Salinity Effects on Some Physiological Characteristics of *Allium ampeloprasum* L.

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Summary

Water and agricultural soils salinity is the major limiting factors influencing vegetable production in most Iranian areas and climates. *Allium ampeloprasum* L. as a leafy vegetable has been of great interest for vegetable producers in most parts of Iran. However, due to gradual increase in soil and water salinity especially at Northwest Iran, the production of this vegetable has been faced with many production constraints. For the study of the salinity effects on some growth related and physiological traits of *Allium ampeloprasum,* an experiment was conducted as a factorial based on RCBD with five NaCl concentrations (0, 40, 80, 120 and 160 mM) levels and two local clones ('Tabriz' and 'Isfahan') with three replications. The results revealed that there were interaction effects of salinity and clonee considering proline content and K^+/Na^+ ratios. The highest amounts of chlorophylls a and b, total chlorophyll content, and leaves fresh weight were recorded in control plants. Na^+ accumulation, MDA, H_2O_2 levels, soluble sugars content and ion leakage rate were the highest with 160 mM NaCl levels. With salinity level added, the proline accumulation in the plants was concomitantly increased.

Key words

Allium ampeloprasum L., enzym activity, phenolic compounds

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Introduction

Allium ampeloprasum L. (Iranian Leek) is monocotyledonous perennial herb that belongs to the Alliaceae family with the common name of Tarreh. This plant and its products have antioxidant, anti-tumor, diuretic, antitussive and expectorant properties. Furthermore, the plant has high vitamin C content and greatly reduces blood pressure (Akabri et al., 2012). Morpho-cytogenetic characteristics of this plant have great similarity to leek (*Allium ampeloprasum* var. porrum Gay) (Akabri et al., 2012). Iranian native *A. ampeloprasum* is a common highly consumed herb in quite all region in Iran and its cultivation dates back to many hundred years ago. However, there are not enough studies evaluating the growth responses of this plant to environmental and cultural stimuli. The preliminary studies clearly show that the yield and quality attributes of this plant are dependent upon the environmental factors and are influenced by abiotic stressors (Akabri et al., 2012).

Plants have diverse physiological mechanisms (Na^+ accumulation in vacuoles to reduce its toxic effects, increased production of osmolytes such as beta-glycine, proline, soluble sugars and phenolics) to overcome the salinity effects (Kafi et al., 2013). The most accepted reason for the deteriorative salinity effects is the ROS molecules over-production. Chloroplast and mitochondria are the major sites for electron transport and energy domains in plant cells and they are more exposed to ROS molecules deterioration. ROS (reactive oxygen species) radicals predominantly damage the macromolecules such as RNA and DNA, denature the cell membranes and impact the vital biosynthetic enzymes (Ashraf and Ali, 2008). MDA (malondialdehyde) content in the plant tissues would be a nice bio-marker showing the damage on the tissues and the cell membranes peroxidation rate (Bhattacharjee and Mukherjee, 2002). The study of the effects of NaCl salinity on *Allium cepa* revealed that with salinity increase, the total protein, carbohydrate, and proline content of seedlings were higher than in control (Joshi and Sawant, 2012). Salinity stress invariably has been a major crops production restraint in Northwest Iran. The limitation in fresh water resources in the region as well as the expanded climatic problems such as dry season and soils salinity are the main reasons that make it inevitable to thoroughly study the salinity effects on the growth characteristics of the plants. This is the only way to have the appropriate information on the plant responses to salinity and to select the more suitable cultivars for the salinity faced soils and waters. With the present study, we aimed to evaluate the growth and some physiological responses of *Allium ampeloprasum* L. to salinity stress.

Material and methods

This experiment was conducted as factorial based on RCBD (Randomized Complete Block Design) with tree replications at the research greenhouse of Azarbaijan Shahid Madani University of Tabriz, Iran during 2015 growing season. Two native *Allium* clones (Tabriz and Isfahan) were employed. The seeds were disinfected by sodium hypo-chloride (10%) and were cultivated in plastic pots (20×30 cm) containing perlite in early April. Each pot was containing 15 seeds. The growing conditions in the greenhouse were: light intensity 450 $\mu molm^{-2}$ s^{-1}, 16:8 (light: darkness), day: 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 two true leaves (10 May), the salinity treatments including control, 40, 80, 120 and 160 mM NaCl were applied on the plants. Samples for traits assay were taken 30 days later.

Total chlorophyll and carotenoids content. Chlorophyll and carotenoids content were calculated according to Prochazkova et al. (2001).

Proline content. Proline content was quantified by Fedina et al. (2002). Toluene was employed as reference standard reagent.

Soluble solid content. Soluble solid was assayed by method used in Siram et al. (2002) and d-glucose was the standard.

Na^+ and K^+ content. Na^+ and K^+ amounts were assayed by Flame-photometric methods as Emami (1997).

Ion leakage. Ion leakage was measured by Akbari et al. (2013).

Leaf relative water. Leaf relative water content was calculated by the methods used by Geranpaieh et al. 2015.

MDA and H_2O_2 content. MDA and H_2O_2 content were measured by the methods used by Najjar-Kodabakhsh and Chaparzadeh (2015).

Statistical analysis. The data were analyzed by SPSS and MSTATC statistical programs (Version 9.2). Mean comparisons were done by LSD.

Results and discussion

The results obtained by ANOVA revealed the interaction effects of clone and salinity on proline and K^+/Na^+ ratio (Table 1 and 2). For the other traits except leaves relative water content (RWC), sugars content and MDA (Table 1) clone type effect was non-significant. In contrast, salinity had significant effects on all the traits studied (Table 3).

Table 1. ANOVA for the effects of clone type and salinity levels on some physiological traits of *Allium ampeloprasum* L.

SV	df	H_2O_2 content	MDA content	Ion leakage	K^+/Na^+	Na^+	K^+	Proline content	Soluble sugars	RWC	Chlorophyll b	Chlorophyll a	Total Chlorophyll	Leaf fresh weight
Replication	2	0.004*	0.03**	109.2*	0.008ns	59.5**	1.7ns	150.4**	0.05ns	26.5**	0.33*	0.08ns	0.68*	0.004ns
Clone	1	0.0002ns	0.009**	0.158ns	0.30**	9.7ns	0.02ns	0.36ns	0.46**	241.4**	0.0005ns	0.09ns	0.09ns	0.001ns
Salinity level	4	0.05**	0.04**	2247.4**	20.3**	1450.3**	119.8**	2209.2**	0.29**	119.4**	1.48**	1.59**	6.09**	0.101**
Clone×Salinity	4	0.0005ns	0.0004ns	7.56ns	0.16**	5.9ns	1.25ns	19.3*	0.03ns	11.6ns	0.14ns	0.01ns	0.08ns	0.001ns
Error	18	0.001	0.0004	22.7	0.01	3.91	0.69	5.02	0.01	4.11	0.062	0.06	0.14	0.002
CV%		6.6	5.5	11.5	8.8	8.1	5.44	5.3	5.2	5.1	12.4	9.4	8.2	9.3

ns non-significant, * significant at $P \leq 0.05$, ** significant at $P \leq 0.01$

Table 2. Mean comparison for the interaction effects of clone type and salinity on proline content and K^+/Na^+ ratio of *Allium ampeloprasum* L.

Clone	Salinity level (mM)	K^+/Na^+	Proline (μg^{-1} Fwt)
Tabriz	0	4.25^{b}	18.0^{e}
Tabriz	40	1.04^{c}	290.4^{d}
Tabriz	80	0.59^{d}	42.4^{c}
Tabriz	120	0.40^{de}	54.1^{b}
Tabriz	160	0.22^{e}	67.9^{a}
Isfahan	0	5.02^{a}	19.2^{e}
Isfahan	40	1.24^{c}	30.13^{d}
Isfahan	80	0.64^{de}	45.0^{c}
Isfahan	120	0.39^{de}	47.6^{c}
Isfahan	160	0.20^{e}	68.8^{a}
LSD (1%)		0.28	5.26

Similar letters in the columns are non-significant based on LSD test.

Leaves fresh weight

The highest and the lowest data for leaves fresh weight belonged to control and 160 mM NaCl, respectively (Table 3). This is in line with the findings of Najjar-Khodabash and Chaparzadeh (2016) on water-cress, where they reported quite the same results in response to salinity. Ion hyper-accumulation in the growing media affects the growth and productivity of the plants. Roots are the initial barriers to combat the negative effects of salinity and to inhibit the transportation of salinity induced ions towards the leaves. So, the leaf size and area are related to the cells number and size. The early stages in organogenesis, in case here the aerial parts formation (cell division stage) is non-sensitive to the abiotic stress. However, the leaf area expansion period is completely sensitive to the salinity stress. Under saline sodic conditions, the leaves cell turgor is greatly reduced and this drastically influences the leaf area expansion and aerial parts growth (Sheidaei et al., 2010).

Relative water content

Clone type had significant effects on the leaves relative water content. Tabriz clone had more relative water content compared to Isahan (Table 4). Moreover, the highest relative water content was recorded with control and 40 mM NaCl treatment (Table 3).

Chlorophyll content

The highest chlorophyll a, b and total chlorophyll content were determined in control plants and 40 mM NaCl salinity level (Figure 1). Increasing salinity correspondingly decreased the chloroplast content in the plants and the lowest data were recorded with 160 mM NaCl salinity level (Figure 1). Geranpaieh et al. (2015) and Akbari et al. (2011) reported that with salinity, the chlorophyll a and b content were decreased in water-cress and Iranian Leek. Deterioration of chloroplast structure and the un-stability in protein–pigment complexes, chlorophyll breakdown and variations in the content and ratios of carotenoids are the immediate damaging effects of the salinity. Chlorophyll b content reduction under salinity condition was due to the over-production and activity of chlorophyllase (Bertrand and Schoefs, 1999). Another effect of the salinity is the accelerated leaf senescence again due to the chlorophylls breakdown. All these salinities related deteriorations lead to the reduced photosynthetic apparatus dynamics and hence the intensified side effects of salinity (Quasin et al., 2003).

MDA content

The results showed the effects of clone (Table 4) and salinity (Table 3) on MDA content. The highest MDA content was determined for Isfhan clone (Table 4). The highest MDA content was correlated with the high salinity (160 mM) level in line with the finding of Najjar-Khodabakhsh and Chaparzadeh (2016). Delavari Parizi et al. (2004) reported the same pattern of MDA increasing content in response to the salinity levels (up to 200 mM) in *Ocimum basilicum* L. plants. They noted that the membrane dissociation under salinity stress, denaturation of cell membrane lipids and MDA accumulation could be considered as a reliable bio-marker to assay the plants responses to the salinity conditions (Derniral and Turkan, 2004).

H_2O_2 content

Salinity increased the H_2O_2 accumulation in plant tissue (Table 3). The highest H_2O_2 accumulation was determined at 160 mM NaCl salinity level. Ashraf and Ali (2008) reported that salinity induced the activity of peroxidase in the rapeseed leaves and relatively compensated the deteriorative salinity effects. Sairam et al. (2002) reported that Na^+ accumulation under saline sodic condition increased the membrane damage in wheat cultivars. Meanwhile, the several studies say that peroxidases have great role in the scavenging of ROS molecules induced by salinity and in reducing the Na^+ accumulation in the leaf tissues. Peroxidases are enzymes derived from the ascorbate-gluthation reductase cycle and has the potential to dissociate H_2O_2 into water molecules. The activity of the enzymes from this cycle has been defined as the key factors in protection of the plants against environmental stress.

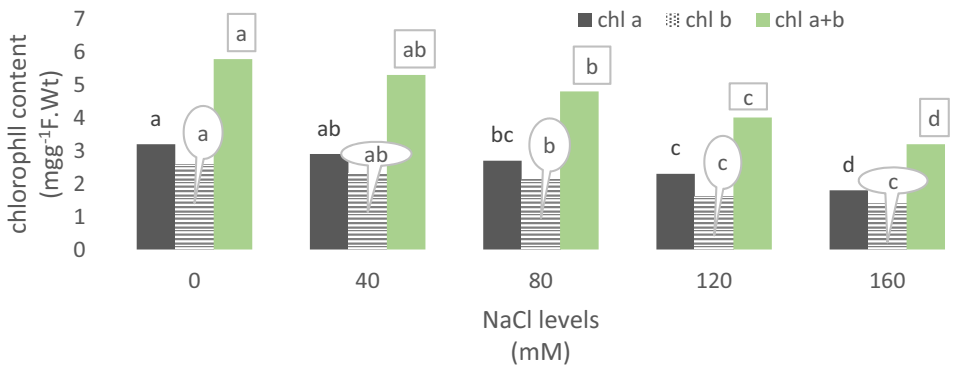

Figure 1. Mean comparison of the effects of NaCl salinity and clone type on chlorophyll content of *Allium ampeloprasum* L. leaves (Similar letters on the bars are non-significant based on LSD test).

Proline content

The interaction effects of the clone and salinity level on proline content are presented in Table 2. The highest proline content in both analyzed clones was determined in plants treated with 160 mM NaCl levels. Control plants had the lowest proline content. Geranpaieh et al. (2015) reported that salinity concomitantly increased the proline content in *Lepidium sativum*. Ali et al. (1999) noted that under salinity conditions, when the ionic balance in the cells is un-equilibrated, proline helps to maintain the osmotic potential of the cells and to compensate the salinity damages and has a regulatory action under salinity condition. This means that carbohydrates accumulation in the leaves is a response to the salinity stress inside the plants. Proline acts as a reagent to equilibrate the osmotic potential between cytoplasm and vacuole and also protects the plants against ROS molecules. During the stressful conditions, proline as an energy, carbon and nitrogen source helps to refurbish the damaged plants tissues (Najafi et al., 2010). Reduced proline usage for the protein synthesis during the stress conditions is seemingly the other reason for the over-accumulation of proline (Mudgal et al., 2009; Soussi et al., 1999).

Soluble sugars content

The soluble sugars content was increased with incresed salinity. The highest data was determined at 160 mM NaCl level (Table 3). Isfahan clone had more sugars content than Tabriz (Table 4). Najafi et al. (2010) and Geranpaieh et al. (2015) in their study reported that, with salinity increasing, the soluble sugar and proline content in plants also increased. The soluble sugars regulate the osmotic potential and increase the cell membrane stability and proteins integrity. Under salinity conditions, un-soluble polysaccharides such as starch is degraded to produce soluble sugars, so that, the cell can maintain the osmotic potential and reduce the cells dehydration (Parvaiz and Satyawati, 2008).

Na^+ content

Na^+ content was linearly increased with the salinity levels. The highest Na^+ content was traced with 160 mM NaCl (Table 3). Munns et al. (2006) reported that during the saline condition K^+ ions were substituted with Na^+ and that leads to the ionic toxicity. The specific effects of salinity stress on plants metabolism and specially on the leaf senescence is related to the accumulation of toxic ions such as Na^+ and Cl^- and the simultaneous out of cells leakage of K^+ and Ca^{2+} (Derniral and Turkan, 2004). The inhibition of plant growth under saline condition is presumably due to the reduced osmotic potential and the reduced water availability as well as a response to the Na^+ and Cl^- over-accumulation and their toxic levels (Kaya et al., 2006).

Table 3. Mean comparison for the effects of NaCl salinity on some traits of *Allium ampeloprasum* L.

Clone	MDA ($\mu molg^{-1}Fwt$)	TSS ($\mu mg^{-1}Fwt$)	RWC (%)
Tabriz	0.037^b	2.30^b	41.13^a
Isfhan	0.04^a	2.59^a	36.26b
LSD (1%)	0.06	0.03	0.52

Similar letters in the column are non-significant based on LSD test.

Potassium content

Mean comparisons revealed that K^+ content was significantly ($P \leq 1\%$) affected by the salinity levels. Control plants had the highest K^+ content (Table 3). K^+ is the most important inorganic ion; its accumulation in the roots helps to reduce the osmotic potential in plant root cells and is a pre-requisite for the transportation of soluble compounds induced by the turgor pressure in xylem route. By this way, K^+ aids in the water equilibrium within the plant and reduces the physiological damage caused by salinity stress (Grattan and Grieve, 1999). In the saline environments, increased Na^+ concentration in the leaves greatly reduces K^+ content due to the relative similarity of these ions, and Na^+ may sometimes substitute K^+ and may simulate its action. Plants need high amounts of K^+ to have balanced growth and development. Na^+ uptake and accumulation may help in the turgor pressure maintenance. Na^+ cannot thoroughly substitute K^+ and this fake substitution is working just for a short period of time. K^+ is specifically in need for the protein biosynthesis and also is essential for the normal function of some key metabolic enzymes (Grattan and Grieve, 1999).

K^+/Na^+ ratio

Table 2 shows the interaction effects ($P \leq 1\%$) of cultivar and salinity level on K^+/Na^+ ratio. Mean comparisons showed that with salinity getting high, the K^+/Na^+ ratio was declined. Derniral and Turkan (2004) reported that in cases of salinity stress, secondary stresses such as oxidative stresses, due to the over-expression of ROS molecules, decompose the proteins and lipids and lead to the cell damage and death. Growth reduction under saline condition is the immediate response of plants to the damages induced by salinity and ionic toxicity. The deteriorative salinity effects are

Table 4. Mean comparison for the effects of clone on some traits of *Allium ampeloprasum* L.

Salinity levels (mM)	Na+ ($mgg^{-1}DWt$)	K+ ($mgg^{-1}DWt$)	RWC (%)	Leaf fresh weight (g)	Ion leakage (%)	TSS ($mgg^{-1}Fwt$)	MDA ($\mu molg^{-1}Fwt$)	H_2O_2 ($\mu molg^{-1}Fwt$)
0	4.61^e	21.35^a	44.70^a	0.7^a	20.3^e	2^e	0.02^e	0.31^e
40	10.60^d	17.75^b	42.38^a	0.59^b	28.6^d	2.2^d	0.03^d	0.33^d
80	24.48^c	14.86^c	38.70b	0.51^c	38.6^c	2.6^c	0.04^c	0.36^c
120	31.85^b	13.65^d	35.60^{bc}	0.44^c	48.9^b	3^b	0.05^b	0.45^b
160	45.42a	9.82e	34.03c	0.37d	70.0a	3.3^a	0.06^a	0.54^a
LSD (1%)	3.29	1.38	3.36	0.196	7.98	1.7	0.9	1.0

Similar letters in the column are non-significant based on LSD test.

dominantly caused by salinity induced water scarcity, ionic toxicity and ionic imbalances. Na^+ over-absorption increases Na^+/K^+ ratio. Na^+ causes damages on the integrity and stability of cell membrane and the within cell components (Cicek and Cakilar, 2002). Huge K^+ content reduction and the related K^+/Na^+ ratio reductions under saline sodic condition are major salinity related negative effects that restrain the suitable K^+ role in plants (Tabatabaei, 2007).

Ion leakage

The highest recorded data for ion leakage was determined for 160 mM NaCl salinity level (Table 3). Intensified competition in Na^+ and K^+ absorption leads to the reduced K^+ absorption under saline sodic conditions and excessive K^+ leakage from the cells. Since, the similar thermodynamic relations are working in the absorption of Na^+ and K^+, these two ions are entering the cells by the same membrane proteins, and so Na^+ will be able to enter the cells by the K^+ canals (Kaya et al., 2006). The higher ratio for K^+/Na^+ in the aerial parts of salinity tolerant genotypes is mainly due to the higher potential in the prevention of Na^+ entrance to the roots, higher K^+ relation and maintenance in the aerial tissues and also the higher and better adaptation of the genotypes to the saline conditions (Bibordi et al., 1389).

Conclusion

Salinity influences the growth and yield components of plants by the great reduction of osmotic potential and by interference in the absorption and transportation of K^+, Ca^{2+} and NH_4^+. Increased ions absorption and the subsequent ionic toxicity affect the cell functions and damages the primary metabolic pathways. NaCl salinity imposed on the Iranian leek decreased chlorophylls a and b content, K^+/Na^+ ratio and plant fresh weight as well as drastically increased proline, MDA, H_2O_2 and soluble sugars content. The way to overcome the problems in salinity faced environments is to have the help of classical and molecular breeding methods to develop new cultivars capable of producing reasonable yield and productivity under salinity conditions.

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