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Effect of Salinity on Growth, Ions Distribution and Accumulation and Chlorophyll Concentrations in Two Canola (*Brassica napus* L.) Cultivars

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Abstract: Salinity is a major environmental factor that influences rapeseed production in arid and semi-arid regions of central and southern Iran. In this study, effect of applied NaCl on shoot and root growth and distribution and accumulation of Na, Cl, K, Ca and Mg in the canola plant (*Brassica napus* L. cv: Hayola 308 and cv: PP 401) was investigated. The experiment was arranged in a completely randomized design (CRD) under the greenhouse condition. Sodium chloride was applied at the rates of 0, 4, 8 and 12 ds m^{-1} NaCl. Shoot and root growth of the canola plants was inhibited by salinity and NaCl significantly decreased shoot and root dry mass of canola plants. Leaf number and shoot height were inhibited by increased salt treatments in both cultivars, although Hayola 308 was more salt-tolerant than PP 401. In 8 and 12 ds m^{-1} salt treatments, Hayola 308 had significantly higher root and shoot dry weights per plant than PP 401. The addition of NaCl in the soil decreased total chlorophyll concentration in plants. The total chlorophyll in Hayola 308, however, was considerably greater (50 and 35%) than in PP 401 at 8 and 12 ds m^{-1} salt treatments, respectively. Concentrations of Na and Cl ions significantly increased in parallel to amount of NaCl. On the contrary K concentrations in canola plant shoots and roots were significantly decreased with increasing salinity. The Ca concentration in the roots declined with NaCl application while Ca increased in the shoots. Mg ion concentration in root at first decreased and then increased. In general, it seems that at high salinity levels PP 401 cultivar was more sensitive than Hayola 308 cultivar, because of more sodium and chlorine in the shoot and more calcium and manganese in the root.

Key words: Salt Stress · Canola · Ions accumulation · Growth · Chlorophyll

INTRODUCTION

Salt stress is one of the most important factors that limit crop production in arid and semi-arid regions [1]. More than 800 million hectares of land throughout the world are salt-affected (including both saline and sodic soils), equating to more than 6% of the world's total land area [2]. Furthermore there is also a dangerous trend of a 10% per year increase in the saline area throughout the world. Soil salinity may be robbing the country of about 25% of its crop production [3]. Iran is one of the countries that suffer from sever salinity problems. For example 18 million ha or 10% of total land area in Iran is salinity or sodicity soil. Need to develop crops with higher salt tolerance have increased strongly due to increased salinity problems [4].

Canola (*Brassica napus* L.) is an important oil crop, ranking third only to soybean and palm oil in global production. It is a member of the family Brassicacea (syn.Cruciferae). It is a winter or spring crop and is amenable to growth in cooler climates. Once considered a specialty crop for Canada, it is now a global crop. Many other countries including the USA, Australia and those in Europe also grow cano1a. However, Canada and the United States account for most of the canola crop [5]. Its oil also has potentially developed in the biodiesel

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market. In addition to oil production, the leaves and stems of canola provide high quality forage matter because of their low fiber and high protein content [6] and can be milled into animal feeds [7].

Salinity has three potential effects on plants: lowering of the water potential, specific ion toxicity (sodium and chloride) and interference in the uptake of essential nutrients [8]. Soil salinity is shown to increase P, Mn and Zn and decrease K and Fe concentrations of plants [9]. Shoots are generally more sensitive to cation disturbances than roots and there are great differences among plant species in the ability to prevent or tolerate the excess salt concentrations [10, 11].

Salt stress affects many physiological aspects of plant growth. Shoot growth was reduced by salinity due to inhibitory effect of salt on cell division and enlargement in growing point [10]. Early flowering reduced dry matter, increased root: shoot ratio and leaf size caused by salinity may be considered as possible ways of decreasing yield in wheat under salt stress condition [11-13]. Toxic effects of salts may change enzymatic activity and hormonal balance of plants. It is reported that high transpiration rate at leaf surfaces cause high accumulation of salt in leaves which kill them before full maturity [14]. Net photosynthesis was decreased due to the reduction in photosynthesis and increasing in respiration per unit of leaf area. Studies on physiological salt tolerance mechanisms revealed that plants may reduce detrimental effects of salts by the control of salt uptake [15] reducing damage under excessive ion uptake [16, 17] and osmotic adjustment [18]. Growth inhibition by salt stress also occurs due to the diversion of energy from growth to maintenance [19]. To maintain under salt stress plants need to regulate ion concentration in various organs and within the cells by synthesis of organic solutes for osmoregulation or protection of macromolecules for maintenance of membrane integrity [14]. It has been reported that increasing salt concentration in growing medium of pea plants increased roots and stems respiration [19, 20]. However, results of many cases show that respiration decreases under water stress condition, though decrease in respiration is much less than photosynthesis. Salt stress was also showed to increase the activity of an alterative pathway along with the cytochrome pathway. A salt tolerant wheat cultivar was showed to produce more ATP than a salt sensitive one [21]. When the salt concentration of the soil solution increases, water potential decreases, the turgor potential of plant cells declines and cells ultimately cease to grow. Under these water stress conditions, in general, stomata close resulting in the reduction of photosynthesis. Protein breakdown is enhanced and plants show poor growth. The low osmotic potential of saline soils makes it necessary for plants growing on them to maintain a lower intracellular osmotic potential; otherwise, they would experience water stress due to the movement of water osmotically from the plant tissue into the soil. In order o achieve a lower osmotic potential, osmotic adjustment under saline conditions can occur in plants due to uptake of inorganic ions from the saline growth medium [22], or by internal synthesis of osmotically active organic solutes [23].

The present study was undertaken to study the response of two canola cultivars to different levels of salinity and to determine the effect of NaCl on shoot and root growth, distribution and accumulation of Cl, Na, K, Ca and Mg in shoot and root of the canola plant.

MATERIAL AND METHODS

Soil Material: The experimental soil taken from Aridisol great soil group was calcareous (198 g Kg^{-1}), silty clay in texture, slightly alkaline (pH: 7.57 and EC: 0.924 dS m^{-1}). The soil sample had 36.7 mg kg^{-1} exchangeable Na and water extractable Cl was 6.41 mg kg^{-1}.

Pot Experiment: The experiment was conducted under greenhouse conditions (humidity 75-85%, air temperature 25-30°C and neutral light intensity 290-460 µmol $m^{-2}s^{-1}$) in a factorial as Completely Randomized Design (CRD) in Islamic Azad University of Ramhormoz, Khuzestan, Iran in October 2012. The soil (6000 g) was placed into pots and was salinised with NaCl at the rates of 0, 4, 8 and 12 ds m^{-1} NaCl. For basal fertilizers, 100 mg N kg^{-1} as ammonium nitrate and 80 mg P kg^{-1} as triple super phosphate were applied to the pots. Five canola (*Brasica napus* L. cv:Hayola 308 and cv:PP 401) seeds were sown into each pot which were thinned to three after emergence. Plants were harvested six weeks after germination and divided into shoot and root. Dry weight measurements of all plant samples were taken after being washed with distilled water. The shoot: root ratio was estimated.

Chemical Analysis: After grinding, all plant samples were digested with HNO3:HClO4 acid mixture (4:1) in order to determine Cl, Ca, Na, K and Mg in the shoots and roots [24]. Na, K and Ca were determined by using Eppendorf Elex 6361 model flame photometry [25]. Chloride was analyzed by precipitation as AgCl and titration [26]. Mg was determined by atomic absorption spectrometry (Shimadzu model AA-670) [27].

Data analysis: Data were analyzed using the GLM procedure of SAS program [28]. Significant differences between treatments were determined using Duncans multiple range test at 0.05 level.

RESULTS AND DISCUSSION

Leaf Number and Shoot Height: Leaf number and shoot height (Table 1 and 2) were inhibited by increased salt treatments in both cultivars, although Hayola 308 was more salt-tolerant than PP 401 (Fig 1). In 8 and 12 ds m^{-1} salt treatments, Hayola 308 had significantly higher shoot height and leaf number per plant than PP 401 (Fig 1 and 2).

Shoot and Root Dry Weights: NaCl application inhibited the growth of canola plant and caused to decrease both shoot and root dry weights (Table 1 and 2). Canola plants grown at the low levels of NaCl (0 and 4 ds m^{-1}) reached relatively higher dry weights and did not imply toxicity symptoms, however, the growth was significantly reduced at higher levels of salinity (8 and 12 ds m^{-1}) indicating the symptoms of salt toxicity as growth depression. The concentrations of NaCl that significantly reduced shoot and root dry weights were 8 and 12 ds m^{-1} by 60.12 and 240% for shoots and by 100 and 400% for roots, respectively, in comparison to the control. The shoot: root ratio was found to decline with increasing salinity (Table 1 and 2). Shoot dry weight plant was most affected by salt in both cultivars. In 8 and 12 ds m^{-1} salt treatments, Hayola 308 had significantly higher root and shoot dry weights per plant than PP 401 (Fig 3 and 4).

Salinity affects both water absorption and biochemical processes resulting in reduction of plant growth [29] and a decline in the rates of net photosynthesis significantly by negatively affecting CO2 assimilation and leads to decrease largely nutrient uptake and finally growth of plants is getting reduce [30-32]. Although many researchers [33-35] reported that low levels of applied NaCl reduced the dry weight of experimental plants, but the results obtained from our study showed that the high levels of NaCl (8 and 12 ds

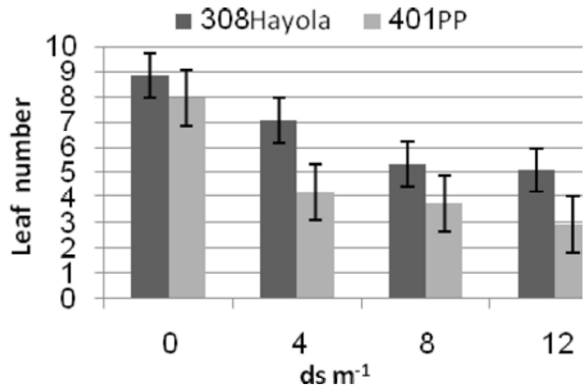

Fig. 1: Effect of salinity levels on leaf number of canola cultivar

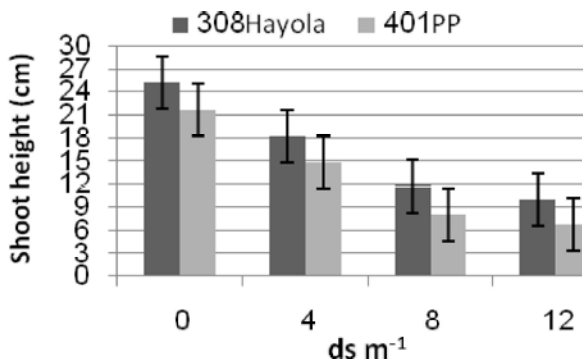

Fig. 2: Effect of salinity levels on shoot height of canola cultivar

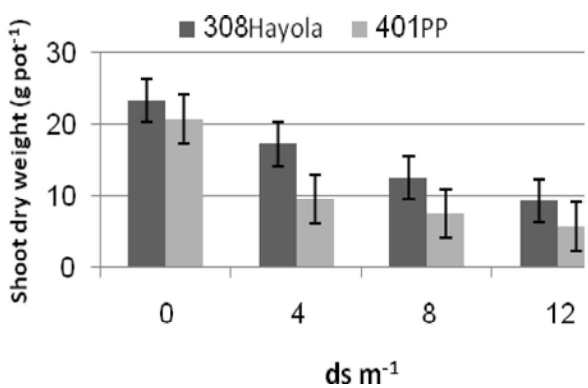

Fig. 3: Effect of salinity levels on shoot dry weight of canola cultivar

Table 1: Analysis of variance for the traits investigated in two canola cultivars in response to salinity stress.

Sources of variation	df	Leaf number	Shoot height	Shoot dry weight	Root dry weight	Shoot: Root Ratio	Total Chlorophyll	Cl	Na	K	Ca	Mg
Cultivar	1	*	**	*	*	**	**	**	**	**	**	**
Salt stress	4	*	*	**	*	**	*	**	**	*	**	**
Cult × S. stress	4	*	*	*	*	*	*	**	**	*	**	**

ns Non- significant, * Significantly at $p < 0.05$, ** Significantly at $p < 0.01$.

Table 2: Mean values of vegetative traits for two canola cultivars and different salinity stress

Treatment	Leaf number	Shoot height (cm)	Shoot dry weight (g pot^{-1})	Root dry weight (g pot^{-1})	Shoot: Root Ratio	Total Chlorophyll (mg g^{-1} fresh weight)
Cultivar						
PP 401	7.1 a	17.2 b	18.62 a	7.23 a	2.57 a	3.93a
Hayola 308	6.9 a	20.6 a	17.24 a	6.59 a	2.61 a	3.22 b
Salinity level ds m^{-1} NaCl						
0	7.6a	17.3 a	16.25a	8.12a	2.58 a	4.89 a
4	7.2a	13.5 b	15.32 a	7.18 a	2.32 b	3.86 b
8	6.6b	12.8 b	10.14 b	4.36 b	2.31 b	3.12 c
12	3.4c	7.1 c	6.62 c	2.56 c	2.00 c	1.87 d

Means with similar letters in each column are not significantly different at 5% level of probability (Duncan).

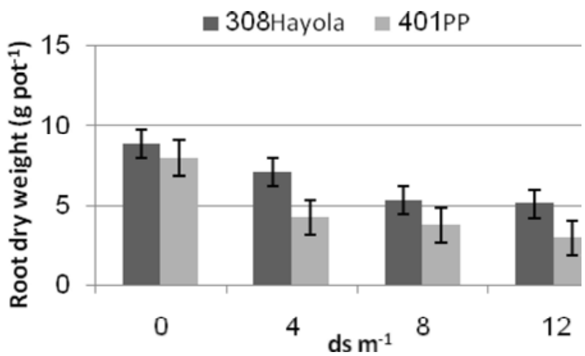

Fig. 4: Effect of salinity levels on root dry weight of canola cultivar

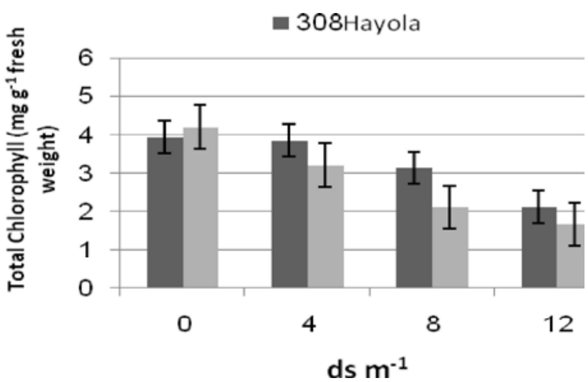

Fig. 5: Effect of salinity levels on total chlorophyll of canola cultivar

m^{-1}) inhibited shoot and root growth of the canola plants. The suppression of plant growth under saline conditions may either be due to osmotic reduction in water availability or to excessive ion levels which is known by the specific ion effect [36]. As findings of this study, the shoot growth was much more affected by salinity than was the root growth. These findings are in agreement with Huck and Schroeder [37] and Esechie *et al*. [38], who reported that roots seemed to be more resistant to salinity than were plant foliage. It may be explained that in the higher levels of NaCl, the osmotic effect could be inhibited by the growth of the shoot.

Total Chlorophyll: Total chlorophyll of canola plants was also affected significantly ($P<0.05$) by salinity (Table 1). The addition of NaCl in the soil decreased total chlorophyll concentration in plants (Table 2). The total chlorophyll in Hayola 308, however, was considerably greater (50 and 35%) than in PP 401 at 8 and 12 ds m^{-1} salt treatments, respectively (Fig 5).

Salinity decreased the total chlorophyll concentration of two maize varieties. The reduction in photon yield in the salt stressed seedlings of maize was positively correlated to net photosynthetic rate; in which the significant drop in Pn of salt stressed seedlings resulted in considerable growth reduction [32].

Ion Concentrations and Distribution: Concentrations of Cl and Na ions significantly increased in parallel to amount of NaCl ($p<0.01$) (Fig 6 and 7). On the contrary K concentrations in canola plant shoots and roots were significantly decreased with increasing salinity ($p<0.01$) (Fig 8). The Ca concentration in the roots declined with NaCl application while Ca increased in the shoots ($p<0.01$) (Fig 9). Mg ion concentration in root at first decreased and then increased (Fig 10). In general, it seems that in high salinity levels PP 401 cultivar was more sensitive than Hayola 308 cultivar, because of more sodium and chlorine in the shoot and more calcium and manganese in the root (Fig 6, 7, 9 and 10).

Increasing levels of NaCl induced a progressive absorption of Na and Cl in both shoot and root [39, 40]. Excessive Na concentration in the plant tissue hinders nutrient balance, osmotic regulation and causes specific ion toxicity [41, 42]. Accumulation of Cl in the root tissue is disruptive to membrane uptake mechanisms and these results in increased translocation of Cl to the shoots [43]. When NaCl was applied to the soil, NaCl decreased K concentrations in the Shoot and root in according with an antagonism between Na and K [44- 46]. Cramer *et al*. [47] showed that excess NaCl leads to the loss of potassium due to membrane depolarization by sodium ions. As a result of salinity, therefore, potassium accumulated in

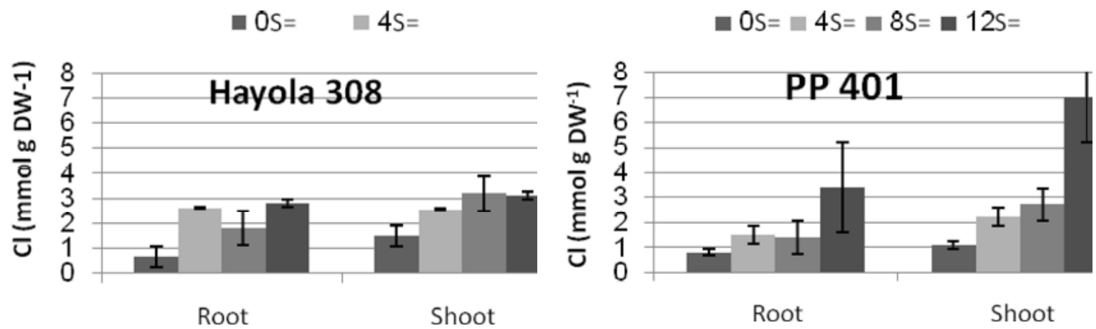

Fig. 6: Effect of salinity levels on chlorine (Cl) concentration in root and shoot of canola cultivar

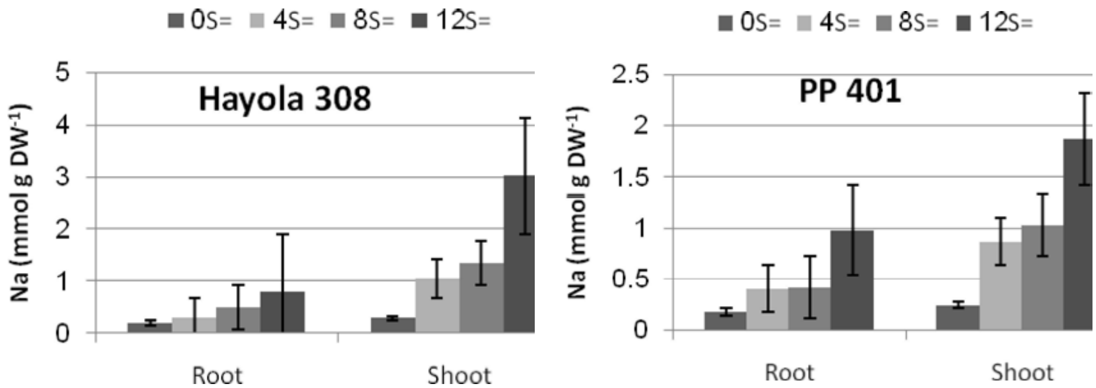

Fig. 7: Effect of salinity levels on sodium (Na) concentration in root and shoot of canola cultivar

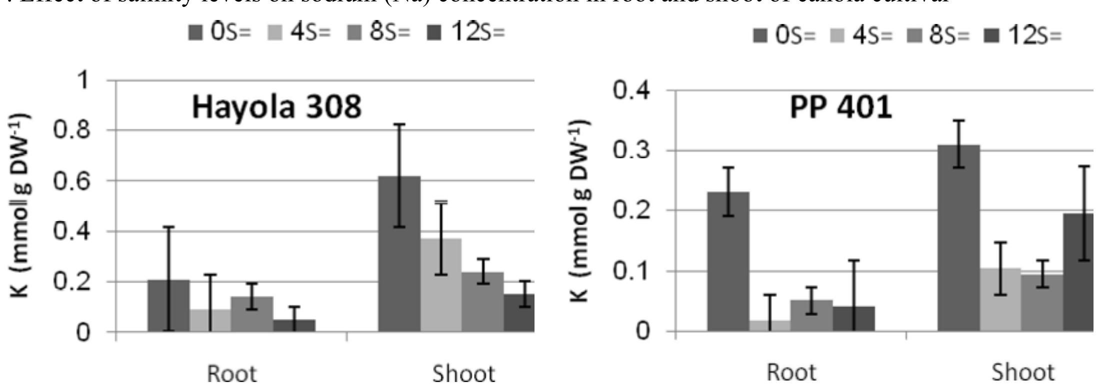

Fig. 8: Effect of salinity levels on potassium (K) concentration in root and shoot of canola cultivar

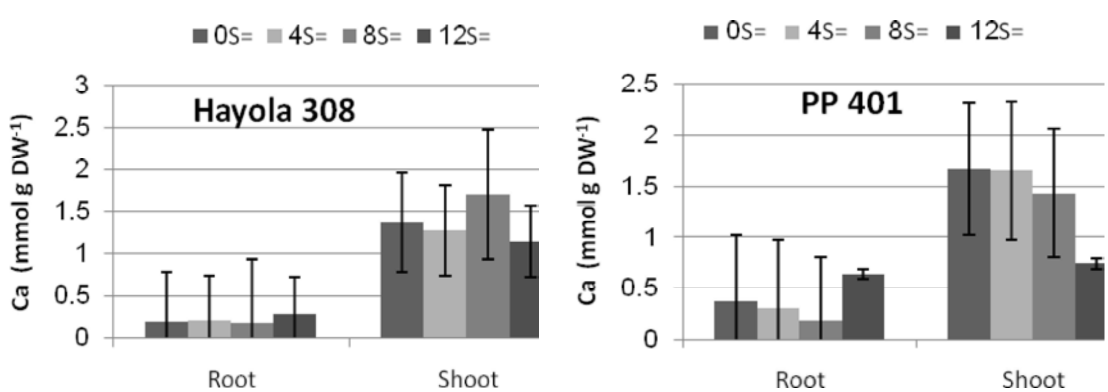

Fig. 9: Effect of salinity levels on potassium (Ca) concentration in root and shoot of canola cultivar

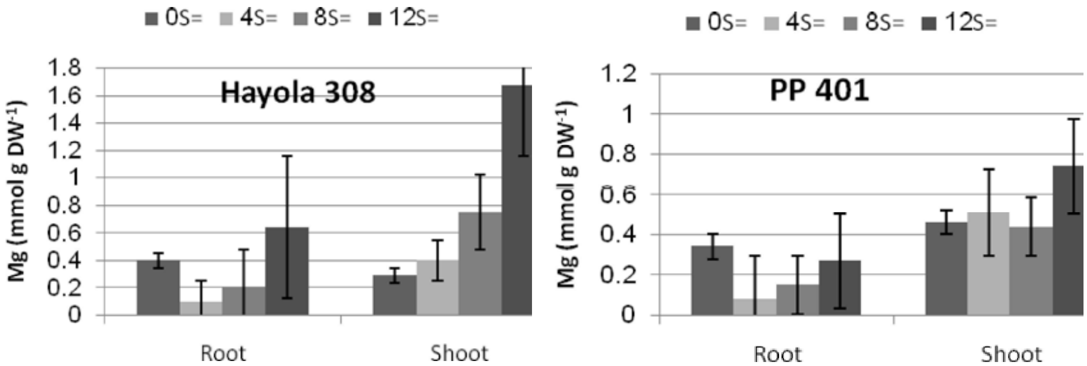

Fig. 10: Effect of salinity levels on potassium (Mg) concentration in root and shoot of canola cultivar

shoot rather than in root by salinity effect. K accumulated in the shoot also reported by Siegel *et al*. [48] and Karmoker *et al*. [46]. High Na concentration in the substrate or soil inhibits uptake and transport of $Ca2^{+}$ and may therefore; induce calcium deficiency in plants [49]. Applying NaCl increased manganese concentrations in the shoot and root. Many researchers were reported similar results [39, 50, 51]. The present study showed that low levels of NaCl did not affect the growth of canola plants. But high levels of NaCl inhibited the growth and caused to decreased dry weight both organs. NaCl caused to decrease, potassium and calcium in the shoot tissue. Na and Mn accumulated in the root tissue in accordance with applied NaCl.

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