

Foliar application of potassium on antioxidant enzyme activities of tomato plants under drought stress

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: Water stress negatively affects productivity in crops, while the foliar application of potassium-containing compounds may be helpful in reducing the drought effects. This study evaluated the efficacy of foliar applied potassium chloride (control - distilled water spray -, 3 and 6 mM⁻¹) on tomato plants under drought stress. Three irrigation levels were maintained at 100, 75 and 50% according to evapotranspiration designated as well watered, moderate and severe drought stressed. Increasing drought stress significantly reduced plant growth and yield. The foliar applied KCl produced maximum leaf area, stem diameter and length, plant yield under each drought stress conditions compared to control. The minimum of growth factors were obtained by control under severe stress. Highest yield per plant was also recorded for foliar applied KCl under moderate condition than other treatments. Foliar applied KCl alone decreased the SOD, CAT and PPO in well-watered condition but KCl application on tomato plants under drought stress induced the antioxidant enzyme activities more than control well-watered treatment.

1. Introduction

The major limitation for plant growth and crop production in arid and semi-arid regions is soil water availability. Plants that are continuously exposed to drought stress can form ROS (Reactive oxygen species), which leads to leaf damage (Foyer *et al.*, 2002; Oerke and Dehne, 2004; Cakmak, 2005) and, ultimately, decreases crop yield. The decrease in soil water potential causes alteration in minerals uptake by plant roots and reduction in leaf expansion under drought stress conditions (Kaminek *et al.*, 1997; Pospisilova *et al.*, 2000).

Drought is becoming a serious threat to crop production worldwide resulting in 67 and 82% reduction in K uptake under mild and severe water stress conditions (Baque *et al.*, 2006). During drought stress, root growth and the rates of K⁺ diffusion in the soil towards the roots were

both restricted, thus limiting K acquisition. The resulting lower K concentrations can further depress the plant resistance to drought stress, as well as K absorption, which ultimately leads to reduction in the fruit size, plant yield, lack in red color of tomato and fruit quality (Bidari and Hebsur, 2011; Afzal *et al.*, 2015), and deteriorated photosynthesis, enzyme activation in plants (Marschner, 1995; Garg *et al.*, 2004; Afzal *et al.*, 2015). Maintaining adequate plant K is, therefore, critical for plant drought resistance.

When plants were supplied with different K⁺ concentrations and then subjected to drought stress, their stomatal conductance was more markedly reduced in normal K plants than in low K plants (Wang *et al.*, 2013). During drought stress, the stomata cannot function properly in K⁺-deficient plants, resulting in greater water loss. Drought stress did not decrease water use efficiency (WUE), whereas it did increase WUE by rapid stomata closing during water deficit (Egilla *et al.*, 2005). Adequate levels of K nutrition enhanced plant drought resistance, water relations, WUE and plant growth under drought conditions (Wang *et al.*, 2013). Besides various adaptive mechanisms; potassium (K) sprayed under drought condition can improve the tolerance of crop plants to various types of abiotic stresses, and it also improved subsequent growth and yield. Mengel and Kirkby (2001) reported that K improves physiological processes by the regulation of turgor pressure and photosynthesis; translocation of cations and enzymes activation, while, Cakmak (2005) also observed that plant suffering from drought stress required more internal K. Yield limiting effect of water deficit could be overcome by increasing K supply (Damon and Rengel, 2007). In legumes, devastating effects of drought can be alleviated by rich K supply (Sangakkara *et al.*, 2000). A close relationship between K nutritional status and plant drought resistance has been demonstrated.

The bottom lines of the reviewed results in this section indicate that under drought stress conditions, yield losses can be minimized by the sufficient supply of K. However, its application effect at tomato growth stages is not well understood yet. The objective of present work was to study the possible role of K applied on tomato plant under drought, in mitigation of stress in terms of physiological components and antioxidant enzyme values.

2. Materials and Methods

This experiment was designed to observe the effect of KCl on tomato (*Solanum lycopersicum* L.) seedlings under drought stress. Seeds of tomato were sown in plastic trays and maintained in a greenhouse up to 4 real leafy stages, at Department of Horticulture, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran. The experimental design was a split plot design with three water stress plot as main plot and 3 KCl treatments (0, 3 and 6 mM⁻¹) as subsidiary plot with 3 replications. The subsidiary plot area was 1 m² (1×1 m) and consisted of four plants per plot. After 70 days from sowing, seedlings with uniform growth were transplanted to an experimental field with a 50 cm inter-seedling spacing. According to evapotranspiration (ET_c = crop evapotranspiration), the water stress was conducted on tomato plants at three levels well-watered (100% ET_c), moderate and severe drought stress (75 and 50% ET_c, respectively). According to Penman-Monteith equation, the crop evapotranspiration, ET_c (Formula 1), is calculated by multiplying the reference crop evapotranspiration, ET_o, by a crop coefficient, K_c and ET_o (Formula 2) is calculated by E_p and K_p factors.

$$ET_c = K_c ET_o \quad [1]$$

Where ET_c = crop evapotranspiration [mm d⁻¹], K_c = crop coefficient [1.15 for tomato], ET_o = crop evapotranspiration [mm d⁻¹].

$$ET_o = E_p K_p \quad [2]$$

Where K_p = Pan coefficient (0.77), E_p pan evaporation.

The foliar spray was applied five times (during two month) to tomato plants during growth and fruit set with KCl at 0, 3 and 6 mM⁻¹ dose.

Growth and plant analysis

Plant height and stem diameter were measured at the end of the harvesting season and presented as cm and mm respectively. The total yield of tomato fruits were measured by gram scales (g) in different harvesting times per plant. The leaf area (LA) measured by Windias (Delta-T Co, England) and presented as (mm²).

The integrated water-use efficiency (formula 3) is typically defined as the ratio of biomass produced (D, kg h⁻¹) to the rate of total water irrigation (W_i, m³ha⁻¹) and rainfall (W_p, mm) during the drought stress treat-

ment.

$$WUE = D / (W_p + W_i) \quad [3]$$

Assays of enzymatic and non-enzymatic antioxidants

Fruits were randomly selected from each treatment, at the end of the experiment. Total soluble proteins were quantified by following the protocol devised by Bradford (1976). For determination of enzymatic antioxidants, fruit samples were extracted in 50 mM phosphate buffer (pH 7.8). The extract was centrifuged at 15,000 rpm 4°C and the supernatant was used for further assay of catalase (CAT) Chance and Maehly, 1955 and poly phenol oxidase (PPO) (Siriphanich and Kader, 1985) and super oxide dismutase (SOD) activities (Giannopolitis and Ries, 1977). Tomato juice was squeezed from the fresh tomatoes onto a digital refractometer (PR-100, Atago Co. Ltd., Tokyo, Japan) to measure total soluble solids (TSS) and the results were expressed in Brix according to AOAC method 932.12 (2005). For measured the proline content, leaves were randomly selected and experimented according to Bates *et al.* (1973) method.

Statistical analysis

Effects of water stress treatments; KCl and corresponding interactions 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 Least Significant Difference (LSD, $p \leq 0.05$) according to method described by Tukey HSD. Analysis showed a significant interaction between KCl and watering treatment for the some measured parameters. The graphs draw by using excels 2010 software.

3. Results

Increasing drought stress (DS) levels significantly reduced plant growth and yield, but foliar applica-

tions of KCl improved the harmful effects of drought stress.

Comparison of means indicate that leaf area (LA) was gradually decreased with increasing drought stress (Table 1). The minimum leaf area was obtained under extreme drought conditions (50% ETc). Water stress decreased LA but exogenous application of KCl ameliorated the negative effects of water stress significantly than water spray only (Fig. 1a). However, highest LA in each treatment was recorded for foliar applied KCl than control under well-watered, moderate and severe drought stress. A minimum LA was recorded under severe drought stress, especially control \times 50% ETc treatment (Fig. 1a).

The increasing water stress decreased stem length (Fig. 1b). A maximum stem length was observed under well-watered conditions by exogenous application of 6 mM⁻¹KCl while in moderate and severe drought, foliar applied KCl had no significant effects. Minimum stem length was noted under extreme water stress without foliar KCl application. Stem diameter decrease in response to DS (Table 1). Foliar KCl application helps to plant tolerance with increasing stem diameter under DS condition. Data showed (Fig. 1c) the increasing in KCl doses (0 up to 6 mM⁻¹) lead to increasing in stem diameter in all of treatments.

The tomato yield displayed a significant reduction in response to the increasing levels of water deficit treatments. For example, under effect of 50% ETc condition, the yield decreased by 21% (Table 1) compared to control. In the other hand, plants had more vegetative growth and less yield in well-watered condition (100% ETc) than moderate DS (75% ETc). In non-DS condition, foliar spray of KCl showed similar fruit yield under well-watered, moderate and severe drought conditions. But foliar KCl application had effective enhancement on plant yield under drought stress (Fig. 1d). Tomatoes irrigated with 50% ETc without KCl foliar application also produced fruits with significantly higher juice brix value than other

Table 1 - Effects of different drought stress levels on tomato plants

Drought	LA (mm ²)	St. Length (cm)	St. Diameter (mm)	WUE	Yield plant ⁻¹	Brix ^o	Proline (µg/g.fw)	Protein (mg g ⁻¹ fw)	SOD (IU mg ⁻¹ min ⁻¹ protein)	CAT (IU mg ⁻¹ min ⁻¹ protein)	PPO (IU mg ⁻¹ min ⁻¹ protein)
Control	7555.156 a	52.66 a	14.69 a	4.360 b	1225.861 a	7.00 b	6734.877 c	1.405 a	967.28 a	178.35 c	80.777 b
75% ETc	5464.41 b	47.44 b	14.41 a	6.187 a	1404.72 a	7.11 b	9468.355 a	1.540 a	1240.31 a	196.63 b	142.66 b
50% ETc	3294.32 c	42.66 c	11.82 b	2.462 c	963.62 b	7.722 a	8161.222 b	1.382 a	1133.79 a	207.83 ab	258.11 a

Means in each column followed by similar letters are not significantly different at 0.05 probability level, using LSD (Least Significant Difference) test.

treatments (Fig. 1e).

The results in figure 1 (f) revealed that DS on tomato had higher significantly record in water use efficiency than those normally irrigated. Because of fewer yield in control treatment than 75% ETC, the results affirmed that the treatment 75% ETC and then control showed the highest water use efficiency than 50% ETC treatments. Foliar application of KCl had no significant effects of WUE under non-DS condition. Finally, the foliar application of KCl × 75% ETC showed the highest significant record in WUE in plants subjected to DS, respectively. Meanwhile, the treatment 6 mM⁻¹ KCl × 75% ETC recorded the highest

significant WUE compared to other studied treatments under well-watered and sever DS.

Leaf proline concentration responded differently to drought and K supply. Drought stress (Table 1) increased proline concentration in leaves but exogenous KCl decreased the proline content under none DS conditions (Table 2). The tomato leaves were sprayed with KCl had more proline content under drought stress than control conditions (Fig. 2 a). At 0 mM⁻¹KCl level with no DS, the proline content in leaves was 1168.4 μM/g F.wt. which increased to 13368.5 and 12111.5 μM/g F.wt. with increasing DS stress in 50% ETC with 3 and 6 mM⁻¹ KCl foliar application, respectively.

Increase in KCl doses enhanced the total soluble proteins in tomato fruits (Table 2). Maximum fruit protein content was evident at moderate stress with 3 mM⁻¹KCl followed by 0 mM⁻¹KCl with minimum value under well-watered conditions (Fig. 2b). Foliar spray with KCl had impressive effects on protein contents under well-watered and drought stress. However, the exogenous application of KCl performed better than control for fruit total soluble protein.

A rise in drought stress also amplified the antioxidants status of tomato fruits (Fig. 2c, d, e). Results showed that the enzymatic antioxidant contents in tomato fruits were sprayed by KCl, were decreased. So, the maximum SOD with no significant differences were recorded for foliar applied 0 and 3 mM⁻¹KCl under severe drought and 0 mM⁻¹KCl under moderate conditions (Fig. 2c). Increased CAT activities with no significant differences were found under medium and severe drought stress (Fig. 2d). Decreasing water level increased PPO contents for all treatments (Fig. 2e) and the highest value of PPO was obtained under sever DS but increasing in foliar application of KCl doses from 0 to 6 (mμ⁻¹) caused to decrease the PPO fruit content (Fig. 2e).

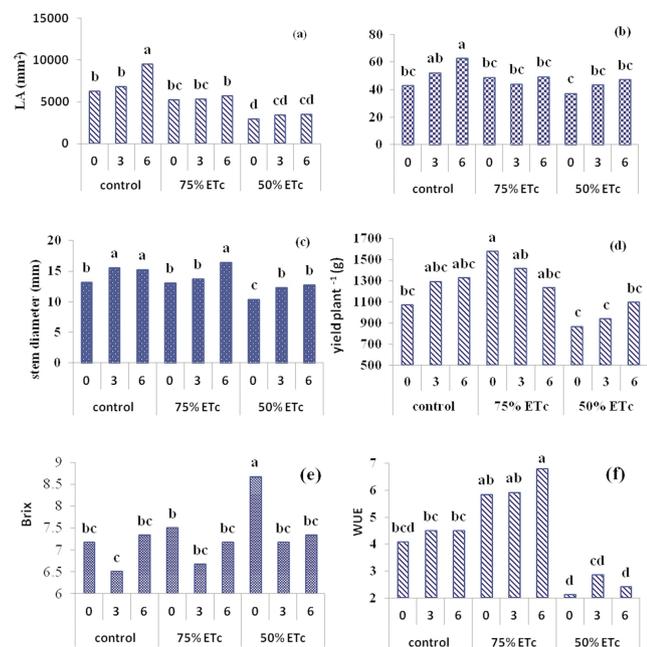


Fig. 1 - Effect of exogenous application of different KCL level on leaf area (a), stem length (b), stem diameter (c), yield (d), Brix (e), and WUE (f) of tomato under drought conditions. (LSD, p<0.05).

Table 2 - Foliar application effects of different KCl levels on tomato plants

KCl	LA (mm ²)	St. Length (cm)	St. Diameter (mm)	WUE	Yield plant ⁻¹	Brix ^o	Proline (μg/g fw)	Protein (mg g ⁻¹ fw)	SOD (IU mg ⁻¹ min ⁻¹ protein)	CAT (IU mg ⁻¹ min ⁻¹ protein)	PPO (IU mg ⁻¹ min ⁻¹ protein)
Control	4836.40 b	43.11 b	12.23 c	4.014 a	1168.46 a	7.777 a	8953.16 a	1.286 b	1539.26 a	230.56 a	167.44 ab
3	5197.64 b	46.55 b	13.88 b	4.426 a	1211.41 a	6.777 ab	7590.68 c	1.562 a	761.61 b	196.29 ab	204.66 a
6	6279.84 a	53.11 a	14.81 a	4.014 a	1214.32 a	7.277 b	7820.60 b	1.479 ab	1040.52 ab	155.96 b	109.44 b

Means in each column followed by similar letters are not significantly different at 0.05 probability level, using LSD (Least Significant Difference) test.

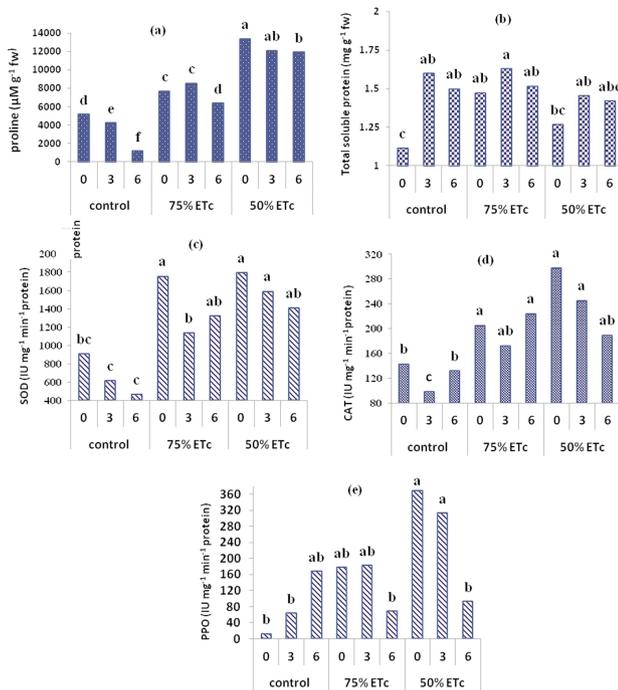


Fig. 2 - Effect of exogenous application of different KCl levels on enzymatic and non-enzymatic antioxidants; proline (a), total soluble protein (b), superoxide dismutase (c), catalase (d) and polyphenol oxidase (e) of tomato fruits under drought conditions. (LSD, $p \leq 0.05$).

4. Discussion and Conclusions

Increasing water stress has direct impact on crop growth and yield reduction and similar observations for reduction in growth and fruit yield of tomato were found in present study. Reduction in tomato yield under restricted water availability might have been due to reduction in photosynthetic area such as leaf area and leaf number (Chaves *et al.*, 2011; Khan *et al.*, 2015). In the present study, yield, leaf area and stem length were drastically reduced due to drought effect, whilst KCl foliar application improved these characters in tomato plants.

It has been reported that the foliar application of KCl improved the growth factors in agronomy crops (Ahmad and Jabeen, 2005; Yasmeen *et al.*, 2013). Besides adaptation role, positive effect of KCl on protein contents was also pronounced under drought stress. Proline is a well-known amino acid that generally accumulates when plants are exposed to environmental stresses (Kavi-Kishor *et al.*, 2005). Enhanced proline synthesis is a common response of tomato plants to drought and may determine the stress tolerance (Doan and Maurel, 2005; Khan *et al.*,

2015). Proline is believed to act as a signaling molecule that initiates adaptation to the stress (Maggio *et al.*, 2002), acts as an osmolyte for osmotic adjustment (Hayat *et al.*, 2012), helps in stabilizing membranes/proteins and scavenges free radicals (Ashraf and Foolad, 2007). Thus, it decreases the adverse effects of cytoplasmic acidosis and maintains proper NADP⁺/NADPH ratios (Liang *et al.*, 2013). In plants grown under drought conditions, proline induces the expression of drought stress responsive genes and, thus, decreases the damage due to excessive Na⁺ ions accumulation (Chinnusamy *et al.*, 2005). Proline acts as a compatible solute in the plants (Mansour, 2000) and, generally, increases with increase in both the salinity stress and drought stress duration (Kishor and Sreenivasulu, 2014). Thus, it is likely to observe enhanced proline synthesis with increasing drought stress duration. Antioxidant enzyme activities were considered as indicators of scavenging ROS and reducing oxidative stress (Dionisio-Sese and Tobita, 1998; Lin and Kao, 2002). For example, SOD may convert superoxide radicals into H₂O₂ and H₂O₂ was further decomposed by CAT and POD (Redman *et al.*, 2011). In this study, drought stress significantly increased the antioxidant enzyme activities. The results of many researchers' studies proved that adequate external K supply significantly decreased antioxidant enzyme activities and proline in drought stressed plants might be caused by enhancing plant physiological metabolism and reducing ROS production and MDA content (Wei *et al.*, 2013; Yasmeen *et al.*, 2013; Bahrami-Rad and Hajiboland, 2017). In the other hand, the combination of drought stress and exogenous KCl application improved the antioxidant activities and proline content than well-watered conditions. So, rise in drought stress with exogenous application of KCl induce tolerance in crops (Yasmeen *et al.*, 2013). A similar trend was followed by all the treatments in case of antioxidant enzyme activities under each water stress treatment.

The results of this study clearly demonstrated that water deficit at any critical crop growth stage severely affected the physiological and antioxidant and non-antioxidant parameters of tomato. Foliar application of K on a drought stressed plants at all growth stages improved the physiological performance and plant tolerance but reduced antioxidant enzyme activities. All these findings lead us to recommend that for tomato crop under drought farmers should spray the crop with 6 (μM^{-1}) KCl to minimize the negative effect of drought. This can have a dual benefit:

improving the physiological performance of tomato and supplying K nutrient to plants. For the foliar spray on a small scale, a common hand-boom sprayer can easily be used, whereas on large scale use of a mechanical boom-sprayer is advised.

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