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Morpho-physiological Responses of Strawberry (*Fragaria×ananassa*) to Exogenous Salicylic Acid Application under Drought Stress

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ABSTRACT

Salicylic acid has been found to play an important role in the regulation of plant growth and development in response to environmental stresses. In the present study, the effect of salicylic acid (SA) (0 and 0.1 mM) on growth parameters and some physiological characteristics of two strawberry cultivars ("Kurdistan" and "Queen Elisa") was investigated under drought stress treatments (-0.1 and -1 MPa). Experimental design was factorial based on completely randomized design with three replications. Results showed that drought stress decreased leaf area, leaf dry matter, shoot dry matter, total dry matter, relative water content, stomatal conductance, yield, and fruit weight. Salicylic acid application increased total leaf area and leaf and shoot dry matter in both cultivars. Leaf number, root dry matter, total dry matter, yield and fruit weight were increased by SA application in "Kurdistan" under drought stress. "Kurdistan" cultivar had higher *RWC* than "Queen Elisa" and SA application increased *RWC*, proline and soluble carbohydrate in "Kurdistan" compared to "Queen Elisa" under drought stress conditions. *RWC*, proline and soluble carbohydrate of "Queen Elisa" did not change with SA spray. Total soluble protein was not affected in "Kurdistan" under drought stress, while "Queen Elisa" showed a reduction in total protein. SA application increased catalase and peroxidase activity in both cultivars under drought stress. In conclusion, "Kurdistan" was less susceptible to drought stress than "Queen Elisa" because "Kurdistan" showed less reduction in leaf number and area, crown, and total dry matter compared to "Queen Elisa". SA application altered some physiological responses of both cultivars under drought stress. The effect of SA was more pronounced in "Kurdistan" than "Queen Elisa".

Keywords: Antioxidant enzymes, Dry matter, Proline, Soluble carbohydrate, Yield.

INTRODUCTION

Drought is one of the main environmental stresses that adversely affect plant growth, economic outcome, and environmentally sustainable productivity. To conserve both the quality and quantity of water, appropriate strategies should be developed to avoid the risk of future water supply shortages. Therefore, the question of improvement of crop drought resistance effectively has become a matter of concern in agricultural production and research. In strawberry plants, deficit irrigation is generally associated with reduction in fruit size and yield (Liu *et al.,* 2007). Deficit irrigation could have considerable effect on fruit physiology and biochemistry in strawberry and different responses of strawberry cultivars to drought stress have been reported (Giné Bordonaba and Terry, 2010).

A major challenge in agricultural practice and research today is how to cope with the environmental stresses, including drought, in

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an economically and environmentally sustainable approach. Salicylic acid (SA), which is an endogenous plant growth regulator and influences different physiological and biochemical functions in plants, is a well-known and naturally occurring signaling molecule that has important role in establishing and signaling a defense response against various biotic and abiotic stress (Arfan *et al.,* 2007; Wang *et al.,* 2010). Plant physiological processes, growth, development, productivity, and responses to abiotic stresses are affected by SA application (Arfan *et al.,* 2007). Antioxidant enzymes activities is altered by SA and play an important role in protecting plants against oxidative damage by detoxifying super oxide radicals (Munns and Tester, 2008). High activities of antioxidant enzymes improve plant resistance to oxidative damage caused by reactive oxygen species (Gapinska *et al.,* 2008)

Hayat *et al.* (2010) reported that exogenous application of SA affect productivity, growth, photosynthesis, plant water relations, and antioxidant enzyme activities of plants exposed to various biotic and abiotic stresses. SA effectively alleviated the toxic effects generated in plants due to the exposure to various abiotic stresses (Hayat *et al.,* 2010). SA enhanced the leaf area and dry matter production in corn and soybean (Khan *et al.,* 2003), *Brassica juncea* (Fariduddin *et al.,* 2003), wheat (Hayat *et al.,* 2005; Hussein *et al.,* 2007) and strawberry (Karlidag *et al.,* 2009). Increasing of root and shoot fresh weight and yield of strawberry by SA application has been reported (Jamali *et al.,* 2011). Moreover, Demiralay *et al.* (2013) reported that foliar application of SA increased proline and soluble carbohydrate content. The lower concentrations of salicylic acid, when applied exogenously, provided tolerance against the damaging effects of drought in tomato and bean plants, whereas, higher concentrations did not show fruitful results (Senaratna *et al.,* 2000). Dry matter accumulation was significantly enhanced in *Brassica juncea*, when lower concentrations of salicylic acid (0.01 mM) were sprayed (Fariduddin *et al.,* 2003). However, higher concentrations of SA had an inhibitory effect. Demiralay *et al.* (2013) reported that 0.01 mM SA treatment induced antioxidant enzyme activities in *Ctenanthe setosa* under osmotic stress.

Strawberry has a shallow root system, large leaf area, and high water content of fruits, therefore, it uses large amounts of water (Klamkowski and Treder, 2006). Variation between 10 (Grant *et al.,* 2010) and 3 (Ghaderi and Siosemardeh, 2011b; Klamkowski and Treder, 2008) strawberry cultivars has been found in response to drought stress. Variation between strawberry cultivars in response to SA application under limited water availability has been little explored. Exogenous application of the lower concentrations of salicylic acid proved to be beneficial in enhancing photosynthesis, growth, and various other physiological and biochemical characteristics of plants (Hayat *et al.,* 2010). At higher concentrations, SA itself may cause a high level of stress in plants (Hayat *et al.,* 2010). The effect of SA on growth and physiological responses of strawberry under drought stress conditions has not yet been well studied. Therefore, this experiment focused on the effect of 0.1 mM SA on plant growth, yield, soluble carbohydrate, proline, total soluble protein, and some antioxidant enzymes activity and leaf water content of strawberries under deficit irrigation.

MATERIALS AND METHODS

Two June-bearing (short day) strawberry (*Fragaria*×*ananassa* Duch.) cultivars were used in order to determine their responses to deficit irrigation. The "Kurdistan" cultivar is widely cultivated in west of Iran (Kurdistan Province) and “Queen Elisa” cultivar is cultivated only recently in this region. In the first year of study, at the end of September, well-rooted strawberry daughter plants were selected for similar size and cultivated in containers filled with

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soil (50% soil, 25% sand, and 25% manure). After planting, sufficient water was applied until the plants were well established. Exogenous application of the lower concentrations of salicylic acid is useful for physiological and biochemical characteristics of plants (Hayat *et al.,* 2010). Therefore, when plants were flowering, they were placed in a completely randomized design under two irrigation regimes and two salicylic acid (SA) treatments (0 and 0.1 mM). Water stress was imposed by withholding water from the plants until soil water potential reached -0.1 (control) and -1 MPa (drought stress) during the experiment. Soil water content was determined through gypsum block. Salicylic acid (SA) was dissolved in absolute ethanol, then, concentration of 0.1 mM was made up with distilled water containing 0.02% tween (Williams *et al*., 2003). SA was sprayed on the foliage of control and the plants were grown under drought stress at a concentration of 0.1 mM SA with a hand sprayer. The volume of the spray was 50 mL per pot. Control group of plants were sprayed with ethanol/water. Plants were treated with SA at the beginning of flowering and 15 days after the first time.

For five days after the onset of drought stress, 6 plants were randomly collected from each treatment to measure the dry mass of leaves, petioles, crowns and roots, total and single leaf area, and leaf number per plants. The root, crown, petiole, and leaf were placed in an oven at 70ºC for 48 hours, then, their dry weights were measured. Stomatal conductance (g_s) was measured on fully expanded upper canopy leaves (two leaves per plant) from 11:00 to 14:00 h at photosynthetic active radiation (PAR) > 1,000 mmol m^{-2} s^{-1} with a portable porometer system (LP4, Delta T, UK). Leaf samples were collected at midday (11:00 to 14:00 solar time). Leaf relative water content (RWC) was estimated gravimetrically according to the method of Galmes *et al.* (2007). Leaves samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis. A sample of 0.1 gr of leaves (fresh weight) was used for total soluble carbohydrates determination by phenol-sulfuric acid method (Khochert, 1987). Total soluble protein was estimated by Bradford method (Bradford 1976).

Peroxidase activity (POD) was measured by the method of Hemeda and Klein (1990). The reaction mixture contained 90 µL 1% guaiacol, 90 µL 0.3% hydrogen peroxide, 780 µL 50 mmol potassium phosphate buffer (pH 6.6), and 40 µL crude extract, in a total volume of 1.0 mL. Activity was determined by the increase in absorbance at 470 nm due to guaiacol oxidation (E026.6 mM^{-1} cm^{-1}). Ascorbate peroxidase activity (APX) was assayed according to Nakano and Asada (1981). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.5 mM ascorbate, 0.1 mM H_2O_2 and suitable aliquots of the enzyme extract in a total volume of 1.0 mL. The reaction was initiated by adding H_2O_2 and the absorbance decrease was recorded after every 30 seconds for 3.0 minutes at 290 nm. Catalase (CAT) activity was determined according to the method of Aebi (1984), by decrease in absorbance at 240 nm for 3 minutes following the consumption of H_2O_2 (ε= 39.4 mM–1 cm–1). The reaction mixture contained 250 µL 100 mM potassium phosphate buffer (pH 7.0), 250 µL 10 mM H_2O_2, 494 distilled water, and 6.6 µL of enzyme extract in a 1.0 mL volume.

Statistical analysis was performed by SAS software (SAS Institute Inc., 1990) according to a factorial design on the basis of Completely Randomize Design (CRD). Means were compared by Duncan test at a significance level of 0.05 and presented as treatment mean±SE of three measurements. From the data collected, charts and curve fittings were performed using Microsoft Excel software. Relationships between variables were determined using Pearson's correlation coefficient test.

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RESULTS AND DISCUSSION

Results showed that "Kurdistan" in general had a greater number of leaves, and drought significantly reduced the number of leaves in both cultivars. Application of 0.1 mM SA resulted in increasing of leaf number in "Kurdistan" under drought conditions and in "Queen Elisa" under control (Table 1). Drought stress reduced single leaf area and total leaf area per plant in both cultivars. Application of 0.1 mM SA increased total leaf area per plant in both cultivars under drought stress. Single leaf area in "Queen Elisa" was significantly higher than the "Kurdistan". In general, "Kurdistan" had a lower total leaf area per plant at control and maintained a larger total leaf area per plant than "Queen Elisa" in drought stress conditions (Table 1). With the exception of roots, the dry matter of plant organs decreased in response to drought stress in "Queen Elisa" (Table 1). Petiole (Table 1) crown, and root dry matter (Table 2) were not affected by drought stress in "Kurdistan". Drought stress reduced leaf dry matter in both cultivars. Under drought stress conditions, application of 0.1 mM SA increased leaf dry matter, irrespective of the cultivar. Salicylic acid significantly increased crown dry matter in control conditions and root and total dry matter under drought conditions in "Kurdistan". "Queen Elisa" had higher root dry weight compared to "Kurdistan", except in drought+salicylic acid treatment. Salicylic

Table 1. Influence of drought stress and salicylic acid on leaf number, leaf area, leaf dry matter, and petiole dry matter of the two strawberry cultivars [a].

Treatments	Leaf number (Number plant^{-1})	Single leaf area (cm^2 leaf^{-1})	Leaf area (cm^2 plant^{-1})	Leaf dry matter (g plant^{-1})	Petiole dry matter (g plant^{-1})
"Kurdistan"					
Control[b]	26±1.1547a	38.25±1.1745bc	991.6±14.8125b	10.74±0.175a	1.94±0.1135bc
SA0.1[d]	24.67±1.453a	40.68±0.7175bc	1003±59.899b	9.72±0.8603a	2.23±0.1885ab
Drought[c]	20.67±0.6667b	20.73±0.7524d	427.6±9.3412e	4.31±0.0935cd	1.78±0.1277c
Drought+SA0.1	25.33±0.3333a	32.4±2.9961cd	819±67.0017c	6.92±0.358b	1.69±0.1732c
"Queen Elisa"					
Control[b]	15.33±0.3333c	78±3.8929a	1167±41.4229a	10.4±0.595a	2.15±0.0872a
SA0.1[d]	18.67±0.6667b	67±10.4892a	1202±9.6383a	10.86±0.8984a	2.33±0.1447a
Drought[c]	11.67±0.6667d	39.67±2.6301bc	459.3±6.5073e	4.013±0.8841d	0.94±0.0551c
Drought+SA0.1	11.67±0.6667d	51.88±3.8586b	602.1±33.1563d	6.027±0.553bc	1.07±0.1947c

[a] Different letters indicate significant differences for similar plant organs by Duncan tests at $P \leq 0.05$. Data are Mean±SE. [b] -0.1 soil water potential; [c] -1 MPa soil water potential, [d] Salicylic acid 0.1 mM.

Table 2. Influence of drought stress and salicylic acid on crown, root, shoot and total dry matter (g plant^{-1}) and root: shoot dry matter of the two strawberry cultivars[a].

Treatments	Crown dry matter	Root dry matter	Total dry matter[e]	Shoot dry matter	Root: Shoot
"Kurdistan"					
Control[b]	2.043±0.1331c	8.489 ± 0.6562e	23.21±1.0347c	14.72±0.3948c	0.575±0.0305e
SA0.1[d]	2.477±0.0617b	9.733±0.6957de	24.16±1.6726c	14.43±0.9796c	0.674±0.0063de
Drought[c]	2.137±0.1386c	8.46±0.8585e	16.68±0.9153d	8.223±0.0636d	1.027±0.0969c
Drought+SA0.1	2.153±0.1192c	11.1±0.9295cd	21.86±0.5202c	10.76±0.424c	1.041±0.1266c
"Queen Elisa"					
Control[b]	3.017±0.0467a	14.8±1.0243b	30.37±1.4037b	15.57±0.5362b	0.95±0.0549cd
SA0.1[d]	2.96±0.1212a	18.67±0.4784a	34.82±1.3826a	16.15±0.9091a	1.16±0.0354bc
Drought[c]	1.87±0.0513c	13.63±1.0068b	20.45±1.9318c	6.823±0.944c	2.039±0.1602a
Drought+SA0.1	1.94±0.0436c	12.54±0.5263bc	21.58±0.2135c	9.037±0.7384c	1.419±0.1887b

[a] Different letters indicate significant differences for similar plant organs by Duncan tests at $P \leq 0.05$. Data are Mean±SE. [b] -0.1 soil water potential; [c] -1 MPa soil water potential; [d] Salicylic acid 0.1 mM, [e] Total dry matter= Root dry matter+ Crown dry matter+Petiole dry matter+Leaf dry matter.

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acid application increased root and total dry matter of "Queen Elisa" under control conditions. The total shoot dry matter was reduced at the drought treatment compared to the control and was not differ significantly between the two cultivars. The application of 0.1 mM SA increased shoot dry matter in "Kurdistan" under drought stress treatment and in "Queen Elisa" under control conditions. The root to shoot ratio of dry matter increased under drought stress in both cultivars (Table 2), which can be attributed to a large decrease in leaf dry matter with relatively little reduction in root dry matter under drought conditions. "Queen Elisa" had the greatest total dry matter (biomass) in control conditions compared to "Kurdistan" (Table 2). Total dry matter decreased under drought stress and was similar in both cultivars in drought+salicylic acid treatment.

Both cultivars responded to deficit irrigation by a reduction in *RWC*, g_s, numbers of leaves, leaf area and leaf and root dry matter. Reduction in leaf area and fruit yield was reported under slightly more severe deficit irrigation (Liu *et al.,* 2007), and Grant *et al.* (2010) report similar results for 10 strawberry cultivars. Razavi *et al.* (2008) also found reductions in number of leaves, leaf area, and leaf dry matter as a result of drought stress. In the present study, "Kurdistan" had lower reduction in leaf number and area, crown, and total dry matter compared to "Queen Elisa". Reduction in leaf area due to drought is related to less cell division and elongation. The cultivar that is sensitive to drought shows more decline in leaf emergence and leaf area. Ghaderi *et al.* (2011a) reported that number of new leaves produced under water stress in drought-tolerant grape cultivar was higher compared to susceptible cultivars. Grant *et al.* (2010) found reduction in total number of new leaves and the leaf area between cultivars and between the two irrigation treatments (well watered and drought). "Kurdistan" has small and hairy leaves that could be important for low transpiration rates and tolerance to drought. Reducing leaf area reduces total plant transpiration and can therefore be seen as a method of drought resistance (Grant *et al.,* 2010). Hsiao and Xu, (2000) reported that altering biomass allocation and modifying growth is seen as an acclimation response to deficit irrigation.

These results indicated a positive influence of salicylic acid on leaf area, number and dry matter, shoot dry matter, root dry matter and total dry matter under drought stress. It is similar to the results obtained by Jamali *et al.* (2011) in strawberry under normal irrigation. Some reports suggested that salicylic acid has antioxidant defense role in protecting plants organs. Salicylic acid has anti-senescence influence on plant organs and its application increased leaf area in strawberry (Jamali *et al.,* 2011). Hussein *et al.* (2007) reported an improvement in growth characteristics by foliage application of SA leading to higher productivity. Salicylic acid significantly enhanced the growth and root dry matter of carrot under stress (Eraslan *et al.,* 2007). Drought stress decreased leaf area and plant height in wheat and exogenous applications of salicylic acid reduced these harmful effects considerably (Pirasteh *et al.,* 2012). Karlidag *et al.* (2009) reported that root and shoot fresh and dry weight of strawberry plants under salt stress decreased, and then increased by salicylic acid application.

Drought treatment decreased relative water content (RWC) and stomatal conductance (g_s) in both cultivars compared to the control. *RWC* and g_s in "Kurdistan" and "Queen Elisa" were equal under drought condition, but "Kurdistan" had higher *RWC* than "Queen Elisa" under drought+SA treatment. The effect of SA under control conditions did not significantly affect the *RWC* and g_s (Figure 1). Application of SA resulted in the increase of soluble carbohydrate by 35.1% in "Kurdistan" and 21.22% in "Queen Elisa" under drought conditions. Drought stress increased proline content by 34.9 and 34.75% in "Kurdistan" and "Queen Elisa", respectively, compared to the control. Plants treated with SA exhibited leaf proline content higher than the

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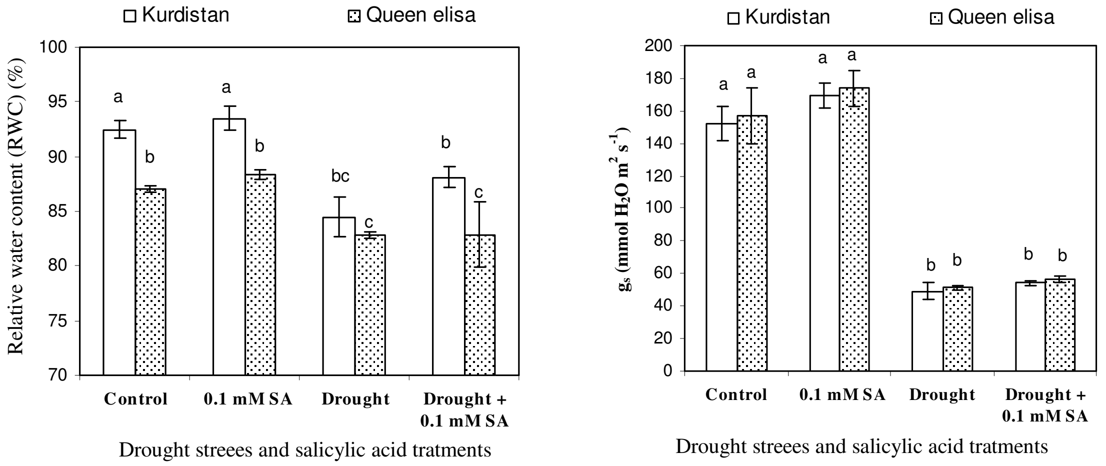


Figure 1. Interaction effects of drought stress and salicylic acid (SA) on relative water content and stomatal conductance in two strawberry cultivars. Control: -0.1 and drought -1 MPa soil water potential, and SA0.1: Salicylic acid 0.1 mM. Different letters indicate significant differences by Duncan tests at $P \leq 0.05$.

control in "Kurdistan". The increase in proline content under drought stress and SA application was 53.1% in "Kurdistan" and 30.18% in "Queen Elisa" compared to the control (Figure 2). Amount of soluble protein reduced in "Queen Elisa" under drought stress and SA application, but drought and SA treatment did not significantly affect soluble protein content in "Kurdistan". Drought stress reduced soluble protein content by 20.72% in "Queen Elisa" (Figure 3). Yield was significantly related to soluble carbohydrate (r= 0.500, P< 0.05), proline (r= 0.808, P< 0.01) and *RWC* (r= 0.866, P< 0.01) (Table 3).

It has been found that plants treated with SA generally exhibit better resistance to drought stress (Karlidag *et al.*, 2009). SA is reported to induce accumulation of proline and soluble carbohydrates in drought stressed plants (Demiralay *et al.,* 2013). Drought stress decreased *RWC* and g_s in "Kurdistan" and "Queen Elisa" in comparison to the control. Amount of *RWC* and g_s was similar in both cultivars under drought conditions. SA application (0.1

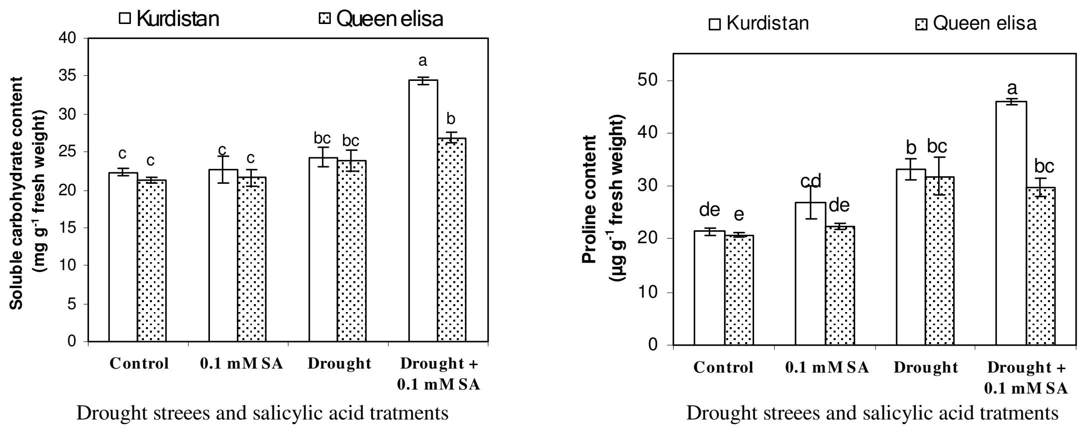


Figure 2. Interaction effects of drought stress and salicylic acid on soluble carbohydrate and proline in two strawberry cultivars. Control: -0.1 and drought -1 MPa soil water potential, and SA0.1: Salicylic acid 0.1 mM. Different letters indicate significant differences by Duncan tests at $P \leq 0.05$.

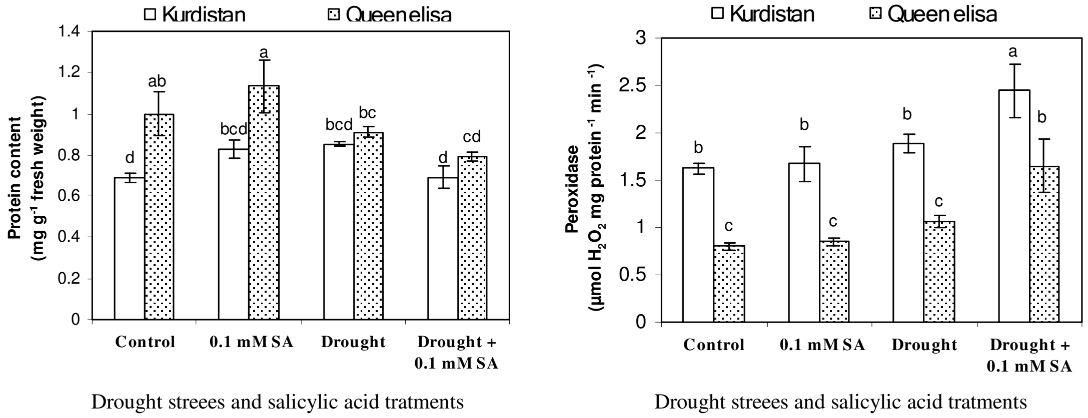


Figure 3. Interaction effects of drought stress and salicylic acid on soluble protein content and peroxidase activity in two strawberry cultivars. Control: -0.1 and drought -1 MPa soil water potential, and SA0.1: Salicylic acid 0.1 mM. Different letters indicate significant differences by Duncan tests at $P \leq 0.05$.

Table 3. Linear correlations among some physiological parameters and fruit weight (FW), fruit number (FN), Yield (Y) under drought stress and salicylic acid application in two strawberry cultivars [a].

	CAT [b]	POX [c]	AXP [d]	SC [e]	P [f]	RWC [g]	Y	FN	FW
FW	0.141^{ns}	-0.294^{ns}	-0.017^{ns}	0.222^{ns}	-0.319^{ns}	0.505^{*}	0.533^{**}	-0.879^{**}	1
FN	0.293^{ns}	0.638^{**}	0.276^{ns}	0.162^{ns}	0.630^{**}	0.802^{**}	0.868^{**}	1	
Y	0.581^{**}	0.734^{**}	0.445^{*}	0.500^{*}	0.808^{**}	0.866^{**}	1		

[a] The correlation coefficient (r) and significant differences are given: **: $P< 0.01$, * : $P< 0.05$, ns: Not significant. [b] Catalase activity; [c] Peroxidase activity; [d] Ascorbat peroxidase activity; [e] Soluble carbohydrate; [f] Proline, [g] Relative water content.

mM) under drought stress increased *RWC* to a greater extent in “Kurdistan” than “Queen Elisa”. Increase in *RWC* by SA spraying has been reported in chrysanthemum (Vahdati Mashhadian *et al.,* 2012). In these conditions, amount of proline and soluble carbohydrate in “Kurdistan” were higher than “Queen Elisa”. Interactions between SA and the accumulation of proline and soluble carbohydrate and increasing of *RWC* could be related to osmotic adjustments by proline and soluble carbohydrates accumulation. Demiralay *et al*., (2013) reported that under osmotic stress, soluble carbohydrate and proline content were significantly increased by exogenous SA treatment, which can lead to osmotic adjustment. In many plant species, there are high positive correlation between increased concentration of intracellular proline and the ability of plant to survive under high salt or water deficit conditions (Chaves and Oliviera, 2004).

The activity of antioxidant enzymes increased under drought stress and SA application in both cultivars. Antioxidant enzyme activity was higher in “Kurdistan” than “Queen Elisa”. Drought stress increased catalase (CAT) and ascorbate peroxidase (APX) activity in “Queen Elisa”. Activity of antioxidant enzyme was not affected by drought stress in “Kurdistan”. Application of SA under non-drought conditions did not change antioxidant enzyme activity in both cultivars and “Kurdistan” had higher antioxidant enzyme activity in non-drought conditions. Activity of antioxidant enzymes was induced with SA plus drought. Application of SA plus drought increased CAT, APX and POD activity by 34.31, 26.3, and 33.62% in “Kurdistan” and 53.5, 43.84, and 51.55% in “Queen Elisa”, respectively, compared to the control. Activity of POD in "Kurdistan" was higher than "Queen Elisa" in all treatments (Figures 3 and 4). Positive and significant correlations were determined between CAT and yield (r= 0.581, $P< 0.01$) and POX and yield (r= 0.734, $P< 0.01$) and AXP and yield (r= 0.445, $P< 0.05$) (Table 3).

The exposure of plants to abiotic stresses often leads to the generation of reactive oxygen species (ROS) (Ashraf and Akram, 2009). Increasing ROS levels cause oxidative damage to cell components such as lipids, proteins, and nucleic acids (Smirnoff, 1993). When plants are exposed to stress, antioxidant systems become active and begin to scavenge ROS. Antioxidant defense systems play vital roles in helping plants tolerate stressful conditions. The induction of antioxidant enzymes (POD, CAT, and APX) by SA treatment under drought and salinity (Hayat *et al.,* 2008; Yusuf *et al.,* 2008) stresses is known. Literature review indicated that SA plays an important role in providing plant tolerance to water stress (Hayat *et al.,* 2010). In the present study, we investigated the activity of certain enzymes (POD, CAT, and APX) to determine whether SA may have a role in the regulation of antioxidant defense in strawberry under drought stress. Drought stress decreased leaf water content in both cultivars. This may induce oxidative stress. The application of SA (0.1 mM) increased POD, CAT, and APX activity under drought stress in both cultivars. Exogenous SA application improved plant environmental stress tolerance by increasing the activities of antioxidant enzymes (Tasgin *et al.,* 2006; Pirasteh *et al.,* 2012). "Kurdistan" showed higher antioxidant activity, which enabled this cultivar to detoxify ROS more efficiently than "Queen Elisa". Increase in antioxidant activity compared to the control was higher in "Queen Elisa" than "Kurdistan". Drought stress decreased leaf water content and was lower in "Queen Elisa" compared to "Kurdistan". This induced maximum oxidative stress in "Queen Elisa" under drought stress and could lead to the generation of ROS and oxidative stress. ROS acts as a signal molecule and plants initiate antioxidant mechanism for protection against ROS (Khan *et al.,* 2003). The application of 0.1 mM SA to drought-stressed plants maximally induced POD activity in "Kurdistan", exhibiting greater potential in this cultivar to detoxify ROS. Plants that have high antioxidant enzymes activities have shown considerable resistance to oxidative damage caused by ROS (Khan *et al.,* 2007; Gapinska *et al.,* 2008).

Drought treatment decreased yield and fruit weight in both cultivars compared to

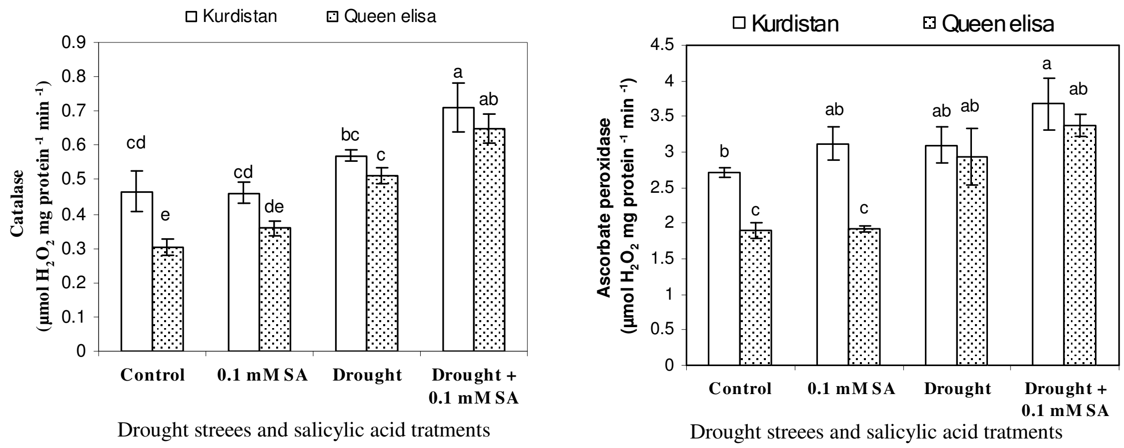
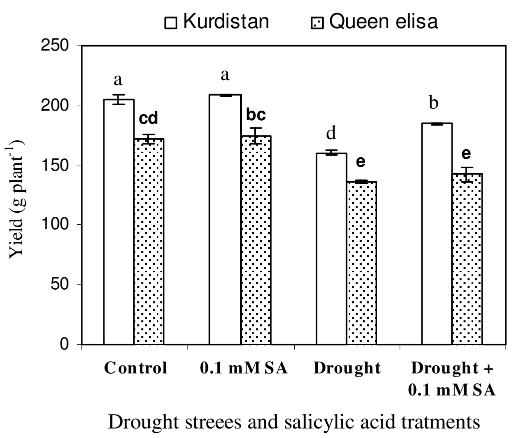


Figure 4. Interaction effects of drought stress and salicylic acid on catalase and ascorbate peroxidase activity in two strawberry cultivars. Control: -0.1 and drought -1 MPa soil water potential, and SA0.1: Salicylic acid 0.1 mM. Different letters indicate significant differences by Duncan tests at $P\leq 0.05$.

the control (Figure 5). Application of 0.1 mM SA resulted in increasing of yield and fruit weight in "Kurdistan" under drought stress. Previously conducted studies have shown a positive effect of SA on yield of strawberry (Jamali *et al*., 2011). In addition, a significant positive correlation between yield and fruit weight and number under drought stress and salicylic application (Table 3) was observed (r= 0.533 and 0.868, P< 0.01). This implies a close linkage between yield and fruit number and weight. Significant positive correlations between antioxidant enzyme activities and yield were determined (Table 3). Significant and positive correlations were determined between fruit number and POX (r= 0.734, P< 0.01), fruit number and proline (r= 0.630, P< 0.01) and fruit number and *RWC* (r= 0.802, P< 0.01) (Table 3).

In conclusion, drought stress had several adverse effects on both cultivars, including decreases in leaf area, leaf dry matter, shoot dry matter, total dry matter, relative water content, and stomatal conductance. There is variation between the two cultivars in leaf area, leaf dry matter, shoot dry matter, total dry matter and relative water content. "Kurdistan" was less susceptible to drought stress than "Queen Elisa" because "Kurdistan" showed less reduction in leaf number and area, crown and total dry matter compared to "Queen Elisa". Drought stress also increased the levels of proline in both cultivars and APX and CAT activities in "Queen Elisa". Low sensitivity of "Kurdistan" to drought stress was due to small and hairy leaves that could be important for low transpiration rates and tolerance to drought. This cultivar also exhibited higher activity of CAT, APX, and POD. These traits helped to remove drought-induced ROS in this cultivar more efficiently than "Queen Elisa". These results indicated that SA application alleviated the drought-induced decrease in organs dry matter and yield in "Kurdistan" more than "Queen Elisa". Salicylic acid application increased total leaf area, leaf dry matter, and shoot dry matter in both cultivars. Leaf number, root dry matter, and total dry matter were increased by SA application in "Kurdistan" under drought stress. Application of SA under drought conditions increased *RWC* in "Kurdistan" compared to "Queen Elisa". In these conditions, amount of proline, soluble carbohydrate, and POD activity in "Kurdistan" were higher than "Queen Elisa". Although application of SA affected some traits in "Queen Elisa", due to more sensitivity to drought stress, application of higher concentrations of SA to alleviate the effects of drought on growth and yield may be necessary.



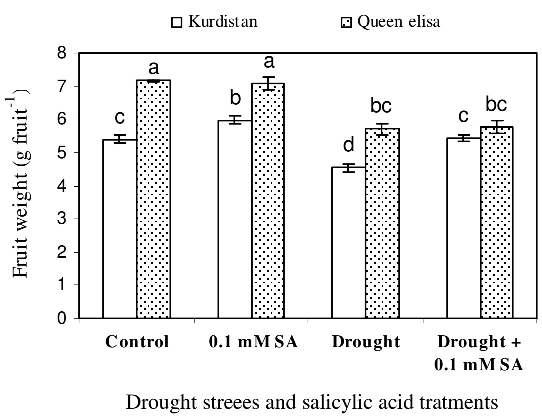


Figure 5. Interaction effects of drought stress and salicylic acid on yield and fruit weight in two strawberry cultivars. Control: -0.1 and drought -1 MPa soil water potential, and SA0.1: Salicylic acid 0.1 mM. Different letters indicate significant differences by Duncan tests at $P \leq 0.05$.

REFERENCES

1. Aebi, H. 1984. Catalase *In vitro*. *Method Enzymol.*, **105:** 121-126.
2. Arfan, M., Athar, H. R. and Ashraf, M. 2007. Does Exogenous Application of Salicylic Acid through the Rooting Medium Modulate Growth and Photosynthetic Capacity in Two Differently Adapted Spring Wheat Cultivars under Salt Stress. *J. Plant Physiol.,* **6(4):** 685-694.
3. Ashraf, M. and Akram, N. A. 2009. Improving Salinity Tolerance of Plants through Conventional Breeding and Genetic Engineering: An Analytical Comparison. *Biotech. Advances.,* **27**: 744-52.
4. Bradford, M. M. 1976. A Rapid and Sensitive Method for the Quantification of Microgram Quantities of Protein Utilizing the Principle of Protein-dye Binding. *Anal. Biochem.,* **72:** 248-254.
5. Chaves, M. M. and Oliveira, M. M. 2004. Mechanisms underlying Plant Resilience to Water Deficits: Prospects for Water-saving Agriculture. *J. Exp. Bot.,* **55:** 2365–84.
6. Demiralay, M., Saglam, A. and Kadioglu, A. 2013. Salicylic Acid Delays Leaf Rolling by Inducing Antioxidant Enzymes and Modulating Osmoprotectant Content in *Ctenanthe setosa* under Osmotic Stress. *Turk. J. Biol.,* **37:** 1205-16.
7. Eraslan, F., Inal, A., Gunes, A. and Alpaslan, M. 2007. Impact of Exogenous Salicylic Acid on Growth, Antioxidant Activity and Physiology of Carrot Plants Subjected to Combined Salinity and Boron Toxicity. *Sci. Hort.,* **113:** 120–128.
8. Fariduddin, Q., Hayat, S. and Ahmad, A. 2003. Salicylic Acid Influences Net Photosynthetic Rate, Carboxylation Efficiency, Nitrate Reductase Activity and Seed Yield in *Brassica juncea*. *Photosynthetica,* **41:** 281–284.
9. Galmes, J., Flexas, J. and Robert, S. 2007. Water Relations and Stomatal Characteristics of Mediterranean Plants with Different Growth Forms and Leaf Habits: Responses to Water Stress and Recovery. *Plant Soil,* **290:** 139-155.
10. Gapinska, M., Sklodowska, M. and Gabara, B. 2008. Effect of Short- and Long-term Salinity on the Activities of Antioxidative Enzymes and Lipid Peroxidation in Tomato Roots. *Acta Physiol. Plant.,* **30:** 11–8.
11. Ghaderi, N., Talaie, A. R., Ebadi, A. and Lessani, H. 2011a. The Physiological Response of Three Iranian Grape Cultivars to Progressive Drought Stress. *J. Agr. Sci. Tech.*, **13:** 601-610
12. Ghaderi, N. and Siosemardeh, A. 2011b. Response to Drought Stress of Two Strawberry Cultivars (cv. Kurdistan and Selva). *Hortic. Environ. Biotech.,* **52(1):** 6-12.
13. Giné Bordonaba, J. and Terry, L. A. 2010. Manipulating the Taste-related Composition of Strawberry Fruits (*Fragaria ananassa*) from Different Cultivars Using Deficit Irrigation. *Food Chem.,* **122:** 1020–1026.
14. Grant, O. M., Johnson, A. W., Davies, M. J., James, C. M. and Simpson, D. W. 2010. Physiological and Morphological Diversity of Cultivated Strawberry (*Fragaria*×*ananassa*) in Response to Water Deficit. *Environ. Exp. Bot.,* **68:** 264–272.
15. Hsiao, T. C. and Xu, L. K. 2000. Sensitivity of Growth of Roots *versus* Leaves to Water Stress: Biophysical Analysis and Relation to Water Transport. *Environ. Exp. Bot.,* **51:** 1595–1616.
16. Hayat, S., Fariduddin, Q., Ali, B. and Ahmad, A. 2005. Effect of Salicylic Acid on Growth and Enzyme Activities of Wheat Seedlings. *Acta Agron. Hungarica.*, **53:** 433–437.
17. Hayat, S., Hasan, S.A., Fariduddin, Q. and Ahmad, A., 2008. Growth of Tomato (*Lycopersicon esculentum*) in Response to Salicylic Acid under Water Stress. *J. Plant Interaction*, **3(4):** 297–304.
18. Hayat, Q., Hayat, S., Irfan, M. and Ahmad, A. 2010. Effect of Exogenous Salicylic Acid under Changing Environment: A review. *Environ. Exp. Bot.,* **68:** 14–25.
19. Hemeda, H. M. and Klein, B. P. 1990. Effects of Naturally Occurring Antioxidants on Peroxidase Activity of Vegetable Extracts. *J. Food Sci.,* **55:** 184–185
20. Hussein, M. M., Balbaa, L. K. and Gaballah, M. S. 2007. Salicylic Acid and Salinity Effects on Growth of Maize Plants. *Res. J. Agric. and Biol. Sci.,* **3(4):** 321–328.
21. Jamali, B., Eshghi, S. and Tafazoli, E. 2011. Vegetative and Reproductive Growth of Strawberry Plants cv. Pajaro Affected Salicylic Acid and Nickel. *J. Agric. Sci. Techn.,* **13:** 895-904.
22. Karlidag, H., Yildirim, E. and Turan, M. 2009. Salicylic Acid Ameliorates the

Adverse Effect of Salt Stress on Strawberry. *Sci. Agricola. Piracicaba Braz.,* **66(2):** 180-187.
23. Khan, W., Prithviraj, B. and Smith, D. L. 2003. Photosynthetic Responses of Corn and Soybean to Foliar Application of Salicylates. *J. Plant Physiol.,* **160:** 485–492.
24. Khan, N. A., Samiullah, S., Singh, S. and Nazar, R. 2007. Activities of Antioxidative Enzymes, Sulphur Assimilation, Photosynthetic Activity and Growth of Wheat (*Triticum aestivum*) Cultivars Differing in Yield Potential under Cadmium Stress. *J. Agron. Crop Sci.*, **193:** 435–444.
25. Khochert, G. 1987. Carbohydrate Determination by Phenol-solphoric Acid Methods. In: *The Handbook of Physiological Methods*", (Eds.): Hellebust, J. A. and Garigie, J. S.. Cambridge University Press, London, PP. 95-97.
26. Klamkowski, K. and Treder, W. 2008. Response to Drought Stress of Three Strawberry Cultivars Grown under Greenhouse Conditions. *J. Fruit Ornamental Plant Res.*, **16:** 179–188.
27. Klamkowski, K. and Treder, W. 2006. Morphological and Physiological Responses of Strawberry Plants to Water Stress. *Agric. Conspectus Sci.,* **71:** 159–165.
28. Liu, F., Savic , S., Jensen, C. R., Shahnazari, A., Jacobsen, S. E., Stikic , R. and Anderson, M. N. 2007. Water Relations and Yield of Lysimeter-grown Strawberries under Limited Irrigation. *Sci. Hort.,* **111(2):** 128–132.
29. Munns, R. and Tester. D. 2008. Mechanisms of Salinity Tolerance. Ann. *Rev. Plant Biol.,* **59:** 651-681.
30. Nakano, Y. and Asada, K. 1981. Hydrogen Peroxide is Scavenged by Ascorbate Specific Peroxidase in Spinach Chloroplast. *Plant Cell Physiol.,* **22:** 867–880.
31. Pirasteh, A. H., Eman, Y., Ashraf, M. and Foolad, M. R. 2012. Exogenous Application of Salicylic Acid and Chlormequat Chloride Alleviates Negative Effects of Drought Stress in Wheat. *Advanced Studies Biol.,* **4(11):** 501 – 520.
32. Razavi, F., Pollet, B., Steppe, K. and Van Labeke, M. C. 2008. Chlorophyll Fluorescence as a Tool for Evaluation of Drought Stress in Strawberry. *Photosynthetica*, **46:** 631–633.
33. Senaratna, T., Touchell, D., Bunn, E. and Dixon, K., 2000. Acetyl Salicylic Acid (Aspirin) and Salicylic Acid Induce Multiple Stress Tolerance in Bean and Tomato Plants. *Plant Growth Regul.* **30:**157–161.
34. Smirnoff, N. 1993. The Role of Active Oxygen in Response of Plants to Water Deficit and Desiccation. *New Phytol.,* **125:** 27–58.
35. Tasgin, E., Atici, O., Nalbantoglou, B. and Popova, L. P. 2006. Effects of Salicylic Acid and Cold Treatments on Protein Levels and on the Activities of Antioxidants Enzymes in the Apoplast of Winter Wheat Leaves. *Phytochem.,* **67:** 710-15.
36. Vahdati Mashhadian, N. Tehranifar, A., Bayat, H. and Selahvarzi, Y. 2012. Salicylic and Citric Acid Treatments Improve the Vase Life of Cut Chrysanthemum Flowers. *J. Agr. Sci. Tech.,* **14:** 879-887
37. Wang, L. J., Fan, L., Loescher, W., Duan, W., Liu, G. J., Cheng, J. S., Luo, B. and Li, S. H. 2010. Salicylic Acid Alleviates Decreases in Photosynthesis under Heat Stress and Accelerates Recovery in Grapevine Leaves. *BMC Plant Biol.,* **10:** 34.
38. Williams, M., Senaratna, T., Dixon, K. and Sivasithamparam, K. 2003. Benzoic Acid Induces Tolerance to Biotic Stress Caused by *Phytophthora cinnamomi* in Banksai Attenuate. *Plant Growth Regul.*, **41:** 89–91.
39. Yusuf, M., Hasan, S. A., Ali, B., Hayat, S., Fariduddin, Q. and Ahmad, A. 2008. Effect of Salicylic Acid on Salinity Induced Changes in *Brassica juncea*. *J. Integrative Plant Biol.*, **50(8):** 1–4.

پاسخهای مورفوفیزیولوژیکی توت‌فرنگی (*Fragaria×ananassa*) به کاربرد اسید سالیسیلیک تحت تنش خشکی

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چکیده

تحقیقات نشان داده که اسید سالیسیلیک در تنظیم رشد و نمو گیاهان در پاسخ به تنش‌های محیطی نقش دارد. در آزمایش حاضر اثر اسید سالیسیلیک (۰ و ۰/۱ میلی‌مولار) بر خصوصیات رویشی و برخی صفات فیزیولوژیکی دو رقم توت‌فرنگی (کردستان و کوئین‌الیزا) تحت تنش خشکی (۰/۱- و ۱- مگاپاسکال) ارزیابی گردید. آزمایش بصورت فاکتوریل بر اساس طرح کاملاً تصادفی با سه تکرار اجرا گردید. نتایج نشان داد که تنش خشکی بر هردو رقم تاثیر داشته است. از جمله اثرات آن کاهش سطح برگ، ماده خشک برگ، ماده خشک شاخساره ماده خشک کل، محتوای نسبی آب برگ، هدایت روزنه‌ای، عملکرد و وزن میوه بوده است. کاربرد اسید سالیسیلیک سطح برگ و وزن خشک برگ و شاخساره را در هردو رقم افزایش داد. تعداد برگ، وزن خشک ریشه، وزن خشک کل، عملکرد و وزن میوه در رقم کردستان در اثر کاربرد اسید سالیسیلیک در تیمار تنش خشکی افزایش یافت. رقم کردستان دارای محتوای نسبی آب برگ بیشتری در مقایسه با رقم کوئین‌الیزا بود و کاربرد اسید سالیسیلیک در شرایط تنش خشکی محتوای نسبی آب برگ، کربوهیدراتهای محلول و پرولین را در رقم کردستان افزایش داد. محتوای نسبی آب برگ، میزان کربوهیدراتهای محلول و پرولین در رقم کوئین‌الیزا در اثر کاربرد اسید سالیسیلیک تغییر نکرد. میزان پروتئین‌های محلول در اثر تنش خشکی در رقم کردستان تغییر نکرد ولی در رقم کوئین‌الیزا کاهش یافت. کاربرد اسید سالیسیلیک میزان فعالیت آنزیم‌های کاتالاز و پراکسیداز را در هر دو رقم افزایش داد. بطور کلی نتایج نشان داد که رقم کردستان دارای حساسیت کمتری به تنش خشکی در مقایسه با رقم کوئین‌الیزا بود زیرا کاهش کمتری در تعداد و سطح برگ، وزن خشک کل و وزن خشک طوقه را نشان داد. کاربرد اسید سالیسیلیک توانست میزان محصول و برخی خصوصیات فیزیولوژیکی و رویشی را در دو رقم تغییر دهد. اسید سالیسیلیک اثر بیشتری بر رقم کردستان در مقایسه با رقم کوئین‌الیزا داشت.

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