



## Impact of sea salt stress on growth and some physiological attributes of some soybean (*Glycine Max* L.) varieties

Khalil M. Saad-Allah\*

Botany Department, Faculty of Science, Tanta University, Tanta, Egypt

### Abstract

The impact of three levels of sea salt (0.0, 8.0, and 16.0 mS/cm<sup>2</sup>) on six varieties of soybean (Crawford, G21, G22, G35, G82, and G83) was studied. Growth criteria, photosynthetic pigments, soluble sugars, soluble protein, free amino acids, free proline, and protein profile of soybean varieties were investigated under sea salt stress. Results of this study showed a considerable decrease in growth criteria (shoot height, root depth, and leaflet area), photosynthetic pigments (Chl a, Chl b and carotenoids), soluble sugars, and soluble proteins with the increase in salinity level. The total free amino acids and free proline contents were increased with the increase in sea salt level. SDS-PAGE of the seedling proteins showed a significant variation in the protein profile of soybean and the variation is a function of varieties and sea salt level.

*Keywords:* *Glycine max*; salinity; growth; pigments; metabolites; protein pattern

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### Introduction

Soybean (*Glycine max* L. Merrill) is an annual crop of *Fabaceae* and is considered as the most important legume crop in the world (Ferguson and Gresshoff, 2009). Also, soybean is the most broadly used oil crop grain in the world, offering high-quality protein, oil, phospholipids, hormones, and antioxidants (Choi and Rhee, 2006). Soybean also provides phenolic compounds, tocopherols, organic acids, sugars and fatty acids (Kumar et al., 2009), volatile compounds and sterols (Boue et al., 2003). In addition to its nutritional value, soybean cultivation results in increased input of combined nitrogen as well as carbon into the soil.

It is well recognized that the amount and quality of irrigation water available in the arid and semi-arid regions of the world are the main limiting factors to the extension of agriculture (Munns, 2002). Saline irrigation water together with the low annual rainfall and high evaporation and transpiration rates in the arid and semi-arid regions caused the accumulation of soluble ions in the soil solution (especially sodium ions) on exchange sites, which can modify the structure and, accordingly, affect the soil hydraulic conductivity (Sameni and Morshedi, 2000). Soil salinity imposes ionic toxicity and osmotic stress which consequently results in nutrient deprivation and oxidative stress (Zhu, 2003). Soil salinity exerts noxious effects on plants and causes a significant drop in yield and crop production in 7% of arable soil worldwide, including the majority of irrigated soils (Halperin et al., 2003). In particular, exposure

\*Corresponding author

E-mail address: khalil.saadallah@science.tanta.edu.eg

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to salinity challenges the plant water status and triggers specific strategies for cell osmotic adjustment and control of water uptake and loss (Fricke and Peters, 2002). The most significant consequence of salinity on plants was observed in photosynthesis and photosynthetic apparatus through inducing stomata closure, leading to a reduction of intercellular CO<sub>2</sub> concentration (Kaymakanova and Stoeva, 2008). Also, salt stress has negative effects on photosynthetic enzymes, chlorophylls, and carotenoids (Stepien and Klobus, 2006) and hence reduces the ability of plants to make use of water and causes a decrease in plant growth rate, as well as alternations in metabolic processes of the plant (Munns, 2002) and suppresses leaf growth which eventually reduces photosynthetic area and dry matter production (Mansour and Salama, 2004).

Soybean is frequently considered as a moderately salt sensitive plant and therefore can be severely affected by highly saline soils (Katerji et al., 2000). Some studies showed the adverse effect of high salt levels on soybean through inhibiting seed germination and seedling growth, decrease in crop yield (Essa, 2002), reduction in the number and weight of root nodules (Lauter et al., 1981), leaf area, root/shoot ratios (Rahman et al., 2008), shoot height and fresh and dry weights (Cicek and Cakırlar, 2008). Also, soybean plants grown in saline conditions exhibit symptoms of leaf chlorosis, stunting and biomass reduction as a result of chloride induced toxicity (Hamayun et al., 2010). The aim of the present study was to evaluate the impact of three sea salt stress levels (0.0, 8.0, and 16.0 mS/cm<sup>2</sup>) on six soybean varieties commonly grown in Egypt through evaluating the impact of the used sea salt levels on the morphological and some physiological aspects associated with the plants under salinity stress at seedling stage.

## Materials and Methods

Seeds of six varieties of soybean (*Glycine max* L. Merrill) were obtained from the Agricultural Research Center (ARC), El-Doki, Giza, Egypt. The obtained varieties are named Crawford, Giza 21, Giza 22, Giza 35, Giza 82, and Giza 83. The seeds were surface sterilized with 0.5% sodium hypochlorite for 5 minutes and

washed 3 times with tap water and once with deionized water. The seeds were sown in plastic pots of 45 cm diameter and 25 cm depth containing 8 kg clay sandy soil (2:1 ratio) and irrigated with tap water. At full emergence, after five days, the pots received sea salt solutions (EC = 0.0, 8.0, and 16.0 mS/cm<sup>2</sup>) prepared by dissolving the sea salt in tap water. The pots were arranged in spilt plot design in a randomized complete blocks arrangement with three replications. The plants were left to grow under the natural day/night conditions (14 h light/ 10 h dark) at 28±2 °C in the greenhouse of Botany Department, Faculty of Science, Tanta University during the summer season of 2014. The pots were irrigated whenever needed with the salt solutions to the end of the growth period (30 days). After that, plants were harvested for the determination of shoot height, root depth as well as leaflet area measurements. Some samples were kept fresh for chemical and molecular analysis, the remaining samples were dried for further phytochemical analysis.

Photosynthetic pigments (Chl a, Chl b, and carotenoids) were estimated in the fresh leaf samples (0.1 g) by extraction in 85% cold acetone according to the method of Metzner et al. (1965) and the pigment concentrations were calculated as µg/g f.wt.

Total soluble carbohydrates were estimated by phenol-sulphuric acid method (Dubois et al., 1956) and expressed as mg/g d. wt. using previously prepared calibration curve using glucose.

The total soluble proteins content was estimated quantitatively according to the method described by Bradford (1976) and the protein content was calculated as mg/g d.wt using a prepared calibration curve by Bovine Serum Albumin (BSA) protein.

Free amino acids content of plant leaves were analyzed by Ninhydrin assay using Glycine as a standard according to Lee and Takahashi (1966) and the amino acids content was calculated as mg/g d.wt. using a prepared calibration curve by Glycine.

Proline content was measured quantitatively according to Bates (1973) in the dried leaf tissues and the proline content was

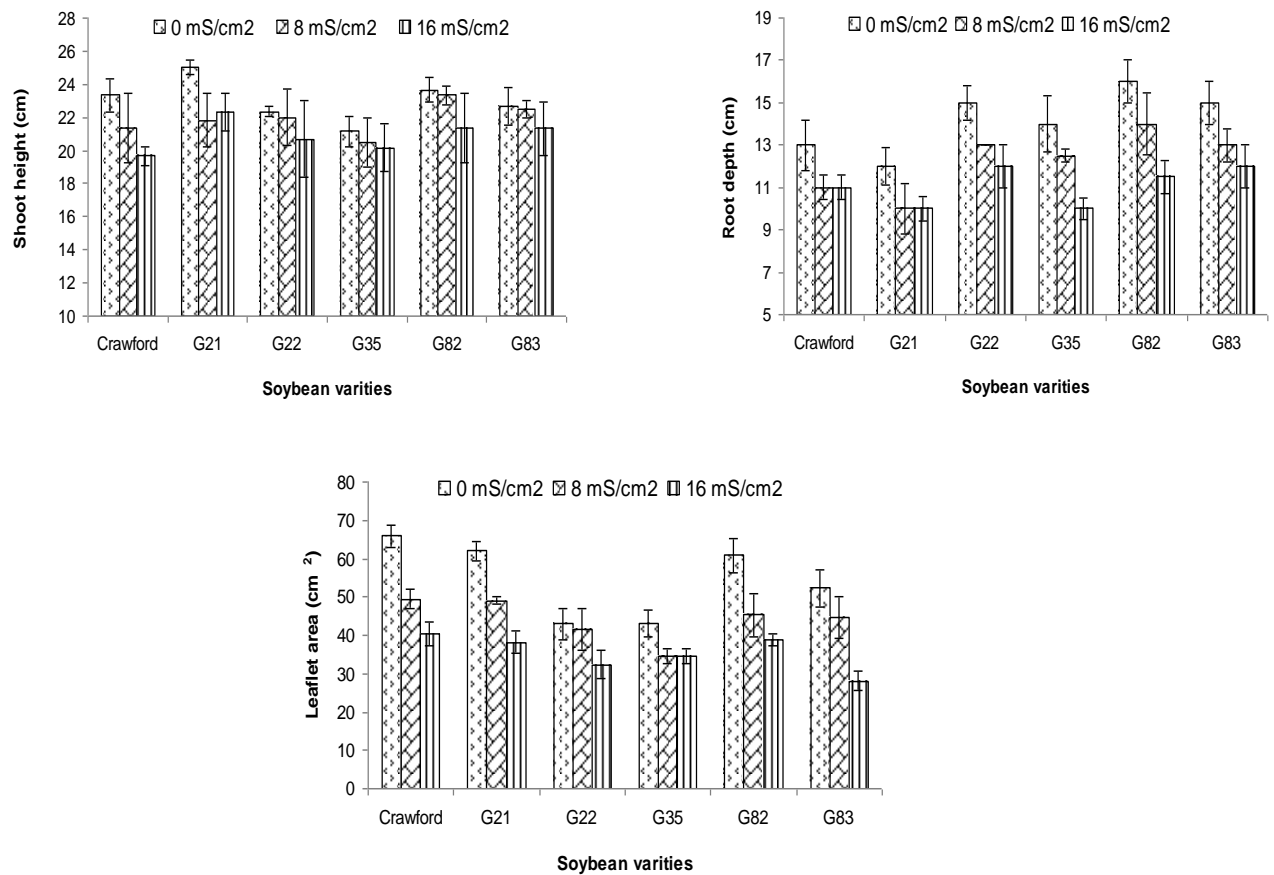


Fig. 1. Growth criteria (shoot height, root depth and leaflet area) of six soybean varieties grown under 0.0, 8.0 and 16.0 mS/cm<sup>2</sup> sea salt salinity for 30 days.

calculated as mg/g d.wt. using a prepared calibration curve by proline.

The protein pattern of the seedlings was studied using Sodium-Dodecyl-Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) as described by Laemmli (1970). The gel was then photographed with digital camera and the molecular weights of protein bands were calculated using Lab Image software version 2.7 produced by Kaplan GmbH, Germany.

## Results

The effect of three sea salt salinity levels (0.0, 8.0 and 16.0 mS/cm<sup>2</sup>) on the growth criteria

(shoot height, root depth and leaflet area) of six varieties of soybean at the early growth stage is represented in Fig. (I). It is evident from the data that all the measured growth criteria demonstrate gradual decline with the increase of salinity level from 0.0 to 16.0 mS/cm<sup>2</sup> in all the studied soybean varieties. However, the varietal differences are apparent, where the data revealed that the maximum reduction in the shoot height was prominent in Crawford variety, in root depth this was in G21 variety and in leaflet area it was in G83 variety. Interestingly, the data revealed that the minimum reduction in shoot height was recorded in G21 and G82 varieties, in root depth was in G82 and G83, and in leaflet area was in G82, G21 and

Crawford varieties. So, it can be concluded from the above data that the inhibitory effect of salinity stress on the growth criteria increase with the increase in the salinity level and the most salt tolerant varieties were G21 and G82 relative to the growth criteria.

The photosynthetic pigments content of soybean leaves was significantly influenced by the level of sea salt it received and the degree of susceptibility depends mainly on the variety (Fig. 2). A significant reduction in the total photosynthetic pigments (Chl a, Chl b and carotenoids) was observed in all soybean varieties under salinity. The reduction in photosynthetic pigments was a function of salinity treatment and varieties. The highest pigment content was recorded in G22 variety followed by G35 variety under unsalinized treatment (0.0 mS/cm<sup>2</sup>). However, G83 variety possessed the lowest photosynthetic pigment content under the unsalinized conditions. Pigment contents of soybean leaves decreased considerably with the increase of the added sea salt concentration. The most pronounced reduction in Chl a and Chl b was observed in Crawford variety due to 16.0 mS/cm<sup>2</sup> salinity level. However, the most pronounced reduction in carotenoids due to the same treatment was recorded in the variety G83. Also, it could be concluded from the provided data that photosynthetic pigments of G35 and G21 varieties are less affected by salinity compared to the other varieties.

The effect of three different levels of sea salt salinity on soluble sugars content of six soybean varieties is illustrated in Fig. (3). The content of soluble sugars decreased proportionally to the increase in the added salt in all soybean varieties. The most recorded decrease was due to 16.0 mS/cm<sup>2</sup> treatment in all varieties except G82 which showed more reduction at 8.0 mS/cm<sup>2</sup> than the higher treatment. The most recorded reductions in soluble sugars content due to the highest salinity level was observed in G21, G22 and G35 varieties. Under the non saline conditions (0.0 mS/cm<sup>2</sup>), the highest soluble carbohydrates was recorded for Crawford and G22

varieties, but the lowest one was recorded for G82.

Total soluble protein content in salt stressed soybean varieties was slightly decreased depending on salt concentration (Fig. 4). When grown without salt stress (0.0 mS/cm<sup>2</sup>), the highest soluble protein content was recorded in G83, followed by G82 then G35 varieties, however the lowest one was recorded in G21 variety. After salt stress, the soluble protein fraction decrease gradually as a function of salinity level. The lowest protein content was recorded in Crawford variety at 16.0 mS/cm<sup>2</sup> sea salt concentration, however the least affected by this salinity level was G82 variety which possessed the highest protein content under this treatment.

The variation in total free amino acids content with the change in salinity level in six soybean varieties is illustrated in Fig. (5). The data revealed that the uppermost free amino acids content was recorded in G35 variety under control and salinity levels, but the lowest content was recorded in G21 variety. Sea salt salinity has resulted in increasing the free amino acids in all studied soybean varieties and the increase was directly proportional to the increase in salinity level.

The results of free proline determination are shown in Fig. (6). The total free proline content showed a positive correlation with increasing sea salt concentration in all soybean varieties during sea salt stress. The lowest proline content under control treatment was observed in G21 variety, however the highest content was observed in G82 and Crawford varieties. The maximum free proline accumulation was found in the case of 16.0 mS/cm<sup>2</sup> salinity level in the whole varieties.

The protein patterns of six soybean varieties grown under different sea salt salinity levels (0.0, 8.0 and 16.0 mS/cm<sup>2</sup>) for 30 days were studied and showed different protein bands with molecular weights from 146 to 17 kD (Fig. 7). In general, all varieties contained major distinct bands under the non-salinized treatment with molecular weights of 17, 26, 42, 65, 85 and 120 kD. However, some soybean varieties contained unique and characteristic bands as 30 kD band in G22 and G83 varieties, 56 kD band in

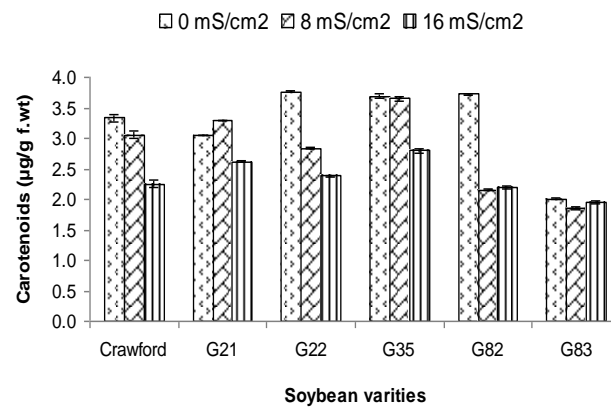


Fig. II. Photosynthetic pigments (Chl a, Chl b and Carotenoids) of six soybean varieties grown under 0.0, 8.0 and 16.0 mS/cm<sup>2</sup> sea salt salinity for 30 days.

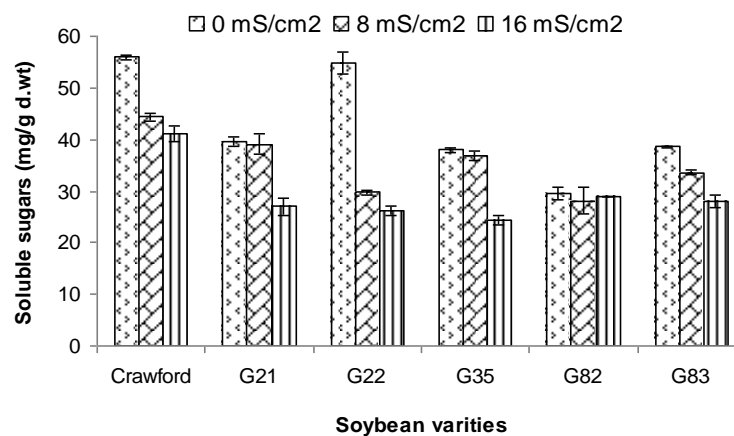


Fig. III. Soluble sugar content of six soybean varieties grown under 0.0, 8.0 and 16.0 mS/cm<sup>2</sup> sea salt salinity for 30 days.

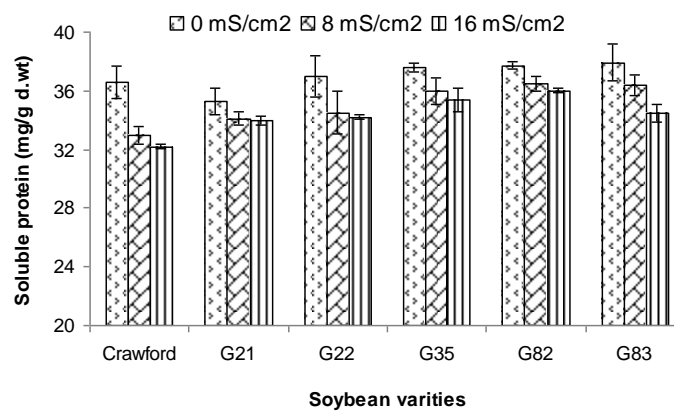


Fig. IV. Soluble protein content of six soybean varieties grown under 0.0, 8.0 and 16.0 mS/cm<sup>2</sup> sea salt salinity for 30 days.

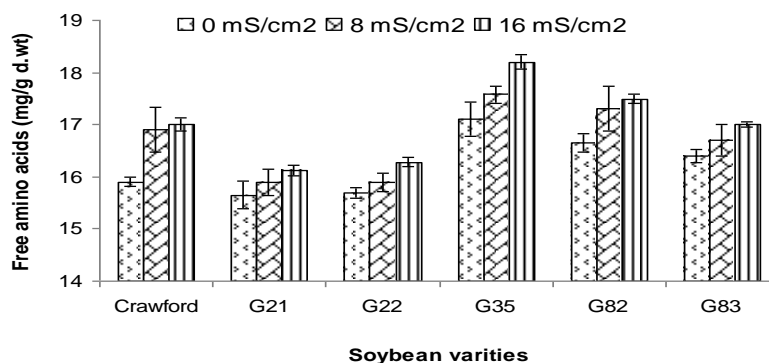


Fig. V. Free amino acids content of six soybean varieties grown under 0.0, 8.0 and 16.0 mS/cm<sup>2</sup> sea salt salinity for 30 days.

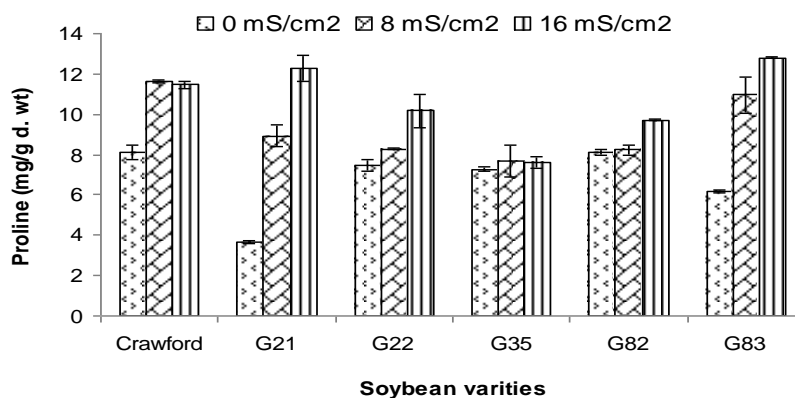


Fig. VI. Free proline content of six soybean varieties grown under 0.0, 8.0 and 16.0 mS/cm<sup>2</sup> sea salt salinity for 30 days

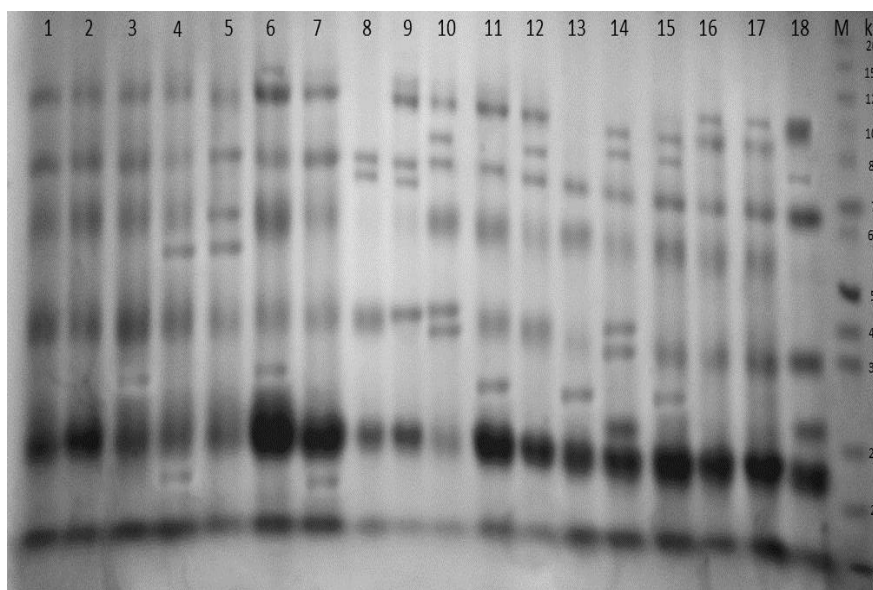


Fig. VII. SDS-PAGE protein profile of six soybean varieties grown under 0.0, 8.0 and 16.0 mS/cm<sup>2</sup> sea salt salinity for 30 days. Lanes: (1) Crawford, (2) G21, (3) G22, (4) G35, (5) G82, (6) G83, (7) Crawford+8 mS/cm<sup>2</sup>, (8) G21+8 mS/cm<sup>2</sup>, (9) G22+8 mS/cm<sup>2</sup>, (10) G35+8 mS/cm<sup>2</sup>, (11) G82+8 mS/cm<sup>2</sup>, (12) G83+8 mS/cm<sup>2</sup>, (13) Crawford+16 mS/cm<sup>2</sup>, (14) G21+16 mS/cm<sup>2</sup>, (15) G22+16 mS/cm<sup>2</sup>, (16) G35+16 mS/cm<sup>2</sup>, (17) G82+16 mS/cm<sup>2</sup>, (18) G83+16 mS/cm<sup>2</sup> and (M) protein marker.

G35 and G82 varieties and high molecular weight band of 146 kD in G83 variety. There is variation in the band numbers and intensities of the different soybean varieties with over-expression in the band with M.wt. of 26 kD in all varieties.

The treatment with 8.0 mS/cm<sup>2</sup> sea salt resulted in some apparent changes in the protein profile of soybean varieties, for example a new band with M.wt. of 22 kD appeared in Crawford variety. However, in G21 variety two bands with M.wt. of 120 and 65 kD were disappeared and a new band with M.wt. of 45 kD was appeared. In G22, two unique bands with M.wt. of 135 and 77 kD were appeared, and in G35 two other bands with M.wt. of 96 and 40 kD were appeared. G82 variety showed an appearance of two bands with M.wt. of 96 and 29 kD and the disappearance of 56 kD band, but G83 showed an appearance of 108 kD band and the disappearance of 146 and 85 kD bands.

Also, the treatment with 16.0 mS/cm<sup>2</sup> has a pronounced effect on soybean protein profile. It had resulted in the absence of the largest band (120 kD) from all soybean varieties. In addition, it caused the absence of other specific bands and the presence of new ones. For example, the newly synthesized band with M.wt. 28 kD in Crawford variety; 98 and 33 kD in G21; 95, 71 and 56 kD in G22; 107 and 92 kD in G35 and G82 and 103 and 90 kD in G83 variety.

## Discussion

The negative effects of increased salinity level on root and shoot development reported in present study is in agreement with previous studies of Dash and Panda (2001); Munns (2002) and Delgado and Sanchez-Raya (2007). The harmful effect of salinity on the root and shoot growth may be due to the toxic effect of salts as well as inhibition of cytokinesis and cell expansion (Kurum *et al.*, 2013). As well, the decline in hormones that stimulate plant growth and increase in hormones that inhibit growth (Taiz and Zeiger, 1998). Kao *et al.* (2003) conducted that salinity decline soybean growth due to excessive accumulation of NaCl in chloroplasts, which is associated with a decrease in the electron transport and inhibition of PSII activity. Nikee *et al.*

(2014) reported that salinity stress hinders plant growth due to generation of osmotic stress leading to reduction in water absorbance and cell division and differentiation.

The decline in chlorophyll and carotenoids content following salinity exposure reported in this study agree with a number of studies reported in a number of glycophytes (Gadallah, 1999; Agastian *et al.*, 2000 and Mitra and Banerjee, 2010). Decrease in total chlorophyll content by salinity was also reported by Khan *et al.* (2009) in six different genotypes of wheat, where the decline was considerable in sensitive genotypes compared to tolerant genotypes. The decrease in chlorophyll content under salt stress may be due to membrane deterioration (Djanaguiraman *et al.*, 2006). Yamane *et al.* (2004) reported that the generated H<sub>2</sub>O<sub>2</sub> and OH<sup>-</sup> are responsible for damaging effects of salt stress on chlorophyll content and chloroplast ultra structure. Also, the decrease of chlorophyll content in the plants developed under saline conditions may be attributed to both of the increased degradation and the inhibited synthesis of chlorophyll (Garsia-Sanchez *et al.*, 2002) and instability of the chloroplast membrane and pigment protein complex (Djanaguiraman and Ramadass, 2004). Aldesuquy *et al.* (2014) reported that the adverse effect of seawater salinity on pigment content could be attributed to the reduced activity of 5-aminolevulinic acid dehydratase (ALA-D) which catalyzes the formation of the porphyrin ring in chlorophyll structure. The decrease in carotenoids under salt stress was due to degradation of β-carotene and formation of zeaxanthins, which are concerned with the protection against photoinhibition (Sharma and Hall, 1991). Also, Babu *et al.* (2011) reported that the reduction in carotenoids content of tomato plants under salt stress might be due to decreased expression of carotenoid biosynthetic genes.

The decrease of carbohydrates following the exposure to salinity was reported by some workers (Mostafa, 2004; Sarwat and El-Sherif, 2007). However, Alamgir and Ali (1999) observed that salinity reduced sugar content in four rice varieties but increased it in other five varieties. In contrast, our results disagree with many authors who reported that salt stress stimulate sugar accumulations in many plant species, such as

barley (Ahmad *et al.*, 2006), sorghum (Almodares *et al.*, 2008), eggplant (Abbas *et al.*, 2010) and sweetleaf (Rathore *et al.*, 2014). Poustini (2002) suggested that salinity causes increasing of respiration rate which lead to the decrease in the content of carbohydrate. Also, the decrease in carbohydrate content in soybean following sea salt salinity exposure may be due to the deleterious effects of salinity on the photosynthetic machinery, closure of stomata, inhibition of key enzymes in Calvin cycle and the depletion of carbohydrates in providing the carbon skeleton for the stress defense molecules as proline and glycinebetaine.

It is well known that soluble protein content is a significant indicator of physiological status of plants. Kosova *et al.* (2013) stated that salinity stress induces signaling events leading to modification in gene expression, relative protein abundance and activity. The reduction in protein content followed the exposure to high salinity was reported by many authors (Agastian *et al.*, 2000; Amirjani, 2010 and Mahboobeh and Akbar, 2013). Golezani and Noori (2011) reported that protein content of three soybean cultivars was decreased by increased salinity levels and they attributed that to the disturbance in nitrogen metabolism. Also, the decrease in protein content by salt stress may be due to the effects of sodium chloride on protein synthesis (Omar *et al.*, 1993). Nasir Khan *et al.* (2007) concluded that high salinity lowers water potential and induces ionic stress, resulting in secondary oxidative stress which affects different metabolic processes such as CO<sub>2</sub> assimilation and protein synthesis. Another possible reason for the decline in protein content under salinity stress is the hydrolysis of proteins into free amino acids and the disorder in nitrate and ammonium assimilation.

The free amino acids change as a result of sea salt stress varied between soybean varieties. Even though salt stress had increased the free amino acids content in the shoot system of all varieties, this increase was more conspicuous in G35 and G82 varieties. Our results are in accordance with those of Panneerselvam *et al.* (1998) and Ramana *et al.* (2012) who stated that free amino acids increased with increasing NaCl salinity in soybean. The accumulation of free amino acids during salinity stress have been

suggested as important contribution to osmotic adjustment (Hare *et al.*, 1998), free radical scavenging, maintenance of protein and membrane integrity (Keutgen and Pawelzik, 2008) and may considered as a detoxification mechanism of the ammonium formed in plants subjected to stress (El-Beltagi *et al.*, 2008). Also, Gilbert *et al.* (1998) hypothesized that the high accumulation of amino acids during salinity stress suggest that these compounds may be acting as sinks for excess nitrogen in relation to the decreased growth occurring during the imposed stress. A number of explanations for free amino acids accumulation in plants under salt stress have been suggested. These include increase of *de novo* synthesis of amino acids, inhibition of amino acids degradation, inhibition of protein synthesis and increase of protein degradation (Mansour, 2000 and Neto *et al.*, 2009).

Proline occurs normally in the cytosol of higher plants and accumulates in larger amounts than other amino acids to regulate the accumulation of useable nitrogen (Abraham *et al.*, 2003) and contribute considerably in osmotic adjustment of the cytoplasm (Ketchum *et al.*, 1991). Proline serves as an important compatible osmolyte contribute to membrane stability, alleviate the effect of NaCl on cell membrane disturbance (Mansour, 1998), reduce cellular water potential to avoid deleterious toxicity of high ionic strength and act as reactive oxygen species scavenger (Verbruggen and Hermans, 2008). Abd Elhamid *et al.* (2014) observed that proline precursors, glutamate and arginine, were greatly increased in two genotypes of wheat subjected to salinity stress. Proline alleviates NaCl stress induced enhancement in oxygenase as well as carboxylase activities of Rubisco (Sivakumar *et al.*, 2000). Also, Maggio *et al.* (2002) stated that proline may act as a signaling/regulatory molecule able to activate multiple responses that are component of the adaptation process and stabilize the structure of membranes and proteins to minimize the damage of cells under salt stress.

Proline accumulation under salinity stress could be due to decrease of proline degradation, increase of proline biosynthesis, decrease of protein synthesis and increase of protein hydrolysis (Charest and Phan, 1999). The higher accumulation of proline could be due to enhanced



activities of ornithine aminotransferase (OAT) and pyrroline-5-carboxylate reductase (P5CR), the enzymes involved in proline biosynthesis (Kohl *et al.*, 1990), as well as due to inhibition of proline catabolizing enzymes, proline oxidase and proline dehydrogenase (PDH) (Kandpal *et al.*, 1981).

The changes in protein profile as a result of salinity exposure may be due to the adaptation of the plant to sea salt salinity, that induced a considerable variation in the protein patterns among the studied soybean varieties. This variation has been attributed to the expression of some newly synthesized polypeptides, the absence of others and over-expression of a third class of polypeptides. Several newly synthesized proteins in response to abiotic stresses were reported as stress-proteins (Patharkar and Cushman, 2000). Yen *et al.* (1997) reported that five polypeptides with molecular weights of 40, 34, 32, 29 and 14 kD were accumulated in the callus of *Mesembryanthemum crystallinum* under salt stress. Also, Tamas *et al.* (2001) reported that salinity induced accumulation of four polypeptides in maize roots with molecular weights of 61, 51, 39 and 29 kD, and Rangan and Swaminathan (2002) reported that out of 58 bands detected by 2-D PAGE in *Potresia coarctata*, 35 are affected by salt stress. Barakat (2003) concluded that the proteins with molecular weights of 68, 52, 46, 43, 35, 33, 18 and 11 kD could play an important role in sever salinity tolerance. It has been suggested that these peptides have a role in osmoprotection (Dure, 1993) or protect cellular structures (Close and Lammers, 1993).

Plants act in response to salt stress by modifying gene expression, which ultimately leads to the restoration of cellular homeostasis, removing toxic substances and improvement of growth (Xiong and Zhu, 2002). Changes in protein synthesis under salt stress may be due to changes in the efficiency of mRNA translation or the regulation of RNA transcription, transport and constancy (Barakat, 2003). However, Sohrabi *et al.* (2011) attributed the decrease in protein content by salinity to the decreased rate of protein synthesis, the increased activities of hydrolyzing enzymes, the decreased availability of amino acids, or the denaturation of the enzymes concerned with amino acids and protein synthesis.

## Conclusion

Considering the data of the present study, it can be concluded that sea salt salinity treatments induced a considerable variation in growth criteria, photosynthetic pigments, carbohydrates, nitrogenous compounds (protein, free amino acids and proline) contents, and the SDS-PAGE pattern among the six soybean varieties. The data confirmed that salinity stress resulted in an inhibitory effect on all studied soybean varieties and the degree of inhibition differ between the varieties. Also, our results confirmed that salinity stress resulted in the formation of new bands and the disappearance of others in the protein pattern as an adaptive strategy to salt stress. In summary, the results presented here confirm that soybean is a moderately salt-sensitive plant.

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