

PAPER DETAILS

TITLE: Comparative Physiological Study of Soybean (Glycine Max L.) Cultivars Under Salt Stress

AUTHORS: Ayman EL SABAGH, Abd OMAR, Hirofumi SANEOKA, Celaledin BARUTÇULAR

PAGES: 269-284

ORIGINAL PDF URL: <https://dergipark.org.tr/tr/download/article-file/205114>

Araştırma Makalesi/Research Article (Original Paper)

Comparative Physiological Study of Soybean (*Glycine Max* L.) Cultivars Under Salt Stress

Ayman EL SABAGH¹, Abd Elhamid OMAR¹,
Hirofumi SANEOKA², Celaleddin BARUTÇULAR³

¹: Department of Agronomy, Faculty of Agriculture, Kafrelsheikh University, Egypt

²: Plant nutritional physiology Lab., Graduate School of Biosphere Science, Hiroshima University, Japan

³: Department of Field Crops, Faculty of Agriculture, University of Cukurova, Turkey
e-posta:aymansabagh@gmail.com

Abstract: Soybean (*Glycine max* L.) is one of the main crops and is widely cultivated in the world. Egypt has a semi-arid country with limited fresh water resources. In order to supply to irrigation water demand, it needs to use of non-conventional water resources such as saline or brackish water for irrigation. With this in mind, an pot experiments was conducted at plant nutritional physiology laboratory, Hiroshima University, Japan in 2010 to investigate the effect of irrigation with saline water on three Egyptian soybean cultivars (Giza-35, Giza-82, and Giza-111) under a non-saline (control) and saline (10 mM NaCl) conditions. The effects of salinity on crop was studied by measuring plant growth parameters. The water relations were estimated by studying the relative water contents and electrolyte leakage. The response to salinity stress was analysed by estimating the chlorophyll content, nitrogen, K⁺, Na⁺, proline content of plant tissue. The results showed a negative relationship between salinity stress and most of the measured plant growth parameters. In contrast, proline, Na⁺ and electrolyte leakage increased with irrigation water salinity. Results indicated that Giza-111 cultivar surpassed other cultivars in all characters under study. The highest value of crop dry weight, relative water content and accumulation of N, K⁺ and proline observed in Giza-111 with the compare to another cultivars. On the other hand, electrolyte leakage and Na⁺ ions accumulations were increased in the shoot under salinity in all cultivars. However, the lowest accumulation of Na⁺ ions was occurred in Giza-111. Similarly, Giza-111 was more stablited membrane to leakage. As a result, Giza-111 cultivar showed more capability and appropriate to survive under salinity condition compared with another cultivars regarding of almost all plant traits.

Key words: Soybean, Ion content, Salt stress, Proline.

Tuzluluk Stresi Altındaki Soya Çeşitlerinin Fizyolojik Olarak Karşılaştırılması

Özet: Soya (*Glycine max* L.), dünyada geniş alanlarda tarımı yapılan tarla bitkilerinden biridir. Mısır, sınırlı tatlı su kaynakları ile yarı kurak bir ülkedir. Sulama suyu ihtiyacını karşılamak için, geleneksel olmayan tuzlu ya da atık suların da sulamada kullanılması gerekmektedir. Bu amaçla, Mısır kökenli üç soya çeşidinde (Giza-35, Giza-82, and Giza-111), tuzlu olmayan (kontrol) ve tuzlu (10 mM NaCl) sulama suyunun etkileri, 2010 yılında Japonya'nın Hiroşima Üniversitesi, bitki besleme fizyolojisi laboratuarında yürütülen saksı çalışmaları ile araştırılmıştır. Tuzluluğun bitki üzerindeki etkileri, bitki büyüme parametreleri (BBP) ile belirlenmiştir. Su ilişkiler ise oransal su içeriği ve elektrolit sızıntısı ile saptanmıştır. Tuzluluk stresine tepkiler, bitki dokularındaki klorofil, azot, K, Na ve prolin içeriğinin analizi ile saptanmıştır. Sonuçlar, tuz stresi ile BBP arasındaki ilişkilerin negatif olduğunu göstermiştir. Buna karşın, Prolin, Na⁺ ve elektrolit sızıntısı, tuzluluk ile birlikte artmıştır. Bu çalışmadaki sonuçlar, Giza-111 çeşidinin incelenen tüm özelliklerinin diğer çeşitlere göre üstün olduğunu göstermiştir. Bitkideki kuru ağırlık, oransal su içeriği ile N, K⁺ ve prolin biriminin en yüksek değerlerin, çeşitler karşılaştırıldığında Giza-111 çeşidinde olduğu saptanmıştır. Diğer yandan, tuzluluk, tüm çeşitlerin saplarında elektrolit sızıntısı ve Na⁺ iyonu birikimini artırmıştır. Bununla birlikte en düşük Na⁺ birikimine Giza-111 çeşidinde rastlanılmıştır. Benzer şekilde, Giza-111 çeşidinin sızıntıya karşı membranlarının daha kararlı olduğu bulunmuştur. Sonuç olarak, çeşitlerin, tüm özellikleri karşılaştırıldığında Giza-111 çeşidinin tuzlu koşullarda yaşama uyum kabiliyetinin diğer çeşitlere göre daha iyi olduğu görülmüştür.

Anahtar kelimeler: Soya, İyon miktarı, Tuz stresi, Prolin

Introduction

Abiotic stresses, are serious threats to agriculture and result in the deterioration of the environment. They are the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Boyer 1982 and Bray et al. 2000). Soil salinization has become a global ecology and resource problem which poses a great threat on agricultural development around the world. Up to now, more than 800 million hectares of lands have been affected by salinity in the world (Munns and Tester 2008). Salinity is one of the major environmental stresses affecting the performance of many crop plants. Salinity has various effects on plant physiological processes such as increased respiration rate and ion toxicity, decreased leaf net CO₂ assimilation rate (Hajlaoui et al. 2006). High salinity is a common abiotic stress factor that seriously affects crop production in some parts of the world, particularly in arid and semi-arid regions (Neumann 1997). There are about of 8 million hectare agricultural land exposure to salt stress in the world (Munns 2005). Salt stress affects many physiological aspects of plant growth. Shoot growth and dry matter are reduced by salinity, root: shoot ratio is increased (Rahman et al. 2008). Salinity can affect germination of seeds either by creating osmotic potential which prevent water uptake, or by toxic effects of ions on embryo viability. Shoot growth was reduced by salinity due to the inhibitory effect of salt on cell division and enlargement in the growing point (McCue and Hanson 1990). It is well known that the halophytes can survive in saline lands, and some special physiological mechanisms may help them to adapt to this adverse environment. Therefore, screening valuable halophytes and exploring the physiological mechanisms of stress tolerance is utmost importance.

Soybean is a major food and oil crop in the most countries where salinity problems exist or might develop. Large areas of formerly arable land are being removed from crop production every year due to increasing soil salinity. Therefore, it is necessary to evaluate the physiological responses of crop plants to salt stress in order to develop appropriate strategies to sustain food production under adverse environmental conditions. Leaf photosynthetic capacity is suggested to be a key parameter determining crop yield (Jiang et al. 2002; Zhang et al. 2007). However, cultivated soybean is identified as a salt-sensitive species (Lauchli 1984). Its growth is severely suppressed and yield decreases dramatically under salt stress (Katerji et al. 2003). As more and more agricultural lands are affected by soil salinity, soybean production is being threatened. Thus, it is very important to breed salt-tolerant soybean cultivars (Lee et al. 2009). The number and quality of root nodules determine the nutritional status of the whole plant. Salt stress affects the nodulation of soybean, reduces the efficiency of nitrogen fixation, and decreases the number and biomass of root nodules (Singleton and Bohloul 1984; Delgado et al. 1994; Elsheikh and Wood 1995). In addition, salt stress strongly inhibits the deformation of root hair in perception of Nod factors and thus hampers the initiation of the symbiosis process (Duzan et al. 2004). The salt tolerance on soybean nodulation is thought to be mediated by solute signals in both the aerial part and roots (Abd- Alla et al. 1998). Even though the role of salinity in limiting the productivity of the crop plants has been studied, limited information regarding the response of the soybean plants to salinity stress is available.

The present study has been undertaken to compare the salinity stress adaptations in three different soybean cultivars, which are commonly cultivated in Egypt, in order to identify the soybean cultivars with a better performance even under saline conditions and providing a reference for breeding salt-tolerant soybean cultivars.

Material and Methods

Plant Material and Culture Conditions

The experiment was conducted at plant nutritional physiology laboratory, Hiroshima University in Japan. The seeds of soybean (*Glycine max* L) cultivars, Giza-111, Giza-82 and Giza-35 were obtained from Egypt Agricultural Research Center. The pots of soil was contained a mixture of granite regosol soil, perlite and peat moss in a volume ratio of 4:2:1 and irrigated daily to keep the moisture at field capacity. The seeds of all cultivars were surface sterilized with 5% Thiophanate-methyl for 5 mins and air dried. The seeds were sown into plastic pots (20cm at the top and 15.5 cm at the bottom in diameter, and 22.5 cm in depth) were used and filled with 3.25 kg of mixture of granite regosol soil and perlite (2:1:1, v/v). Pots were kept under greenhouse conditions. Plants were irrigated with nutrient solution at each watering using an irrigation system. The basal nutrient solution contained 8.3 mM NO₃-N, 0.8 mM NH₄-N, 0.5 mM P₂O₅, 2.2 mM K₂O, 0.7 mM MgO, 2.1 mM CaO, 11 µM MnO, 5 µM B₂O₃, and 13 µM Fe. After

sowing, plants were subjected to treatments every day until water drained from the bottom of the pot. Each pot was fertilized at a rate of 40 kg N ha⁻¹, 120 kg P₂O₅ ha⁻¹ and 100 kg K₂O₅ ha⁻¹ using fertilizer mixture and soil pH was adjusted to 6.0 with dolomitic calcium carbonate (300 kg ha⁻¹). The seeds were inoculated with old soils from field after soybean and therefore the roots were modulated with soil. The experiment was established as a completely randomized design with five replication. All pots were randomly located in the greenhouse in order to avoid positional effects.

Treatments

Saline water was prepared artificially by dissolving calculated amount of commercially available salt (NaCl) with tap water to make 10 mM NaCl solution. Tap water was used as control. The electrical conductivity (EC) of tap water and the respective solutions was equivalent to 0.1 and 1.0 dS/m, respectively. Saline application was started from sowing to harvest of V6 growth stage (six weeks) of cultivars.

Dry Matter

Plants were harvested after 6 weeks (V6 growth stage) from the sowing and separated into the leaves, stem and roots. The separated segments were wiped with tissue towel paper to remove moisture and their fresh weights were measured. The fresh samples were kept frozen in liquid nitrogen, and then freeze-dried and we measured the dry weight.

Relative Water Content (RWC)

The RWC was measured as described by (Saneoka et al. 1995), leaf fresh weight (FW), fully turgid weight after soaking leaves in water for 24h (TW), and dry weight (DW) were measured, and RWC was defined as follow:

$$\text{RWC (\%)} = [(FW - DW) / (TW - DW)] \times 100$$

Total Chlorophyll Content (SPAD)

Chlorophyll meter readings as a SPAD values (Konica-Minolta, Japan) were repeatedly taken at two trifoliated of fully expanded youngest leaves throughout the experiments end of 6 weeks.

Electrolyte Leakage Rate (ELR)

Membrane permeability can be reflected by electrolyte leakage rate (ELR) which was measured with the method described by (Lutts et al. 1995). Fresh leaves (1 g) were cut into pieces of 5-mm length and equally placed in to test vials containing 30ml deionized water. The vials were incubated at 25°C on a rotary shaker for 12 h, and then the initial electrical conductivity (EC₁) was measured using a DDS-11C conductivity meter (Hongyi Company, Shanghai, China). Then the vials were autoclaved at 120°C for 20 min to release all electrolytes and finally cooled to 25°C for the measurement of the electrical conductivity (EC₂). ELR can be defined as follows:

$$\text{ELR (\%)} = (EC_1/EC_2) \times 100$$

Determination of Total Nitrogen, Na⁺ and K⁺ Content

The freeze-dried stem and leaves were ground into a fine powder using a vibratory sample mill (Model T1-100, Heiko Seisakusho Co., Ltd., Japan). Around 20 mg dry weighed powder were digested by nitric acid-hydrogen peroxide. Na⁺ and K⁺ concentrations were determined using a flame photometer (ANA-135, Eiko Instruments Inc., Tokyo, Japan) according to (Kushizaki 1968). Plant nitrogen concentration was determined by the Kjeldahl method after digestion with sulfuric acid.

Measurement of Proline in Leaves

The proline was extracted by methanol and then measured following the ninhydrin method described by (Bates et al. 1973) using L-proline as a standard.

Statistical Analysis

Data were examined by one-way ANOVA analysis of variance. Multiple comparisons of means of data between different salinity treatments within the plants were performed using Duncan's test at the 0.05 significance level.

Results and Discussion

Plant Fresh Weight

Salinity has a direct influence on plant biomass production of a crop. In this study Salinity decrease dry and fresh weight of soybean but this decrease was lower in Giza-111 cultivars. The leaf and stem fresh weight of soybean cultivars declined with saline stress. However, the Giza-82 and Giza-35 produced significantly decrease leaf and stem fresh weight than Giza-111 (Figure 1).

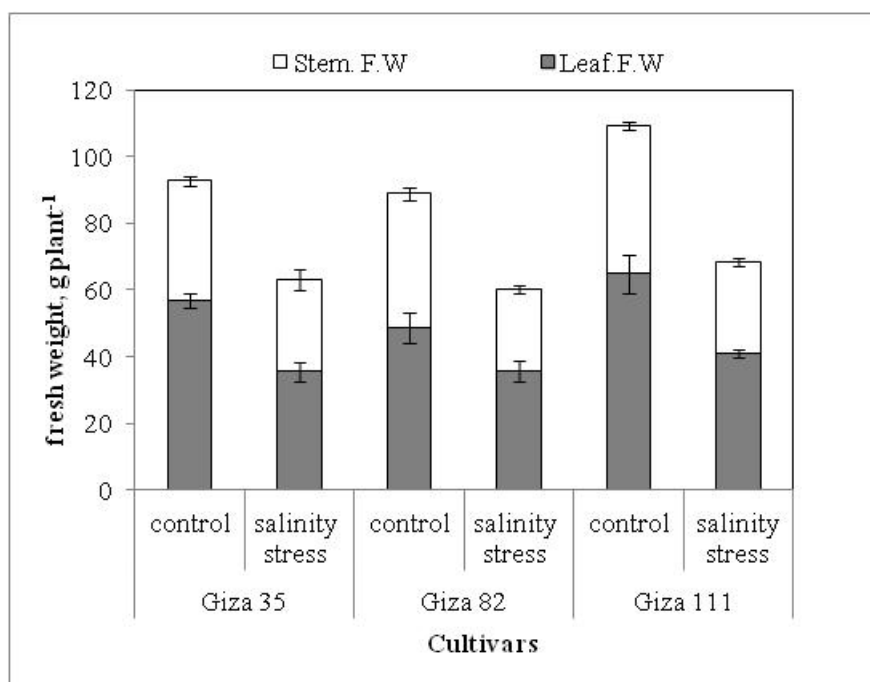


Figure 1. The effects of salinity on fresh weigh (Leaves, stem) in soybean cultivars.

Changes of plant dry weight were similar to that of plant fresh weight. (Siddique and Kumar 1985) stated that both shoot and root growths were significantly reduced with the increasing salinity levels in pea. Morphologically, the most typical symptom of saline injury to a plant is retarded growth due to inhibition of cell elongation (Bandeoglu et al. 2004). Researchers reported that accumulation of salts and ions in plant growth environment causes osmotic and stress leading to decrease of water absorption by plant tissues. Decrease of tissue water content results in reduction of cellular growth and development. Therefore, restriction of water absorption and its consequences for cellular growth and development is one of the most important causes of decreased growth of stem and root (Ashraf, M. and Mecneilly 1988; Cavalcanti 2007; Delauney and Verma 1993; Lee et al. 2001; Munns 2002).

Plant Dry Weight

Leaf and stem dry weight was significantly reduced by salinity stress. Giza-111 showed a smaller reduction than Giza-82 and Giza-35 (Figure 2).

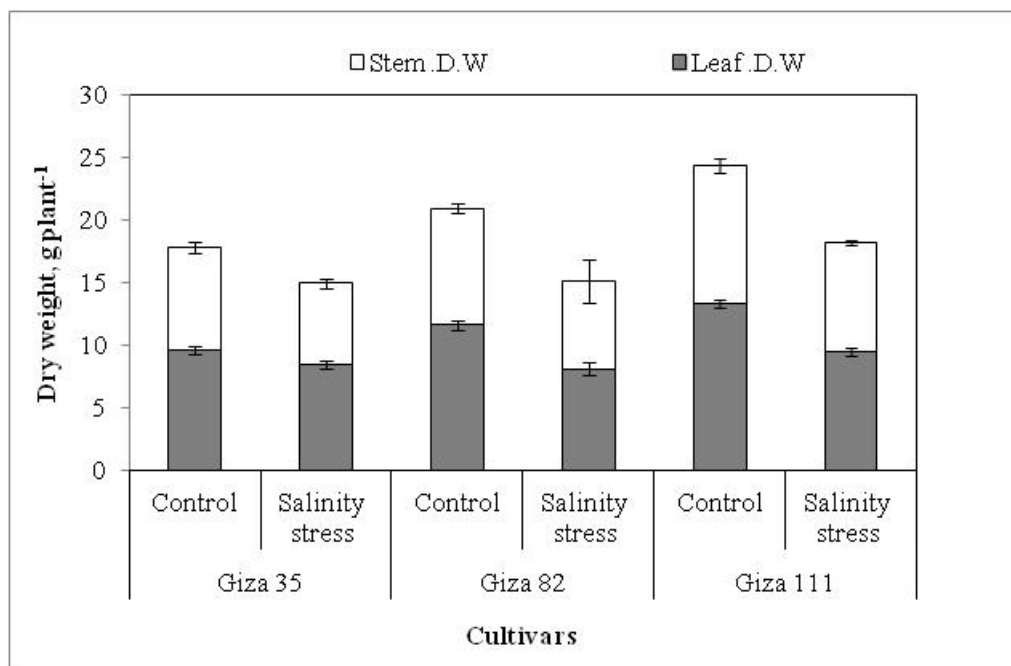


Figure 2. The effects of salinity on dry weigh (Leaves, stem) in soybean cultivars.

Salinity stress reduced the plant growth irrespective of the cultivar is evident from the decline in dry weights of shoots with salinity stress. As stated by (Munns and James 2003), suppression of plant growth under saline conditions may either be due to the decreasing availability of water or to the increasing toxicity of sodium chloride associated with increasing salinity. The reduction in dry weight under salt stress may be attributed to inhibition of hydrolysis of reserve synthesizing food and its translocation to the growing shoots (Singh et al. 2000). Hence, reduction in shoot dry mass with a decline in root mass was a normal growth reaction. Shoot growth is a complex phenomenon and several factors other than reduced root growth are involved. According to (Cheeseman 1988). According to the previous values, the cultivars were arranged as following: Giza111 > Giza82 > Giza35 (Figure 2). Our results are in agreement with the results of other researchers. For example, (Hussein et al. 2009) reported that a negative relationship was detected between vegetative growth parameters and salinity stress. The same results were also obtained by other researchers (Mansour et al. 2005). The decrease of shoot and root dry weight might be due to some reasons such as (i) salt stress reduced photosynthesis per unit leaf area which turned intolimited supply of carbohydrate needed for shoot growth, (ii) reduced turgor resulting in lower water potential and (iii) disturbance in mineral supply might have directly affected growth. In addition, salinity affected final cell size as well as rate of cell production and thereby resulting in reduced shoot and root dry weight. The results are in agreement with the findings of (Alam et al. 2004; Mahmood et al., 2009).

Nodule Dry Weight

Data presented in Figure 3 showed that salinity stress decreased Nodule dry weight of the tested cultivars. The percent reduction of Nodule dry weigh was greater in Giza 82 and Giza 35 than in Giza-111. The total nodule weight per plant was significantly decreased ($P < 0.05$) by salinity. This was reflected in the smaller size nodules formed at the salt stress. (Tu 1981) reported that the development of new nodules by soybean and nitrogen fixation by the existing nodules were greatly inhibited by salinity, with a resulting decline in plant nitrogen content. The failure of nodule formation at high salinity might be attributed to shrinkage of root hairs; consequently, the reduction in plant growth under conditions of high salinity could only be partly accounted for by the reduction or failure in nodulation. The processes of nodule initiation in soybean was reported to be extremely sensitive to NaCl. A reduction in nodule number and weight of 50% occurred with (3.1 dS m^{-1}) in the rooting medium (Singleton and Bohlool 1984). Average nodule weight of faba bean was found to increase with salinity (Yousef and Sprent 1983). (Zahran and Sprent 1986) found a reduction in average nodule weight using the same salinity levels. Such differences

were attributed to differences in the environmental conditions. this increase may partly compensate for reduced specific nitrogenase activity (Yousef and Sprent 1983).

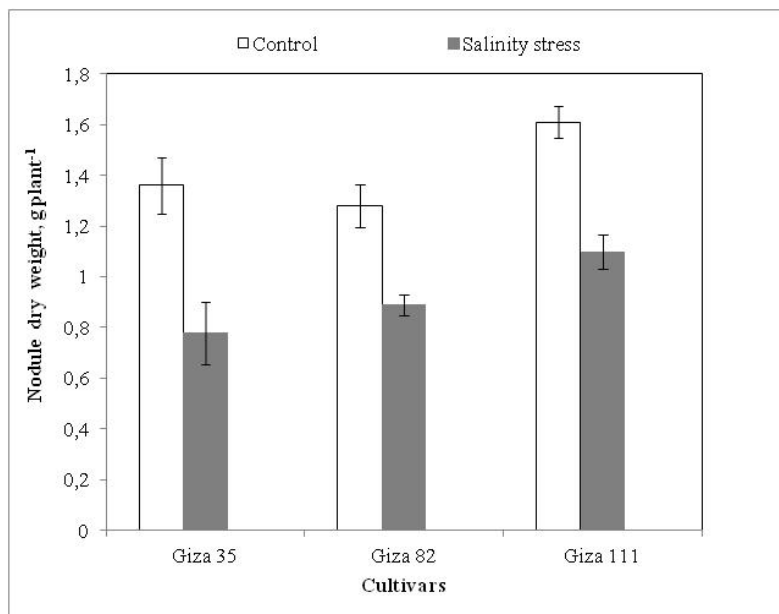


Figure 3. The effects of salinity on nodule dry weight in soybean cultivars.

Nodule Number

Data presented in (Figure 4) showed that Salinity stress significantly reduced the total nodule number per plant of the tested cultivars. The percent reduction of Nodule number was greater in Giza 82 and Giza 35 than in Giza-111. In general, the total nodule number increased with time in the control with compare salt treatments. These results are in agreement with the results of (Hafeez et al. 1988) who reported that the nodulation of *Vigna radiata* was reduced by about half at a salinity level of 5.0 dS m⁻¹ when compared to 1.4 dS m⁻¹. However, they also found that nodulation was completely depressed when salinity was raised to 10.0 dS m⁻¹, regardless of the plant growth stage.

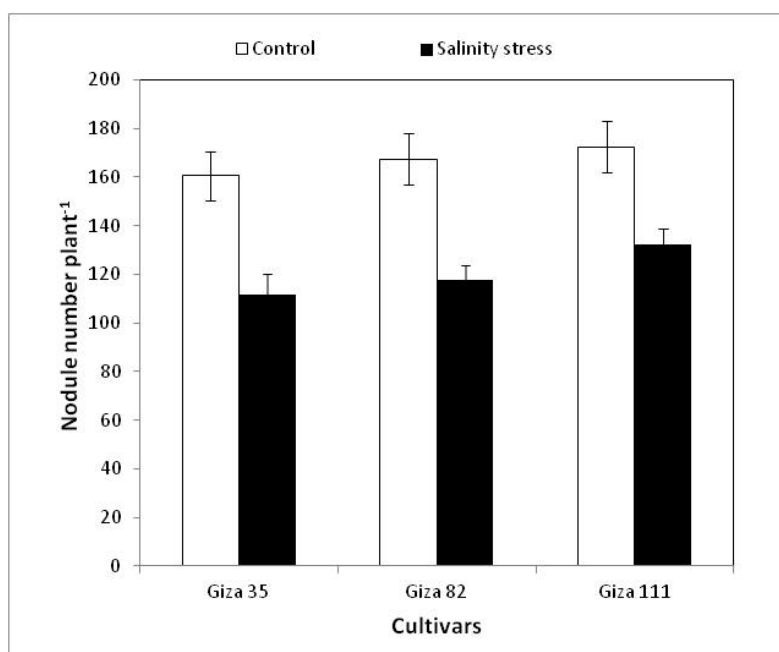


Figure 4. The effects of salinity on nodule number in soybean cultivars.

Chlorophyll Content

Data presented in (Figure 5) showed that salinity stress decreased chlorophyll content for the tested cultivars. The percent reduction of chlorophyll content was greater in Giza 82 and Giza35 than in Giza-111. Several investigators reported that chlorophyll and total carotenoid contents of leaves decrease, in general, under salt stress. The ability of plants to tolerate salt is determined by multiple biochemical pathways that facilitate retention and/or acquisition of water, protect chloroplast function and maintain ion homeostasis (Parida and Das 2005). The marked decrease of chlorophyll content was observed in all varieties,. Leaf chlorophyll content is considered to be a good indicator of photosynthetic capacity. Lower chlorophyll content would limit photosynthetic potential and lead to a decrease in biomass and production (Naumann et al. 2008). Decreasing chlorophyll content index (CCI) of soybean leaves with salinity stress (Figure 5) Could be related to increasing the activity of chlorophyll degrading enzyme: chlorophyllase (Jamil et al. 2007), and the destruction of the chloroplast structure and the instability of pigment protein complexes (Singh and Dubey 1995).

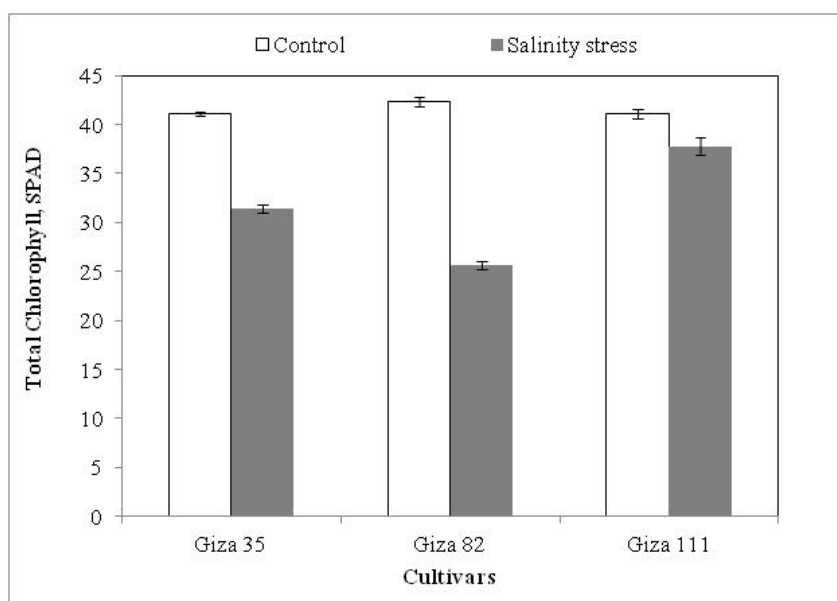


Figure 5. Effects of salt stresses on total chlorophyll (SPAD) of soybean cultivars.

Electrolyte Leakage Rate (ELR)

The ELR is a good strain index as it reflects the degree of plant injury by salt stress. The REL of the leaf tissue was significantly increased as salinity stress. The presence of saline stresses in the rooting medium induced a significant increase in electrolyte leakage rate increased in young leaves of all the cultivars (Figure 6). These results indicated that salinity- may have caused more serious injury on membranes, and furthermore, the injury was more serious in Giza-82 and Giza-35 except in Giza-111. The cultivars variations were more pronounced and comparison among the cultivars, ELR (%) was greater in Giza-82 and Giza-35 compare to Giza-111 under the stresses indicated that Giza-82 and Giza-35 were more affected by the treatments. While, Giza-111 was less affected by the stress treatments. Calcium is important in maintaining selective permeability of membranes, while sodium can increase membrane leakage rates (Hansen and Munns 1988).

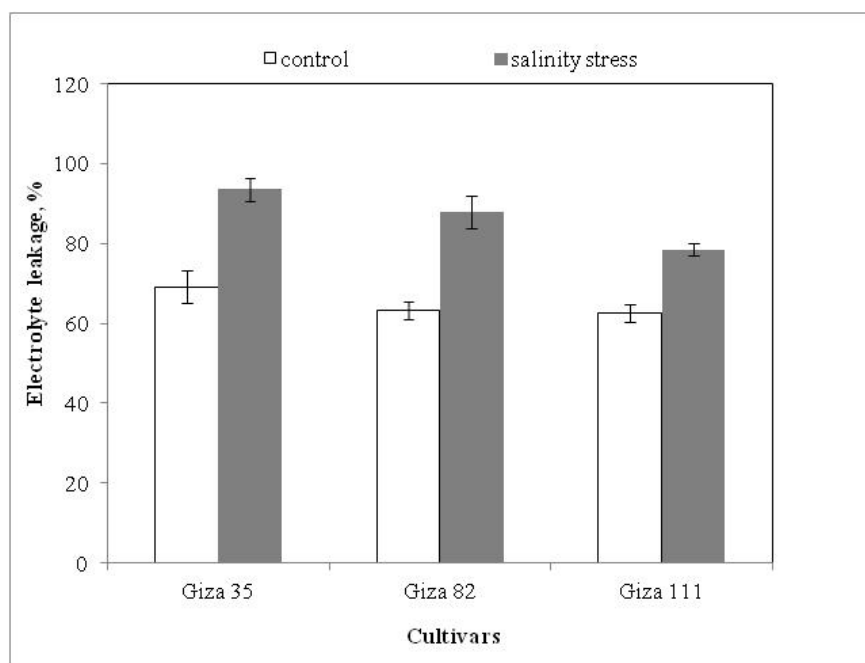


Figure 6. Effects of salt stresses on electrolyte leakage rate of soybean cultivars.

Membrane structure and properties, this enhanced free radical formation and lipid peroxidation under salt stress in salt sensitive cultivars may have also brought about an increase in membrane permeability or loss of membrane integrity, as evidenced by the increase in solute leakage (Figure 6). Salt stress-induced electrolyte leakage has also been previously observed in foxtail millet (Sreenivasulu et al. 2002).

Sodium affected the cell membrane permeability, deconstructed the cell membrane and destroyed the selectivity property (Letey 1993; Makki et al. 1987).

Relative Water Content

RWC significantly was decreased with salinity stress (Figure 7). Salinity stress led to a gradual decrease in the RWC in all cultivars. Stress treatments caused a high significant decrease in leaf relative water content; in addition, reduction rate was higher both Giza-82 and Giza-35 than Giza-111. The relative changes among the cultivars were more pronounced in Giza-82 and Giza-35 than in Giza-111 cultivars. The highest water content was detected in cultivar Giza-111 followed by Giza-82, while the lowest contents were detected in and Giza-35. The decrease in leaf RWC could be related to low water availability under stress conditions (Shalhevet 1993), or to root systems, which are not able to compensate for water lost by transpiration through a reduction of the absorbing surface (Gadallah 2000). Salt stress induced a reduction in the relative water content of the leaves, which indicates a loss of turgor that resulted in limited water availability for cell extension process (Katerji et al. 1997).

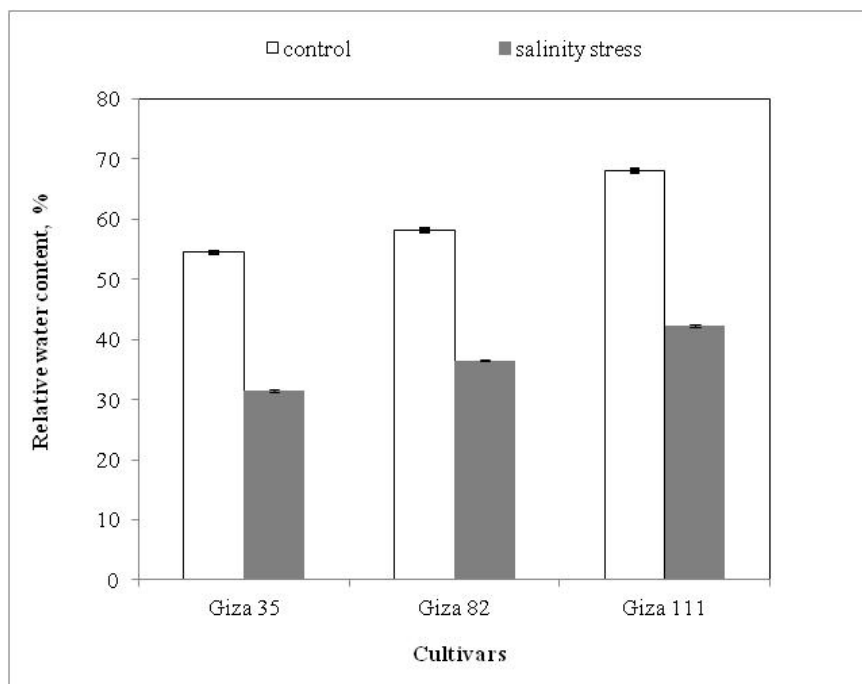


Figure 7. Effects of salt stresses on relative water content of soybean cultivars.

Inorganic Ion Concentrations

Accumulation of inorganic cations, in general, in the cell is one of the main physiological responses of plant to salt stress. The accumulated solutes function as osmolytes, pH adjustment agents and other roles. Most plants stressed by NaCl absorb Na^+ abundantly, whereas their K^+ contents decrease.

Nitrogen Contents

Leaves nitrogen content was significantly reduced with increasing salinity (Figure 8).

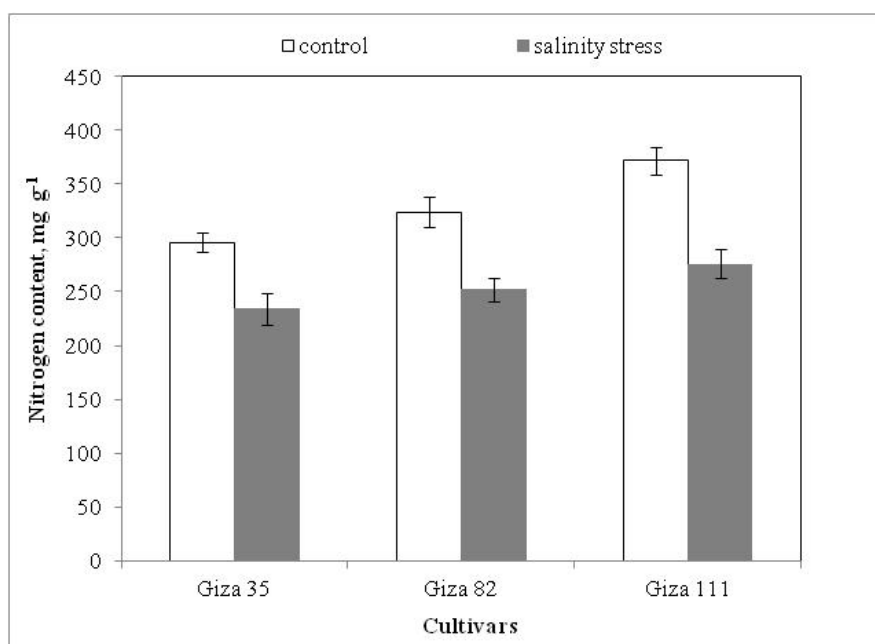


Figure 8. Effects of salt stresses on Nitrogen content ($\text{mg N g dry weight}^{-1}$) of soybean cultivars.

Giza-111 showed a smaller reduction than Giza-82 and Giza-35. This results obtained here in show that the salt stress (NaCl) caused a marked decrease in total nitrogen, total soluble nitrogen and protein-N in mungbean plants such reduction was accompanied by a marked increase in proline amino acid. These results are in agreement with the results obtained by (Singh et al. 1994). The decreases in total-N concomitantly with the decrease in soluble-N and protein-N in salinity stressed mungbean plants can be attributed to the effect of salinity on decreasing the biosynthesis of protein and/or the decrease in nitrogen fixation and/or inhibition in nitrate reductase activity. (Helal and Mengel 1981) have suggested that there is marked decomposition of protein under saline conditions. This may offer an alternative explanation of chlorophyll destruction under saline conditions. Nitrogen accumulation in shoot and root decreased due to different levels of salinity. Similar results were reported by (Wilson et al. 2000). (El-Arquan et al. 2002) reported that nutrients uptake (N, P, K) were decreased by increasing soil salinity levels. This indicates that excessive content of Na^+ and Cl^- ions in growth media has an inhibitory effect on the uptake and translocation processes of essential nutrients (Thalooth et al. 2006).

Sodium

The Na content in (shoots) of the three cultivars increased with salinity stress. It is clear from the results that Giza-111 was the lowest accumulated from Na with the comparison each other (Figure 9). The Na^+ content of leaves and stems increased with salinity. This is consistent with findings in many crop species (Hajibagheri et al. 1987; Schachtman and Munns 1992). Na^+ may help to maintain turgor but it is unable to substitute for specific functions of Ca^{2+} and K^+ , for example enzyme activation and protein synthesis to produce adequate growth. Increasing Na^+ contents and decreasing K^+ contents and K^+/Na^+ ratios in plant leaves can be attributed to the effect of competition between Na^+ and K^+ ions on the absorptive sites of the plant roots (Bhivare and Nimbalkar, 1984).

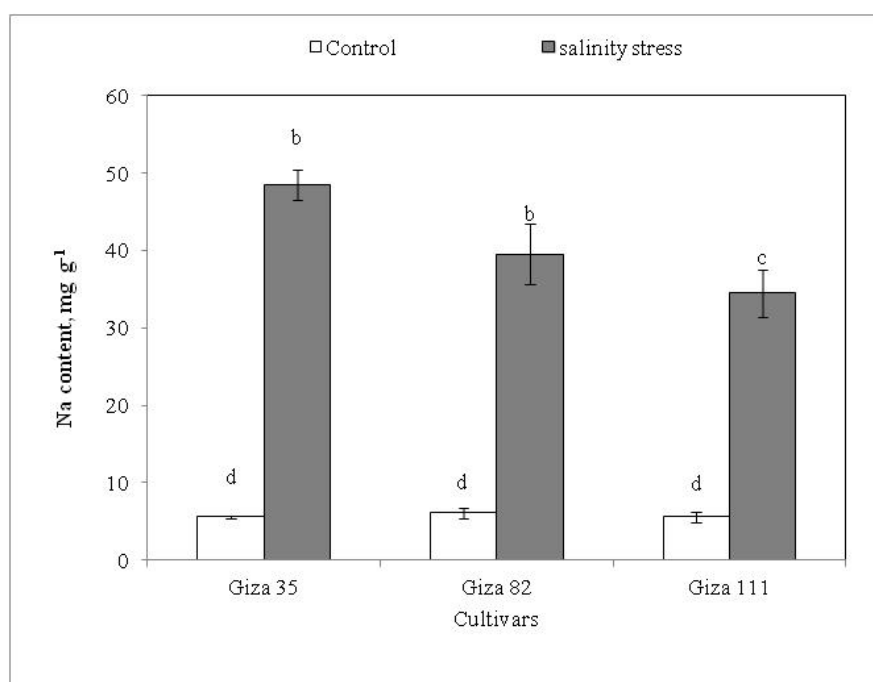


Figure 9. Effects of salt stresses on Na accumulation (mg g^{-1}) of soybean cultivars.

Na^+ and K^+ ratio in the leaves of soybean cultivars differing in salt tolerance agree with the view that there is an inverse relationship between shoot Na^+ and K^+ concentration and salt tolerance (Umezawa et al 2004; Yeo and Flowers 1983). The cultivar Giza-111 was found to be relatively tolerant to salt did not exhibit a significant increase in Na^+ accumulation in the leaves even under salinity stress, whereas both Giza-35 and Giza-82 showed pronounced accumulation. Our results showed that cultivar relatively tolerant to salt has higher K^+ concentration and $\text{K}^+:\text{Na}^+$ rate than salt sensitive cultivars.

On the other hand, this study showed composite correlation between increase of K^+ and decrease of Na^+ with growth of soybean. Ashraf and McNilley (2004) showed that salt stress increase of Na^+ and decrease

of K^+ in shoot of soybean and salt tolerance soybean have higher $K^+ : Na^+$ ratio than salt sensitive cultivars. Chen et al. (1996) studied soybean, wheat, maize and cotton and suggested that Na^+ concentration increased with the increase in salinity level in all of these plants. Root Na^+ content of cotton which is a salt stress tolerant plant was more than soybean root indicating preservation of Na^+ in cotton root and lack of Na^+ transportation to shoot.

Data of this study showed negative correlation between Na^+ concentrations in shoot of soybean and positive correlation between K^+ accumulation and these parameters. There is a relationship between potassium decreasing and sodium increasing in seedling tissue with sensitivity to salinity. Sodium affected the cell membrane permeability, deconstructed the cell membrane and destroyed the selectivity property (Letey 1993; Makki et al. 1987).

Potassium

The K^+ contents of the three cultivars were affected significantly by salinity stress (Figure 10). Generally, the concentration of K^+ in (leaves and stem) of Giza-111 was higher than that in Giza-82 and Giza-35. Salinity can interfere with K nutrition (Rengel 1992).

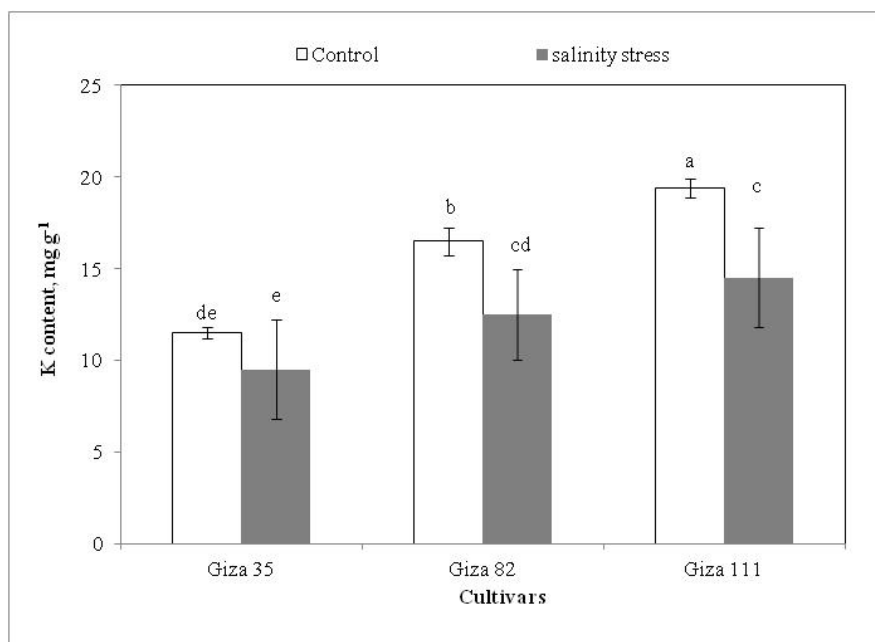


Figure 10. Effects of salt stresses on K accumulation ($mg\ g^{-1}$) of soybean cultivars.

Potassium is an activator of many enzymes that are essential for photosynthesis and respiration, and deficiencies in K would result in inhibition of photosynthesis and eventually reduction in growth (Salisbury and Ross 1992). The maintenance of a high K content may be crucial to sustain the integrity of the photosynthetic system under high salinity (Chow et al. 1990) and thus an important pre-requisite for salinity tolerance. Results from present investigation showed higher Na and lower K contents in (leaves, stem and root) of three cultivars. This result implies that the differential accumulation of leaf Na and K might be responsible for the different sensitivity of the three soybeans to NaCl treatment. The K content in plant tissues represents the main cation in plant cells, and is an important component of the cell osmotic potential.

In our study, under all treatments, leaf K^+ concentrations were lower at salinity stress. Accumulation of inorganic ions for osmotic adjustment is an energy-effective way for higher plants to combine productivity with salt tolerance (Yeo and Flower 1982). The reduction in K^+ concentration could inhibit growth by reducing the capacity for osmotic adjustment and turgor maintenance or by adversely affecting metabolic functions (Helal and Mengel 1979; Greenway and Munns 1980). The reduced contribution of K^+ ions could probably be offset by an enhanced accumulation of Na^+ ions. Rengel (1992) reported that

the main response of the plant to salt stress is a change in Ca^{2+} homeostasis. He attributed the salt tolerance of plants to their ability to avoid Na^+ toxicity and to maintain Ca^{2+} and K^+ concentration.

It is said that K^+ concentration observed in salt stress tolerant plants were more than that of susceptible cultivars led to decreased Na^+ toxicity. Increased Na^+ content led to decrease in seed germination level and seedling fresh weight in such plants (Chen et al. 1996; Chen et al. 1999; Maziah et al. 2009; Sadeghi 2009). Morant et al. (2004), working on Triticale cultivars suggested that $\text{K}^+:\text{Na}^+$ ratio decreased with the increase in salt stress level in growth environment in all of investigated cultivars, however, more increase value was observed among salt stress susceptible plants and reported decreased K^+ absorption in presence of NaCl as the cause of this observation.

Proline Content

Proline is considered to be the most important organic compatible osmolyte and it is also a protectant of biological macromolecules in the protoplasm. The results show that salt stress caused marked highly significant increases in proline contents with salt stress. , the concentration of proline in leaves of Giza-111 was higher than that in cultivars tested (Figure 11). Furthermore, Giza-111 accumulated more proline than Giza-82 and Giza-35. In general, proline accumulation correlates closely with the intensity of the osmotic stress (Shi 1997) caused by salt, alkali stress and others stresses. Proline content increased under saline conditions under salt stress in all cultivars.

In addition to its role as an osmolyte and a reservoir of carbon and nitrogen, proline has been shown to protect plants against free radical induced damage and slow utilization of proline for protein synthesis and stimulation of glutamate conversion to proline during stress may be the possible reason for proline accumulation. Proline is one of the most important osmoprotectant in plants. Under salt stress most plant species exhibit a remarkable increase in their proline content (Bandeoglu et al. 2004; Lee et al. 2001). In our experiments we also observed a similar behavior in soybean cultivars. Supporting findings come from other plants (Tramontano and Jouve 1997; Ashraf and McNilley 2004). Where salt stress resulted in extensive proline accumulation. In support of our observations, recently in rice roots exposed to NaCl stress, a uniform accumulation of proline was shown to be related with increasing

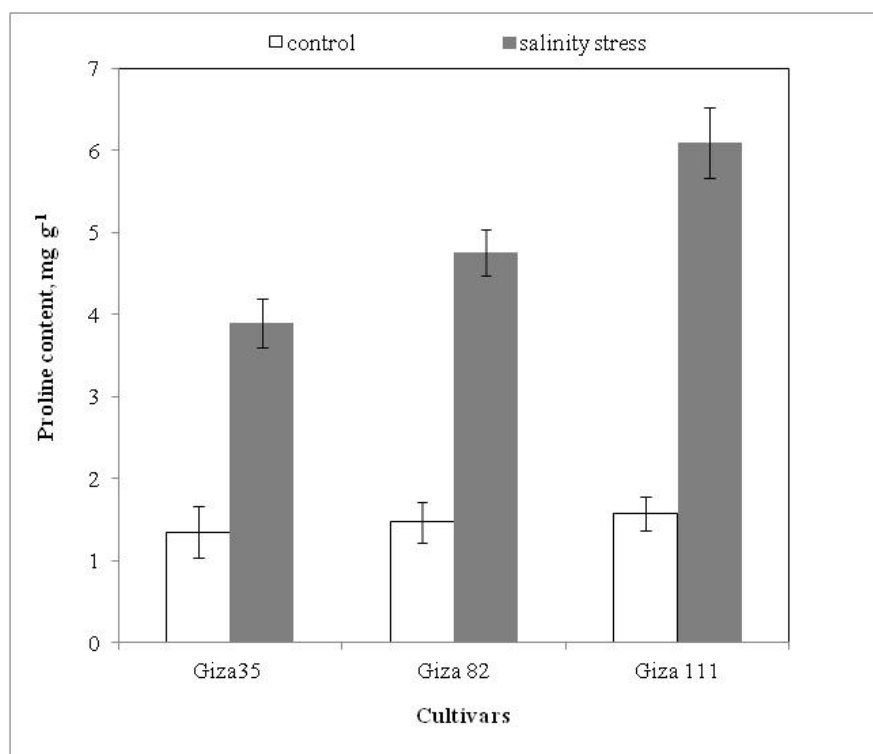


Figure 11. Effects of salt stresses on Proline content (mg g^{-1}) of soybean cultivars

NaCl concentrations (Khan et al. 2002; Morant et al. 2004). Increasing leaf proline content under salinity stress (Figure 11) might be caused by the induction or activation of proline syntheses from glutamate or decrease in its utilization in protein syntheses or enhancement in protein turnover. Thus, proline may be the major source of energy and nitrogen during immediate post stress metabolism and accumulated proline apparently supplies energy for growth and survival, thereby inducing salinity tolerance (Gad 2005).

Conclusion

This study sought to the adaptation of some Egyptian cultivars under salinity condition. The end result of this was drastic reduction in growth of the plants under salinity conditions. Giza-111 in contrast, accumulated much lower Na^+ and lowest of electrolyte leakage rate. On the other hand, observing-high relative water content, high accumulated from K^+ and proline with enhanced of all the above parameters. This results suggest that Giza-111 showed a better performance under salinity stress and more appropriate under salinity condition and recommended to use in breeding program for enhancing soybean production in newly reclaimed soils of Egypt.

Acknowledgements

Sincere thanks to all staff members in Agronomy department, faculty of agriculture, Kafrelshikh University, Egypt and Plant Nutritional Physiology Laboratory, Hiroshima University, Japan.

References

- Abd-Alla MH, Vuong TD, Harper JE (1998). Genotypic differences in dinitrogen fixation response to NaCl stress in intact and grafted soybean. *Crop Sci.* 38, 72–77 .
- Alam MZ, Stuchbury T, Naylor R E L, Rashid M A (2004). Effect of salinity on growth of some modern rice cultivars. *J. Agron.* 3: 1–10.
- Ashraf M, McNilley T (2004). Salinity tolerance in Barcia oil seeds. *Plant Science*, 23(2): 157-172.
- Ashraf M, Mcneilly T (1988). Variability in salt tolerance of nine spring wheat cultivars. *Journal of Agronomy and Crop Science*, 160:14-21.
- Bandeoglu E, Eyidogan F, Yucel M, Oktem A (2004). Antioxidant responses of shoots and roots of lentil to NaCl-salinity stress. *Plant Growth Regulation*, 42: 69-77.
- Bates LS, Waldren RP, Teare ID (1973). Rapid determination of free proline for water stress studies. *Plant and Soil*, 39: 205-207.
- Bhivare VN, Nimbalkar JD (1984). Salt stress effect on growth and nutrition of French beans. *Plant Soil.* 80: 91-98.
- Boyer J S (1982). Plant productivity and environment. *Science* 218: 443–448.
- Bray EA, Bailey-Serres J, Weretilnyk E (2000). Responses to abiotic stresses. In W. Gruissem, B. Buchanan, R. Jones, eds, *Biochemistry and Molecular Biology of Plants*. American Society of Plant Physiologists, Rockville, MD, pp 1158–1249.
- Cavalcanti F, Lima JP, Silva S, Viegas R, Silveria J (2007). Roots and leaves display contrasting oxidative response during salt stress and recovery in cowpea. *Journal of Plant Physiology*, 164: 591-600.
- Cheeseman JM (1988). Mechanisms of salinity tolerance in plants. *Plant Physiol.*, 87: 547-550.
- Chen D, Yu-Renpei D M, Yu DM (1996). Studies of relative salt tolerance of crops. Salt tolerance of some main crop species, *Acta pedologica science*, 33: 121-128.
- Chen K, Hu G, Keutgen N, Janssens MJJ, Lenz F (1999). Effects of NaCl salinity and CO_2 enrichment on pepino (*Solanum muricatum*). II. Leaf photosynthetic properties and gas-exchange. *Scientia Horticulturae* 81: 43-56.
- Chow WS, Ball MC, Anderson JM (1990). Growth and photosynthetic responses of spinach to salinity: implication of K^+ nutrition for salt tolerance. *Aust. J. Plant Physiol.* 17: 563-578.

- Delauney AJ, Verma DPS (1993). Proline biosynthesis and osmoregulation in plants. *Plant Journal*, 4: 215-223.
- Delgado MJ, Ligerio F, Liuch C (1994). Effects of salt stress on growth and nitrogen fixation by pea, faba-bean, common bean and soybean plant. *Soil Biol. Biochem.* 26, 371–376.
- Duzan HM, Zhou X, Souleimanov A, Smith DL (2004). Perception of *Bradyrhizobium japonicum* Nod factor by soybean [*Glycine max* (L.) Merrill] root hairs under abiotic stress conditions. *J. Exp. Bot.* 55: 2641–2646.
- El-Arquan MY, El-Hamdi KH, Seleem EM, El-Tantawy IM (2002). Nutrient uptake of sugar beet as affected by NPK fertilization and soil salinity levels. *Egypt J. Soil Sci.* 42(4):783-797.
- Elsheikh EA, Wood M (1995). Nodulation and N₂ fixation by soybean inoculated with salt-tolerant rhizobia or salt-sensitive bradyrhizobia in saline soil. *Soil Biol. Biochem.* 27: 657–661.
- Gad N (2005). Interactive effect of salinity and cobalt on tomato plants. II. Somephysiological parameters as affected by cobalt and salinity. *Res. J. Agric. Bio. Sci.* 1: 270-276.
- Gadallah MAA (2000) Effects of indole-3-acetic acid and zinc on the growth, osmotic potential and soluble carbon and nitrogen components of soybean plants growing under water deficit. *J Arid Environ.* 44: 451–467.
- Greenway H, Munns R (1980). Mechanism of salt tolerance in halophytes. *Annu. Rev. Plant Physiol.* 31: 149-190.
- Hafeez FY, Aslam J, Malik KA (1988). Effect of salinity and inoculation on growth, nitrogen fixation and nutrient uptake of *Vigna radiata* (L.) Wilczek. *Plant and Soil.* 106: 3-8.
- Hajibagheri MA, Harvey, DMR, Flowers TJ (1987). Quantitative ion distribution within root cells of salt-sensitive and salt-tolerant maize varieties. *New Phyt.* 105: 367–379.
- Hajlaoui H, Denden M, Bouslama M (2006). Effet du chlorure de sodium sur les critères morpho-physiologiques et productifs du pois chiche (*Cicer arietinum* L.). *Institut National de Recherches en Génie Rural, Eaux et Forêts* 8:171–187.
- Hansen EH, Munns DN (1988). Effects of CaSO₄ and NaCl on growth and nitrogen fixation of *Leucaena leucocephala*. *Plant and Soil* 107: 94–99.
- Helal HM, Mengel K (1979) . Nitrogen metabolism of young barley plants as affected by NaCl salinity and potassium. *Plant Soil.* 51, 457-462.
- Helal HM, Mengel K (1981). Interaction between light intensity and NaCl salinity and their effects on growth, CO₂ assimilation and photosynthetic conversion in young broad beans. *J. Plant Physiol.* 67: 265-275.
- Hussain M, Malik MA, Farooq M, Khan MB, Akram M, Saleem MF (2009). Exogenous glycinebetaine and salicylic acid application improves water relations, allometry and quality of hybrid sunflower under water deficit conditions. *J. Agron. Crop Sci.* 195: 98-109.
- Jamil M, Rehman S, Lee KJ, Kim JM, Kim HS, Rha ES (2007). Salinityreduced growth PS II photochemistry and chlorophyll content in radish. *Sci. Agric.* 64: 1-10.
- Jiang H, Wang XH, Deng QY, Yuan LP, Xu DQ (2002). Comparison of some photosynthetic characters between two hybrid rice combinations differing in yield potential. *Photosynthetica* 40: 133-137.
- Katerji N, Hoorn JW, Hamdy A, Mastrorilli M (2003). Salinity effect on crop development and yield, analysis of salt tolerance according to several classification methods. *Agric Water Manage* 62: 37-66.
- Katerji N, Vanhoorn JW, Hamdy A, Mastrorilli M, Mou Karzel E (1997). Osmotic adjustment of sugar beets inresponse to soil salinity and its influence on stomatal conductance, growth and yield. *Agric Water Manage* 34: 57–69.
- Khan MH, Singha K LB, Panda SK (2002).Changes in antioxidant levels in *Oryza sativa* L.roots subjected to NaCl salinity stress. *Acta Physiol. Plant* 24: 145-148.
- Kushizaki M (1968). An extraction procedure of plant materials for the rapiddetermination of Mn, Cu, Zn, and Mg by the atomic absorption analysis. *J. Sci. Soil Manure Japan* 39: 489-490.
- Lauchli A (1984). Salt exclusion: an adaptation of legume crops and pastures under saline conditions. In: Staples RC, Toeniessen GH. (eds) *Salinity tolerance in plants: strategies for crop improvement*. New York: John Wiley and Sons. PP. 171-187.
- Lee DH, Kim YS, Lee CB (2001). The inductive responses of antioxidant enzymes by salt stress in rice (*Oryza sativa* L.). *Journal of Plant Physiology* 158: 737-745.
- Lee JD, Shannon JG, Vuong TD, Nguyen HT (2009). Inheritance of salt tolerance in wild soybean (*Glycine soja* Sieb. and Zucc.) accession PI483463. *J Heredity* 100: 798–801.
- Letey J (1993). Relationship salinity and efficient water use. *Irrig. Sci.* 14:75-84.

- Lutts S, Guerrier G (1995). Peroxidation activities of two rice cultivars differing in salinity tolerance as affected by proline and NaCl. *Biol. Plant* 37: 577-586.
- Mahmood A, Latif T, Khan MA (2009). Effect of salinity on growth, yield and yield components in basmati rice germplasm. *Pakistan J. Bot.* 41: 3035-3045.
- Makki YM, Tahir OA, Asif MI (1987). Effect of drainage water on seed germination and early seedling growth of five field crop species. *Biological waste* 21: 133-137
- Mansour MMF, Salama KHA, Ali FZM, Hadid AFA (2005). Cell and plant responses to NaCl in Zeamays L. cultivars differing in salt tolerance. *Gen. Appl. Plant Physiol.* 31(1-2): 29-41.
- Maziah M, Abdul Rahman Z, Mohd H, Shamsuddin SZ, Subramaniam S (2009). Responses of banana plantlets to rhizobacteria inoculation under salt stress condition, *Am.-Eurasian J. Sustain. Agric.* 3(3): 290-30.
- McCue KF, Hanson AD (1990). Salt inducible betaine aldehyde dehydrogenase from sugarbeet: cDNA cloning and expression. *Trends Biotechnol.* 8: 358-362.
- Morant MA, Pradier E, Tremblin G (2004). Osmotic adjustment, gas exchange and chlorophyll fluorescence of a hexaploid triticale and its parental species under salt stress. *Plantphysiology* 161(1): 25-33
- Munns R (2002). Comparative physiology of salt and water stress. *Plant cell and environment* 25: 239-250.
- Munns R (2005). Genes and salt tolerance: bringing them together. *New Phytol* 167(3): 645-663.
- Munns R, James RA (2003). Screening methods for salinity tolerance: a case study with tetraploid wheat. *Plant and Soil*, 253: 201-218.
- Munns R, Tester M (2008). Mechanisms of salinity tolerance. *Annu Rev Plant Biol* 59: 651-681.
- Naumann JC, Young DR, Anderson JE (2008). Leaf chlorophyll fluorescence, reflectance, and physiological response to freshwater and saltwater flooding in the evergreen shrub, *Myrica cerifera*. *Environ Exp. Bot.* 63: 402-409.
- Neumann P (1997). Salinity resistance and plant growth revisited. *Plant Cell Environ.* 20: 1193-1198.
- Parida AK, Das AB (2005). Salt tolerance and salinity effects on plants: a review. *Ecotoxicology and Environmental Safety* 60: 324-349.
- Rahman M, Soomro UA, Zahoor-ul-Haq M, Gul S (2008). Effects of NaCl salinity on wheat (*Triticum aestivum* L.) cultivars. *World Journal of Agricultural Sciences* 4 (3): 398-403.
- Rengel Z (1992). The role of calcium in salt toxicity. *Plant Cell and Environment* 15: 625-632.
- Robinson RG (1975). Amino acids and elemental composition of sunflower and pumpkin seeds. *Agron. J.* 61: 541.
- Sadeghi H (2009). Effects of different levels of sodium chloride on yield and chemical composition in two barley cultivars, *Am.-Eurasian J. Sustain. Agric.* 3(3): 314-320.
- Salisbury FB, Ross CW (1992). Mineral nutrient. In: *Plant Physiology*. Wadsworth Inc., California, pp. 116-135.
- Saneoka H, Nagasaka C, Hahn DT, Yang W, Premachandra GS, Joly RJ, Rhodes D (1995). Salt tolerance of glycinebetaine-deficient and -containing maize lines. *Plant Physiol.* 107: 631-638.
- Schachtman DD, Munns R (1992). Sodium accumulation in leaves of *Triticum* species that differ in salt tolerance. *Aust. J. Plant Physiol.* 19: 331-40.
- Shalhevet J (1993). Plants under salt and water stress. In: Fowden L, Mansfield T, Stoddart J (ed) *Plant Adaptation to Environmental Stress*, Chapman and Hall, London, Glasgow, New York, Tokyo, Melbourne, Madras, pp. 133-154
- Shi G, Jing J, Shi G, Jing J (1997). Mitigative effect of external Ca^{2+} on the inhibition of hypocotyl elongation in mungbean seedling under salt stress. *Plant Physiol. Communication.* 33: 24-27.
- Siddique S, Kumar S (1985). Effect of salinisation and desalinisation of growth and development of pea (*Pisum sativum* L.). *Indian J. Plant Physiol.* 28: 151-156.
- Singh AK, Dubey RS (1995). Changes in chlorophyll a and b contents and activities of photosystems 1 and 2 in rice seedling induced by NaCl. *Photosynthetica* 31: 489-499.
- Singh AK, Prakash V, Sastry EVD (2000). Variation in seedling growth parameters in wheat under normal and saline environments. *Indian J. Agri. Res.* 34: 185-187.
- Singh SP, Singh BB, Singh MR, Singh M (1994). Effect of kinetin on chlorophyll, nitrogen and proline in mugbean (*Vigna radiata*) under saline conditions. *Indian J. Plant Physiol.* 37: 37-39.
- Singleton PW, Bohlool B (1984). Effect of salinity on nodule formation by soybean. *Plant Physiology* 74: 72-76.

- Sreenivasulu N, Grimm B, Wobus U (2002). Differential responses of antioxidant compounds to salinity stress in salt tolerant and salt sensitive seedling of foxtail millet (*setaria italica*). *Physiological Plantarum* 109: 435-442.
- Thalooth A T, Tawfik M M, Mohamed Mogda H (2006). A comparative study on the effect of foliar application of zinc, potassium and magnesium on growth under water stress conditions. *World J. Agric. Sci.* 2: 37-46.
- Tramontano WA, Jouve D (1997). Trigonelline accumulation in salt stressed legumes and the role of other osmoregulators as cell cycle control agents. *Photochemistry* 44: 1037-1040.
- Tu JC (1981). Effect of salinity on Rhizobium-root hair interaction, nodulation and growth of soybean. *Can. J. Plant Sci.* 61: 231 - 239.
- Umezawa T, Shimizu K, Kato M (2004). Enhancement of salt tolerance in soybean with NaCl pretreatment. *Physiology Plantarum* 110:59-63.
- Wilson C, Lesch SM, Grieve CM (2000). Growth stage modulates salinity tolerance of New Zealand Spinach (*Tetragonia tetragonoides*, Pall) and Red Orach (*Atriplex hortensis* L.). *Ann. Bot.* 85: 501-509.
- Yeo AR, Flowers TJ (1982). Accumulation and localization of sodium ions within the shoot of rice (*Oryza sativa* L.) varieties differing in salinity resistance. *Physiologia Plantarum* 56: 343-348.
- Yeo AR, Flowers TJ (1983). Varietal differences in the toxicity of sodium ions in rice leaves. *Physiologia Plantarum* 59: 189-195.
- Yousef AN, Sprent JI (1983). Effect of NaCl on growth, nitrogen incorporation and chemical composition of inoculated and NH_4NO_3 fertilized *Vicia faba* L. plants. *J. Exp. Bot.* 143: 941- 950.
- Zahran HH, Sprent JI (1986). Effects of sodium chloride and polyethylene glycol on root hair infection and nodulation of *Vicia faba* L. plants by *Rhizobium leguminosarum*. *Planta* 167: 303-309.
- Zhang C, Chu H, Chen G, Shi D, Zuo M, Wang J, Lu C, Wang P, Chen L (2007). Photosynthetic and biochemical activities in flag leaves of a newly developed superhigh yield hybrid rice (*Oryza sativa*) and its parents during the reproductive stage. *J. Plant Res.* 120: 209-217.