

Response of Different Soybean (*Glycine Max L.*) Genotypes Grown in Sand Culture to Salinity Stress

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ABSTRACT

The aim of this study was to evaluate eight soybean genotypes to salinity stress. Plants were grown in sand culture in a greenhouse experiment and irrigated with one-tenth strength modified Hoagland nutrient solution with or without 50 mM NaCl. The experimental design was split plot, arranged in randomized completely block design with three replications. Main plot factor was salt stress level (0 and 50 mM NaCl) and sub-plot factor was soybean genotypes (Giza21, Giza22, Giza35, Giza82, Giza83, Giza111, Clark, and Crawford). After four weeks from sowing, the whole plants were collected. The results indicated that salinity induced significant decrease in plant growth of all soybean genotypes, since, salt stress decreased shoot height, whole plant, shoot and root fresh and dry weight and leaf area of all soybean genotypes. However, salt stress increased shoot/root ratio on fresh and dry weight basis, plant moisture content and electrolyte leakage of all soybean genotypes. Chlorophyll content index no significantly decreased with salt stress. Salt stress increased shoot and root Na⁺ content while decreased K⁺ content and K⁺/Na⁺ ratio of shoot and root for all soybean genotypes. The obtained results showed that the eight soybean genotypes responded differently to salt stress. It seems that Giza82 was more tolerant and Clark was more sensitive to salinity than other genotypes. These genotypes can be arranged with respect to tolerance to salinity in the order: Giza82 > Giza35 > Giza21 > Giza22 > Giza111 > Crawford > Giza83 > Clark.

Keywords: salt stress, soybean genotypes, growth, electrolyte leakage, chlorophyll content index, sodium and potassium content.

INTRODUCTION

Salt stress is one of the major type of abiotic stress that adversely affect growth and development of legumes in arid and semi-arid regions. Over 20–50% of the whole arable land is affected by salt stress every year (Xu et al., 2011). Salinity decreases the productivity of most crops as plant growth is affected in several aspects of its metabolism. Selection of salt tolerant crops may substantially expand the world's food-producing area. Soybean is an important dicot. crop due to the high content of oil and protein in its seeds (Luo et al., 2005 and Sharifi et al., 2007). It is widely used in Egypt for human and poultry consumption. It has been reported that salinity stress inhibits seed germination and seedling growth, reduces

nodulation, and decreases biomass accumulation and yield of soybean (Essa, 2002). Soybean is classified as a moderately salt-tolerant crop and the yield will be reduced when soil salinity exceeds 5 dS/m (Maas and Hoffman, 1977). The adverse effect of salinity on plant is dependent on salt concentration in the substrate, duration of exposure to salinity and stages of plant growth (Blum, 1988; Maas and Poss, 1989; Gill, 1990). Recently, salt stress has become one of the limiting factors that reduce its yield like many other crops. Exposure of plants to salt environment during various developmental stages appears to affect various physiological changes. Reduction of photosynthetic activity, accumulation of organic acids and osmolytes, and changes in carbohydrate metabolism, are typical physiological and biochemical responses to stress (Munns, 2002). Salt stress commonly reduces content of chlorophyll but increase carotenoids in plant leaves and the salinized plants showed the highest values of total soluble sugars, proline, and total free amino acid (Khalafallah et al., 2008). The conductivity test, based on solute leakage, has been proposed as a good indicator of salt tolerance in plants (Ghoulam et al., 2002). The cell membrane often suffered injury associated with the increases in permeability and loss of integrity (Blokchina et al., 2003). The increase in electrolyte leakage was due to the loss of ability to reorganize cellular membranes rapidly and completely (McDonald, 1980). Farhodi et al. (2007) found salt tolerance of canola cultivars have a direct relationship with K⁺/Na⁺ ratio so that the ratio decreased with the increase of salinity level but less decrease is observed in tolerant cultivars. They concluded that K⁺/Na⁺ ratio can be a measure of salt stress tolerance. The response of soybean to salinity stress depends on both genotypes and environmental conditions (Ghassemi-Golezani et al., 2009).

The aim of present study is to evaluation of eight soybean genotypes to salinity stress during seedling stage.

MATERIALS AND METHODS

A pot experiment, using sand culture technique under field conditions, was carried out during summer season 2017 at Faculty of Agriculture, Elbeheira Governorate, Egypt to investigate the effect of salinity

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stress on the early growth of eight genotypes of soybean (*Glycine max L.*) obtained from Agriculture Research Center, Ministry of Agriculture and Land Reclamation (MALR), Egypt. A randomized complete block design in a split-plot array with three replicates was used. The main plot was two salt levels (0 and 50 mM NaCl) and the sub-plot was eight soybean genotypes (Giza21, Giza22, Giza35, Giza82, Giza83, Giza111, Clark, and Crawford).

Ten seeds of every soybean genotype were sown in plastic pot (12 cm inside diameter and 9 cm depth with holes in the bottom for drainage) containing 0.5 kg pre-washed sand as described by Abdelraouf, (2017). Each pot was irrigated three times per week with 100 mL of irrigation solution, which contains both one-tenth strength modified Hoagland and Arnon nutrient solution (Hewitt, 1966), and the tested salt levels (0 or 50 mM NaCl). The concentrations of macronutrients in the irrigation solution were 16.87, 8.47, 11.92, 29.99, 12.00, 4.78, and 6.38 mg L⁻¹ for N-NO₃, N-NH₄, P, K, Ca, Mg, and S, respectively. The concentrations of micronutrients in the irrigation solution were 0.50, 0.11, 0.05, 0.01, 0.01 and 0.005 mg L⁻¹ for Fe, Mn, B, Zn, Cu and Mo, respectively. After 18 days from sowing, the plants were thinned to five plants per pot.

Before plants collecting the chlorophyll contents of fully expanded leaves were measured by the chlorophyll meter device (SPAD-502, Minolta, Japan) according to Abdelraouf, (2017). After four weeks from sowing, the whole plants were collected, washed by distilled water, and then separated into shoots and roots. The fresh weight of shoots and roots, and shoot height were measured. Leaf area was measured by disk method (Radford, 1967). The plant samples were then dried at 70°C for 48 hours and the dry weight of shoots and

roots were measured. The shoot/root ratio on fresh and dry weight basis and moisture content of the whole plant, shoots, and roots were calculated. Shoots and roots oven-dried samples were ground using stainless steel mill, and dry ashing at 550°C for 5 hrs. The ash was dissolved in 2 N hydrochloric acid for 20 min and solution was filtered in 100 ml volumetric flask with distilled water to estimate Na⁺ and K⁺ contents using flame photometer.

The membrane damage was assessed by measuring the leakage of electrolytes from leaf discs according to the method described by Mishra and Choudhuri (1999) and as described by Abdelraouf, (2017). The relative decrease expressed as: (control – treatment) / control X 100 of most parameters was calculated.

The analysis of variance was calculated using the CoStat 6.400 Statistical Analysis Software (CoStat, 2005) and the differences are identified among means by Fisher's Least Significant Difference (LSD) test at 0.05 probability level.

RESULTS AND DISCUSSION

Plant weight

Shoot: The shoot fresh weight of soybean genotypes significantly ($p < 0.05$) decreased with increasing NaCl concentration (Table 1). The mean relative decrease in shoot fresh weight of all soybean genotypes under 50 mM NaCl was 17.2 %. There were significant differences ($p < 0.01$) between soybean genotypes with respect to shoot fresh weight under salt stress. On the other hand, there were no significant differences with respect to shoot fresh weight between the interaction of salt stress and soybean genotypes. The highest relative decrease of the shoot fresh weight was that of Giza83 and the lowest was that of Giza21 (Fig. 1).

Table 1. The fresh and dry weights of plant shoots and roots and shoot/root ratio of the different soybean genotypes grown under salt stress

Treatment	Fresh weight (g plant ⁻¹)			Dry weight (g plant ⁻¹)			Shoot/Root ratio	
	Whole	Shoot	Root	Whole	Shoot	Root	F. W.	D. W.
mM NaCl								
0	3.30	1.94	1.36	0.32	0.24	0.08	1.46	3.06
50	2.21	1.61	0.60	0.19	0.16	0.03	2.78	5.28
LSD	0.32	0.20	0.12	0.04	0.02	0.02	0.58	1.39
Genotypes								
Giza21	3.21	2.06	1.16	0.29	0.22	0.07	2.00	4.08
Giza22	2.83	1.74	1.09	0.27	0.20	0.07	1.89	3.27
Giza35	2.24	1.50	0.74	0.21	0.17	0.04	2.26	4.64
Giza82	2.76	1.76	1.01	0.26	0.20	0.06	1.99	3.94
Giza83	2.53	1.66	0.87	0.24	0.19	0.05	2.19	4.23
Giza111	2.65	1.76	0.88	0.26	0.21	0.05	2.47	4.68
Clark	2.77	1.84	0.93	0.26	0.21	0.05	2.34	4.75
Crawford	3.05	1.89	1.16	0.27	0.21	0.06	1.81	3.80
LSD	0.38	0.24	0.18	0.03	0.02	0.01	0.28	ns

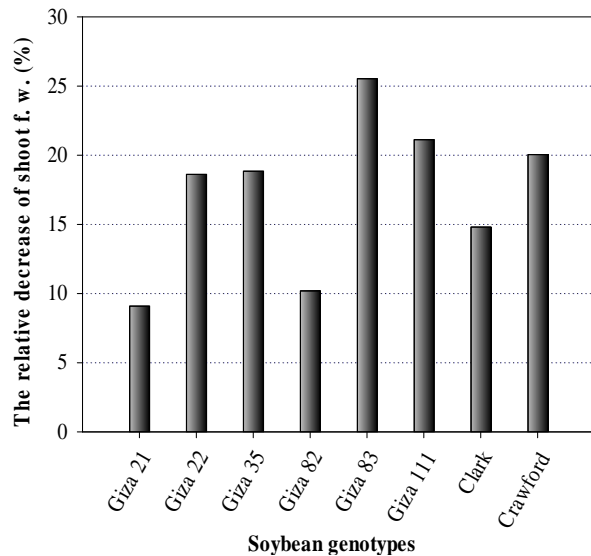


Fig. 1. The relative decrease of shoot fresh weight due to salt stress of the different soybean genotypes

Shoot dry weight of soybean genotypes significantly ($p < 0.01$) decreased due to salt stress, where the mean relative decrease in shoot dry weight of all soybean genotypes was 32.7 %. Also, there were significant differences ($p < 0.05$) between soybean genotypes with respect to shoot dry weight under salt stress. However, no significant differences between the interaction of salinity and soybean genotypes, on shoot dry weight, were observed. The highest relative decrease of the shoot dry weight was that of Clark and the lowest was that of Giza82 (Fig. 2).

Roots: The root fresh weight of soybean genotypes significantly ($p < 0.01$) decreased with salt stress (Table 1). The mean relative decrease in root fresh weight, of all soybean genotypes, was 55.7 %. Also there were significant differences ($p < 0.01$) between soybean genotypes with respect to root fresh weight under salt stress. However, there were no significant differences with respect to root fresh weight between the interaction of salt stress and soybean genotypes. The highest relative decrease of root dry weight was that of Giza111 and the lowest was that of Giza82 (Fig. 3).

Root dry weight of soybean genotypes significantly ($p < 0.01$) decreased due to salt stress, where the mean relative decrease in root dry weight, of all soybean genotypes, was 59.8 %. Moreover, there were significant differences ($p < 0.01$) between soybean genotypes with respect to root dry weight under salt stress. On the other hand, no significant differences were observed between the interaction of salinity and soybean genotypes, on root dry weight. The highest

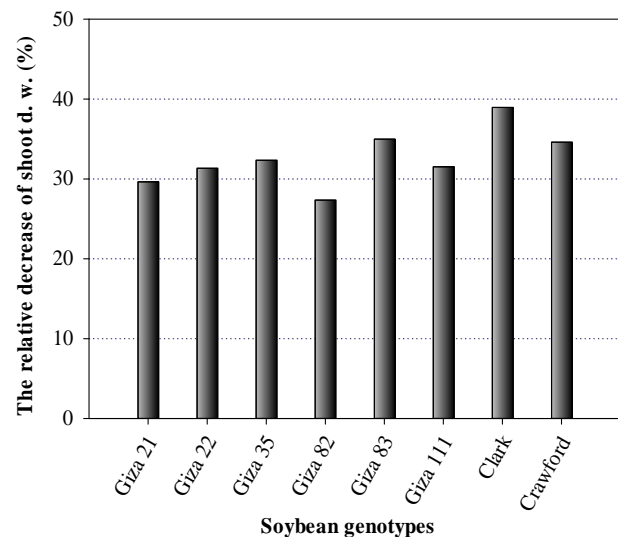


Fig. 2. The relative decrease of shoot dry weight due to salt stress of the different soybean genotypes.

relative decrease of root dry weight was that of Clark and the lowest was that of Giza82 (Fig. 4).

Whole Plant: The whole plant fresh weight of all soybean genotypes significantly ($p < 0.01$) decreased under Salt stress (Table 1). The mean relative decrease of the whole plant fresh weight due to 50 mM NaCl was 33.1 % compared to the control (0 mM NaCl) of all soybean genotypes. Also, there were significant differences ($p < 0.01$) among soybean genotypes with respect to the whole plant fresh weight under salt stress. There were no significant differences between the interaction of salinity and soybean genotypes on the whole plant fresh weight. The highest relative decrease of the whole plant fresh weight was that of Giza83 and the lowest was that of Giza21 (Fig. 5).

The whole plant dry weight significantly ($p < 0.01$) decreased with salt stress of all soybean genotypes (Table 1). The mean relative decrease in whole plant dry weight was 39.5 % of all soybean genotypes. Also, there were significant differences ($p < 0.01$) among soybean genotypes with respect to whole plant dry weight under salt stress. There were no significant differences between the interaction of salinity and soybean genotypes on whole plant dry weight. Accordingly, the relative decrease of whole plant dry weight can be arranged in the order: Giza82 > Giza35 > Giza21 > Giza22 > Giza111 > Crawford > Giza83 > Clark (Fig. 6).

These results are in agreement with those reported by Essa and Al-Ani (2001), Amirjani (2010), El-Rodeny et al. (2012), Farhoudi et al. (2015), Saad-Allah (2015). The reduction of plant growth under saline

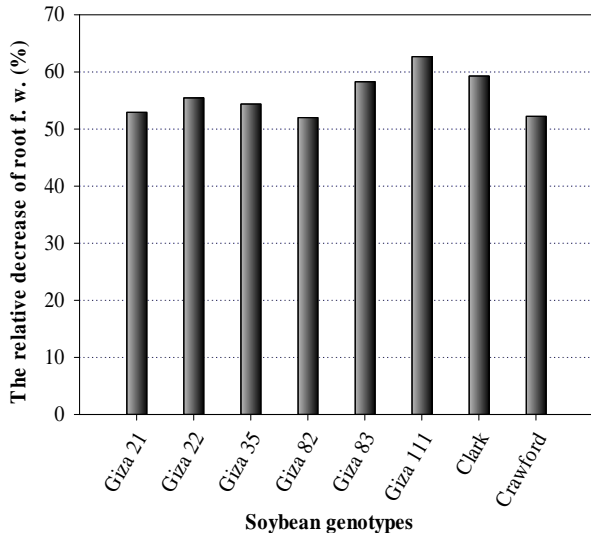


Fig. 3. The relative decrease of root fresh weight due to salt stress of the different soybean genotypes

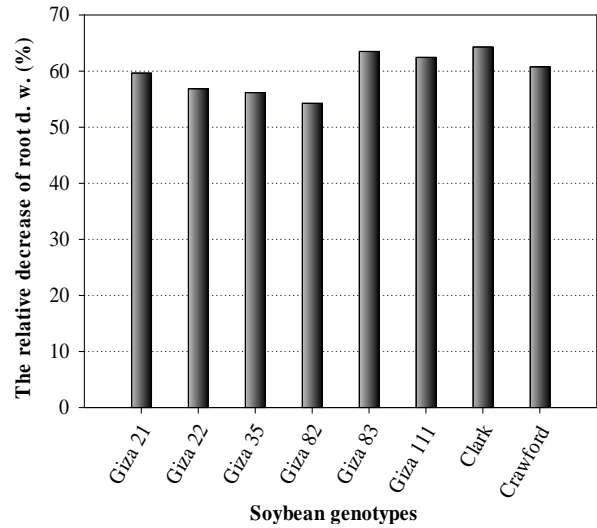


Fig. 4. The relative decrease of root dry weight due to salt stress of the different soybean genotypes

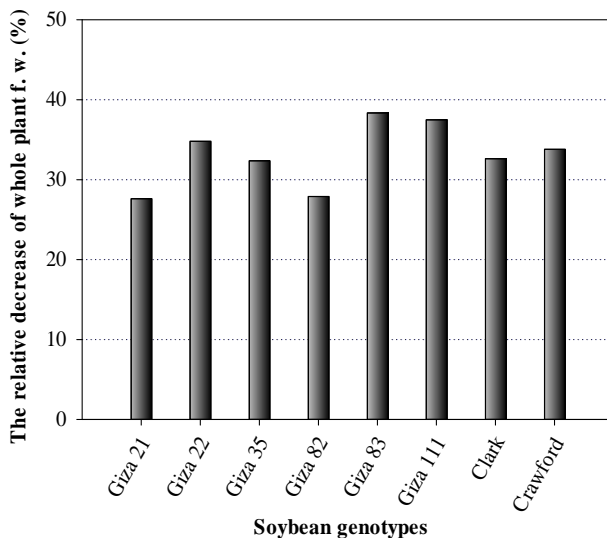


Fig. 5. The relative decrease of the whole plant fresh weight due to salt stress of the different soybean genotypes

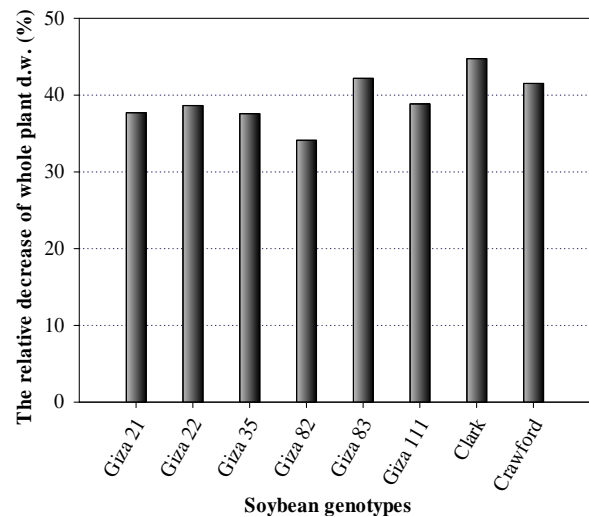


Fig. 6. The relative decrease of the whole plant dry weight due to salt stress of the different soybean genotypes

conditions may either be due to excessive ions, Na⁺ and Cl⁻ accumulation in the plant tissues (Cusido et al., 1987; Gunes et al., 1996; Yousef and Al-Saadawi, 1997). Also, due to generation of osmotic stress leading to reduction in water absorbance and cell division and differentiation (Nikee et al., 2014). It has been reported that salt stress significantly reduced net photosynthetic rates, increased energy losses for salt exclusion mechanism, largely decreased nutrient uptake and finally reduced plant growth (Long and Baker, 1986; Seemann and Sharkey, 1986).

Shoot/Root ratio: The shoot/root ratio, on fresh and dry weight basis, significantly ($p < 0.05$) increased under salt stress (Table 1), where the mean relative increase of shoot/root ratio on fresh and dry weight basis were 90.3 and 72.5 %, respectively. There were significant differences ($p < 0.01$) between soybean genotypes in shoot/root ratio on fresh weight basis. While there were no significant differences in shoot/root ratio on dry weight basis between soybean genotypes. Also, there were no significant differences on shoot/root ratio, on fresh and dry weight basis,

between the interaction of salt stress and soybean genotypes. It could be concluded that the magnitude of reduction of root growth was greater than that of shoot under salinity stress. This indicates that roots of the studied soybean genotypes are more sensitive to salinity stress than shoots. It is also clear that the higher values of shoot/root ratio on dry weight basis than on fresh weight basis is due to higher moisture content in roots than in shoots. This result is in agreement with that obtained by Abdelraouf et al. (2016) who observed that the root growth of broad bean cultivars was more adversely affected by salinity than the shoot.

Shoot height

The shoot height significantly ($p < 0.05$) decreased under salt stress (Table 2). The mean relative decrease in shoot height due to salt stress was 39.0 %. There were also significant differences ($p < 0.01$) between soybean genotypes with respect to shoot height under salt stress. On the other hand, there were no significant differences of shoot height between the interaction of salt stress and soybean genotypes. The relative decrease of shoot height was the lowest for Giza82 and the highest for Clark (Fig. 7). These results are in agreement with those reported by Saad-Allah (2015).

Leaf area

The leaf area of soybean genotypes significantly ($p < 0.01$) decreased under salt stress (Table 2). The mean relative decrease in leaf area under salt stress was 65.8 %. However, there were no significant differences between soybean genotypes under salt stress and between the interaction of salt stress and soybean genotypes on the leaf area since Giza82 was the lowest and Clark was the highest leaf area relative decrease (Fig. 8). These results are in agreement with those obtained by Saad-Allah (2015) and Khan et al. (2014). It is clear that excessive salt uptake can result in the deterioration of leaves and reduces the total photosynthetic leaf area. The reduction in leaf area was attributed to the increasing in leaf senescence and the reduced size of leaves could be due to low turgor under saline stress conditions Khan et al. (2014).

Moisture content

The shoot moisture content of soybean genotypes significantly ($p < 0.01$) increased under salt stress. However, this increase was not significant for whole plant and root moisture content (Table 2). The mean relative increase with respect to whole plant, shoot, and root moisture contents of all soybean genotypes under salt stress were 0.9, 2.5, and 0.4 %, respectively. There were no significant differences between soybean genotypes with respect to moisture content in the whole plant, shoot, and root under salt stress. There were also

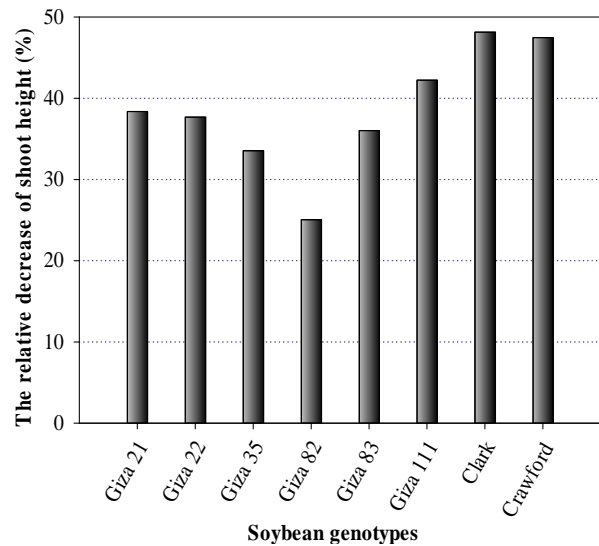


Fig. 7. The relative decrease of shoot height due to salt stress of different soybean genotypes

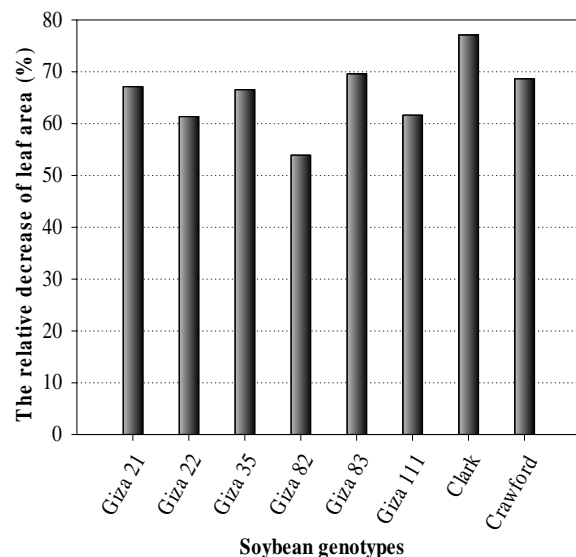


Fig. 8. The relative decrease of leaf area due to salt stress of different soybean genotypes

no significant differences between interaction of salt stress and soybean genotypes with respect to moisture content in the whole plant, shoot, and root.

It is clear that the soybean plants tolerated the adverse effects of salt stress by succulence, which mean the plant increased the shoot and root fresh mass by increasing the moisture content more than the biomass production. These results are in agreement with those reported by Elsokkary et al. (2010, 2011), Abdelraouf et al. (2016), and Abdelraouf (2017).

Chlorophyll content index

Salt stress no significant variations of chlorophyll content index in leaves of all soybean genotypes (Table 2). However, the mean relative decrease in chlorophyll content index of all soybean genotypes was 1.2 %. There were also no significant differences between soybean genotypes for chlorophyll content index and, there were no significant differences between the interaction of salt stress and soybean genotypes for chlorophyll content index. Similar results are obtained by El-Rodeny and EL-Okkiah (2012) and Essa and Al-Ani (2001). The observed reduction in leaf chlorophyll content under NaCl stress could be attributed to the destruction of chlorophyll pigments and the instability of the pigment protein complex (Saad-Allah, 2015). It is also attributed to the interference of salt ions with the de novo synthesis of proteins, the structural component of chlorophyll, rather than the breakdown of chlorophyll (Jaleel *et al.*, 2008; Al-Sobhi *et al.*, 2006).

Electrolytes leakage

Salt stress increased significantly ($p < 0.05$) membrane damage where the leakage of electrolytes from the leaf cells were increased by salt stress of all soybean genotypes (Table 2). The mean relative increase in electrolytes leakage due to salt stress of all soybean genotypes was 43.0 %. However, there were no significant differences between soybean genotypes on membrane damage. There were also no significant effect of the interaction between salt stress and soybean genotypes on membrane damage. Thus, increasing electrolyte leakage was used to assess membrane permeability, and therefore addition of 50 mM NaCl induced membrane damage. Salt stress induced

electrolytes leakage has been previously observed in sugar beet (Abdelraouf, 2017). Mohamed *et al.* (2007) reported that electrolyte leakage from plant leaves was found to increase with higher salinity.

K⁺ and Na⁺ content

Salt stress at 50 mM NaCl significantly ($p < 0.05$) decreased K⁺ content of roots while its decrease in shoot was not significant for all soybean genotypes (Table 3). The mean relative decrease in K⁺ content of shoots and roots of all soybean genotypes under salt stress were 19.8 and 25.3 %, respectively. There were also no significant differences between soybean genotypes and between salt stress and soybean genotypes interaction on K⁺ content of shoots and roots.

Salt stress significantly ($p < 0.01$) increased Na⁺ content of shoots and roots of all soybean genotypes (Table 4). The mean relative increase in Na⁺ content of shoots and roots of all soybean genotypes were 420.8 and 168.5 %, respectively. On the other hand, there were no significant differences between soybean genotypes and between salt stress and soybean genotypes interaction on Na⁺ content of shoots and roots.

Salt stress significantly ($p < 0.05$) decreased K⁺/Na⁺ ratio of shoots and roots of all soybean genotypes (Table 3). The mean relative decrease in K⁺/Na⁺ ratio of shoots and roots of all soybean genotypes under salt stress were 84.6 and 72.3 %, respectively. There were no significant differences between soybean genotypes and between salt stress and soybean genotypes interaction on K⁺/Na⁺ ratio of shoots and roots.

Table 2. The shoot height, leaf area, moisture content, chlorophyll content index and electrolyte leakage of the different soybean genotypes under salt stress

Treatment	Shoot height (cm)	Leaf area (cm ² plant ⁻¹)	Moisture content (%)			Chl. content index (SPAD value)	Elect. leakage (μScm ⁻¹)
			Whole	Shoot	Root		
mM NaCl							
0	10.0	36.3	90.3	87.7	94.1	36.4	78.3
50	6.1	12.4	91.1	89.9	94.5	36.0	112.0
LSD	2.0	3.7	ns	0.9	ns	ns	22.6
Genotypes							
Giza21	7.0	26.0	91.2	89.4	94.4	37.6	108.1
Giza22	7.4	26.0	90.4	88.5	93.4	35.9	88.0
Giza35	6.6	19.7	90.6	88.7	94.5	35.5	94.0
Giza82	9.6	25.6	90.8	88.7	94.4	36.2	82.9
Giza83	7.4	24.5	90.6	88.8	94.2	35.0	92.2
Giza111	9.0	25.7	90.1	88.2	93.9	35.7	119.8
Clark	9.6	22.0	90.7	88.7	94.6	38.2	88.3
Crawford	7.7	25.5	91.3	89.2	94.8	35.7	88.2
LSD	1.2	ns	ns	ns	ns	ns	ns

Table 3. The potassium and sodium content in shoots and roots of the different soybean genotypes under salt stress

Treatments	K ⁺ Content (%)		Na ⁺ Content (%)		K ⁺ /Na ⁺ ratio	
	Shoot	Root	Shoot	Root	Shoot	Root
mM NaCl						
0	3.03	4.75	0.53	2.16	5.72	2.20
50	2.43	3.55	2.76	5.80	0.88	0.61
LSD	ns	1.13	0.87	1.50	2.14	0.79
Genotypes						
Giza21	2.60	4.56	1.53	4.07	3.48	1.31
Giza22	3.17	4.26	2.20	4.32	2.67	1.32
Giza35	2.80	4.94	1.49	3.82	3.01	1.88
Giza82	2.85	4.28	1.56	4.23	3.54	1.23
Giza83	2.46	3.64	1.44	3.92	2.55	1.19
Giza111	2.63	3.71	1.63	4.25	2.72	1.08
Clark	2.55	3.79	1.44	3.72	2.83	1.16
Crawford	2.79	4.00	1.90	3.52	2.91	1.32
LSD	ns	ns	ns	ns	ns	ns

The results showed that Giza82 showed the highest shoot K⁺/Na⁺ ratio but it was not the highest for root K⁺/Na⁺ ratio. According to these results, it can be concluded that the soybean genotype Giza82 is more salt stress tolerant due to its higher shoot K⁺/Na⁺ ratio compared with other studied genotypes. There is a relationship between potassium decrease and sodium increase in plant tissue with sensitivity to salinity. Sodium affected the cell membrane permeability, deconstructed the cell membrane and destroyed the selectivity property (Munns, 2002). Other investigations demonstrated that resistant plants to salinity not only have the higher K⁺/Na⁺ ratio, but also accumulated higher sodium in root tissue and therefore it would inhibit sodium transmit to shoot tissue and inhibit their damage. Very close results were obtained by Amirjani (2010), Khan et al. (2014) and Farhoudi *et al.* (2015).

CONCLUSION

Salt stress (50 mM NaCl) decreased all growth parameters, chlorophyll content index and K⁺/Na⁺ content ratio of shoot and root, while shoot/root ratio, electrolyte leakage and moisture, K⁺ and Na⁺ content of shoot and root were increased for all soybean genotypes. The order of soybean genotypes with respect to tolerance to salinity was Giza82 > Giza35 > Giza21 > Giza22 > Giza111 > Crawford > Giza83 > Clark.

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