Salinity Induced Effects on Growth Parameters, Chemical and Biochemical Characteristics of Two Forage Sorghum (Sorghum bicolor L.) Cultivars

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Abstract: Plant production stability under saline condition compared to normal has had limited success due to lack of knowledge on salt tolerance mechanisms. To evaluate the changes in chemical and biochemical characteristics (content of free proline and total protein, Na' and K') and growth parameters (leaf area, plants height, total tiller number, leaf number, shoot and root weight, percent of emergence, growth rate and growth power) of sorghum under salinity stress, a pot experiment was carried out at Shiraz University, Shiraz, Iran in 2010. Four salinity levels containing 3, 6, 9, 12 ds m⁻¹ using NaCl and without using NaCl (as control) and also two sorghum cultivars consist of Pegah (Iranian cultivar) and Speedfeed were evaluated in a factorial experiment design with three replications. Results showed that salinity decreased accumulation of K', emergence percentage and all growth parameters but increased amount of Na', proline and total protein content in shoot and leaves of treated plants. Multiple regression, stepwise selection, selected root weight and their ratio (shoot/root), leaf area and leaf number per plant, tiller number per plant, photosynthesis rate and also content of K' as most significant factors for contributing in shoot weight. Stepwise selection and correlation coefficient cleared that root weight is the most important factor in plant shoot production under salinity condition and breeding program must be carried out to screen cultivars with higher root density in order to increase resistance to salinity. Based on the results, Speedfeed is proper cultivar to be used in breeding programs to achieve higher salinity resistance by screening or crossing programs.

Key words: Forage sorghum, proline, salinity, total protein, biochemical characteristics

INTRODUCTION

During growth and development, plants often encounter adverse environmental conditions, including drought, salinity, extreme temperature, nutrient deficiency, pathogens and so on (Li et al., 2010). Soil salinity has been recognized as one of the most serious problems for agriculture in arid and semi-arid areas of the world. It is known that one crop may be highly tolerant at one stage of development and sensitive during another stage. Saline soils are estimated to cover about 5-10% of the world’s arable land and the area affected by salinity is increasing steadily, in part due largely to mismanaged irrigation (Krishnamurthy et al., 2007). Soil salinity drastically reduces the productivity of most crops although to a varying extent across species (Munns et al., 2002). Several attempts have been made to overcome the effects of salinity on germination and seedling development of different species such as wheat, sorghum, barley, oat, carrot and tomato.

Sorghum (Sorghum bicolor L.), an important oilseed crop in the global agroecosystem, is the fifth most important grain crop grown worldwide, which is unusually tolerant of low input levels, an essential trait for arid and semi-arid areas in temperate and tropical regions. Recently, its significance is increasing as a biofuel crop for its high yield of biomass and broad adaptation to different kinds of environmental regions (Paterson et al., 2008). It’s also emerging as a genetic model for tropical grasses based on its small and well-characterized genome, low level of gene duplication and close relationship to the larger and more complex genotypes of maize and sugarcane (Paterson et al., 2008, 2009). Also, sorghum is a major grain and forage crop and was previously characterized as moderately tolerant to salinity (Igartua et al., 1995). It is considered relatively more salt tolerant than maize, the cereal crop ranking first in productivity globally (Maas, 1985) and so sorghum has the potential as a crop for salt affected areas (Igartua et al., 1994). The presence of large genotypic variation for tolerance to salinity reported in sorghum

(Maity et al., 1994) offers a good scope for integrating
tolerance characteristics into appropriate breeding
programs to improve crop productivity on saline soils
(Krishnamurthy et al., 2007).

Efforts to enhance crop yields under salinity stress
have had a limited success because available knowledge
of the mechanisms of salt tolerance has not been
completed and also not turned into useful selection of
genotypes. Attempts have been made to evaluate salt
tolerance at germination and emergence stages in grain
sorghum (Igarta et al., 1994) and large genotypic
differences were reported, but this early evaluation
appears to have little relation with overall performance
under saline conditions (Munns et al., 2002).

Though Na' exclusion and grain K'/Na' ratios have
been suggested to be reliable traits for selecting salt
tolerant crops (Munns and James, 2003; Munns et al.,
2002), the value of that trait has not been used in a large
scale in sorghum (Poustiti and Siosemardeh, 2004).

This study was conducted to evaluate effects of
salinity on growth parameters and powers, biochemical
traits and photosynthesis rate of two forage sorghum
cultivars for better understanding of tolerance
mechanisms to salinity.

MATERIALS AND METHODS

The surface layer of soil (0-30 cm) was collected from
Bajgah, located in the province of Fars, south of Iran
(coordinated: 29°43’ N and 52°35’ W). The main
characteristics of soil were: pH 7.09; electrical
conductivity, 0.0513 ds m⁻¹; total organic mäter, 1.046;
Total N, 0.660 g kg⁻¹; Available P, 12.5 µg g⁻¹ and soil
water capacity, 23.27%. Based on the analysis of soil
70 mg N kg⁻¹ soil and 20 mg P kg⁻¹ soil were added to soil.

Experimental procedures: A factorial experiment on the
bases of completely randomized design was established
with two factors and three replications. The first factor
was cultivar that was consisted of Pegah (Iranian cultivar)
and Speedfeed and the second factor was salinity levels
containing without adding NaCl to soil (as control), 3, 6,
9 and 12 ds m⁻¹ salinity.

The pot experiment was carried out at a greenhouse
in Agriculture School, Shiraz University, Shiraz, Iran in
2010. The pots with 23 cm diameters, 20 cm heights were
filled with 3 kg washed and sieved soil. Before planting
seeds were treated with ethanol 98% for about 20 second
and then three times were washed with distilled water.

Four levels of salinity were applied prior to sowing
through a one-time application of deionized water
with 3, 6, 9 and 12 ds m⁻¹ NaCl and without adding NaCl
(as control). Ten seeds of each cultivar were sown in each
pot in equal distance from each others. Pots were
weighted daily and based on decreasing the amount of
water in pots, water was added to pots soils until gain to
soil field capacity (23.2%, w/w).

Plant analyses
Growth parameters: Leaf number at two stage, total tiller
number, plants height with a ruler and leaf area of plants
using following formula: Leaf area of plant = Maximum
leaf heights × maximum leaf diameters × 0.75 were measured.
Shoot and root weight of plants were determined after
their harvesting.

Emergence, growth rate and growth power: After
complete germination period of plants, emergence percent
based on the germinated number of seeds number per pot
ratio was obtained. After calculating the emergence
percent, number of remained plants per pot was counted
and percent of surviving power for plants subjected to
control plant numbers in emergence was calculated at
three stages. For growth power, plants of each pot
visually scaled from 1 (lowest growth power) to 5
(highest growth power). Results of emergence and growth
rate are presented in Fig. 1.

Photosynthesis rate: During the vegetative stage,
Photosynthesis rate was conducted on the youngest fully
expanded leaf at stem elongation stage on two sorghum
cultivars. Net CO₂ assimilation, was assessed on intact
leaves using the LiCor 6400 gas exchange system
(Lincoln, NE, USA). During the reproductive stage
Photosynthesis rate was measured at flowering (65-70 day
after sowing) on flag leaves after 1-2 h of acclimation in
a growth cabinet, under a light intensity of about
1000 µmol m⁻² s⁻¹, relative humidity of 70% and 29°C to
ensure measurement under stable conditions.

Free proline content: Free proline was extracted from
fresh leaves according to the method of Bates et al.
(1973). Leaves samples (0.5 g) were homogenized in 10 mL
of 3% (w/v) aqueous sulphotsalicylic acid then the
homogenate was filtered through Whatman No. 2 filter
paper. Two milliliters of filtrate was then mixed in a test
tube with 2 mL acid ninhydrin and 2 mL glacial acetic acid
and incubated in a 100°C water bath for 1 h. The reaction
was terminated by placing the mixture in an ice bath. It
was then extracted with 4 mL toluene. The absorbance
was recorded at 520 nm and the proline concentration was
determined as (µg g⁻¹ FW) using a standard curve.

Total protein content: The protein content was estimated
according to the method of Bradford (1976), using Bovine
Serum Albumin (BSA) as a standard and observation of
595 nm.
**Determination of Na+ and K+ content:** One hundred and fifty milli-grams of finely ground shoot sample was digested in 4 mL of concentrated sulfuric acid with 0.5% selenium powder at 360°C for 75 min on a block digester and the digest was diluted to 75 mL. Exchangeable K and Na were estimated (Sahrawat et al., 2002) using an atomic absorption spectrophotometer (Varion model 1200, Australia).

**Statistical analysis:** Statistical Analysis Software (SAS 9.1) was applied for all analysis containing, two ways ANOVA, mean comparison, Pearson correlation and multiple regressions. Mean comparison was carried out based on Least Significant Difference (LSD) and multiple regression was based on stepwise selection.

**RESULTS**

**Photosynthesis rate:** Analysis of variance showed statistical significant difference between cultivars and among salinity levels (p<0.01). Photosynthesis Rate (PR) in Pegah cultivar (19.22) was higher than Speedfeed (18.55). Salinity decreased the photosynthesis rate of plants. Highest (PR) was measured for control (34.24) and lowest PR was observed for 9 salinity levels and there was no significant difference between 6 and 9 levels. Fitted regression lines for two cultivars showed that the most variation of data (R² = 0.97 and R² = 0.94, respectively) could interpret by these lines (Fig. 3).

**Chemical and biochemical parameters:** Free proline content (Pr): measuring free proline content showed no significant difference between cultivars and salinity × cultivar interaction but difference among salinity levels was significant (p<0.01). Highest amount of Pr was recorded for 9 salinity level and the lowest one was recorded for control. The difference between 3 and 6 salinity levels was not significant.

**Total protein contents:** Protein content of Pegah (8.17) was significantly lower than content of Speedfeed (9.13). In general, salinity enhanced protein concentration of treated plants but no significant different was observed.

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**Fig. 1(a-d):** Percent of emergence (a) growth rate at first (b) second (c) and third (d) measuring. Different alphabets are significantly different at p<0.01
Table 1: Analysis of variance and mean comparison for main and interaction effect of tiller number and also leaf number and height of plants at two different time of sampling

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Leaf number (first)</th>
<th>Height (cm) (first)</th>
<th>Total tiller number</th>
<th>Leaf number (second)</th>
<th>Height (cm) (second)</th>
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<td>Specified (V2)</td>
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<td>6.50a</td>
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<td>3 (S2)</td>
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<td>15.08b</td>
<td>2.17c</td>
<td>5.17b</td>
</tr>
<tr>
<td></td>
<td>6 (S3)</td>
<td>4.00c</td>
<td>11.75c</td>
<td>1.50c</td>
<td>3.00c</td>
</tr>
<tr>
<td></td>
<td>9 (S4)</td>
<td>2.83d</td>
<td>9.50e</td>
<td>1d</td>
<td>2.67c</td>
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<td></td>
<td>12 (S5)</td>
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Analysis of Variance

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<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>V*S</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
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<td>ns</td>
</tr>
</tbody>
</table>

*ns: Not significant; **: Significant at p<0.001; ***: Significant at p<0.01 and *: Significant at p<0.05. The means with the same letters are not significantly different (Duncan’s multiple ranges test; p<0.05)

between control and 3 ds m⁻¹ level. On the other hand, content of protein in Pegah for control was higher than 3 ds m⁻¹ level of salinity.

K⁺, Na⁺ and K/Na ratio: Main effects of cultivar and salinity levels showed significant difference for both Na⁺ and K⁺ but just salinity showed significant difference for K/Na ratio. The only significant interaction between cultivar-salinity was obtained for Na⁺. Pegah cultivar had higher Na⁺ (7.35%), K⁺ (5.2%) and K/Na (8.47%) ratio than Speedfeed. Salinity decreased amount of K⁺ and K/Na ratio but increased amount of Na⁺. Highest amount of Na⁺ (220.17 mm kg⁻¹), K⁺ (503.10 mm kg⁻¹) and K/Na ratio (5.02) were measured for 9 ds m⁻¹, control and control but the lowest ones were observed in control, 6 and 9, respectively (Table 3).

Growth parameters

Leaf Number (LN): Measuring leaf number in two different times showed that numbers of leaves were decreased from first measuring to second measuring generally. In both time of measuring, Pegah had higher leaf number per plant than Speedfeed but the difference was not significant. Salinity decreased number of leaves per plant at both measurements. Maximum number of leaves per plant was recorded for control and the minimum number was observed in 9 ds m⁻¹ salinity level in both measurements, respectively. At first time measuring, significant difference between 6 and 9 ds m⁻¹ levels of salinity was observed but this difference was not obtained for second measurement (Table 1).

Plant height: Plants height of Pegah was significantly (p<0.01) higher than Speedfeed (17.54 and 13.11%) at first and second measurements, respectively. Control plants showed maximum height (24.75 and 29.64 cm respectively) and 9 ds m⁻¹ stressed plants showed minimum height (9.5 and 10.92 cm respectively). The difference between 6 and 9 ds m⁻¹ salinity levels was not significant at first measuring (Table 1).

Total tiller number per plant: There was no significant difference between two cultivars related to total number of tillers per plant but salinity showed significant difference. Control had maximum tiller number per plant but 9 ds m⁻¹ level of salinity had minimum number (Table 1).

Shoot weight, root weight and shoot/root ratio: Analysis of variance showed no significant difference between cultivars for shoot weight, root weight and shoot/root ratio. With increase salinity level, shoot weight and root weight was decreased. Highest shoot (3.19 g) and root (2.29 g) weight were obtained in control while the lowest ones were measured for 9 level of salinity. Minimum shoot/root ratio was observed in 9 ds m⁻¹ salinity but it had no difference with 3 ds m⁻¹ salinity level. On the other hand, maximum ratio of shoot/root was observed in
control while the difference among control, 3 and 6 salinity levels were not significant (Table 2).

**Leaf Area (LA):** Results of leaf area measuring at two times showed that cultivars had no significant difference at first time but had significant difference at second time measuring and Speedfeed showed higher leaf area (13.5%). Leaf area decreased with increasing salinity levels and during times of measurements. Highest leaf area was observed in control while the lowest was recorded for 9 ds m⁻¹ salinity level. Regression lines separately were fitted for Pegah and Speedfeed cultivars and they could interpret most of the variations at first (Pegah; R² = 0.86 and Speedfeed; R² = 0.83) and also second (Pegah; R² = 0.85 and Speedfeed; R² = 0.96) measuring (Fig. 2).
Growth rate: Results of three times counting plant number after emergence and comparing with emergent percent in control showed that cultivars was not different in growth rate but salinity levels were significantly different. Growth rate in control plants was 100% at all measurements but in other salinity levels was decreased during the measurements. Salinity level of 12 ds m\(^{-1}\) showed 52.77% growth rate at first time measurement but at second time was nearly zero and finely at last time measuring was completely zero. Significant difference between control and other salinity levels except 12 ds m\(^{-1}\) was not significant at first time measurement but significant different between control with 6 and 9 ds m\(^{-1}\) was significant at second and third times (Fig. 3).

Growth power: Recording results of growth power at four times showed that there no significant different between cultivars at all recording while the differences among salinity levels were significant. Average growth power at first time recording for 12 ds m\(^{-1}\) was 1.7 while it had minimum value (1) at other recordings. Maximum value for growth power was recorded for control plants at all recordings. Difference between control and 3 ds m\(^{-1}\) salinity level was not significant at first, second and third times but significant at fourth time. Growth powers for all saline treatments were decreased during recording periods (Fig. 4).

Multiple regressions: Because of being first variable entered in model (p<0.001) based on Stepwise selection for shoot weight, root weight is the most important indicator of shoot weight in this multiple regression. After root weight, second leaf area (p = 0.003), tiller number (p = 0.003), Shoot/Root ratio (p = 0.096), content of K\(^+\) (p<0.001), first leaf number (p = 0.060), first leaf area (p = 0.057) and photosynthesis rate (p = 0.113) entered in model, respectively (Table 4).

Correlation coefficient: Mean of the morphological, biochemical and chemical traits were used for correlation coefficient analyzing. Correlations between shoot weights with other variables except concentration of Na\(^+\) were significantly positive but correlation of shoot weight with Na\(^+\) was negative and not significant (r = 0.07). Highest correlation of traits with shoot weight was belonged to root weight. Content of Na\(^+\) showed significant correlation just with proline content (r = 0.96), shoot per root ratio (r = 0.72) and K\(^+\) (r = 0.68). In general, all correlation among traits except correlations of Na\(^+\) content with shoot and root weight were positive (Table 5).

Emergence, growth rate and growth power
Percent of emergence: Results showed that emergence percent of Speedfeed was more than that in Pegah but the difference was not significant. Maximum emergence (71.67%) was obtained in control plants but the minimum was observed for 12 ds m\(^{-1}\) salinity level (15%). Among 3, 6 and 9 levels of salinity significant difference were not observed (Fig. 3).
Fig. 4(a-d): Growth power of sorghum cultivars under salinity levels at first (a) second (b) third (c) and fourth time measuring. Different alphabets are significantly different at p<0.001

Table 5: Correlation coefficient among measured traits

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<tr>
<th>Trait</th>
<th>LA</th>
<th>LN</th>
<th>height</th>
<th>Root (gr)</th>
<th>Sh/R</th>
<th>proline</th>
<th>K⁺</th>
<th>Na⁺</th>
<th>K/Na</th>
<th>PR</th>
<th>TN</th>
<th>SW</th>
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<td>height</td>
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<td>Sh/R</td>
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<td>0.81**</td>
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<td>0.77**</td>
<td>0.76**</td>
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LA: Leaf area, LN: Leaf numbers, RW: root weight, Sh/R: Shoot per root ratio, PR: Photosynthesis rate, SW: Shoot weight and TN: Tiller numbers

**DISCUSSION**

Results indicate that photosynthesis rate decreased by increase salinity levels. Abiotic stresses such as salinity stress cause impairment in the balance between light capture in leaf of stressed-plants and its utilization. This extra energy of light results in production of Reactive Oxygen Species (ROS) that are so dangerous for chloroplast. ROS damage chloroplast and its photosystems consist of PSEE and PSIT and finally results in decreased photosynthesis. On the other side less decreasing of photosynthesis rate in plants under stress conditions is an indicator for more salinity resistance. Pegah significantly showed more photosynthesis rate that is may be due its higher resistance of its chloroplastic cells to salinity. There are many studies report decreasing photosynthesis rate under salinity stress in crops such as rice (Yeo et al., 1985) sorghum, linseed (Nasir Khan et al., 2007) and other plants species.

Salinity caused increases the amount of free proline in stressed-plants leaves which salinity levels of 9 ds m⁻¹ showed the highest amount. Proline is an osmoregulate and has a key role in osmotic adjustment. Salinity usually is accompanied with declining water absorption in root. This situation causes loss of water and turgor in plants cells that induces osmotic stress. One of the most important
plant responses to osmotic stresses is higher accumulation of osmoregulators such as proline. Accumulation of proline in plants cells induces higher osmotic adjustment and inhibiting water loss on cells. In addition to this proline can detoxify some kind of ROS such as singlet oxygen. Thus, proline is an important component for stress protection in plants. Higher amount of proline in salinity treatments of this study indicate accruing osmotic stress in this condition. On the other side, there was no significant difference between cultivars implying that both of them have same response to salinity related to proline. Free proline found to be increased in studies on wheat (Goudarzi and Pakniyat, 2009), tobacco (Kuznetsov and Shevyakova, 1997), Arabidopsis (Nanjo et al., 1999) and other plants.

Salinity increased total protein content and Speedfeed showed higher amount of protein. With occurring stresses, plants respond by expression or over-expression of some stress related genes and since the final product of most of these genes are different proteins, higher accumulation of protein can caused more protection in plants. Higher protein content could be an appropriate indicator for higher resistance of plants and because of higher protein in Speedfeed, this cultivar is an eligible cultivar to achieve breeding aims for higher resistance to salinity.

Stressed-plants had higher accumulation of Na⁺ indicates higher uptake of Na⁺ in this situation, although higher levels of salinity causes higher uptake of Na⁺. On the other hand, salinity decreased amount of K⁺ in plants implying that higher levels of salinity and Na⁺ have a negative effect on absorption and accumulation of K⁺. Both K⁺ and Na⁺ are ions that have important effect in osmotic adjustment. Higher accumulation of Na⁺ has positive effect on osmotic adjustment but extra amount of this ion can be very dangerous for plant cells and therefore, one of important negative effects of salinity in plants is high amount of this ion. Also, K⁺ has an osmoregulating role and decreasing content of this ion under salinity stress has negative effects on plants cells. Salinity reduced K/Na ratio implying negative effect of salinity on K⁺ and its positive effects on Na⁺ uptakes. Speedfeed cultivar showed higher amount of K⁺ and lower content of Na⁺ in its leaves that is may be due to higher controlling of ion uptake in this cultivar. Mahmood et al. (2010) reported higher accumulation in Na⁺ and lower accumulation in K⁺ under saline condition.

All growth parameters were decreased with respect to enhancing salinity levels. Decreasing the amount of growth parameters under saline condition in this study probably is due to inhibiting effects of salinity on photosynthesis and also higher use of energy to control the ion uptake in root and higher accumulation of proline. Comparing effects of salinity on emergence and growth parameters indicated that salinity had lower effects on emergent percentage than growth parameters. Pegah cultivar had higher stem height but the leaf area of Speedfeed was higher. On the other hand, the difference between cultivars for other growth parameters was not significant. Due to importance of leaves in photosynthesis, leaf area is more proper factor than height and therefore, Speedfeed is more appropriate than Pegah under saline condition.

Percent of emergence for 3, 6 and 9 ds m⁻¹ salinity levels were not different but 12 ds m⁻¹ salinity level and control had minimum and maximum percent, respectively. Plants growth rate in the first time of measuring was not significant between control and 3, 6 and 9 ds m⁻¹ salinity levels but 12 ds m⁻¹ showed different growth rate. During salinity period, growth rate of plants under salinity levels were decreased and plants under 12 ds m⁻¹ salinity level treatment were completely destructed. On the other hand, growth power of plants under saline condition showed significant difference with control in all measuring times. The results indicate that severity and lasting of the salinity are of important factors for plants in order to response to this stress. Speedfeed cultivar showed higher emergence percent and also percent of growth and growth rate than Pegah indicating higher resistance of this cultivar to salinity that is may be due to higher control in ion (Na⁺ and K⁺) uptakes.

Results of multiple regressions based on stepwise selection showed that most important factor for shoot weight of sorghum under salinity condition is root weight factor. As well as root weight, shoot per root ration entered in the model as an effective variable affects shoot weight of sorghum. Salinity causes limiting available water in soils by increasing content of Na⁺ and other substance in soil and then, absorbing water from soil becomes hard for plants roots. Higher density of root under saline condition can be so helpful for more resistance of plants bye increasing absorbing surface of root. Two stages of leaf area and first stage measuring leaf number and also photosynthesis rate were entered to the model that is implying the importance of photosynthesis rate and ability to continue of plants on producing higher shoot weight. Stepwise selection also indicated important effect of higher K⁺ accumulation by entering this variable in the model. Results of correlation coefficient confirmed results of multiple regressions since highest coefficient correlation of traits was belonged to shoot and root weights.
CONCLUSION

Salinity showed deleterious effects on growth parameters and caused higher accumulation of Na⁺ and proline and also total protein content but lower accumulation of K⁺ and decreased photosynthesis rate, indicating that severity of salinity can cause high negative effects on productivity of plants. Stepwise selection and correlation analysis showed that root density is the most important factor for plant productivity. Speedeed in general had higher power of survive, growth and emergence and it can be a valuable cultivar for selecting or crossing programs with Pegah in order to gain more salinity resistance.

REFERENCES


