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Response of coriander to salinity stress

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Abstract

Salinity in the soil and irrigation water is an environmental problem and a major constraint for crop production. *Coriandrum sativum* as a leafy vegetable is great interest for vegetable producers in the world. However, due to gradual increase in soil and water salinity, especially in Northwest Iran, the production of this vegetable has been faced with many constraints. This experiment was conducted to study the effect of salinity on some morphological and physiological traits of *Coriandrum sativum*. The factors were arranged as factorial based on randomized complete block design with five NaCl levels (0, 50, 100, 150 and 200 mM NaCl) and local ecotypes (Tabriz, Malayer) with three replications. The results revealed that there was interaction of salinity levels with ecotypes in terms of proline content and relative water content. The highest amounts for proline content (77 μg^{-1} FWt) was recorded for Tabriz clone under 200 mM NaCl. The greatest relative water content was obtained by Tabriz clone × NaCl 0 and Malayer × NaCl 0 and 50 mM combinations. The highest dry weight of leaves, K^+/Na^+ ratio and K^+ belonged to the non-salinity condition. Highest amounts of Na^+ accumulation, MDA and H_2O_2 level and ion leakage were attained with 200 mM NaCl level. Soluble sugars content were affected by both salinity and ecotype. The highest content of soluble sugars was achieved by Malayer ecotype. With increasing salinity level, the content of soluble sugars increased and the highest amount of soluble sugars content was recorded at 200 mM NaCl.

Keywords: *Coriandrum sativum*; H_2O_2; MDA; Salinity; Yield

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Introduction

Salinity is an important abiotic factor which adversely affects the production of crop species. Salt stress impacts various biochemical and physiological processes in plants. NaCl may act directly via osmotic or ionic sensory mechanisms, or it may act indirectly through mediators to affect existing metabolic pathways, gene expression, and results in a coordinated response to osmotic stress (Thomas *et al.* 1992). Plants suffer from oxidative damage under environmental stresses. Reactive oxygen species (ROS) are regarded as one of the main factors that contribute to the deteriorative effects of salinity (Ashraf and Ali 2008). Since ROS are cytotoxic, they can react with lipids, proteins, nucleic acids and result in protein denaturing, lipid peroxidation and DNA mutation (Ashraf and Ali 2008). Peroxidation of plasmalemma leads to the leakage of cellular contents, rapid desiccation and cell death (Scandalios 1993). Plants are equipped with antioxidant molecules to reduce the adverse effects of ROS. Super oxide dismutase reacts with the superoxide radical to produce H_2O_2. H_2O_2 is scavenged by catalase and peroxidase (Berwal and Ram 2018). Ali *et al*. (2004) reported that yield per plant, chlorophyll concentration, fertility percentage, number of productive tillers, panicle length and number of primary braches per panicle of rice were reduced by salinity.

Coriandrum sativum is dicotyledonous herb which belongs to the Apiaceae family. All parts of the plant are edible, but generally its fresh leaves and dried seeds are used (Önder 2018). Coriander's essential oil is a stimulant of gastric secretion (Jabeen *et al.* 2009). It has benefits as a carminative, eupeptic, estrogen and spasmolytic and also has antibacterial and antifungal effects (Önder 2018). Furthermore, the plant reduces blood pressure (Jabeen *et al.* 2009).

Climatic problems such as dry season and soil salinity invariably have been the major crop production restraints in Northwest Iran. Studying the salinity effects on plant morphology and physiology is an appropriate way to obtain information about the responses of plant to salinity and to select desirable genotypes under saline conditions. There are limited reports about the responses of coriandrum to environmental stresses. Therefore, the objective of the present investigation was to study the effects of salinity on enzymatic activity, yield and some physiological characteristics of *Coriandrum sativum*.

Material and Methods

This experiment was conducted as factorial based on randomized complete block design with tree replications at the research greenhouse of Azarbaijan Shahid Madani University of Tabriz, Iran during 2016 growing season. Two native *Coriandrum sativum* ecotypes (Tabriz and Malayer) were employed. The seeds were cultivated in plastic pots (20 × 30 cm) containing perlite. The growing condition in the greenhouse was as follows: light intensity of 450 µmol m^{-2} s^{-1}, 16:8 light and darkness, day and night temperature regime of 25:20 and relative humidity of 65%. Half-strength Hoagland's nutrient solution was used to feed the plants. After the plants were emerged and when they had three real leaves, the salinity treatments including control, 50, 100, 150 and 200 mM NaCl were applied on the plants. Samples for measurement of the traits were taken 40 days later.

Chlorophyll content

Chlorophyll (Chl) was extracted from 0.1 g leaf material by adding 5 ml of dimethyl sulphoxide (Hiscox and Israelstam 1979). Absorbance was recorded by spectrophotometer ($T80^+$, China) at 645 and 665 nm, respectively. Chla and Chl b contents were calculated as described by Lichtenthaler and Wellburn (1983).

Proline, soluble solids, Na^+ and K^+ content

Proline content was measured according to Bates *et al*. (1973). Toluene was employed as the reference standard reagent. Soluble solids were assayed according to Yemm EW and Willis (1954) and D-glucose was the standard. The Na^+ and K^+ content in leaves were determined by the flamephotometric method (Emami 1997).

Electrolyte leakage

0.3 g fresh leaf was incubated in 10 mL deionized water in a water bath at the constant temperature of 32 °C for 2 h and the initial electrical conductivity of the medium (EC_1) was determined. In order to release all electrolytes, the samples were autoclaved at 121 °C for 20 min, cooled to 25 °C and the final electrical conductivity (EC_2) was measured (Dionisio-Sese and Tobita 1998). Electrolyte leakage (EL) was calculated by the following formula:

$$EL = (EC_1 / EC_2) \times 100.$$

Relative water content

Relative water content (RWC) was measured on the leaves of each plant and then calculated by the following equation:

$$RWC\ (\%) = 100 \times (FW - DW)/(TW - DW)$$

where, FW, DW and TW are leaf fresh, dry and turgid weight, respectively (Xu and Leskovar 2014).

MDA and H_2O_2 content

A leaf sample (0.5 g) was used for determination of malondialdehyde (MDA). MDA content was determined using the thiobarbituric acid reaction. The absorbance value at 532 nm and the value for nonspecific absorption at 600 nm were detected to calculate the MDA content using the extinction coefficient of 155 mM^{-1} cm^{-1} (Sahu and Sabat 2011).

The H_2O_2 concentration was measured according the method of Alexieva *et al*. (2001). A 0.5 g leaf sample was homogenized in 5 mL ice-cold 3% trichloroacetic acid, then centrifuged at 12000 × g for 20 min at 4 °C. One mL supernatant was transferred to a new tube and mixed with 0.1 mL phosphate buffer. The absorbance of the solution was read at 390 nm.

Statistical Analysis

Three replications was used for each treatment. After analysis of variance, means were compared using Duncan's multiple range test at 5% probability level. The data were analyzed by SPSS (Version 21) and MSTATC (Version 9.2).

Results and Discussion

Analysis of variance revealed the significant interaction clones with salinity for proline and RWC (Tables 1 and 2). Clone type and salinity levels affected soluble solids. Salinity had significant effects on H_2O_2, MDA, ion leakage, K^+/Na^+ ratio, K^+, Na^+, chl a, chl b, and leaf dry weight (Table 1).

Leaf dry weight

The highest (0.8 g) and lowest (0.27 g) leaf dry weight belonged to the control and 200 mM NaCl, respectively (Table 3). The results of this experiment are in line with the findings of Zakery- Asl *et al*. (2014) on *Suaeda aegyptiaca* and Sheidaei *et al*. (2010) on safflower where they reported the adverse effects of salinity on these plants. Na^+ accumulation in the media adversely affects the growth and yield of the plants.

Table 1. Analysis of variance for the effects of clone type and salinity levels on some physiological traits of *Coriandrum sativum*.

SOV	df	H_2O_2	MDA	Ion leakage	K^+/Na^+	Na^+	K^+	Proline	Soluble solids	RWC	Chloro-phyll b	Chloro-phyll a	Total chloro-phyll	Leaf dry weight
Replication	2	0.008^{*}	0.02^{**}	119.2*	0.006^{ns}	55.5^{**}	2.1^{ns}	121.3^{**}	0.04^{ns}	28.4^{**}	0.43^{*}	0.06^{ns}	0.88^{*}	0.006^{ns}
Clone	1	0.0001^{ns}	0.003^{ns}	124.1^{ns}	0.09^{ns}	7.3^{ns}	0.02^{ns}	0.24^{ns}	0.64^{**}	241.4^{**}	0.001^{ns}	0.04^{ns}	0.07^{ns}	0.001^{ns}
Salinity	4	0.07^{**}	0.06^{**}	1243.2^{**}	22.1^{**}	1235.1^{**}	119.8^{**}	2579.2^{**}	0.27^{**}	119.4^{**}	1.37^{**}	2.39^{**}	0.09^{ns}	0.31^{**}
Salinity × Clone	4	0.0004^{ns}	0.0004^{ns}	8.01^{ns}	0.16^{ns}	4.9^{ns}	1.45^{ns}	27.3^{*}	0.023^{ns}	27.6^{**}	0.10^{ns}	0.01^{ns}	0.08^{ns}	0.002^{ns}
Error	18	0.002	0.0003	19.8	0.91	2.7	0.98	4.13	0.05	4.11	0.051	0.08	0.13	0.003
CV (%)		7.6	6.4	10.1	8.8	7.3	4.44	6.1	4.9	5.1	9.78	8.3	9.2	9.3

ns, * and ** not significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively; MDA: malondialdehyde; RWC: relative water content.

Relative water content

The highest RWC was obtained in Tabriz (41.6%) and Malayer (45.02%) clones in non-saline condition (Table 2). However, there was no significant difference between control and 50 mM NaCl salinity in Malayer clone. Qin *et al*. (2010) reported that water status is the main factor affecting the *Shepherdia argentea* plants' growth. Significant reduction of RWC in leaves of plants treated with 400 and 600 mmol NaCl indicated that salinity also resulted in dehydration at cellular level and dehydration symptoms were greater in the higher NaCl concentration because of the increasing cellular water loss. As already understood, salinity leads to reduced water accessibilities and/or absorption and therefore lowers leaf turgor and leads to stomata closure. The final result of these nonstandard conditions would be the reduction of NADPT or NADPH, H^+ ratio and increased ROS production (Esfandiari *et al*. 2007).

Chlorophyll content

The highest chlorophyll b content (1.02 mg g^{-1} FWt) belonged to the control plants. For chlorophyll a content, the highest amount was observed at control (1.840 mg g^{-1}FWt) and 50 mM NaCl salinity level (1.59) (Table 3). Increasing salinity decreased the chloroplast content of the plants and the lowest amount was recorded with 200 mM NaCl salinity level (Table 3). Qin *et al*. (2010) reported that with increasing salinity level, the chlorophyll content decreased in *Shepherdia argentea*. Decrease in Chl content of the leaves may be attributed to both the inhibited synthesis of this pigment and damaged photosystem antenna which also accelerates leaf senescence (Mudgal *et al*. 2009).

MDA content

The results obtained from Table 3 showed that with increasing salinity levels, MDA content was significantly increased as compared to the control plants. The highest MDA content (0.06 µmol g^{-1} FWt) was recorded with 200 mM NaCl. This could be an indication of the cell membrane damage (oxidative damage) and reduction in cell membrane stability (Table 3). The result of this experiment was in line with the finding of Najjar-Khodabakhsh and Chaparzadeh (2015) in water–cress. According to Sairam *et al*. (2002), Na^+ accumulation under saline condition increased the membrane damage. Demiral and Turkan (2004) reported the membrane dissociation and the denaturation of cell membranes lipids because of salinity. Hence, MDA accumulation could be an appropriate bio-marker to assay the plants reaction to salinity conditions (Kumar *et al.* 2017).

H_2O_2 content

With increasing salinity stress H_2O_2 in the plant tissue was increased (Table 3). The highest H_2O_2 accumulation belonged to 200 mM NaCl salinity level. The activity of enzymes has been mentioned as the key factor that protects the plants against environmental stressors. It has been frequently reported that salinity stress significantly increased the activity of H_2O_2 in plants grown under salt stress condition (Sairam *et al*. 2002; Perveen *et al*. 2011).

Proline content

Proline content increased in both clones as salinity level increased. However, the highest proline content (77 μg^{-1}FWt) belonged to Tabriz clone at 200 mM NaCl level (Table 3). Control plants had the lowest proline content (Table 3). Accumulation of proline in response to excess NaCl has been described in several plants (Giannakoula and Ilias 2013; Zakery-Asl *et al*. 2014; Geranpayeh *et al*. 2017). Proline protect the plants against ROS molecules (singlet oxygen and superoxide) (Vass and Ur Rehman 2018). Hong *et al*. (2000) indicated that proline content reduce free radical levels in response to osmotic stress in tobacco.

Soluble solids content

Soluble solids in the leaves of both clones progressively increased with increasing salinity stress. The highest amount (3.48 mg g^{-1} FWt) was obtained at 200 mM NaCl level (Table 3). Clone type also had significant effect on the content of soluble solids and Malayer clone had significantly higher soluble solids than Tabriz clone (Table 4). Reports also show that salinity stress has increased soluble solids in potato (Levy *et al*. 1998) and *Suaeda aegyptiaca* (Zakery-Asl *et al*. 2013).

Na^+ content

Na^+ content linearly increased with the increase in salinity. The highest Na^+ content (46.2 mg g^{-1} DWt) was observed with 200 mM NaCl (Table 3). Melo *et al*. (2017) also observed the increase in Na^+ content in the leaves and roots of pineapple seedlings under saline condition. Qin *et al*. (2010) reported that Na^+ content in the leaves of *Shepherdia argentea* plants was 4.6, 9.8 and 15.6 times of the controls at 200, 400 and 600 mmol/l

Table 2. Mean comparison for the treatment combinations of clone type and salinity on proline content and K^+/Na^+ ratio of *Coriandrum sativum*.

Clone	Salinity level (mM)	RWC (%)	Proline (μm g^{-1} FWt)
Tabriz	0	41.60^a	17.1^e
Tabriz	50	33.6^b	35.4^d
Tabriz	100	30.4^b	42.4^c
Tabriz	150	25.63^c	59.1^b
Tabriz	200	21.3^c	77.0^a
Malayer	0	45.02^a	24.2^e
Malayer	50	41.3^a	30.1^d
Malayer	100	36.6^b	48.1^c
Malayer	150	30.3^b	45.2^c
Malayer	200	28.20^d	58.3^b

Means with similar letters in each column are not significantly different at 0.05 probability level based on Duncan's multiple range test.

Table 3. Means of some traits of *Coriandrum sativum* as affected by NaCl salinity levels.

Salinity levels (mM)	Na+ (mg g^{-1} DWt)	K+ (mg g^{-1} DWt)	K^+/Na^+	Leaf dry weight (g)	Chl a (mg g^{-1} FWt)	Chl b (mg g^{-1} FWt)	Ion leakage (%)	Soluble solids (mg g^{-1} FWt)	MDA (μmol g^{-1} FWt)	H_2O_2 (μmol g^{-1} FWt)
0	4.1^e	22.3^a	5.43^a	0.80^a	1.87^a	1.02^a	16.3^e	1.90^c	0.030^e	0.21^e
50	11.4^d	16.7^b	1.42^b	0.69^b	1.59^a	0.87^b	27.3^d	1.98^c	0.038^d	0.30^d
100	20.8^c	15.1^c	0.70^c	0.49^c	1.04^b	0.80^b	38.1^c	2.19^b	0.043^c	0.32^c
150	29.7^b	16.5^d	0.55^{dc}	0.44^c	0.98^b	0.68^{bc}	58.9^b	2.76^b	0.059^b	0.38^b
200	46.2^a	10.2e	0.22^{dc}	0.27^d	0.79^c	0.51^c	73.1^a	3.48^a	0.063^a	0.60^a

Means with similar letters in each column are not significantly different at 0.05 probability level based on Duncan's multiple range test; MDA: malon dealdehyde.

Table 4. Total soluble solids (TSS) of two *Coriandrum sativum* clones averaged over NaCl salinity levels.

Clone	TSS (μmg^{-1} F.Wt)
Tabriz	2.30[b]
Malayer	2.59[a]

Mean with similar letters are not significantly different based on t test.

salinity, respectively. They indicated that *S. argentea* seedlings were not efficient in restricting sodium movement to the photosynthetic parts of plants. Khajeh-Hosseini *et al*. (2003) stated that soil salinity may affect the germination of soybean seeds by preventing water uptake through osmotic potential, or toxic effects of Na+ and Cl− ions.

Potassium content

K^+ content was significantly affected by salinity. Control (22.3 mg g^{-1} DWt) and 150 mM NaCl (16.5 mg g^{-1} DWt) had the highest and the lowest K^+ content (Table 3). Potassium (K^+) is an essential ion that has important roles in enzyme activation, osmotic adjustment and regulation of membrane potential (Barragan *et al.* 2012). Moreover, it is known that toxic effects of Na^+ are mainly due to its ability to compete with K^+ for the binding sites necessary for cell function (Bhandal and Malik 1988).

K^+/Na^+ ratio

Table 3 shows the effect of salinity on K^+/Na^+ ratio. As salinity increased, the K^+/Na^+ ratio declined. Baibordi *et al.* (2010) stated that the higher K/Na ratio in aerial parts of some rapeseed genotypes is probably due to their ability to prevent sodium entry into the roots and maintain potassium in shoots and also the better adaptation to stress conditions. The osmatic effect of salinity retard the growth and development of plant species. High concentration of Na+ or Cl- may result in accumulation of them in leaves and cause the firing of leaves and appearance of symptoms similar to nutritional deficiency in plants (Shannon and Grieve 1998). Similar results were reported by Melo *et al*. (2017) in *Ananas comosus*. They indicated that ionic imbalance in this plant damages the cell membrane and increases ion leakage.

Electrolyte leakage

The highest electrolyte leakage (73.1 %) belonged to 200 mM NaCl salinity level (Table 3). Under saline sodic conditions, Na^+ absorption leads to the reduced K^+ absorption (Cramer *et al.* 1986). Zakery-Asl *et al*. (2014) reported that salinity stress increases electron leakage in *Suaeda aegyptiaca*, due to cell damage. Similar result was reported by Melo *et al*. 2017 in *Ananas comosus*.

Conclusions

Plants use complicated mechanisms to cope with the adverse effects of salinity stress by balancing the growth and physiological processes. Our results indicated the damaging effects of salinity stress on physiological traits of *Coriandrum sativum*. NaCl salinity imposed on the *Coriandrum sativum* decreased the chlorophylls a and b content, K^+/Na^+ ratio and plant dry weight and increased proline, MDA, H_2O_2 and soluble sugars content. Although, based on our results, *Coriandrum sativum* clones under study were relatively sensitive to the salinity stress but further experiments are necessary to evaluate other clones at different salinity levels to possibly select the salinity tolerant clones in this species.

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پاسخ گیاه گشنیز به تنش شوری

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چکیده

شوری درآب و خاک یک مشکل محیطی بوده و از مهمترین عوامل محدود کننده در تولید محصول می‌باشد. گشنیز به عنوان سبزی برگی مورد توجه تولیدکنندگان سبزی در دنیا است. به دلیل افزایش تدریجی شوری در آب و خاک در شمال غرب ایران، تولید این محصول با محدودیت مواجه شده است. به منظور بررسی اثر تنش شوری به برخی ویژگی‌های فیزیولوژیک و مورفولوژیک گشنیز آزمایشی بر مبنای فاکتوریل بر پایه بلوک‌های کامل تصادفی با پنج سطح شوری کلرید سدیم (صفر، ۵۰، ۱۰۰، ۱۵۰ و ۲۰۰ میلی مول) و دو کلون محلی (تبریز و ملایر) طراحی شد. نتایج نشان دهنده وجود اثرات متقابل معنی‌دار سطوح شوری و کلون بر محتوای پرولین و آب نسبی برگ بود. بیشترین میزان پرولین (۷۷ میکروگرم بر گرم وزن تر) در تیمار کلون تبریز × ۲۰۰ میلی مول نمک ثبت شد و بیشترین میزان آب نسبی برگ در کلون تبریز در شرایط بدون تنش شوری و کلون ملایر در شرایط بدون تنش شوری و تنش شوری ۵۰ میلی مول به دست آمد. بیشترین میزان وزن خشک برگ، نسبت پتاسیم به سدیم و پتاسیم متعلق به تیمار بدون شوری (شاهد) بود. بیشترین تجمع سدیم، مالون دی آلدئید، پراکسید هیدروژن و نشت یونی در تیمار ۲۰۰ میلی مول نمک مشاهده شد. محتوی مواد جامد محلول به وسیله تیمار شوری و رقم تحت تاثیر قرار گرفت و بیشترین میزان مواد جامد محلول در کلون ملایر به دست آمد. با افزایش تنش شوری بر محتوی مواد جامد محلول افزوده شد و بیشترین میزان آن در تیمار ۲۰۰ میلی مول حاصل شد.

واژه‌های کلیدی: پراکسید هیدروژن؛ شاهی؛ عملکرد؛ مالون دی آلدئید