SHORT COMMUNICATION

Abscisic acid-mediated leaf phenolic compounds, plant growth and yield is strawberry under different salt stress regimes

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ABSTRACT: The present research was conducted to evaluate the interaction effects of exogenous abscisic acid (ABA) and salt stress on phenolic compounds, growth and yield of two short day strawberry cvs "Queen Elisa" and "Kurdistan". Plants were subjected to control, gradual salt stress (up to 20 mmol L^{-1} over 5 weeks) and salinity shock (20 mmol L^{-1}). ABA treatments included: 0 (control), 5, 10, 20 and 40 µmol L^{-1}. The experiment was carried out based on a complete randomized design in factorial experiment. The highest level of ferulic acid was observed by applying 40 µmol L^{-1} ABA in "Queen Elisa" under salt stress shock but effective increase in caffeic acid and p-coumaric acid was shown at gradual salt stress for both cultivars at the same ABA level. Maximum level of ABA led to the highest gentisic acid and gallic acid at gradual salt stress in "Kurdistan". Methyl gallate and flavonoid content showed a striking increase at the same conditions in "Queen Elisa". The interaction effect of salt stress regimes and ABA resulted in an increase in ellagic acid content for both cultivars. The interaction effect of ABA and salt shock caused higher reduction in root and shoot fresh dry weights and decrease in fruit yield in "Kurdistan". The results of this experiment accounts for the important role of exogenous ABA in the activation of antioxidant defense mechanism, growth and yield maintenance under gradual salt stress in strawberry.

KEYWORDS: ABA, *Fragaria*, phenolics, salinity, fruit.

Salinity is one of the major constraints that limit crop productivity in many important agricultural areas (Quesada et al. 2000). It is anticipated that about 50% of arable lands of the world will be affected by salt stress by 2050 (Blumwald and Grober 2006). Strawberry (*Fragaria* × *ananassa* Duch.) has recently been in a great demand in Iran both for fresh market and fruit processing industry. Strawberry plants are considered to be extremely sensitive to salts and have a threshold EC_e (electrical conductivity of saturated soil extract, a traditional soil salinity measurement) of 1 dS m^{-1} and 33% loss in yield for every 1 dS m^{-1} increase beyond this threshold value (Maas 1990).

The adaption to salinity may involve the interplay or interaction of a large number of responses. Acclimation to external environmental changes can occur in plants via internal adjustments within the tissues and cells, enabling plant metabolism to proceed under these altered conditions (Demmig-Adams et al. 2008). When plants respond to stress, a pre-existing program enables survival while maintaining the original developmental program (Amzallag and Lerner 1995). In nature, plants are typically subjected to a gradual build up of salt due to: (1) application of the fertilizers required for crop growth and (2) increase in salt concentration in the soil solution as soil water is depleted (Eilers et al. 1995). This gradual increase in salt concentration is ideal for salt acclimation that might occur in the soil, while salt shock is uncommon in nature (Maas and Grattan 1999).

Most researches on salt stress responses involve short-term exposure to relatively high concentration of salt on non-acclimated

plants but there are few studies on acclimation to salt stress compared to the extensive literature using salt shock. Regardless of the fact that irrigation waters and agricultural soil solutions are comprised of multiple combinations of cations and anions, the vast majority of salinity experiments on plants use NaCl as the sole salinizing salt (Lauchli and Grattan 2007). There are few studies on acclimation to salt stress compared to the extensive literature using salt shock. For instance, Amzallag et al. (1990) reported that Sorghum pre-treated with 150 mM NaCl at the seedling stage for 20 days could then survive and produce seeds in the presence of salt concentrations that were lethal to non-acclimated plants. Enhanced salt tolerance following pre-treatment with low concentrations of NaCl was observed in maize seedlings (Rodriguez et al. 2005), cowpea (Silveira et al. 2001) and rice (Djanaguiraman et al. 2006).

The phytotoxicity of NaCl is likely due to its ability to generate reactive oxygen species represented predominantly by superoxide radicals (O_2^-), hydroxyl radicals (OH·), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and nitric oxide (NO·) (Valderrama et al. 2007). A sudden and dramatic increase in cellular ROS production leads to protein and lipid oxidation and DNA single- and double- strand breakage (Miller et al. 2008), thus upsetting the homeostasis of the cell. Plants produce a high diversity of natural products or secondary metabolites containing three main groups: terpenes, nitrogen containing compounds and phenolics (such as phenolic acids, coumarins, lignans, stilbenes, flavonoids, tannins and lignin) (Agostini-Costa et al. 2012). Phenolics are characterized by at least one aromatic ring (C6) bearing one or more hydroxyl group. They are mainly synthesized from cinnamic acid, which is formed from phenylalanine by the action of L-phenylalanine ammonia-lyase (EC 4.3.1.5), the branch point enzyme between primary (shikimate pathway) and secondary (phenylpropanoid) metabolism. Phenols are divided into several different groups, distinguished by the number of constitutive carbon atoms in conjugation with the structure of the basis phenolic skeleton (simple phenols, benzoic acids, phenylpropanoids and flavonoid) (Michalak 2006). Phenolic acids including hydroxybenzoic acid and hydroxycinnamic acid derevatives are chemical compounds with at least one aromatic ring bearing one or more hydroxyl groups (Hounsome et al. 2008). Phenolics are important in the protection against salt stress. Phenolic hydroxyl groups are good H-donating antioxidants, which scavenge reactive oxygen species and break the cycle of generation of new radicals. The radical scavenging antioxidants inhibit free-radical-mediated oxidation of lipids, proteins and DNA. Phenolics act as antioxidants by inhibiting enzymes involved in radical generation (Jaganath and Crozier 2010, Castellano et al. 2012).

Reports have demonstrated that the application of exogenous abscisic acid (ABA) provides tolerance to salt stress conditions. ABA is known to play an important role in enhancing plant water use efficiency under environmental stress. ABA exhibits both fast effects (modulation of ion flows resulting in stomatal closure) and relatively long-term effects on gene expression pattern (Vankova 2010). Although rapid production of ABA in response to drought and salt stresses is essential to define ABA as a stress hormone, an equally rapid catabolism of ABA when such stresses are relieved is also essential in that role. ABA acts as a long distance signal mediating whole plant responses to drought and salt stresses. It is also a cellular signal mediating the expressions of genes responsive to these stresses (Zhang et al. 2006). It was suggested that acclimation to salinity may be achieved via enhancing the antioxidant defense system (Amzallag and Lerner 1995, Saha et al. 2010). The aim of this study was to evaluate the role of exogenous ABA in different salt stress regimes in the case of phenolic compounds, growth and yield of two short day strawberry cultivars, "Queen Elisa" and "Kurdistan". To our knowledge, the effects of ABA on inducing phenolic compounds production at saline conditions are still ambiguous.

Strawberry plants (*Fragaria* × *ananassa* Duch., cvs. "Queen Elisa" and "Kurdistan") were singly planted in 2 L pots filled with a mixture of perlite and cocopeat (1:1) and then fed continuously with 250 mL of half strength Hoagland nutrient solution [Macronutrients: $NH_4H_2PO_4$ (115.03 g L^{-1}), KNO_3 (101.10 g L^{-1}), $Ca(NO_3)_2.4H_2O$ (236.15 g L^{-1}), $MgSO_4.7H_2O$ (246.47 g L^{-1}), Micronutrients: KCl (3.728 g L^{-1}), H_3BO_3 (1.546 g L^{-1}), MnSO4. H_2O (0.845 g L^{-1}), $ZnSO_4.7H_2O$ (0.575 g L^{-1}), $CuSO_4.5H_2O$ (0.125 g L^{-1}), $H_3MoO_4.H_2O$ (0.09 g L^{-1}), FeEDDHA (9.31 g L^{-1})] from March 2012 to May 2012.

Sodium chloride (NaCl) was incorporated into the nutrient solution as the source of salinity. Gradual salt treatment was performed up to 20 mmol L^{-1} over 5 weeks. (First week: 0 mmol L^{-1} NaCl, second week: 5 mmol L^{-1}, third week: 10 mmol L^{-1}, fourth week: 15 mmol L^{-1}, fifth week: 20 mmol L^{-1}). Plants were subjected to 0 (control) and 20 mmol L^{-1} NaCl for salt stress shock treatment. Salt treatments started four weeks after planting when plants were well established. All the treatments were performed three times a week and double distilled water was added once a week, to all pots to prevent salt deposition in the culture media. This strategy was chosen to reduce the effect of water deficit and to focus on the salt-specific impact of NaCl stress at the levels used in the experiment.

Plants were sprayed by ABA, 24 hours after the first salt treatment. ABA (Sigma Aldrich) treatments included:

0 (control), 5, 10, 20 and 40 µmol L^{-1} and were dissolved in ethanol 5% and 0.05% Tween 20 was used as wetting agent. Control plants were sprayed with ethanol 5% and 0.05% Tween 20. Plants were located randomly in a greenhouse with day/night temperature of 25/16°C and relative humidity of 75–80%. In order to measure phenolic acids, methyl gallate and flavonoids, six leaves were selected from each replication, three weeks after ABA application. Leaves (1 g) were boiled in 0.1 N HCL for 25–30 min. The filtrate was then partitioned with ethyl acetate and dissolved in water and the portion insoluble in water dissolved in 80% methanol and filtered through a Millex HA 0.45 µm filter (Milipore Crop.) before injection. For free ellagic acid analysis, 1 g of each samples were extracted with methanol (90%) at room temperature for 30 min under a constant shaking. The suspension was filtered and the residue was re-extracted under the same conditions. The supernatants were combined with methanol (Koponen et al. 2007). Chromatographic separation was performed on a Hypersil ODS 5 µm column (4.6 × 250 mm) at 25°C. Chromatography was performed with a Crystal 200 series HPLC pump (Unicam, Cambrige, UK) equipped with a UV-Vis detector, regulated at 254 nm. The mobile phase consisted of potassium dihydrogen phosphate and acetonitrile (80:20, v:v). The flow rate was 1 mL min^{-1}. For ellagic acid separation, Lichrocart 100-RP-18 (125 × 14 mm, 5 µm i.d.) column was used and the mobile phase consisted of acetonitrile/methanol (85:15 v/v) with 1 mL min^{-1} flow rate. Standard acids (ferulic, caffeic acid, p-coumaric acid, p-hydroxybenzoic acid, gentisic acid, ellagic acid and gallic acid) and methyl gallate were purchased from E. Merck. Stock solutions of the standard acids were prepared in a concentration of 1 g 100 mL^{-1} in pure methanol (Vekiari et al. 2008).

Aluminum chloride colorimetric method was used for flavonoids determination (Chang et al. 2002). Each plant extract (0.5 mL of 1:10 g mL^{-1}) in methanol were separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. It remained at room temperature for 30 min and the absorbance of the reaction mixture was measured at 415 nm with a double beam UV/Visible spectrophotometer (Cary 100- USA). Quercetin was used to make the calibration curve. Ten milligrams of quercetin was dissolved in methanol and then diluted to 12.5, 25, 50 and 100 µg mL^{-1}. At the end of the experiment, the plants from each replicate were harvested and root and shoot fresh and dry weights were determined. In order to measure dry weights, plant material was placed into the oven at 70°C for 48 h. Mean fruit fresh weight was measured at harvest time to determine yield.

The experiment was carried out based on a complete randomized design in factorial experiment with three replicates per treatment and three plants per replicate. The statistical analysis was carried out by multifactorial analysis of variance (ANOVA) with SAS 9.1.3 software.

The results of analysis of variance in this experiment showed significant differences for individual effects of salinity and ABA. The effect of cultivar was not significant for ferulic acid, caffeic acid and methyl gallate. Interaction effect of salinity and ABA was significantly different for all the studied parameters. Similar results were noticed for ABA and cultivar interaction. Although salinity significantly effects gallic acid and ellagic acid, but gallic acid and ellagic acid were not significantly different in the interaction of salinity and cultivar. This shows that cultivars did not strengthen or attenuate the salinity effects for those two phenolic acids. The interaction effect of salinity, ABA and cultivar showed significant differences for all the studied parameters.

Acclimation can be induced rapidly if the stress is applied progressively. Not all genotypes have the capacity to acclimate (Amzallag and Lerner 1995). The results obtained from this experiment showed that the concentrations of three hydoxycinnamic acids, ferulic acid, caffeic acid and p-coumaric acid were increased by ABA at saline conditions for both cultivars. The highest level of ferulic acid was observed by applying 40 µmol L^{-1} ABA in "Queen Elisa" under salt stress shock. With 20 and 40 µmol L^{-1} ABA in "Queen Elisa" and 40 µmol L^{-1} ABA in "Kurdistan" no significant difference under gradual salt stress (Table 1). In contrast to our results, application of 1 µmol L^{-1} ABA to roots of wheat seedlings reduced the amount of wall-bound ferulic acid as the coleoptiles grew (Wakabayashi et al. 1997). It has been proposed that ferulic acid protects cucumbers against dehydration stress by decrease of lipid peroxidation by activation of antioxidant enzymes and increase of proline and soluble sugar content in leaves (Li et al. 2013). It is suggested that higher concentrations of ABA might be more effective in ferulic acid enhancement and the interaction of salt stress shock and 40 µmol L^{-1} ABA in "Queen Elisa" is more effective in ferulic acid enhancement in comparison with "Kurdistan".

No significant differences were noticed between cultivars at 40 µmol L^{-1} ABA applications for caffeic acid. The treatment of 40 µmol L^{-1} ABA caused maximum level of caffeic acid and p-coumaric acid at gradual salt stress in "Kurdistan". P-coumaric acid in "Kurdistan" showed a higher increase in comparison with "Queen Elisa" at gradual salt stress. Gradual salt stress was more effective in increasing caffeic acid and p-coumaric acid content for both cultivars (Table 1). Similar to our results, Berli et al. (2010) reported that concentrations of these two hydroxycinnamic acids were increased by application of 10 µmol L^{-1} ABA in grape leaf tissues. Rezazadeh et al. (2012)

Table 1. The interaction effect of salinity and exogenous abscisic acid (μmol L^{-1}) on leaf phenolic acids and methyl gallate (mg 100 g^{-1} DW) in "Kurdistan" and "Queen Elisa" strawberries

ABA (μmol L^{-1})	Control		Gradual salt stress		Salt stress shock	
	Kurdistan	Queen Elisa	Kurdistan	Queen Elisa	Kurdistan	Queen Elisa
			Ferulic Acid			
0	3.54 hi	2.35 j	3.81 fghi	3.32 i	3.70 ghi	3.55 hi
5	3.61 hi	3.36 i	3.93 efgh	3.94 efgh	3.96 efgh	3.71 fghi
10	3.64 hi	3.39 i	4.18 def	4.29 cde	4.14 defg	4.85 ab
20	3.69 ghi	3.56 hi	4.43 bcd	4.82 ab	4.30 cde	4.66 abc
40	3.75 fghi	3.67 ghi	4.85 ab	4.51 abcd	4.44 bcd	4.95 a
			Caffeic acid			
0	3.76 hi	2.81 i	3.73 hi	3.69 hi	3.38 hi	3.74 hi
5	3.87 hi	4.16 h	5.48 fg	5.34 fg	3.59 hi	4.50 gh
10	4.32 gh	5.38 fg	7.60 bcd	6.38 ef	4.55 gh	5.45 fg
20	5.48 fg	6.51 def	8.28 ab	7.81 bc	6.49 def	6.81 cde
40	7.39 bcde	7.55 bcde	9.32 a	8.40 ab	7.64 bcd	7.53 bcde
			p-Coumaric acid			
0	124.00 k	127.26 k	157.35 j	163.16 i	127.40 k	124.82 k
5	124.84 k	128.12 k	187.88 g	184.21 gh	181.68 h	186.24 gh
10	188.13 g	185.13 gh	206.99 e	188.84 g	196.29 f	194.75 f
20	187.66 g	187.69 g	208.80 e	205.85 e	209.54 e	205.71 e
40	188.78 g	187.23 g	299.95 a	287.61 b	220.09 d	226.99 c
			Gentisic acid			
0	17.64 defg	17.35 efg	17.10 fg	17.35 efg	17.70 defg	14.60 h
5	18.74 cde	16.59 g	18.75 cde	17.73 defg	18.24 cdef	16.66 g
10	18.28 cde	17.69 defg	19.43 c	19.05 cd	18.70 cdef	17.65 defg
20	18.63 cdef	17.85 cdefg	21.16 b	21.06 b	18.92 cde	18.44 cdef
40	19.00 cd	18.42 cdef	23.82 a	21.73 b	19.01 cd	18.61 cdef
			Gallic acid			
0	2.15 j	2.18 ij	2.16 j	2.17 ij	2.15 j	2.16 j
5	2.18 ij	2.46 efg	2.40 fg	2.16 j	2.18 hi	2.18 ij
10	2.48 efg	2.47 efg	2.61 cd	2.52 def	2.38 gh	2.40 fg
20	2.51 def	2.42 fg	2.77 ab	2.65 c	2.58 cde	2.51 def
40	2.66 bc	2.50 def	2.83 a	2.64 c	2.55 cde	2.50 def
			Ellagic acid			
0	4.27 j	4.28 j	4.31 j	4.31 j	4.28 j	4.27 j
5	5.47 i	5.53 h	5.48 i	5.46 i	5.64 g	5.56 h
10	6.67 e	6.67 e	6.66 e	6.29 f	6.76 d	6.76 d
20	7.78 b	7.75 b	7.98 a	7.57 c	7.54 c	7.59 c
40	7.95 a	7.75 b	7.97 a	7.98 a	7.96 a	7.94 a
			Methyl gallate			
0	12.11 g	12.32 g	12.36 g	12.27 g	12.93 g	12.72 g
5	12.34 g	13.46 fg	15.52 bcd	12.70 g	12.80 g	12.75 g
10	12.63 g	13.53 efg	15.77 bcd	14.87 def	14.74 def	15.36 cd
20	15.37 cd	14.58 def	17.05 ab	15.09 cde	14.85 def	15.26 cd
40	15.82 bcd	15.79 bcd	17.37 a	17.43 a	15.38 cd	16.73 abc

Different letters in each column for each compound indicate significant differences between the mean values according to the Duncan's Multiple Range Test (p>0.01). ABA: abscisic acid.

have observed that caffeic acids in artichoke plants were at their highest level when subjected to moderate salinity.

The role of secondary metabolites as antioxidants and antiradicals, assisting the plants to cope with oxidative stress has been confirmed (Gould et al. 2002). Significant increase in the accumulation of caffeic acid and p-coumaric acid help to reduce oxidative pressure, since caffeic acid and p-coumaric acid show high radical scavenging activity due to their hydroxyl nature (Rezazadeh et al. 2012). The hydroxycinnamic acid derevatives are more potent antioxidants than their hydroxybenzoic acid counterparts. This is due to an increase in the possibilities for delocalization of the phenoxyl radical by hydroxycinnamic acid derevatives. They are also able to donate hydrogen and electrons to stabilize other free radicals (Steenkamp et al. 2013). Hydroxybenzoic acids measured in this experiment included p-hydroxybenzoic acid, gentisic acid, ellagic acid and gallic acid. Trace amounts of p-hydroxybenzoic acid was determined in the leaves of both cultivars and considered to be negligible. Although gentisic acid has very similar chemical structure to p-hydroxybenzoic acid, but gentisic acid amount was more expressible in this experiment. Higher levels of this compound was found in the interaction effect of gradual salt stress and 20 and 40 µmol L^{-1} ABA in both cultivars. Maximum level of ABA led to the highest gentisic acid content at gradual salt stress in 'Kurdistan' and leaves subjected to shock stress were not significantly different after adding 40 µmol L^{-1} ABA in both cultivars (Table 1). Hydroxy-group containing compounds such as hydroxybenzoic acids help in reactive oxygen species scavenging activity by electron transfer interactions (Edreva et al. 2008). Results showed that increasing in ABA level enhanced ellagic acid content in both cultivars. The interaction effect of salt stress regime and and 40 µmol L^{-1} ABA did not show significant difference with control plants at the same ABA level for "Kurdistan" (Table 1). It has been reported that exogenous ABA increased ellagic acid, myricetin, quercetin and kaempferol contents of muscadine grape skins (Sandhu et al. 2011). Due to our results, it is suggested that ABA has an important role in ellagic acid enhancement for both cultivars.

Interaction effect of gradual salt stress and 40 µ mol L^{-1} ABA led to the highest level of gallic acid in "Kurdistan" (Table 1). Based on a research upon salt stress effects on phenolics, it has been shown that under 75 mmol L^{-1} NaCl stress, gallic acid content doubled in Canadian sweet marjoram shoots (Baatour et al. 2013). Salts and esters of gallic acid are termed gallates. As measured in this experiment, 40 µmol L^{-1} ABA resulted in the highest level of methyl gallate in "Kurdistan" leaves subjected to both gradual and shock stress. The striking interaction effect of ABA and salinity on methyl gallate content in "Queen Elisa" leaves was observed after adding 40 µM ABA at gradual saline condition but not at shock stress (Table 1). However, it has been found that, free radical scavenging potential of methyl gallate was lower than the standard antioxidant compound, i.e. gallic acid and the lower effect was related to substitution of OH group by CH_3 (Kaur et al. 2011).

The highest level of flavonoid content was measured after adding 40 µmol L^{-1} ABA at gradual stress in "Queen Elisa" leaves (Table 2). Enhanced flavonoid level in two salt tolerant varieties of Indica rice under 100 mmol L^{-1} NaCl stress has been reported by Chutipaijit et al. (2009). In addition to free radical scavenging role because the hydroxyl groups present in flavonoids structure, their modifications, i.e. glycosylation, prenylation and metylation, could affect their antioxidant properties, thus they may help inhibit lipid peroxidation in stressed plants (Potapovich and Kostyuk 2003). Chelation of transition metals (Fe) by flavonoids, such as quercetin, interferes with the generation of reactive oxygen species via fenton reaction, thus contributing to a powerful antioxidant performance (Leopoldini et al. 2006).

Involvement of exogenous ABA in sugar accumulation has been reported by Arenas-Huertero et al. (2000). Production of phenolic compounds depends on leaf carbon amount. Whenever carbon production is more than the metabolic demand for growth, polyphenols accumulation begins (Hichem et al. 2009). There is also evidence that ABA induces accumulation of reactive oxygen species in plant cells as secondary messengers for the activation of defensive responses (Sakamoto et al. 2008). This ABA signal also induces the expression of genes encoding superoxide dismutase, catalase and ascorbic peroxidase (Jiang and Zhang 2001), as well as the enhancement of non-enzymatic defense systems (antioxidant molecules – Jiang and Zhang 2002). In grape, ABA application was also shown to induce synthesis of polyphenols (Jeong et al. 2004). A differential regulation of carbon flux or utilization was identified upon salt and ABA treatment. As depicted with the raiffinose oligosaccharide pathway, the expression of salt responsive metabolite genes mainly depends on ABA while ABA only triggers a partial metabolic reaction. Salt stress induces complex re-adjustment of carbohydrate metabolism and that ABA triggers the initial steps of carbon mobilization (Kempa et al. 2008). Normally, the growth habit in "Queen Elisa" plants was stronger than "Kurdistan" but the reduction rate in root fresh weight was higher in "Queen Elisa" compared to "Kurdistan" at gradual stress and salt shock regimes (21.35% reduction in "Queen Elisa" compared to 15.76% in "Kurdistan" at gradual salt stress and 27.90% reduction in "Queen Elisa" compared to 17.27% reduction in "Kurdistan" at salt shock). ABA treatment caused the lowest (21.22%) shoot fresh

Table 2. The interaction effect of salinity and exogenous abscisic acid (µmol L^{-1}) on leaf flavonoids (mg QE 100 g^{-1} DW) of root and shoot fresh and dry weight (g) and fruit yield (g), in "Kurdistan" and "Queen Elisa" strawberries

ABA (µmol L^{-1})	Control		Gradual salt stress		Salt stress shock	
	Kurdistan	Queen Elisa	Kurdistan	Queen Elisa	Kurdistan	Queen Elisa
			Flavonoids			
0	422.34 i	422.92 i	568.88 e	583.77 e	551.71 e	564.48 e
5	441.41 i	446.15 hi	551.97 e	584.77 e	636.93 d	649.13 d
10	487.35 fg	475.55 gh	642.66 d	661.58 d	754.03 c	757.14 c
20	481.17 g	432.47 i	776.11 bc	803.01 b	756.66 c	767.21 c
40	515.00 f	495.71 fg	759.77 c	839.45 a	781.56 bc	800.71 b
			Root fresh weight			
0	21.33 e	20.04 h	20.55 fg	25.48 b	20.55 fg	28.27 a
5	20.05 h	20.03 h	20.04 h	23.04 c	20.55 fg	25.13 b
10	20.05 h	18.84 i	20.03 h	20.95 ef	17.07 k	22.10 d
20	20.03 h	18.00 j	17.31 k	20.07 h	17.02 k	22.09 d
40	17.02 k	18.01 j	17.31 k	20.04 h	17.00 k	20.38 gh
			Shoot fresh weight			
0	42.44 cde	53.98 b	44.52 c	64.22 a	43.36 c	64.66 a
5	35.80 g	53.37 b	43.15 cd	55.34 b	40.44 ef	43.17 cd
10	35.39 g	42.10 de	39.52 f	42.13 de	35.22 g	35.36 g
20	35.27 g	29.18 h	36.12 g	38.25 f	22.55 j	25.63 i
40	22.24 j	25.67 i	35.07 g	38.26 f	22.77 j	25.63 i
			Root dry weight			
0	4.77 g	6.29 b	5.33 c	6.35 a	5.28 d	5.33 c
5	4.23 h	5.26 de	4.97 f	5.26 de	4.96 f	5.27 de
10	4.21 h	4.21 h	4.97 f	5.24 e	4.98 f	4.20 h
20	3.95 i	4.20 h	4.20 h	4.97 f	3.87 j	4.20 h
40	3.94 i	4.20 h	4.19 h	4.97 f	2.96 k	3.87 j
			Shoot dry weight			
0	11.23 e	13.26 c	11.23 e	15.33 a	10.64 f	14.74 b
5	10.56 f	13.25 c	10.56 f	14.98 b	10.22 g	12.88 d
10	10.56 f	13.25 c	9.90 h	14.74 b	9.87 h	12.88 d
20	9.89 h	12.92 d	9.77 h	14.76 b	7.23 i	10.56 f
40	9.88 h	12.88 d	9.71 h	11.26 e	7.20 i	10.45 fg
			Yield			
0	89.62 d	103.12 b	98.89 c	102.66 b	98.57 c	107.82 a
5	88.42 de	97.99 c	85.14 fg	102.99 b	98.63 c	107.41 a
10	85.63 fg	98.51 c	84.73 fg	101.43 b	85.58 fg	97.96 c
20	83.19 g	85.55 fg	73.07 h	98.22 c	68.79 i	98.28 c
40	67.96 i	86.36 de	73.02 h	98.16 c	67.88 i	98.26 c

ABA: abscisic acid.

weight reduction rate in "Kurdistan" at gradual salt stress and the highest (60.36%) reduction rate in "Queen Elisa" at salt shock regime. Interestingly, root dry weight showed the maximum reduction rate (43.93%) in "Kurdistan" at salt shock.

Individual effect of ABA resulted in higher root dry weight decrease (33.22%) in "Queen Elisa" compared to the interaction of ABA and both salinity regimes (32.58% at gradual salinity and 27.39% at salt shock). The interaction effect of ABA application and gradual salinity in "Queen Elisa" showed higher (26.54%) rate of shoot dry weight reduction compared to "Kurdistan" (13.53%) (Table 2). ABA showed striking effect in root dry weight but not shoot dry weight reduction. The effects of ABA on plant growth regulation are complicated. Different responses exist between shoot and root. ABA has dual roles in physiological regulation. It shows inhibitive role functions when it is accumulated in large amount under stress to help plant survival and, at normal level, it has been shown essential for vegetative growth and primary root growth (Zhang et al. 2006). From the results of growth parameters in this experiment, salt shock was more impressive in decreasing root and shoot fresh weight for both cultivars and the rate of reduction was more obvious in "Queen Elisa" compared to "Kurdistan". Interaction effect of 20 and 40 μmol L^{-1} ABA and salt shock led to a decrease in fruit yield in "Kurdistan". Individual effect of 40 μmol L^{-1} ABA also showed a sensible reduction in fruit yield (31.13%). Individual effect of ABA showed higher reducing effect in "Kurdistan" (24.16%) compared to "Queen Elisa" (16.25%). Salt stress affects the plant growth and development thereby affecting the yield quantity and quality (Sattar et al. 2010). A successful salt tolerant cultivar should exhibit salt tolerance without compromising its yield potential. Although "Queen Elisa" showed higher rate of growth reduction after ABA application at salt stress regimes, the rate of decrease in fruit yield was lower in this cultivar (Table 2). An important role of ABA on fruit physiology is to stimulate sugar accumulation in fruits, thus improving fruit yield and quality (Zhang et al. 2001).

Salt responses in crops is highly challenging as it involves participation of multiple physiological and biochemical pathways. From the above results, it can be concluded that exogenous ABA enables plant metabolism to proceed more favorable under altered conditions. It seems that ABA application in "Kurdistan" and "Queen Elisa" strawberry plants increases the ability to tolerate salt stress under gradual salt stress more effective than salt shock conditions. Overall the interaction effect of gradual salinity and exogenous ABA showed higher contents of three hydroxycinnamic acids, three hydroxybenzoic acids and methyl gallate, an ester of gallic acid in both cultivars at gradual salinity conditions, but ABA was more impressive in "Kurdistan" and led to a higher content of phenolic acids. Enhanced flavonoid content was also the result of ABA application at gradual salt stress for "Kurdistan". Our results confirm the effective role of ABA on production of phenolic compounds and activation of antioxidant defense mechanism. Resistance reflects the capacity of the plant to express its original developmental program under stress conditions and due to the results better acclimation exists at gradual salinity. Totally the interaction effect of ABA and salt shock caused higher reduction in root and shoot fresh weight but root dry weight was mostly affected by the individual impression of ABA in "Queen Elisa". It is suggested that ABA might be more successful in fruit yield protection under gradual salt stress compared to salt shock. Measuring phenolic compounds after 48 h of gradual salt exposure up to the end of the salt treatments is suggested and might result in more accurate observations. On the other hand, comparing the role of ion toxicity and osmotic stress in strawberry plant acclimation at different salinity regimes is still unclear.

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