Antioxidant enzyme and plant productivity changes in field-grown tomato under drought stress conditions using exogenous putrescine

Akram Farzane¹, Hossein Nemati²*, Mahmoud Shoor³ and Hossein Ansari³

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¹PhD student, Department of Horticulture and Landscape Engineering, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.
²Department of Horticulture and Landscape Engineering, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.
³Department of Water Science and Engineering, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.
*Corresponding author; E-mail: nemati@um.ac.ir

Abstract
Scarcity of water is a severe environmental constraint and the growth and development of tomato (Lycopersicon esculentum Mill.) seedlings are strongly inhibited under drought stress. Putrescine (PUT) is involved in drought stress tolerance. There is little information about the exogenous application of PUT on tomato seedlings under drought stress. This experiment was carried out to examine the application of putrescine on some physiological and biochemical characteristics of tomatoes under water stress. Plants were exposed to three water conditions: 100% (ET₀) as the non-drought treatment (or control), 75 and 50% of ET₀ as the water-stress treatments. Foliar application of PUT was done using 0.5 and 1 mgL⁻¹ concentrations, while control plants were sprayed with distilled water. Drought stress significantly affected plant growth and productivity, and reduced leaf area (LA), plant height and fruit yield at 50% ET₀ compared to the control treatment. Also antioxidant enzymes such as SOD, PPO and CAT were increased by increasing the drought stress level. Moreover, the activity of PPO, CAT and SOD enzymes were elevated by the PUT foliar application. Results showed that appropriate PUT doses persuade biosynthesis of important metabolites, which may reduce the negative effects of drought stress on tomato plants.

Keywords: CAT; ET₀; PPO; SOD; Tomato.


Abbreviations
APX: Ascorbate peroxidase
CAT: Catalase
ET₀: Crop evapotranspiration
LA: Leaf area
MDA: Malondialdehyde
PAs: Polyamines
POX: Peroxidase
PPO: Polyphenol oxidase
PUT: Putrescine
ROS: Reactive oxygen species
SOD: Superoxide dismutase
Spd: Spermidine
Spm: Spermine

Introduction
Drought is one of the most significant abiotic stresses that limit the growth and productivity of crop plants (Petrozza et al. 2014). About one-third of the world’s agricultural land is located in low water areas (Klunklin and Savage 2017). Drought
is the single most common cause of severe crop production reduction in developing countries, and global warming is predicted to further exacerbate drought's impact (Ribaut et al. 2012). Hassan et al. (2013) reported that drought stress had negative effects on herb and oil yields, RWC, chlorophyll and N, P and K percentages compared to the non-drought stress condition.

Drought stress significantly increased ROS production such as H$_2$O$_2$ and O$_2$ and caused oxidative stress to lipids assessed by the increase in MDA content (Talaat et al. 2015). Polyamines (PAs) biosynthesis was induced under abiotic stress, especially, drought stress (Yang et al. 2007; Kubis 2008). So, some mechanisms occurred in plants in the face of drought stress (Groppa and Benavides 2008).

PAs improved drought tolerance in terms of dry matter yield and net photosynthesis was associated with the maintenance of leaf water status and improved water use efficiency. (Farooq et al., 2009). Also, Pottosin et al. (2014) reported that PAs has an important role in plant growth and development and stress tolerance. There has been much research on the effects of PAs on improving plant resistance to environmental conditions such as heavy metal, salt and drought stress (Zapata et al. 2004; Xu et al. 2011; Ghosh et al. 2012; Kubis et al. 2014; Li et al. 2015; Sanchez-Rodriguez et al. 2016). Exogenous Spd effectively alleviated the harmful effects of drought stress, as demonstrated by lower O$_2$ generation rate, H$_2$O$_2$ and MDA content, higher relative water content, chlorophyll content and antioxidant enzyme activities (SOD, POX, CAT, APX) as compared to untreated plants (Li et al. 2015). In another study, proline and MDA accumulation were increased under drought stress in cucumber. Also, exogenous application of PAs effectively reduced the membrane injury, lipid peroxidation and elevated the proline accumulation. Hydrogen peroxide and superoxide radical contents were also reduced in the stressed plants after Spd pretreatment (Kubis et al. 2014). Talaat et al. (2015) suggested that foliar application of Spm enhanced the antioxidant enzyme activities which confined the agglomeration of both MDA and ROS, therefore, diminished the negative effects of drought stress (Talaat et al. 2015). Radhakrishnan and Lee (2013) reported that the application of Spm could be exploited to alleviate a moderate level of osmotic stress through the regulation of stress-related components such as photosynthetic pigments, plant hormones and antioxidants.

There are many studies of PAs that were carried out on vegetative crops (Sanchez-Rodriguez et al. 2016) but, available information about the effects of PAs on tomato (Lycopersicon esculentum Mill.) plants is very restricted. Tomato is a major vegetable crop that has achieved tremendous popularity over the last century. It is grown practically in every country of the world in outdoor fields, greenhouses and net houses. Aside from being tasty, tomatoes are a very good source of vitamins A and C (Farooq et al. 2005).

Generally, tomato physiology and the synthesis of secondary metabolites such as phenolic acids, flavonoids and terpenoids are affected by environmental stresses (Barbagallo et al. 2013). Yin et al. (2010) reported that tomato fruit quality characters such as soluble solids (organic acids, amino acids, sugars), which are
major compounds that accumulate in the fruit, increased during the drought stress. The quality of tomato fruits depends on the amount of soluble solids (which provide water, taste and flavor) and antioxidant production (Apple and Hirt 2004) such as enzymatic (CAT, glutathione reductase, SOD, etc.) and non-enzymatic (carotenoids, flavonoids, vitamins E and C, phenolic compounds) systems.

The aims of this study were to: (i) characterize the response of tomato seedlings grown under drought stress and foliar PUT spray; and (ii) investigate their interactions on the growth characteristics and antioxidant contents in tomato plants.

Material and Methods

Plant materials and treatments

This experiment was designed to observe the effect of putrescine on tomato seedlings under drought stress. Seeds of tomato were sown in plastic trays and maintained in a greenhouse up to the 4-leaf stage, at the Department of Horticulture, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran. The experimental design was a split-plot design with three water stress levels as the main plots and three PUT treatments (0, 0.5 and 1 mg L⁻¹) as subplots with three replications. The subplot area was 1 m² (1 m × 1 m) and consisted of four plants per plot. After 70 days from sowing, seedlings with uniform growth were transplanted to the experimental field with a 50 cm inter-seedling spacing. Based on evapotranspiration (ET₀), water stress was conducted on tomato plants at three levels (100, 75 and 50% ET₀). The foliar spray was applied five times (during two months) to tomato plants during growth and fruit set with PUT at 0, 0.5 and 1 mg L⁻¹.

Plant height, leaf area and fruit yield

Plant height was measured at the end of the harvesting season. The total yield of tomato fruits per plant was measured at different harvesting times. The LA was measured by Windias (Delta-T Co, England) leaf area meter.

Assays of enzymatic and non-enzymatic antioxidants

Frozen samples (200 mg) were homogenized in 3 mL of 25 mM Na-Pi buffer (pH 6.8) and centrifuged at 15,000 g for 15 min at 4°C. The supernatant was used for the assay. The activity of CAT was measured in a reaction mixture consisted of 10 mM H₂O₂, 25 mM Na-phosphate buffer (pH 6.8), and diluted enzyme extract in a total valium of 3 mL. The decomposition of H₂O₂ was followed by the decline in absorbance at 240 nm within 1 min, using a spectrophotometer (T90, Beijing Karaltay Scientific Instruments, China). CAT activity was expressed based on changes in the absorbance against mg of protein of the extract. Protein content was determined by the method of Bradford (1976), using BSA as the standard (Azimi et al., 2016).

To assay the SOD activity, frozen samples (200 mg) were homogenized in 3 mL of 50 mM K-Pi buffer (pH 7.8). The homogenate was centrifuged at 15,000 for 15 min. All operations were performed at 4 °C. Reaction mixture (3 mL) consisted of 50 mM buffer K-Pi (pH 7.8), 0.1 mM EDTA, 13 mM-methionine, 75 lM NBT, 20 lM riboflavin and enzyme extract. One unit of SOD
activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of NBT reduction measured at 560 nm and was expressed as mg of protein (Azimi et al., 2016).

To determine the activity of antioxidants, 100 mg of the fresh leaf was homogenized with ethanol 96% and the mixture was moved later to the centrifuge equipment at 3500 rpm for 5 minutes. Then approximately 0.02-0.06 mg mL$^{-1}$ of plant extracts was melted and added to the 800 μM of DPPH (0.05 M pH 7.4) to measure the absorbance at 517 nm (ABE et al. 1998). The total antioxidant activity was measured according to the following formula:

$$\text{Antioxidant activity} = \left(\frac{\text{Ai} - \text{At}}{\text{Ai}}\right) \times 100$$

where Ai is the absorbance of the control and At is the absorbance of test treatment.

To determine the PPO, one mL of the reaction mixture was used which contained 20 μl enzyme extract and 10 mmol/L phosphate buffer, pH 7.0. Each sample was aerated for 2 min in a small test tube followed by the addition of catechol as the substrate at a final concentration of 20 mmol/L. PPO activity was presented as the change in one unit of absorbance at 420 nm per minute per gram fresh weight of the sample (Zheng et al. 2005).

**Statistical analysis**

Effects of water stress, PUT and their interaction were determined by analysis of variance according to the general linear model procedure of SAS (version 8.2; SAS Institute, Cary, N.C.). Means were compared using Tukey’s HSD test ($p \leq 0.05$). The graphs were drawn by the Excel 2007 software.

**Results**

**Effect of PUT on physiological traits of tomatoes under drought stress**

The growth characters, yield and antioxidant activities exposed to drought stress and PUT application were significantly affected, except for SOD and CAT under drought stress and leaf area under PUT application. Interaction of drought stress with PUT was significant for all traits under study (Table 1). The main effects of drought stress and foliar PUT application on tomato plants are presented in Tables 2 and 3, respectively. The highest and lowest amounts of plant height (51.88-44.66 cm) and LA (6250.66-3489.03 mm$^2$) were observed in 100% and 50% ET$_0$ treatments, respectively (Table 2). The increase in drought stress level was associated with a decrease in plant growth characteristics (plant height and LA). Increasing the PUT level had a positive effect on plant height but there were no significant differences between 0.5 and 1 mg L$^{-1}$ PUT levels (Table 3). Also, LA was not affected under PUT treatments, however, PUT sprays caused a slight increase in LA. As shown in Figure 1a, plant height was affected by the interaction of drought stress with PUT. At no PUT, plant height was highest under 75% ET$_0$. At 0.5 mg L$^{-1}$ PUT the highest plant height was obtained under normal condition, but at 1 mg L$^{-1}$ PUT, the plant height under 75% ET$_0$ was close to the normal condition. Although LA was improved by the application of PUT, however, the effect of PUT was more pronounced under 50% ET$_0$ (Figure 1b). Fruit yield is a vital character reflecting the PUT and drought stress effects. Results showed an increase of about 24 and
27% in fruit yield at 1 and 0.5 mg L\(^{-1}\) PUT levels, respectively, as compared to the control (Table 3).

**Table 1.** Analysis of variance for the studied traits in tomato plants under three drought stresses (DS) and sprayed with putrescine (PUT).

<table>
<thead>
<tr>
<th>SV</th>
<th>df</th>
<th>Plant height</th>
<th>LA</th>
<th>Fruit yield</th>
<th>SOD</th>
<th>PPO</th>
<th>CAT</th>
<th>Antioxidant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>187.1**</td>
<td>7932175.5**</td>
<td>359783.2*</td>
<td>1589082.3**</td>
<td>37041.3**</td>
<td>33354.7**</td>
<td>37.8ns</td>
</tr>
<tr>
<td>DS</td>
<td>2</td>
<td>141.4**</td>
<td>17713614.0**</td>
<td>494200.7*</td>
<td>122983.4ns</td>
<td>26481.8*</td>
<td>2668.9ns</td>
<td>422.5**</td>
</tr>
<tr>
<td>Error a</td>
<td>4</td>
<td>10.2</td>
<td>721102.9</td>
<td>44264.0</td>
<td>209000.2</td>
<td>1944.4</td>
<td>559.4</td>
<td>12.8</td>
</tr>
<tr>
<td>PUT</td>
<td>2</td>
<td>258.3**</td>
<td>210857.7ns</td>
<td>267305.5*</td>
<td>1234474.5**</td>
<td>22650.1*</td>
<td>16544.3**</td>
<td>304.9**</td>
</tr>
<tr>
<td>DS x PUT</td>
<td>4</td>
<td>32.4*</td>
<td>258845.2*</td>
<td>196877.6**</td>
<td>872623.6**</td>
<td>45289.2**</td>
<td>22717.7**</td>
<td>485.6**</td>
</tr>
<tr>
<td>Error b</td>
<td>12</td>
<td>6.5</td>
<td>78977.8</td>
<td>60325.6</td>
<td>122166.2</td>
<td>4140.6</td>
<td>1644.6</td>
<td>12.9</td>
</tr>
</tbody>
</table>

LA: Leaf area; SOD: Superoxide dismutase; PPO: Polyphenol oxidase; CAT: Catalase; ns, *, **: Not significant and significant at p ≤ 0.05, respectively.

**Table 2.** Effects of different drought stress levels on morphological and biochemical characters of tomato plants.

<table>
<thead>
<tr>
<th>Drought stress (ET(_0))</th>
<th>Plant height (cm)</th>
<th>LA (mm(^2))</th>
<th>Fruit yield (g per plant)</th>
<th>SOD (U g(^{-1}) FW)</th>
<th>PPO (U g(^{-1}) FW)</th>
<th>CAT (U g(^{-1}) FW)</th>
<th>Antioxidant activity (U g(^{-1}) FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 % (Control)</td>
<td>51.88a</td>
<td>6250.66a</td>
<td>1420.72ab</td>
<td>1139.14a</td>
<td>117.77b</td>
<td>179.36a</td>
<td>78.16c</td>
</tr>
<tr>
<td>75 %</td>
<td>51.11a</td>
<td>5299.47b</td>
<td>1568.50a</td>
<td>1172.74a</td>
<td>174.66ab</td>
<td>167.73a</td>
<td>85.10b</td>
</tr>
<tr>
<td>50 %</td>
<td>44.66b</td>
<td>3489.03c</td>
<td>1109.44b</td>
<td>1356.31a</td>
<td>226.22a</td>
<td>201.62a</td>
<td>91.85a</td>
</tr>
</tbody>
</table>

LA: Leaf area; SOD: Superoxide dismutase enzyme activity; PPO: Polyphenol oxidase enzyme activity; CAT: Catalase enzyme activity; Values followed by the same letter within each column do not differ significantly at p ≤ 0.05 based on Tukey's test.

**Table 3.** Effects of different putrescine (PUT) levels on morphological and biochemical characters in tomato plants.

<table>
<thead>
<tr>
<th>PUT (mg L(^{-1}))</th>
<th>Plant height (cm)</th>
<th>LA (mm(^2))</th>
<th>Fruit yield (g per plant)</th>
<th>SOD (U g(^{-1}) FW)</th>
<th>PPO (U g(^{-1}) FW)</th>
<th>CAT (U g(^{-1}) FW)</th>
<th>Antioxidant activity (U g(^{-1}) FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>43.1b</td>
<td>4836.4a</td>
<td>1168.5b</td>
<td>1008.3b</td>
<td>125.66b</td>
<td>147.47b</td>
<td>78.58b</td>
</tr>
<tr>
<td>0.5</td>
<td>51.4a</td>
<td>5106.1a</td>
<td>1484.4a</td>
<td>1009.5b</td>
<td>167.46ab</td>
<td>170.66b</td>
<td>86.66b</td>
</tr>
<tr>
<td>1</td>
<td>53.1a</td>
<td>5096.6a</td>
<td>1445.8ab</td>
<td>1650.4a</td>
<td>225.55a</td>
<td>230.56a</td>
<td>89.87a</td>
</tr>
</tbody>
</table>

LA: Leaf area; SOD: Superoxide dismutase enzyme activity; PPO: Polyphenol oxidase enzyme activity; CAT: Catalase enzyme activity; Values followed by the same letter within each column do not differ significantly at p ≤ 0.05 based on Tukey's test.
Figure 1. Effect of interaction of drought stress with putrescine on tomato plant height (a) and leaf area (b).

Figure 2. Effect of interaction of drought stress with putrescine on tomato fruit yield per plant.
However, there was an interaction between water stress and PUT in terms of fruit yield (Figure 2). Application of PUT increased fruit yield under both 100 and 50% ET₀, but not under 75% ET₀. The lowest fruit yield was obtained under 50% ET₀, but the fruit yield was close to 100 and 75% ET₀ at 1 mg L⁻¹ PUT.

Drought stress had a significant (p≤0.05) effect on PPO and antioxidant activities and increased their values. Although the SOD and CAT activities increased under drought conditions, the change was not significant (Table 2). The PUT application increased the rate of all antioxidant enzymes significantly. The amount of increase as compared to the control treatment was as follows: SOD (63.68%), PPO (79.49%), CAT (56.34%) and antioxidant activity (14.36%). Therefore, PUT spray treatment had the highest effect on PPO and SOD enzyme activities (Table 3).

There were interesting results in examining the interactions of drought stress with PUT spray on tomato plants in terms of enzyme activity. According to Figure 3 (b and c), simultaneous increasing of water stress and PUT concentration enhanced the antioxidant and CAT activities. On the other hand, application of 1 mg L⁻¹ PUT increased PPO only under 100 and 75% ET₀ (Figure 3a) and application of 0.5 mg L⁻¹ PUT increased SOD activity under all water levels (Figure 3d). However, at higher levels of drought condition (50% ET₀) and PUT concentration (1 mg L⁻¹), PPO (Figure 3a) and SOD (Figure 3d) activities dropped.
Discussion

The results demonstrated that drought stress decreased plant height and LA as compared with the non-drought conditions. Other researchers presented the negative effects of drought stress on growth characteristics in vegetable crops and our results were in harmony with their results (Farooq et al., 2009 and Hassan et al., 2018). Reduction in nutrient uptake under water stress is one of the most important reasons to describe the reduction in growth characters (Hassan et al. 2018). However, regarding the fruit yield per plant, the plants irrigated under 75% ET₀ had higher (although not significant) fruit yield as compared to 100% ET₀ because of higher plant growth characters (stem height and LA) in the control condition (100 % ET₀). Plants confront water stress by pursuing various tactics including regulation in photosynthetic factors, cumulating of suitable solutes (glycine, proline, betaine) and activating genes to produce the non-antioxidant and antioxidant enzymes (Farooq et al. 2009; Morshedloo et al. 2017; Mohammadi et al. 2018). Researchers have reported the increase in CAT, SOD, PPO and antioxidant activities in plants under drought stress conditions (Li et al. 2013, Palma and Carvajal 2016; Hassan et al. 2018) which are in close agreement with our results.

When the seedlings were treated with different PUT levels (0.5 and/or 1 mg L⁻¹), the fruit yield and stem height enhanced significantly (Table 2). So foliar application of PUT improves the growth of tomatoes (Amin et al. 2008). Mohammadi et al. (2018) reported almost similar results on Thymus vulgaris L. seedlings when sprayed with PUT. Talaat et al. (2005) indicated that exogenous application of putrescine on periwinkle transplants considerably increased plant growth at successive developmental stages. Youssef et al., (2004) presented similar results in Datura species. Amin et al. (2011) reported that foliar application of PUT up to 100 mg L⁻¹ significantly increased plant height, the number of leaves/plant, fresh weight of leaves/plant, fresh weight and dry weight/plant, LA, LA/plant, and bulb length and diameter compared to untreated control plants.

The seedlings that were treated with 1 mg L⁻¹ PUT had the highest amount of SOD, PPO, CAT and antioxidant activities as compared to the control treatment on the average of three water stress conditions. Furthermore, application of PUT (0.5 and/or 1 mg L⁻¹) increased the antioxidant enzyme activities (SOD and CAT) in the drought-stressed treatments in most cases as compared to the non-drought condition. Toro-Funes et al. (2013) stated that due to the potential antioxidant activity of PAs, SOD activity increased in thyme plants when treated with PUT under water stress conditions. According to Li et al. (2015), the application of exogenous Spd increased SOD, POX, CAT, APX activities and reduced the undesirable effects of drought stress. Based on Hassan et al. (2018), proline content, and CAT and
SOD enzyme activities were improved by applying Spm or Spd. H$_2$O$_2$ production was restricted and MDA accumulation was limited and hence the membrane stability was retained and the water stress damage was alleviated accordingly. In another study, Spm led to more effective ROS scavenging (less tissue damage) in tomato fruits and contributed to higher dehydration tolerance in this crop (Sanchez-Rodriguez et al. 2016).

Conclusions
According to the results of this study, foliar application of PUT prevented the fruit yield reduction under drought stress conditions. Also, the amount of SOD at 0.5 mg L$^{-1}$ PUT and the rate of CAT at 0.5 and 1 mg L$^{-1}$ of PUT increased under both drought stresses as compared to non-drought conditions. Although in the present study, plant height and fruit yield were diminished due to drought stress, the foliar application of PUT alleviated these reductions.

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Conflict of Interest
The author declare that they have no conflict of interest with any organization in relation to the subject of the manuscript.

References


اثر محلول پاشی پوترسین بر تغییر آنزیم‌های آنتی اکسیدانی و عملکرد گیاه گوجه فرنگی مزرعه‌ای تحت شرایط تنش خشکی

آکرم فرزانه، سید حسین نعمتی، حسین انصاری

چکیده

رشد و نمو گوجه فرنگی (Lycopersicon esculentum Mill.) تحت تنش خشکی به شدت کاهش می‌یابد. هورمون پوترسین نقش مهمی در تحمل گیاه به تنش آبی ایجاد می‌کند. اما در حال حاضر، اطلاعات محدودی در زمینه اثر استفاده خارجی از هورمون پوترسین روی گیاهان، و یکی از آنها گیاه گوجه فرنگی وجود دارد. این تحقیق بر پایه بررسی اثرات کاربرد خارجی پوترسین (محلول پاشی) روی ویژگی‌های بیوشیمیایی و فیزیولوژیکی گیاه گوجه فرنگی تحت شرایط تنش خشکی اجرا شد. گیاهان تحت سطح تنش آب شامل شاهد یا 100% (به عنوان شرایط بدون شریک)، 75% و 50% (نیاز آبی گیاه ET) قرار داده شدند. پوترسین به صورت محلول پاشی برگی در غلظت‌های 0/5 و 1/0 میلی لیتری مورد استفاده قرار گرفت و برای تیمار شاهد نیز از آب مقطر استفاده شد. تنش خشکی موجب اختلال در صفات رشدی و عملکرد میوه گوجه فرنگی شد و عملکرد میوه طول طول زمان و قیمت میوه در تیمار تنش خشکی 50% نیاز آبی نسبت به شرایط بدون تنش (شاهد) کاهش یافت. همچنین با افزایش سطح تنش خشکی، عملکرد میوه به طور قابل توجهی کاهش یافت. به‌طوری‌که استفاده از هورمون پوترسین و یک نوع زیست‌محیطی روی گیاهان تحت تنش خشکی (به‌طوری‌که عملکرد میوه در گیاهان کاهش یافته تحت تنش خشکی را بهبود بخشید. افزایش سطح پاشی پوترسین باعث افزایش فعالیت آنزیم‌های CAT، PPO و SOD در گیاهان تحت تنش خشکی شد. نتایج حاصل از این تحقیق نشان داد که استفاده از محلول پاشی پوترسین در غلظت‌های مناسب عامل محور مناسبی برای پوشش گیرنده‌های انرژی از طریق بهبود عملکرد گیاهان تحت تنش خشکی را روي گوجه فرنگی جیران کند.

واژه‌های کلیدی: گوجه فرنگی، نیاز آبی گیاه، CAT، PPO، SOD، ET 12020, 10(1): 29-40