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Effects of Salinity on Some Physiological Characteristics of *Lepidium sativum* L.

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Abstract
Salinity is one of the major environmental stresses, which has deleterious effect on growth, development and yield of crops. Due to the gradual increase in soil and water salinity in the East Azarbaijan province of Iran, the garden cress cultivation in this region has always been associated with many problems. In order to evaluate the tolerance of this plant to different levels of salinity through some physiological characteristics, the present experiment was conducted using randomized complete block design with five treatments consisting of 0, 50, 100, 150 and 200 mM NaCl concentrations and three replications. The results showed that with enhancement in salinity levels, sodium, proline, soluble sugars and carotenoids content increased but potassium content, potassium to sodium ratio and amounts of chlorophyll a and b declined. Salinity had no significant effect on chlorophyll a+b content, chlorophyll a/b ratio and relative water content. Plants were destroyed at 200 mM concentration after 21 days. Since potassium to sodium ratio was lower than 1 at 100 and 150 mM concentrations, continuing of salinity would has presumably led to the destruction of plants in these treatments.

Keywords: *Lepidium sativum* L.; Physiological characteristics; Salinity

Introduction

Salinity is one of the most important environmental stresses, which has adverse effect on growth, development and yield of plants by the physiological dehydration, ion imbalance, oxidative stress, cell membranes damage and photosynthesis decline. Plants cope with salinity stress through different mechanisms such as ionic homeostasis, osmolytes production, maintenance of relative water content, keeping up potassium to sodium ratio and stabilizing chlorophylls structure (Cornic and Massacci 1996).

Garden cress (*Lepidium sativum* L.) is a plant with nutritional and pharmaceutical values (Radwan *et al.* 2007; Swatsitang and Wonginyoo 2008), but it's cultivation in Iran is limited and hence comprehensive research on different characteristics of this plant as well as the effects of environmental stresses on these features has not been carried out. Although salinity creates many problems in the production of various crops including garden cress in major parts of East Azarbaijan Province, especially with increasing dryness of Lake Urmia in recent years, but due to lack of cultivation areas, use of this region's lands with salty soil and/or water is unavoidable. Thus, the study of salinity effects on different characteristics of crops is necessary and, therefore, the present study was conducted to examine garden cress tolerance to different salinity levels considering some physiological characteristics.

Materials and Methods

Plant materials and initiation of the experiment

Seeds of garden cress were obtained from Pakan Bazr Corporation, Esfahan, Iran. The seeds were surface sterilized with 1% hydrogen peroxide for 20 min, washed thoroughly with distilled water and then planted on perlite in pots with 20×30 cm dimensions feeding by the Hoagland's solution (Hoagland and Arnon 1950). Nutrient solutions were renewed every three days. Composition of this solution was: (mM) 1 calcium nitrate [$Ca(NO_3)_2$, $4H_2O$]; 0.1 mono potassium phosphate (KH_2PO_4); 0.5 potassium sulfate (K_2SO_4); 0.5 magnesium sulfate ($MgSO_4$) and (μM) 10 boric acid (H_3BO_3); 20 manganese sulfate ($MnSO_4$, H_2O); 0.5 zinc sulfate ($ZnSO_4$, $7H_2O$); 1 copper sulfate ($CuSO_4$, $5H_2O$); 0.1 molybdenum trioxide (MoO_3); 100 iron sulfate ($FeSO_4$, $7H_2O$). Seedlings were grown in a greenhouse at a 16-h light: 8-h dark photoperiod with a light intensity of 450 μmol m^{-2} s^{-1} supplied by combined fluorescent and incandescent lamps, 25/20 ˚C day/night temperature and 70% relative humidity.

Induction of salt stress

Salt stress treatments were imposed on four weeks old plants by adding 50, 100, 150 and 200 mmol NaCl to the nutrient solution by the order of 25 mM at first, 25 mM one day later and 50 mM every three days to avoid osmotic shock until the final concentration of 200 mM NaCl was achieved. A nutrient solution without NaCl addition served as the control. After three weeks of salinization, the leaves were sampled, transferred to the liquid nitrogen and maintained at −70 ˚C until the measurement of variables. At the same time, samples for the relative water content (RWC) assays were collected and brought to the laboratory in ice buckets.

Evaluation of salt injury

Potassium and sodium content

Potassium and sodium contents were measured by the flame photometry method. Leaf samples were dried and pulverized. One gram of powdered leaf material was kept at 560 ºC for 4 h for ash preparation. To these samples, 20 ml of 1 N hydrochloric acid (HCl) was added and the mixtures were heated at 90 ºC to drive off the hydrochloric acid. The digested ash was dissolved in 100 ml distilled water and then filtered. The filtrate was stored in a refrigerator until analysis. Concentrations of potassium and sodium ions were estimated by referring to 0, 5, 10, 20 and 30 ppm standard working solution. The test solution was diluted if its signal was above that of the highest standard. Content of the elements were calculated by using the following equation (Bandehhag *et al.* 2004):

$$E = [(C \times V \times D) / (M \times 10^6)] \times 100$$

Where E is the element (either potassium or sodium) content of the test sample, expressed in %, C is the element mass of the test solution, expressed in mg/l, read from the calibration graph, V is the volume, in ml, of the digested solution (V= 100). D is the dilution factor of the test solution carried out during the measurement step. M is the mass, in g, of the test sample used in the procedure.

Chlorophyll content

For the measurement of chlorophyll content, dried leaf samples (0.5 g) were incubated in 5 ml of

dimethyl sulphoxide (DMSO) at 65 °C for 4 h. Absorbance was recorded at 645, 665 and 470 nm, and chlorophyll a (chla), chlorophyll b (chlb), chla/chlb and total chlorophyll contents were calculated (Prochazkova *et al.* 2001).

Proline content

Leaf samples (0.5 g) were homogenized in 5 ml of 3% (w/v) sulfosalycylic acid using mortar and pestle. About 2 ml of extract was taken in test tube and 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent were added. The reaction mixture was boiled in water bath at 100 °C for 30 min. After cooling the reaction mixture, 6 ml of toluene was added and then transferred to a separating funnel. After thorough mixing, the chromophore containing toluene was separated and absorbance was read at 520 nm in spectrophotometer (T_{80}, UK) against toluene blank. Concentration of proline was estimated by referring to a proline standard curve (Fedina *et al.* 2006).

Total soluble sugars content

Total soluble sugars were estimated by using anthrone reagent. Leaf extracts (0.05 ml) were taken in test tubes and the volume was made up to 1 ml. To this solution, 4 ml of anthrone regent was added and the mixture was heated in boiling water bath for 8 min followed by cooling. Optical density of green to dark green color was read at 630 nm (Sairam *et al.* 2002).

Relative water content

Leaf samples (0.5 g) were incubated in 100 ml of distilled water for 4 h. After this period, the turgid weights of leaf samples were measured. The leaf samples were packed in butter paper bags and oven dried at 70 °C for 48 h. The dry weights of the samples were taken after confirming that the samples were completely dried out. The RWC was determined by the following formula (Xu *et al.* 2005):

$$RWC = [(\text{Fresh wt.} - \text{Dry wt.}) / (\text{Turgid wt.} - \text{Dry wt.})] \times 100$$

Statistical analysis

Data were subjected to analysis of variance using the MSTATC program. The mean differences were compared by the LSD test at the $p \leq 0.05$ levels.

Results and Discussion

Analysis of variances showed that salinity had significant effect on Na^+ and K^+ concentrations, potassium to sodium ratio and chlorophyll a, chlorophyll b, carotenoid, proline and soluble sugars contents (Table 1). Furthermore, all seedlings were died at 200 mM of salt solution (Table 2).

Salinity increased sodium concentration, so that significant difference observed between the treatments. The minimum and maximum concentrations of sodium were derived from the control and 150 mM of salt, respectively (Table 2). Increase in the amount of sodium in response to salinity stress was reported by Al Karaki (2000) in tomato, De Lacerda *et al.* (2003) in sorghum and Ekiz and Yilmaz (2003) in barley. Munns (2002) believed that the increase in sodium content in plants and its accumulation in cytoplasm under salt stress makes the potassium to be replaced by sodium. Replacement of potassium by sodium in specific sites of cytosol,

decreases concentration of this ion to do functional processes (Zhu 2003). A negative correlation was not observed between aggregation of sodium and salt tolerance in plants in the study of Flowers (2004). Accordingly, sodium accumulation in vacuoles could be an effective mechanism for plant cells to efficient use of salt for osmoregulation, prevention of its toxicity in cytosol and induction of the salinity tolerance (Paranychianakis and Chartzoulakis 2005).

Based on Table 2, potassium content reduced by the enhancement of salt concentration. Although difference between the control and 50 mM and between 100 and 150 mM were not significant, the highest content of potassium was obtained from the control (6.59%) and the lowest concentration acquired from 150 mM salt (2.65%). Reports about the decrease in potassium content at high levels of salt were provided by Reggiani *et al.* (1995) in durum wheat, Baalbaki *et al.* (2000) in pea and Elbaz *et al.* (2003) in cucumber. Maintaining an adequate amount of potassium is necessary for survival of plants in salty areas. Potassium accumulation in roots by keeping low osmotic potential, causes solutions transport in xylems through turgor pressure, helps to maintain water balance in plant and decreases effects of drought caused by salinity stress. This element also plays a role in the opening/closing stomata and specifically it is required for the synthesis of some proteins, activation of several enzymes and increasing efficiency of photosynthesis (Grattan and Grieve 1999). Therefore, keeping high levels of potassium is considered as a tolerance mechanism under salt conditions (El Hendawy *et al.* 2005).

Table 1. Analysis of variance of physiological variables of Garden Cress leaf under salt stress

Source	df	Mean squares										
		Na^+	K^+	K^+/Na	Chl a	Chl b	Chl a+b	Chl a/b	Car	Proline	SS	RWC
Replication	2	0.31ns	0.02ns	0.094ns	11.67ns	20.7ns	9.6ns	0.24ns	171.6ns	188.9ns	0.7ns	0.001ns
Treatment	3	15.01**	10.07**	6.000**	196.95**	206.0**	211.7 ns	1.84 ns	802.8**	2060.8**	0.5**	0.007ns
Error	6	0.068	0.13	0.035	14.47	7.5	67.7	0.911	70.0	73.3	0.003	0.02
C.V. (%)		6.3	8.1	11.4	15.2	19.3	21.9	20.2	12.0	4.6	2.4	29.3

ns and **: Non-significant and significant at 1% probability level, respectively; Chl: Chlorophyll; Car: Carotenoids; SS: Soluble Sugars; RWC: Relative water content

Although there was no significant difference between 100 and 150 mM in terms of potassium to sodium ratio, but this ratio decreased by increasing salt levels, so that it reached from 3.57 in the control to 0.37 in the 150 mM salt level (Table 2). Although Chinnusamy *et al.* (2005) recognized that high potassium to sodium ratio is necessary for cellular functions execution of plants exposed to salt stress, the decline in potassium to sodium ratio in response to salinity were reported in rice (Asch *et al.* 1999), sorghum (De Lacerda 2003) and durum wheat (Azizpour *et al.* 2010). Wyn Jones *et al.* (1979) argued that the ratio of potassium to sodium in non-halophytes should be more than 1 to continue the vital processes in normal environmental conditions, which was not observed at concentrations of 100 and 150 mM in our experiment and perhaps if the experiment was continued, it could lead to destruction of plants in these treatments.

Table 2. Means of physiological variables of Garden Cress leaf under salt stress

Salt (mM)	Na^+	K^+	K^+/Na	Chl a	Chl b	Chl a+b	Chl a/b	Car	Proline	SS	RWC
0	1.89a	6.59a	3.57a	33.17a	26.47a	49.56a	1.27a	50.11a	153a	2.00a	46a
50	2.86b	5.58a	1.69b	29.64a	11.9b	37.25a	2.88a	66.17ab	176ab	2.19b	53a
100	4.70c	3.43b	0.72c	22.27ab	9.77b	31.99a	2.71a	74.21ab	194bc	2.58c	45a
150	6.97d	2.65b	0.37c	14.94b	8.67b	31.51a	1.57a	89.34b	215c	2.92d	44a
200	-	-	-	-	-	-	-	-	-	-	-

In each column, means with different letters are significantly different at 0.05 probability level, respectively; Chl: Chlorophyll; Car: Carotenoids; SS: Soluble Sugars; RWC: Relative water content

With the enhancement in salt concentration to the 150 mM level, chlorophyll a and chlorophyll b contents reduced 54.9 % and 67.2% as compared to the control, respectively. Higher sensitivity of chlorophyll b to salinity (Sultana *et al.* 1999) or converting it to chlorophyll a in order to hold amount of chlorophyll a at the high level, could be the reason for the reduction of chlorophyll b more than chlorophyll a (Fang *et al.* 1998). Many studies referred to the decline in chlorophyll content due to salinity (Heuer and Nadler 1998; Erylmaz 2006; Jampeetong and Brix 2009) which is an important factor affecting the loss of photosynthesis rate in plants. Decrease in chlorophyll content may be associated with the effects of toxic ions on reducing uptake of elements related to the chlorophyll biosynthesis, such as nitrogen, magnesium and iron (Neocleous and Vasilakakisi 2006), high activity of chlorophyllase enzyme which is responsible for degradation of chlorophylls (Bertrand and Schoefs 1999) and rapid aging of leaves in the presence of salt (Qasim *et al.* 2003).

Increase in salinity, enhanced the amount of carotenoids, so that the minimum and maximum values of this variable were obtained from the control and 150 mM treatments, respectively (Table 2). Sairam *et al.* (2002) reported that salt stress increased carotenoids content of wheat. They believed that high levels of carotenoids can be the indication of relative tolerance to salinity. Under salinity, these pigments protect photosynthetic apparatus against photoinhibition (Schroeder and Johnson 1993), adjust $NADPH,H^+/NADP^+$ ratio (Ort 2001) and turn off triplet chlorophyll (Havaux 1998) and consequently, prevents generation of reactive oxygen species that effect chloroplasts membranes through lipid peroxidation.

Proline content increased with the increase in salt stress intensity from the control to 150 mM salt level (Table 2). Gadallah (1999) in cotton, Azizpour *et al.* (2010) in durum wheat and Farkhondeh *et al.* (2012) in sugar beet reported that proline content increased with the enhancement in salt concentration. Proline accumulation in plants exposed to salinity, causes osmotic regulation, helps to absorption of water from root zone and plays an important role in keeping intracellular turgor pressure (Ali *et al.* 1999; Misra and Gupta 2005; Demiral and Turkan 2006). Due to the consumption of $NADPH,H^+$ in the biosynthesis of proline, the $NADPH,H^+/NADP^+$ ratio may be adjusted and production of the oxygen free radicals can be declined (Matysik *et al.* 2002; Sanchez *et al.*

2003; Ashraf and Foolad 2007). Proline also helps the stability of membranes, macromolecules and enzymes structure (Nayyr 2003).

Salinity significantly increased the amount of soluble sugars (Table 2). Silva *et al.* (2003), Azizpour *et al.* (2010) and Farkhondeh *et al.* (2012) reported that with increasing in salt concentration, the amount of soluble sugars increased in cowpea, durum wheat and sugar beet. Drought stress induced by salinity usually leads to conversion of polysaccharides to soluble monosaccharides which reduces cell osmotic potential to lose less water or absorb more of it. Soluble sugars also bound to membrane proteins and phospholipids in order to make their structure stable and eventually help to maintain integrity of membranes (Parvaiz and Satyawati 2008).

There was no significant effect of salinity on leaf relative water content (Table 2). It seems that ionic homeostasis by absorption and transport of sodium to vacuoles and also increase in proline and soluble sugars content could justify high levels of this variable. High percentage of relative water content in the tolerant cultivars could be due to reduction in water loss through stomata closure, more water absorption through expansion of roots and efficient osmoregulation through osmolytes production such as glycine betaine, proline and soluble sugars (Cornic and Massacci 1996).

The results showed that with any increase in salinity levels, sodium, proline, soluble sugars and carotenoides increased but potassium, potassium to sodium ratio, chlorophyll a and chlorophyll b declined. Salinity had no significant effects on chlorophyll a+b content, chlorophyll a/b ratio and relative water content. According to our results, garden cress cultivation as a vegetable is possible at 150 mM concentration of salt, however, since the potassium to sodium ratio was lower than 1 at 100 and 150 mM concentrations, it is most likely that higher levels of salinity stress can lead to destruction of plants and makes the seed production impossible.

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