Study effect of salinity on some physiologic and morphologic properties of two grape cultivars

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Abstract: Salinity is a phenomenon challenging the plantation and growth of grape in arid and semiarid regions. During the present research, tolerance of two grape cultivars (Soltanin and Fakhri) was evaluated against various sodium chloride salinity levels (zero, 50, 100, 150, 200 and 250 mM), which was conducted based on factorial experiment in the form of Randomized Complete Design (RCD) with three replications at Agricultural and Natural Resource Researches Center (ANRRC) of East Azerbaijan, during 2011. Based on the obtained results, the cultivar and salinity levels were significantly effective on morphological and physiological traits. Moreover, the results from analysis of variance revealed the significant effects of salinity levels on rates of chlorophyll a and b; rate of chlorophyll a + b; photosynthesis and transpiration rate; stomatal conductance; dry weight of stem and root; concentrations of elements such as nitrogen, phosphorus, potassium, Chloride; plant height; leaf area; and relative water content (RWC). Furthermore, increased salinity levels led to significant decrease in values of majority of the abovementioned parameters. In contrast, the proline content, sodium and chloride concentrations increased as a result of increasing salinity. In addition, "Salinity × cultivar" interaction also proved significantly effective on traits such as plant height, leaf area, dry weight of stem, proline content, chlorophyll a and b, chlorophyll a + b, photosynthesis and transpiration rate, stomatal conductance, dry and fresh weights of stem and root, nitrogen and sodium content of leaf and RWC. More specifically, the lowest values for the abovementioned parameters were measured at 250 mM sodium chloride salinity level for Fakhri cultivar. Without salinity application Soltanin produced the best values for physiological and morphological indices. In general, Soltanin cultivar proved more tolerant against salinity than Fakhri cultivar did.

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Introduction:

Salinity or increased concentration of soluble salts in cultivated soils is one of the main challenges for sustainable agriculture, with a decreasing effect on plant growth and specifically on horticultural crops yield. Roughly 15% of lands in East Azerbaijan are considered as saline and brackish and are threatened by salinity. Unless their productivity and crop yield from them do not meet the economical expectations, cultivation in these lands should not be ignored due to limiting effect of salinity on plant growth. Undoubtedly, any research addressing the effect of salinity on the physiological characteristics of the grape will contribute greatly to a rational advance in grape production. As the salinity in most of the fertile lands such as regions surrounding the Orumiyyeh Lake is affected by sodium chloride, this experiment will be able to identify salinity tolerance rate for widely cultivated native grape species within the prospect to expand the planting area of grape in the province. Furthermore, in addition to fresh consumption and raisin production from grape, its leaves also are used to prepare various Dolmas (foods). As the mineral content of grape leaves is important for a human and plant balanced nutrition, it will prove highly useful to investigate the effect of various salinity levels on chemical composition of grape leaves also (1).

In saline soils, concentrations of sodium and chloride in soil solution is generally higher than that of most of the other elements and it not only causes osmotic stress and specific ion effect, also leads to disorder in uptake of other elements as well as their translocation into aerial organs of the plant (nutritional diseases) and consequently decreases plant yield (2 and 3). The effects of salinity on both quantity and quality of grape have been researched in multitudes of investigations conducted in and out of the country. Salinity tolerance threshold for this plant reportedly is 1.5 dS.m-1, while at 2.5

dS.m-1 the plant growth decreases by 10% (4 and 5). However, it's worth consideration that cultivars of the species of a given plant vary greatly in terms of their tolerance against salinity.

The first response of glycophytes plants in face of salinity stress is decreased and finally ceased growth of their leaves (name, year). Gomez et al. (2002) demonstrated that decreased total leaf area was the result of decreased number and size of the leaves, as such that the numbers of leaves were decreased to 11 (at 50 mM concentration) and 18 (at 100 mM), whereas their size decreased 29% (at 50 mM) and 46% (at 100 mM), respectively. Moreover, in this research the salinity decreased the dry plant weight through decreasing branch length and total leaf area. Interestingly, the salinity affected more leaf area than branch length.

Biomass of Asghari and Soltanin cultivars were decreased by 34 and 64% at 50 and 10 mM NaCl concentrations, respectively. At lower salinity level (25 mM), decreased percentage of dry weight of aerial organs, which was similar to decreased percentage of dry weight of roots, which suggests that branches and roots are equally susceptible against NaCl salinity. Growth of root was less affected by salinity at 50, particularly 100 concentrations, than that of branches (name year). Singh (year) demonstrated that increasing salinity level led to decreased dry and fresh weights of aerial organs as well as number of stomatas and node interval.

Estion and Harvey (35) conducted an in vitro experiment in order to determine the salinity tolerance in some grape cultivars and demonstrated that salinity tolerant cultivars maintain their growth rate to a relative extent, and are capable of dealing with metabolic disorders such as chlorophyll deficiency. Salinity experiments on cultivars such as Chavosh, Moshkeleh and Soltanin was conducted under laboratorial conditions and by using lateral seedling planting method. During the experiment, germination, growth, chlorophyll content and healthy of vegetative samples decreased as a result of increased concentration of NaCl and extended period of the treatment. In addition, it was found that salinity treatment caused various rate of necrosis in the samples dependent on the cultivar, NaCl concentration and treatment period. In general, Chavosh had the highest tolerance against NaCl salinity, followed by Soltanin and then by Moshkeleh.

Salinity tolerance in fruit trees, particularly in grape tree, is heavily influenced by cultivar. Results from the research revealed that the capacity of cultivars to regulate the absorption of Na+ and Cl- determines their tolerance, i.e. the higher the capacity of plant in preventing the uptake of Na+ and Cl, the higher will be its tolerance. Salinity stress produces both short-term and long-term effects. One or two days after the plant exposure to salinity, it takes only a few hours for the short-term effects to take place, during which a complete cessation of carbon assimilation is resulted. Whereas, the long-term effects after the exposure of plant to salinity for several days and decreased carbon assimilation, happens due to salt accumulation in the leaves (15).

Study by Flexas et al. (19) on the response of 6 cultivars of grapevine against sodium chloride salinity revealed that increasing salinity significantly increased the salt content of plant tissue. Curiously, this research investigated plant response with respect to growth, minerals content of tissues as well as gas trade-offs, and corresponding results showed that growth, dry matters of aerial organs, leaf area and total dry weight decreased significantly at all salinity levels. Growth measurement was achieved through measuring leaf area, leaf number, dry and fresh weights of plant and root; as well as through the measurement of gas trade-offs including leaf photosynthesis, stomatal conductance, respiration rate and intracellular concentration of CO2.

Grapevine trees are relatively susceptible against salinity (18) and the main damages are caused by chloride ions (20). Mass (26) estimated the tolerable chloride concentration for Dogrilgs and Salt creek cultivars of grape to be 80 and 60 M/m3, respectively. It is well established that grapevine response against salinity depends upon various factors such as combination of rootstock and scion, cultivar, irrigation system, type of soil and climate. Shani and Ben-Gal (28) believed that growth decline as a result of salinity is often attributed to such factors as ion toxicity and/or low osmotic potential, which are capable of influencing physiological and biochemical processes. For instance, study by Walker et al. (36) on Soltanin cultivar suggests that photosynthesis and stomatal conductance are heavily influenced by sodium chloride salinity and are directly connected to ratio of high concentration of Cl- to Na+ in the leaves. It's worth mentioning that grapevine cultivars vary in their tolerance against salinity.

This research focuses on evaluating the tolerance of two grape cultivars against various salinity levels.

Materials and methods:

In order to investigate salinity effect on two widely cultivated grape cultivars in East Azerbaijan Province, a factorial experiment based on RCD, with three replications, was conducted at greenhouse of department of soil and water researches of ANRRC, in 2011. First factor was sodium chloride salinity in six levels (zero, 50, 100, 150, 200 and 250 mM), whereas the second factor included two grape cultivars namely Soltanin and Fakhri in four replications. Grape scions were planted in beds containing equal proportions of sand, perlite and vermiculite in 20-L vases. Each vase was supported on a saucer and the electric conductivity (EC) of both incoming and outgoing solutions was controlled. During the first two to three weeks after the plantation, the vases were nourished by half Hoagland nutrient solution. In order to create the desired salinity levels, another half of the Hoagland nutrient solution was salinized with 0, 50, 100, 150, 200 and 250 mM concentrations of sodium chloride and their ECs were measured. After the establishment of scions, the vases were irrigated by the prepared solutions. In addition to EC, pH of leaching water of the vases was measured throughout the growth period. In case the salinity of leaching water was in excess of the salinity levels determined for this study, the beds were irrigated and leached by tap water. Throughout the growth period, measurement was done on growth parameters such as plant height, leaf area, dry and fresh weights of aerial organs and roots. In addition, the chlorophyll index of leaves was measured for at least three stages of plant growth by using chlorophyll Meter (SPAD-504). Concentrations of elements such as N, P, K, Cl-, and Na were measured in leaf throughout the growth season, and in aerial organs and roots after the harvest. Moreover, the photosynthesis was measured using Photosynthesis Meter (Model Da-1000, Wallz Co., Germany), and last but not least, the measurement was conducted between 9 A.M and 2 P.M local time under a fixed light intensity.

Chlorophyll a and b was measured using Arnon method. In this method, as little as a half gram of wet vegetative matter was chopped and thoroughly mashed in liquid nitrogen, in a porcelain mortar. As much as 20mL of 80% aceton was added to the sample, and then the mixture was put into centrifuge device with 6000 rpm speed for 10 minutes. Supernatant was transferred into a glass balloon. Some of the samples in the balloon were read in spectrophotometer for chlorophyll a at 663nm; for chlorophyll b at 645nm; and for Carotenoids at 470nm. Finally, the following formulas were used to calculate chlorophyll a and b and carotenoids contents in mg/g of fresh weight of the sample.

Chlorophyll a = (19.3 * A663 - 0.86 * A645) V/100W

Chlorophyll b = (19.3 * A645 - 3.6 * A663) V/100W

Stem height was measured by a ruler. The leaves were counted and then towards the end of the experiment Leaf Area Meter (Model 200) was used to measure area of the leaves. Using a 0.0001 scale, dry and fresh weight of leaves and dry weight of stem and root were measured. In order to determine the dry weight, prior to weighing the samples were put in 70°C for 72 hours to achieve the desired desiccation.

Moreover, in order to estimate relative water content (RWC) of the leaves, two completely developed leaves were cut and removed from each of plants and 10 leaf disks, 8 mm in diameter each, was cut from the middle part of the blades. The disks were weighed and then put into lidded petri dishes containing distilled water and kept in refrigerator at 4°C under dark conditions for 24 hours. After removing the disks from distilled water, they were blot dried to remove the excessive humidity; then their water saturated weight was measured. Then, they were transferred to a 70°C for 48 hours before being weighed for their dry weights. The following equation was used to obtain RWC of the leaves.

$$RWC = \frac{\text{fresh weight of the leaf disks} - \text{dry weight of the leaf disks}}{\text{inflated weight of the leaf disks} - \text{dry weight of the leaf disks}} \times 100$$

Temperature of the leaf was read and recorded using infrared thermometer (Hi 99550 Hana) from a distance as far as 4 cm from two randomly selected vases from each unit. Chlorophyll indices of the leaves were measured by using Chlorophyll Meter (SPAD – 502 –

Minolta Osaka Model, Japan), whereas Paquin and Lechasseur (1976) method was used to measure the proline content.

As for measuring the proline density, 1 mL of the prepared alcoholic extract was diluted by 10 mL of distilled water before applying 5 mL of Ninihydrin as reagent (the preparation method of Ninihydrin for each sample was: 0.125 g ninihydrin + 2 mL phosphoric acid 6 molar + 3 mL of glacial acetic acid). Furthermore, the application of reagent ninihydrin was followed by adding as much as 5 mL of glacial acid acetic to the solutions, which were stirred for 45 minutes in boiling water bath and cooled off before applying 10 mL of benzene for each samples (32 samples) and then were stirred so intensively that the proline entered benzene phase. Then the samples were left immobile for 30 minutes. Some standards of proline were prepared with density ranging from zero through 0.1 mM/mL (0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.1 mM/mL) and finally the absorption rate of standard solutions and of samples were measured at 515 nm by using spectrophotometer. Data was analyzed using MSTATC software, whereas diagrams were drawn by Excel software.

Results

Based on results from Table of analysis of variance, the salinity levels had a significant effect on traits such as plant height; leaf area; fresh stem weight; proline content; chlorophyll a; chlorophyll b; chlorophyll a + b; photosynthesis and transpiration rate and stomatal conductance; fresh and dry weight of root; dry weight of stem; nitrogen, phosphorus, potassium, chloride, and sodium content of leaf; and RWC (Table 1).

Interaction of "salinity × cultivar" was found to be significant on traits such as fresh stem weight, proline content, chlorophyll a, chlorophyll b, and dry stem weight. The highest plant height (19.5 cm) was produced at control treatment, whereas the lowest value (8.45 cm) for this trait was measured at salinity level of 250 mM. Likewise, the highest leaf area (96.3 cm^2) was measured at control treatment, whereas the lowest value for this trait was produced at 250 mM sodium chloride treatment. Moreover, the increasing salinity levels had a significantly decreasing effect on fresh and dry weights of both root and stem, while the lowest values for these traits were produced in 250 mM sodium level. In contrast, the increasing salinity caused a significant increase in proline content of grape leaf, while the highest value (35.5 mg/g) was measured in 250 mM treatment (Table 2).

Moreover, increasing salinity level had a significantly decreasing effect on contents of chlorophyll a, b and of chlorophyll a + b, while the lowest values were found at 250 mM sodium chloride level. Likewise, photosynthesis and transpiration rate and stomatal conductance declined significantly in the face of increasing salinity levels, while the lowest values for these parameters were obtained at 250 mM sodium chloride salinity level. Furthermore, increasing salinity level led to a significant decrease of nitrogen, phosphorus and potassium content of leaf. Conversely, it led to a remarkable increase in the sodium and chloride content of the leaf. As for RWC, the highest value (88.78%) was produced at without salinity application treatment (control), whereas the lowest (36%) at application of 250 mM sodium chloride (Table 2).

Soltanin cultivar exhibited more efficiency with respect to quantitative factors such as plant height, leaf area, fresh and dry weights of both stem and root than Fakhri cultivar (Table 2). Similarly, highest values for traits such as Proline content, content of chlorophyll a, b and of chlorophyll a + b, photosynthesis and transpiration rate, stomatal conductance and RWC were more in Soltanin cultivar than in Fakhri cultivar. In addition, Soltanin cultivar had the highest concentration of nitrogen, phosphorus and potassium, whereas Fakhri had the highest content of sodium and chloride.

The highest plant height (21 cm) and highest fresh stem weight (85 gr) belonged to Soltanin cultivar in control, whereas Fakhri produced the lowest plant height (6.5 cm) and lowest fresh stem weight (28 g) in 250 mM sodium chloride treatment. As for dry stem weight, the highest (21 g) and lowest (6.5 g) values were obtained in control and 250 mM sodium chloride treatment, respectively, while both belonged to Fakhri cultivar. Soltanin cultivar was able to produce the highest (35 mg/g) proline content in 250 mM sodium chloride treatment. It also produced the highest contents of chlorophyll a (6 mg/g) and chlorophyll b (3.8 mg/g) in the leaf in control, which decreased with the increasing salinity level. Furthermore, the highest rate of photosynthesis (9.5 µmol.m-2.s-1) happened in control in Soltanin cultivar, whereas the lowest rate (5.2 µmol.m-2.s-1) happened in 250 mM sodium chloride treatment and in Fakhri cultivar.

Discussion

Presence excess salt in planting medium was found to be one of the main reasons for decreased number of root, while extreme condition such as a concentration as high as 200 to 250 mM of sodium chloride in the planting medium practically stopped root development. In addition, increased osmotic pressure in the medium combined with the increasing salt presence had a decreasing effect on root development, which subsequently delayed their appearance. Furthermore, increased salt concentration in the medium would lead to more negativity of already negative osmotic pressure in the root growth zone as well as toxic effect of high salt concentration, which would not create a favorable condition for root growth. Interestingly, the increased salt concentration in the environment not only negatively influenced root development, but also stem development.

Authors believe that total decrease in fresh and dry weights of stem may not relate to efficiency of leaf area to produce photosynthetic substances, rather to decreased number of leaves, or more specifically to decreased leaf area. The change in plants as a result of increased salt may reveal as plant's failure to take up more ions under salinity stress condition or failure in quick translocation of ions to leaves and their distribution in the leaf cells. During low concentrations of salt, as the uptake or translocation of the ions are characteristically selective, root begins to take up sodium ion in consistence with increasing salinity; however in high salinity levels this mechanism fails as there is no practical root development. Consequently, grape is classified among the salt-absorbing and salt-storing plants in the face of increasing salts in its planting medium (17).

Grape cultivars differed in their response against various salinity levels. In saline soils, vegetative growth and leaf development are influenced in the first place. Furthermore, the decreased growth and development of plants in saline soils is linked with increased osmotic pressure associated with presence of sodium, chloride, magnesium and sulfate ions, which ultimately makes water less usable by the plant (16).

One of the remarkable effects of salinity is declined vegetative features such as plant height and leaf area of grape. Majority of the authors have related such a decline to mitigated photosynthesis due to increased salinity levels (150-200 mM). In this study, also the decreased chlorophyll index as well as decreased rate of chlorophyll a and b as a result of increased salinity level has led to decreased dry weights of leaf, stem and roots of cultivars. Under stress condition, there is a competition between aerial organs and root in uptake of photosynthetic substances, which negatively influence these organs (15).

In this experiment, increasing salinity level had a decreasing effect on RWC of the leaves. This may be accounted for by status of stomatas and increased transpiration rate of the leaves. Osmotic regulation is an indication of response to osmotic stress and when there is a water limitation caused by salinity stress, osmotic potential is declined and this in turn causes the reduction of RWC of the leaves.

Osmotic regulation depend upon the cultivar as well as on decreased rate of water potential and this is safe to say that one of the mechanisms of tolerance against salinity in grape is to maintain high RWC of the leaf. RWC is mainly positively correlated with leaf area, dry leaf weight, chlorophyll content and other growth indices. Furthermore, increasing salinity level had a decreasing effect on chlorophyll content of the leaf, while this was more evident in the leaves of grape soltani than in cultivar fakhri In contrast, increasing salinity stress had a significantly increasing effect on proline content of the leaves, while this was more evident in cultivar fakhri than cultivar soltani It is known that salinity stress reduces chlorophyll content, because the glutamate which is the primary constituents of chlorophyll and proline is consumed in favor of proline production. Furthermore, salinity stress induce glutamate ligase enzyme to transform glutamate into proline. Another reason for chlorophyll reduction is the increased use of nitrogen for proline synthesis. Proline plays a key part in maintaining the osmotic pressure and cytoplasmic enzymes and protects cell membrane from any damage through absorbing free radicals. Different researchers also believe that decreased chlorophyll content may be due to inhibitory effect of ions accumulated in chloroplast, chlorophyll degradation by oxidative stress caused by salt, activation of chlorophylase enzyme by salinity ions and its negative effect on protophyzine. Increasing salinity level leads to decreased chlorophyll biosynthesis through increased salt. It causes a rise in leaf temperature and consequently the stomatas are closed due to water limitation stress caused by salinity, at the same time due to synthesis of abscisic acid in the root and its translocation to the stomatas. In addition, shrinking of the mezophyllic cells

contribute to synthesis of abscisic acid and its translocation to stomatal cells. Decreased stomatal conductance as a result of this phenomenon leads to a rise in leaf temperature, because, as a rule the leaves get rid of the excessive heat through doing transpiration.

Conclusion

Results from the study revealed that Soltani cultivar was more tolerant against salinity than Fakhri cultivar, because Soltani produced higher values for the majority of morphological indices such as plant height, leaf area, dry and fresh weights of stem and root than Fakhri did. In addition, other mechanisms including RWC and proline concentration makes it a tolerant cultivar for overcoming salinity stress, whereas Fakhri could not potentially employ this mechanism as efficiently as Soltani could, due to lower accumulation of proline.

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Table 1. Analysis of variance on different characteristic of grape affected by sanity and cultivars.

Sources of variation	Degree of freedom	Height	Leaf area	Stem F. W	Proline	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Raet of photosynthesis	Transpiration rate	Stomatal conductance	Stem D. W	N(%)	P(%)	K(%)	Cl (mg.g-1)	Na (mg.g-1)	RWC(%)
Salinity	5	100.85**	2940.03**	1293.15**	354.03**	9.19**	4.02**	25.30**	27.98**	14.80**	0.03**	108.66**	3.02**	0.018**	1.45**	0.97^{ns}	205.41	2405.81**
Cultivars	1	31.37**	1681**	930.86**	930.86^{ns}	2.83**	2.28^{ns}	10.24**	2.20**	9.20**	0.01**	46.58**	0.284^{ns}	0.027**	0.16^{ns}	0.52^{ns}	110.25	171.61**
Salinity x cultivars	5	2.29^{ns}	26.51^{ns}	303.50**	330.52**	5.40**	6.10**	0.82	0.48^{ns}	0.71^{ns}	0.001^{ns}	88.60**	0.035^{ns}	0.001^{ns}	0.035^{ns}	0.054^{ns}	32.25	8.38^{ns}
Error	24	3.74	254.79	187.99	43.41	0.24	0.27	0.78	0.98	1.62	0.001	5.37	0.139	0.002	0.08	2.02	16.01	38.97
C.V (%)	-	14.71	24.79	18.99	16.41	14.21	15.20	17.45	13.52	8.62	18.25	15.37	10.12	9.32	17.41	23.02	16.01	25.97

*, **, ns: significant at 0.05 , 0.01 probability level and no significant

Table 2. Main effect salinity on different characteristics of grape.

parameter / Salinity Levels	Height (cm)	Leaf area (cm^2)	Stem F. W (gr)	Praline	Dry stem weight (gr)	Photosynthesis (µmol.m-2.s-1)	Transpiration rate(µmol.m-2.s-1)	Stomatal conductance	RWC(%)	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	N (%)	P (%)	K (%)	Na (mg.-1g)	CL (mg.-1g)
0NaCl	19.50^{a}	96.33^{a}	74.04^{a}	15.41^{f}	18.47^{a}	9.23^{a}	6.65^{a}	0.36^{a}	88^{a}	5.57^{a}	3.70^{a}	9.27^{a}	4.13^{a}	0.30^{a}	3.61^{a}	4.56^{f}	1.58^{b}
50 mM	16.71^{b}	79.68^{b}	62.08^{b}	18.01^{f}	14.90^{b}	7.75^{b}	5.55^{b}	0.28^{b}	72^{b}	4.57^{b}	3.13^{a}	7.70^{b}	3.60^{b}	0.22^{a}	3.28^{a}	7.58^{e}	2.01^{ab}
100	14.61^{c}	67.81^{c}	53.92^{c}	22.32^{f}	11.33^{c}	6.65^{c}	4.52^{c}	0.27^{b}	63^{c}	3.72^{c}	2.63^{b}	6.35^{c}	3.18^{b}	0.21^{c}	2.93^{b}	10.55^{d}	2.17^{a}
150	11.97^{d}	54.40^{d}	46.12^{d}	26.52^{c}	9.78^{d}	5.53^{d}	3.86^{d}	0.18^{b}	51^{d}	3.17^{c}	2.33^{b}	5.50^{d}	2.75^{c}	0.18^{d}	2.70^{b}	15.93^{c}	2.43^{a}
200	10.55^{e}	47.45^{e}	37.95^{e}	30.88^{b}	8.37^{e}	4.10^{e}	3.03^{d}	0.16^{c}	40^{e}	2.70^{d}	1.92^{c}	4.62^{e}	2.38^{c}	0.15^{e}	2.46^{b}	19.93^{a}	1.61^{a}
250	8.45^{f}	35.25^{f}	36.03^{f}	35.52^{a}	7.29^{f}	3.66^{f}	2.45^{e}	0.15^{c}	36^{f}	2.23^{d}	1.44^{c}	3.66^{f}	2.35^{c}	0.13^{f}	2.33^{b}	16.40^{a}	2.61^{a}

Values within the each column and followed by the same letter are not different at P<0.05 by an ANOVA protected Duncan's Multiple Range- Test

Table 3 Main effect salinity and cultivars on different characteristics of grape.

Parameters / Salinity Levels		Height (cm)	Stem F. W (gr)	Stem F. W (gr)	Proline(mg.-1g)	Chlorophyll a	Chlorophyll b	Photosynthesis rate(µmol.m-2.s-1)	Transpiration rate (µmol.m-2.s-1)	RWC(%)
NaCl mM 0	Soltanin	21.50^{a}	75.40^{a}	21.50^{a}	16.20^{f}	6.10^{a}	4.80^{a}	9.8^{a}	0.49^{a}	98^{a}
	Fakhri	19.25^{b}	65.20^{b}	17.10^{b}	15.30^{g}	5.80^{b}	3.50^{b}	8.7^{b}	0.38^{b}	82^{ab}
50 mM	Soltanni	19.10^{b}	65.30^{b}	17.20^{b}	18.60^{e}	5.10^{b}	3.60^{b}	8.2^{c}	0.38^{c}	78^{b}
	Fakhri	15.20^{c}	56.20^{c}	14.15^{c}	17.20^{f}	4.60^{c}	3.80^{c}	6.8^{d}	0.29^{c}	61^{b}
100	Soltanin	15.20^{c}	55.20^{c}	11.60^{d}	26.60^{d}	4.0^{c}	3.20^{c}	7.20^{d}	0.30^{c}	69^{bc}
	Fakhri	13.30^{d}	46.10^{d}	11.20^{e}	24.40^{e}	3.40^{d}	2.70^{d}	6.2^{e}	0.26^{d}	56^{d}
150	Soltanin	12.40^{d}	48.60^{d}	9.60^{e}	28.40^{c}	3.10^{d}	2.60^{d}	5.1^{f}	0.26^{d}	58^{e}
	Fakhri	11.30^{e}	36.15^{e}	9.20^{f}	27.20^{c}	2.80^{e}	2.50^{e}	4.8^{g}	0.20^{e}	46^{e}
200	Soltanin	11.20^{e}	36.10^{e}	8.20^{g}	32.20^{b}	2.10^{e}	2.10^{e}	3.1^{g}	0.20^{e}	42^{g}
	Fakhri	10.60^{ef}	35.10^{f}	7.40^{g}	31.10^{b}	1.90^{f}	2.00^{f}	2.8^{h}	0.16^{f}	34^{f}
250	Soltanin	6.80^{f}	26.80^{f}	7.10^{g}	36.75^{a}	1.90^{f}	1.40^{f}	1.1^{h}	0.18^{f}	30^{g}
	Fakhri	5.40^{f}	25.10^{f}	6.10^{i}	35.40^{a}	1.60^{f}	1.30^{f}	1.0^{h}	0.14^{g}	26^{h}

Values within the each column and followed by the same letter are not different at P<0.05 by an ANOVA protected Duncan's Multiple Range- Test