological attributes of the plant under stress (He et al., 2009). Moskova et al. (2009) mentioned that treatment with H$_2$O$_2$ did not influence catalase but increased the activity of POD. Similarly, Liu et al. (2010) stated that H$_2$O$_2$ treatments induced POD activity in cucumber leaves. Furthermore, exogenous H$_2$O$_2$ may induce oxidative stress tolerance by enhancing the activities of POD and PPO under various biotic and abiotic stresses (Goud and Kachole (2011).

Exogenous SA could regulate the synthesis and activities of antioxidant enzymes and increase plant tolerance to biotic and abiotic stress (He et al., 2002; Fayez and Bazaid, 2014). SA was found to enhance the activities of antioxidant enzymes such as POD when sprayed exogenously to the drought stressed plants (Hayat et al., 2010) or to the salinity stressed plants (Szepesi et al., 2008), might be due to its regulatory role at transcriptional and/or translational levels (Hayat et al., 2005). In Brassica juncea, Yusuf et al. (2012) reported that SA enhanced the level of antioxidant system (SOD and POD) under stress and stress-free conditions. On the other hand, SA has the ability to inhibit the activity of the CAT that lead to rise in the level of the H$_2$O$_2$ in vivo and stimulate the defense genes (Boukraa et al., 2014). Ahmad et al. (2012) stated that at suboptimal condition of low temperature, priming maize seeds with SA and H$_2$O$_2$ induced activities of scavenging enzymes where, 20 mL L$^{-1}$ H$_2$O$_2$ and 20 mg L$^{-1}$ salicylate seems to be suitable concentration for increasing the chilling tolerance.

Table 3. Effect of H$_2$O$_2$ and salicylic acid on some antioxidant enzyme activities of two tomato cultivars grown under low temperature conditions

<table>
<thead>
<tr>
<th>Treatments</th>
<th>CAT</th>
<th>POD</th>
<th>PPO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µ mole / g fresh weight</td>
<td>µ mole / g fresh weight</td>
<td>Unit / g fresh weight</td>
</tr>
<tr>
<td>H$_2$O$_2$ (0.5 mM)</td>
<td>134.43 cd</td>
<td>142.39 abc</td>
<td>34.74 ab</td>
</tr>
<tr>
<td>H$_2$O$_2$ (1.0 mM)</td>
<td>129.65 d</td>
<td>138.57 bcd</td>
<td>36.21 a</td>
</tr>
<tr>
<td>SA (0.5 mM)</td>
<td>142.07 abc</td>
<td>149.40 a</td>
<td>29.50 ef</td>
</tr>
<tr>
<td>SA (1.0 mM)</td>
<td>138.60 bcd</td>
<td>144.94 ab</td>
<td>34.69 ab</td>
</tr>
</tbody>
</table>

Continued Table 3

<table>
<thead>
<tr>
<th>Treatments</th>
<th>CAT</th>
<th>POD</th>
<th>PPO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µ mole / g fresh weight</td>
<td>µ mole / g fresh weight</td>
<td>Unit / g fresh weight</td>
</tr>
<tr>
<td>H$_2$O$_2$ (0.5 mM)</td>
<td>74.54 cde</td>
<td>76.77 cd</td>
<td>15.21 ef</td>
</tr>
<tr>
<td>H$_2$O$_2$ (1.0 mM)</td>
<td>68.17 f</td>
<td>72.63 def</td>
<td>16.89 cd</td>
</tr>
<tr>
<td>SA (0.5 mM)</td>
<td>77.09 cd</td>
<td>82.82 ab</td>
<td>12.92 h</td>
</tr>
<tr>
<td>SA (1.0 mM)</td>
<td>70.72 ef</td>
<td>78.36 bc</td>
<td>14.78 fg</td>
</tr>
</tbody>
</table>

Data are means of two seasons -Means followed by the same letter for each tested parameter for both cultivars are not significantly different by Duncan’s test (P ≤ 0.05).
Malondialdehyde (MDA) and Electrolyte Leakage (EL)

The content of MDA, a product of lipid peroxidation, has been considered as an indicator of oxidative damage, while electrolyte leakage represents cell membrane injury. Floridat cultivar was characterized by significant decreases in the MDA and EL than streenb cultivar in leaf tissues under all treatments (Table 4). Either H$_2$O$_2$ or SA treatments (0.5 mM and 1.0 mM) caused significant decreases in MDA and EL values in two tomato cultivars relative to control plants. Moreover, H$_2$O$_2$ treatment at 1.0 mM caused the highest significant decrease in the two parameters.

Environmental stresses always result in cellular membrane injuries including the increase of membrane permeability and MDA content due to oxidative damage and they are considered to be sensitive stress markers (He et al., 2009; Moskova et al., 2009). AzvedoNeto et al. (2005) reported that addition of H$_2$O$_2$ to the nutrient solution induced salt tolerance by enhanced activities of antioxidants and reduced peroxidation of membrane lipids in leaves and roots of maize. Likely, He et al. (2009) stated that seed treatment with H$_2$O$_2$ could greatly alleviate the deleterious effects of drought on the membrane integrity and stability in the wheat seedlings through reducing membrane damage rate and MDA content. Liu et al. (2010);Abass and Mohamed (2011);Terzi et al. (2014) summarized that H$_2$O$_2$ treatments at low concentrations enhanced stress tolerance by increasing some metabolite and phytohormone levels as well as decreasing MDA and endogenous H$_2$O$_2$ concentration in plants.

The decrease of MDA and electrolyte leakage in tomato leaf tissues under application of SA is consistent with that reported by Stevens et al.(2006)and Agamy et al. (2013) who mentioned that SA application regulates and maintains the membrane functions of tomato plants. In addition, SA can diminish the injuries in cell membranes through enhancing the antioxidant potential of plant under stress conditions and partly maintained membrane permeability as well as reduced the amount of ion leakage (Tasgin et al., 2006; Orabi et al., 2010; 2013). Kabiri et al. (2014) mentioned that pretreatment with SA was evidenced by a reduction in the level of lipid peroxidation and leakage of electrolytes from plant tissues as well as by more intensive growth processes as compared to control plants.

Table 4. Effect of H$_2$O$_2$ and salicylic acid on malondialdehyde (MDA) and electrolyte leakage (EL) of two tomato cultivars grown under low temperature conditions

<table>
<thead>
<tr>
<th>Treatments</th>
<th>MDA µ mole / g fresh leaf</th>
<th>EL %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0.5 mM)</td>
<td>7.86 d</td>
<td>7.28 e</td>
</tr>
<tr>
<td>H$_2$O$_2$ (1.0 mM)</td>
<td>6.57 f</td>
<td>4.67 h</td>
</tr>
<tr>
<td>SA (0.5 mM)</td>
<td>8.81 c</td>
<td>8.07 d</td>
</tr>
<tr>
<td>SA (1.0 mM)</td>
<td>7.48 e</td>
<td>6.23 g</td>
</tr>
</tbody>
</table>

Data are means of two seasons -Means followed by the same letter for each tested parameter for both cultivars are not significantly different by Duncan’s test (P ≤ 0.05).

Fruits yield

Floridat cultivar was characterized by significant increases in fruits yield (g/plant) than streenb cultivar under all treatments (Table 5). All H$_2$O$_2$ and SA treatments (0.5 mM and 1.0 mM) caused significant increases in yield of the two tomato cultivars relative to control plants. Moreover, H$_2$O$_2$ treatments (0.5 mM and 1.0 mM) caused the highest significant increase in the yield of both tomato cultivars followed by SA treatments (0.5 mM and 1.0 mM).

These results are in good agreement with those reported by Hameed et al. (2004) who stated that exogenous application of H$_2$O$_2$ provided more vigorous root system in wheat, that can be used to increase nitrogen uptake resulting in better growth and yield (Liao et al., 2004).

Regarding SA, foliar application of SA significantly increased yield and its components of maize (Abdel-Wahed et al., 2006) and wheat plants (Iqbal and Ashraf, 2006). Cucumber and tomato fruit yield was enhanced significantly when the plants were sprayed with lower concentrations of salicylic acid (Larque-Saavedra and Martin-Mex, 2007).
Fruits quality

Total soluble solids (TSS)

Fruits of floridat cultivar were characterized by significant increases in the TSS than fruits of streenb cultivar (Table 5). All H$_2$O$_2$ and SA treatments (0.5 mM and 1.0 mM) caused significant increases in TSS in fruits of both tomato cultivars relative to control plants. Moreover, 1.0 mM H$_2$O$_2$ caused the highest significant increase in the TSS followed by 1.0 mM SA. TSS values associated with taste and had significant indication for improvement in yield quality as reported by Vural et al. (2000). Ozaki et al. (2009) reported that application of H$_2$O$_2$ could increase soluble sugar content in melon fruits. Regarding SA effect, Chandra et al. (2007) reported that application of salicylic acid increased total soluble sugar and soluble protein of cowpea plants. Moreover, Abdullahi et al. (2011) show that plant growth and TSS levels were increased as a result of salicylic acid treatment.

Table 5. Effect of H$_2$O$_2$ and salicylic acid on fruit yield, total soluble solids and antioxidant activity of two tomato cultivars grown under low temperature conditions

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fruits yield</th>
<th>Total soluble solids</th>
<th>Antioxidant activity (40µL)</th>
<th>Antioxidant activity (80µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>403.19 f</td>
<td>445.53 e</td>
<td>4.51 g</td>
<td>4.73 f</td>
</tr>
<tr>
<td>H$_2$O$_2$ (0.5 mM)</td>
<td>691.22 b</td>
<td>801.46 a</td>
<td>4.71 f</td>
<td>5.03 e</td>
</tr>
<tr>
<td>H$_2$O$_2$ (1.0 mM)</td>
<td>706.46 b</td>
<td>820.14 a</td>
<td>6.30 b</td>
<td>6.76 a</td>
</tr>
<tr>
<td>SA (0.5 mM)</td>
<td>608.36 d</td>
<td>678.06 bc</td>
<td>4.81 f</td>
<td>5.16 d</td>
</tr>
<tr>
<td>SA (1.0 mM)</td>
<td>640.70 cd</td>
<td>698.53 b</td>
<td>5.20 d</td>
<td>5.60 c</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>77.63 e</td>
<td>85.77 b</td>
</tr>
</tbody>
</table>

Data are means of two seasons -Means followed by the same letter for each tested parameter for both cultivars are not significantly different by Duncan’s test (P ≤ 0.05).

Antioxidant activity

Fruits of floridat cultivar were characterized by significant increases in the antioxidant activity than fruits of streenb cultivar at the two samples (40µL and 80µL) (Table 5). All H$_2$O$_2$ and SA treatments (0.5 mM and 1.0 mM) caused significant increases in antioxidant activity in fruits of both tomato cultivars at two samples (40µL and 80µL) relative to control plants. Moreover, 1.0 mM H$_2$O$_2$ caused the highest significant increase in the antioxidant activity followed by 1.0 mM SA. The increase in antioxidant activity due to H$_2$O$_2$ application is similar to those reported by AzevedoNeto et al. (2005) who stated that addition of H$_2$O$_2$ to the nutrient solution enhanced activities of antioxidants in leaves and roots of maize.

Regarding SA effect, Boukraa et al. (2014) and Fayez and Bazaid (2014) stated that antioxidant activity mainly depends on the dissociation of hydrogen radical from phenolic compounds to form a stable compound with DPPH radical. There is an important link between plant antioxidant ability and the applied doses of the SA (Chen et al., 1997). Salicylic acid activates the resistance system and increases the cell antioxidant capacity (Larque-Saavedra and Martin-Mex, 2007) which leads to cell membrane protection and synthesis of photosynthetic pigments and finally improves the growth indexes and secondary metabolites synthesis (Momeny et al., 2012). Many studies suggest the predominant role of the salicylic acid in the modulation of the response of plants towards abiotic and biotic stresses by induction of the antioxidant ability (Orabi et al., 2013; Boukraa et al., 2014). Asadi et al. (2013) showed that exogenous application of salicylic acid alleviated the toxic action induced by salinity and decreased lipid peroxidation rates with increasing antioxidant activity.

Conclusion

It could be concluded that all H$_2$O$_2$ and SA treatments (0.5 mM and 1.0 mM) have positive significant effect on growth, growth regulators (GA$_3$, IAA and ABA), antioxidant enzymes (POD and PPO) activity, fruits yield quantity and quality (total soluble solids and antioxidant activity) of the two tomato cultivars grown under low temperature conditions in sand-ponic culture. On the other hand, these treatments caused significant decreases in CAT enzyme activity, MDA and EL values. It is worthy to mention that, H$_2$O$_2$ treatment at 1.0 mM was the most pronounced treatment.
References


