Journal of the Saudi Society of Agricultural Sciences (2011) **10**, 7–15

King Saud University

Journal of the Saudi Society of Agricultural Sciences

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Effect of salt stress on plant growth and metabolism of bean plant *Vicia faba* (L.)

Amira M.S. Abdul Qados

Botany Department, Princess Nora Bint Abdul Rahman University, P.O. Box 2508, Safaqes St. Granada eq., Riyadh 13242-07229, Saudi Arabia

Received 25 April 2010; accepted 27 June 2010
Available online 17 December 2010

KEYWORDS

Osmotic potential;
Chlorophyll;
NaCl;
Plant height;
Protein

Abstract The effect of sodium chloride (NaCl) concentrations (0.0, 60, 120, 240 mM) on growth, osmotic potential, chlorophyll content, protein content of (*Vicia faba* L.) seedlings was investigated.

NaCl caused an increase in plant height with low and medium concentrations and a decrease with the highest concentration, in both measurement periods. No significant effect was observed in the number of leaves or leaf area with low concentration, while a decrease was noticed for each, with two higher concentrations and in both measurement periods.

Salinity increased both fresh and dry weights of the shoot in the two measurement periods. Osmotic potential (O.P.) showed a significant decrease with the increase in concentrations, and in the duration of the stress periods.

Salinity significantly reduced chlorophyll 'a' content in both measurement periods. It also significantly reduced chlorophyll 'b', total chl., and carotenoids contents after ten days of treatment.

An increase was observed in the protein content in the two measurement periods due to the impact of salinity stress. A directly proportional relationship was found between protein content and the increase in salt concentrations in the first measurement period, while it was inversely proportional in the second.

1. Introduction

The over salinity of the soil is one of the main factors that limits the spread of plants in their natural habitats. It is an ever-increasing problem in arid and semi arid regions (Shanon, 1986). Fisher and Turner (1978) estimate that arid and semi arid lands represent around 40% of the earth's area.

The property of salinity tolerance is not a simple attribute, but it is an outcome of various features that depend on different physiological interactions, which are difficult to determine. The morphological appearance presented by the plant in response to salinity, may not be enough to determine its effect,

E-mail address: amira_m.s@hotmail.com

Peer review under responsibility of King Saud University.
doi:10.1016/j.jssas.2010.06.002

Production and hosting by Elsevier

so it is important to recognize other physiological and biochemical factors, including toxic ions, osmotic potential, lack of elements and other physiological and chemical disorders, as well as the interactions between these various stresses (Munns, 1993, 2002; Neumann, 1997; Yao, 1998; Hasegewa et al., 2000).

From the results of the studies, which looked at the effect of salt stress on growth, one can notice a connection between the decrease in plant length and the increase in the concentration of sodium chloride (Beltagi et al., 2006; Mustard and Renault, 2006; Gama et al., 2007; Jamil et al., 2007; Houimli et al., 2008; Rui et al., 2009; Memon et al., 2010). Numerous studies showed the affection of leaf area negatively by using different concentrations of NaCl (Raul et al., 2003; Netondo et al., 2004; Mathur et al., 2006; Chen et al., 2007; Zhao et al., 2007; Yilmaz and Kina, 2008; Rui et al., 2009).

The harmful influence of salinity on leaf number, also increases with the increase in concentration, according to the studies held by Raul et al. (2003), Jamil et al. (2005), Gama et al. (2007), Ha et al. (2008).

Many studies have shown that the fresh and dry weights of the shoot system are affected, either negatively or positively, by changes in salinity concentration, type of salt present, or type of plant species (Bayuelo Jimenez et al., 2002; Jamil et al., 2005; Niaz et al., 2005; Saqib et al., 2006; Turan et al., 2007; Saffan, 2008; Rui et al., 2009; Taffouo et al., 2009, 2010; Memon et al., 2010).

Changes in water relations of plants that are stressed by salinity, can be seen in certain studies that confirm that, many plants undergo osmotic regulation when they are exposed to salt stress by increasing the negativity of the osmotic potential of the leaf sap (Rodriguez et al., 1997; Gama et al., 2007, 2009; Kaymakanova and Stoeva, 2008; Kaymakanova et al., 2008).

Many studies confirm the inhibitory effect of salinity on biochemical processes, of which photosynthesis is the most important. The effect on photosynthesis can be gauged from the effect on the photosynthetic pigments. The results of specific studies (Sultana et al., 2000; Tort and Turkyilmaz, 2004; Misra et al., 2006; Murillo-Amador et al., 2007; Taffouo et al., 2010) clearly indicate that salinity reduces the content of photosynthetic pigments in treated plants.

Protein content can also be affected negatively or positively, by salt stress. The results of certain studies (Sultana et al., 2000; Tort and Turkyilmaz, 2004; Beltagi et al., 2006; Chen et al., 2007; Kapoor and Srivastava, 2010) demonstrate a decrease, or increase, in protein content in plants treated with different salt concentrations.

Agriculture plays a pioneering role in economical development in many countries, especially in Saudi Arabia. However, salinity, which affects most areas of the kingdom, represents one of the main obstacles that limit the expansion of the agricultural area or the increase in agricultural production for many crops. High salinity is due to the high concentration of soluble salts in irrigation waters and the high rate of evaporation caused by the high temperatures in Saudi Arabia, inefficient drainage, or soil type. Bean is one of the important economic cereal crops, a cereal used as food for both people and animals, besides its capacity to tolerate salinity. In light of this, our research aims to study the effect of salt stress, using different concentrations of sodium chloride, on the growth and metabolism of *Vicia faba* (L.) and to determine the extent of its tolerance of salinity.

2. Materials and methods

2.1. Plant material

The experiments were carried out in a greenhouse, at princess Nura Bint Abdul-Rahman University, science sections, Riyadh, using (*Vicia faba* L.), Reina Mora; the seeds were obtained from the local market and is one of the types grown in Saudi Arabia.

2.2. Preparing and sowing of seed

Intact seeds, which were homogeneous and identical in size and colour, and free from wrinkles, were chosen then sterilized with 10% Clorox for 10 min. A drop of (tween 20) per 100 ml, was added to the solution, as a scattering material.

Twenty seeds were grown in each plastic pot, 45 cm in diameter and 55 cm height, containing equal quantities of vermiculite washed with filtrated water. The seeds were left to grow inside the greenhouse under natural lighting, (25/15) ± 2 °C (day/night) and 70% relative humidity. The pots were distributed randomly in lines, with each line comprising of all treatments. The number of plants per pot was decreased to 10, and only homogenous seedlings showing the strongest growth, were selected and left to grow until they were 6 weeks of age (from the beginning of germination), then treatments were started. The selection of age was in accordance with Schreiber and Stanberey (1965) and Day and Thompson (1975).

2.3. Treatments

We used different concentrations of sodium chloride (0, 60, 120, and 240 mM). Pots were irrigated every three days, alternatively with filtrated water and a (1× solution) nutrient solution, according to Etherton (1963), until they were 6 weeks old. After that, homogeneous plants were divided into four groups, each group comprising one of the concentrations of sodium chloride. The control plants only received nutrient solution. To avoid osmotic shock due to high concentrations, plants were started on lower concentrations, then the concentration was increased on a daily basis, until each group reached the concentration determined for it; then plants were irrigated with nutrient solution every three days (250 ml per day) with the addition of sodium chloride to the nutrient solution every two weeks. Each pot was washed with 500 ml filtrated water a week before irrigation with saline solution to prevent the increase in osmotic potential resulting from the accumulation of salts by the succession of irrigation procedures.

A total of three replicates were chosen for each morphological and physiological measurement (at an average of three plants per replica). Specimens were collected on the 10th day from the start of treatment, and when 40% of the fourth group of plants on the highest concentration had died. The total age of the plants at that time was 90 days.

2.4. Growth measurements

Growth measurements, for the plants exposed to saline treatments, were taken at times mentioned previously, namely after 10 days of treatment and at the death of 40% of plants at the highest concentration. The three replicates taken for each treatment, were used to calculate the mean of each measurement. The measurements taken were the following:

- Length of the Shoot system.
- Number of plant leaves.
- Leaf area, as measured by an area meter (Area Meter CI, 202).
- Fresh and dry weights of the plants: root system weights were taken immediately, then put in sacks and left to dry in a drying oven at 70 °C until the weight was stable.

2.5. Physiological study

In our study, and for measuring the osmotic potential of the leaf sap, an indirect method was used, namely the measurement of the (refractive index) of the cellular sap after extracting it from the leaf. Samples were quickly frozen in liquid nitrogen in order to break cell walls, then the sap was extracted rapidly by grinding the frozen leaves in a manual mortar. The Measurement of the refraction index of total soluble solids (T.S.S.) was done on a drop of cellular sap, at 20 °C, using a Refractometer. The readings were converted to osmotic potential via the appropriate table, and according to Gusev (1960).

2.6. Chemical content

2.6.1. Photosynthetic pigments

One gram of fresh tissue, taken from the third and fourth leaf, was extracted by grinding in a mortar using 20 ml 80% acetone, a small amount of pure (Silica Quartz), and 0.5 g calcium carbonate to equalize the cellular sap acidity. The extract was filtered using a glass funnel (Sentered glass funnel G4) and collected in a conical flask. The residue was re-extracted using the same method, until it became devoid of color. All the filtrate was collected in a standard flask and the volume completed to a specific amount by adding 80% acetone. The optical density (O.D.) of the extract was measured at wave lengths 663, 645, and 440.5 nm (Smith and Benitez, 1955) to estimate chlorophyll 'a' and 'b', and carotenes respectively, using a Spectrophotometer (Spectronic 21D) and a vitreous cell (thickness of photo route 1 cm). Three replicates were used for each treatment, and the amount of pigment present in each sample was calculated according to the following equations:

$$\text{mg chlorophyll a/g-tissue} = 12.7\ (\text{O.D.})\ 663 - 2.69\ (\text{O.D.})\ 645 \times \frac{v}{w \times 1000}$$

$$\text{mg chlorophyll b/g-tissue} = 22.9\ (\text{O.D.})\ 645 - 4.68\ (\text{O.D.})\ 663 \times \frac{v}{w \times 1000}$$

$$\text{mg total chlorophyll/g-tissue} = 20.2\ (\text{O.D.})\ 645 + 8.02\ (\text{O.D.})\ 663 \times \frac{v}{w \times 1000}$$

$$\text{mg carotenoids/g-tissue} = 46.95\ (\text{O.D. } 440.5 - 0.268 \times \text{chlorophyll 'a' + 'b'})$$

whereas W, the fresh weight by grams for extracted tissue; V, the final size of the extract in 80% acetone; O.D., optical density at specific wave length.

2.7. Protein

2.7.1. Extraction of protein

> Sample (0.5 g) of dry tissue were ground in a mortar with 10 ml filtrated water then transferred quantitatively to test tubes. One millilitre of trichloroacetic acid (10%) was added, then the tubes were placed in an ice bath for 10 min. The supernatant was separated from the precipitate and transferred to a centrifuge and run at 5000 rpm, for 10 min at 4 °C. The precipitate was clarified in 20 ml sodium hydroxide (0.1 N) to dissolve the protein, and the volume was rounded up to the nearest whole number, in accordance with Lowry et al. (1951).

2.7.2. Determination of protein

Protein was determined using the Lowry method (Lowry et al., 1951). In summary:

(1) 5 ml of copper solution was added to tubes containing 0.1 ml of the protein extract. The Copper solution composed of
   (a) 100 ml of sodium chloride (0.1 N) in which were dissolved, 2 g of anhydrous sodium carbonates and 1 ml of sodium tartrate (2.7%).
   (b) 1 ml of copper sulphate (1%).

   (a and b) were mixed immediately before use and the tubes were left for 15 min, then the optical density (O.D.) was measured at 570 nanometers.
(2) The same steps were repeated with the standard solution (of known concentration) of Bovine Serum Albumin.
(3) Steps (1 and 2) were repeated thrice, and the mean value of the 3 readings were compared with the standard curve of Bovine Serum Albumin.

2.8. Statistical analysis

The results were analyzed by comparing (F) values obtained from a one-way ANOVA using the SPSS statistical package (Gerber et al., 1997). The lowest significant (LSD) between the means, at the (5%) level, were determined for various treatments, following the method of Steel and Torrie (1981).

3. Results and discussion

3.1. Effect of salt stress on plant growth

3.1.1. Plant height

By following the lengths of the plants under salt stress during the treatment periods, Table 1 it appears there is a general trend for the increase in the lengths of the plants using the concentrations, 60 mM and 120 mM. More precisely, the increase is correlated inversely, after 10 days of treatment, with the increase of the concentration of salt from 60 to 120 mM, whereas the increase is correlated directly with the same concentrations of salt, at the death of 40% of the plants. Then, the length of plants decreases, in both periods, with the use of the highest concentration (240 mM), however the decrease was significant during the first measurement period only.

In general, statistical analysis demonstrated significant differences, whether an increase or decrease, for the youngest plants (after 10 days of the treatment), specifically when using the concentrations 60 mM and 240 mM, whereas, there were no significant differences between the plants of the control experiment or at a later stage (i.e. at the death of 40% of the plants), for the treated plants.

Other results that support what has been shown here, are those by Hamada (1995) with his study on maize *Zea mays*

Table 1 Effect of treatment with different concentrations of (NaCl) on the height (cm) of bean plants.

Number of days after start of treatment Concentration (mM)	10 days after treatment Mean ± Std. error	At the death of 40% of plants Mean ± Std. error
Zero	50. 33 ± 0.882	86.00 ± 8.718
60	55.67 ± 2.028*	90.00 ± 10.00
120	51.33 ± 0.667	97.00 ± 1.528
240	43.67 ± 0.333*	75.00 ± 2.887
F	18.088	1.814
P value	0.001	0.223

Values are means of three replicates with three plants per replicate.
* $P < 0.05$.

L., Misra et al. (1997) with their study on rice seedlings *Oryza sativa* L. vr. Damodar, Dantus et al. (2005) in their study on cowpea, *Vigna unguiculata* L., and finally by Memon et al. (2010) in their study on *Brassica campestris* L. where they indicated that the use of low concentrations of sodium chloride led to increases in plants lengths, whereas higher concentrations caused shortage. Contrary results were registered as well, including the study done by Mathur et al. (2006) on moth bean *Vigna aconitifolia* L., Jamil et al. (2007) on radish plant, *Raphanus sativus* L., Taffouo et al. (2009) on cowpea *Vigna unguiculata* L., and Kapoor and Srivastava (2010) on *Vigna mungo* L. They found that increasing the concentrations of NaCl developed a decline in the lengths of the plants.

Generally speaking, we may infer that, the elongation of the stem when treated with low concentrations of salts may induce osmotic adjustment activity in the plants which may improve growth. On the other hand, the noticed decrease in the length of the stem, also due to treatment with sodium chloride solution, could be due to the negative effect of this salt on the rate of photosynthesis, the changes in enzyme activity (that subsequently affects protein synthesis), and also the decrease in the level of carbohydrates and growth hormones, both of which can lead to inhibition of the growth (Mazher et al., 2007).

3.1.2. Number of leaves

Results presented in Table 2 showed that higher levels of salinity decrease leaf number throughout the experiment. It was found that the general trend of the treatment reflects a gradual decrease in the number of plant leaves with the increase of salt concentration, compared with the plants of the control experiment, except for the 60 mM treatment, which did not lead to the decrease in the number of leaves on the plants after 10 days of the treatment, whereas the same concentration led to a non significant increase in the number of leaves in the second period (when 40% of the treated plants had died).

Statistical analysis did not show significant differences, whether in the increase or decrease of leaves for plants exposed to salt stress, compared with the control plants of both periods.

These results have been confirmed by the results of Karen et al. (2002), with their study on *Cirer arietinum* L. and Raul et al. (2003), with their study on the leaves of the teprary bean (*Phaseolus acutifolius* L.), cowpea (*Vigna unguiculata* L.), and wild bean (*Phaseolus filiformis* L). They mention that, the treatment of sodium chloride reduced the number of leaves compared with control plants.

One of the studies that supports these results also, is a study by Jamil et al. (2005). They mention that, the treatment of Cabbage *Brassica oleracea capitata* L. and *Brassica oleracea botrytis* L. with the concentrations: zero, 4.7, 9.4, 14.1 dsm^{-1} NaCl, had a negative effect on leaf number of these plants. The results of a study performed by Gama et al. (2007), on beans (*Phaseolus vulgaris* L.), agree with our results, as well. They mention that there is a decrease in the number of leaves when treated with 50, and 100 mM of sodium chloride.

The decrease of leaf numbers may be due to the accumulation of sodium chloride in the cell walls and cytoplasm of the older leaves. At the same time, their vacuole sap cannot accumulate more salt and, thereby decreases the concentration of salt inside the cells, which ultimately leads to their quick death and cut down (Munns, 2002).

3.1.3. Leaf area

Leaf area represents a measure of plant growth, which can be affected by different stresses, including salt stress. The results listed in Table 3 demonstrate the response of the leaf to salt stress. Generally the results showed a decrease in leaf area with increasing salinity in the two periods of treatment, after 10 days and when 40% of plants had died, except for the minimum concentration (60 mM), in the second period. Herein it led to a non-significant increase in the leaf area, compared with the control plants.

Looking at the statistical analysis for those values, it appears that the decrease in leaf area was significant in plants after 10 days of treatment, using the concentrations 120 and 240 mM, while at the death of 40% of plants, it was significant when using the highest concentration, 240 mM.

These results agree with what Mathur et al. (2006) reported, that the stress of the moth bean plant (*Vigna aconitifolia* L.) with increasing concentrations of sodium chloride, led to a decrease in leaf area. This decrease was inversely proportional to

Table 2 Effect of treatment with different concentrations of (NaCl) on the number of leaves of bean plants.

Number of days after treatment Concentration (mM)	10 days after treatment Mean ± Std. error	At the death of 40% of plants Mean ± Std. error
Zero	5.000 ± 0.000	13.667 ± 0.882
60	5.000 ± 0.000	15.000 ± 0.000
120	4.667 ± 0.333	13.333 ± 0.882
240	4.000 ± 0.577	11.333 ± 1.333
F	2.000	2.756
P value	0.193	0.112

Values means of three replicates with three plants per replicate.

Table 3 Effect of treatment with different concentrations of (NaCl) on leaf area (cm^2) of bean plants.

Number of days after treatment Concentration (mM)	10 days after treatment Mean ± Std. error	At the death of 40% of plants Mean ± Std. error
Zero	16.667 ± 0.726	20.833 ± 0.601
60	15.667 ± 0.441	21.500 ± 0.500
120	13.167 ± 0.333*	19.667 ± 0.333
240	12.333 ± 0.167*	18.667 ± 0.882*
F	19.387	4.189
P value	0.000	0.046

Values are means of three replicates with three plants per replicate.
* $P < 0.05$.

the concentrations. Also, a significant decrease in leaf area of sugar cane (*Beta vulgaris* L.) in response to salt stress using concentrations zero, 50, 100, 150 mmol of sodium chloride, has been reported (Jamil et al., 2007).

Other supporting results, include those of Zhao et al. (2007), With their study on oat (*Avena sativa* L.) and Yilmaz and Kina (2008), with their study on *Fragaria x ananssa* (L.). They found that the exposure to salinity by NaCl reduced leaf area.

This notable decrease in leaf area, found in this study as a result of the treatment with increased concentrations of sodium chloride, could be explained by the negative effect of salt on photosynthesis that leads to the reduction of plant growth, leaf growth, and chlorophyll content (Netondo et al., 2004).

3.1.4. Fresh and dry weight

Going through the data in Tables 4 and 5, it is obvious that there is a positive effect for salt stress on the fresh and dry weight of the bean. There is an increase in the fresh and dry weights of the plants in general, and this applies to both measurement periods. The maximum increase in fresh and dry weight, after 10 days of the treatment, was achieved by using the minimum concentration, 60 mM. This increase was significant for fresh weight, whereas a higher increase was achieved during the second measurement period, for both fresh and dry weights, when using the concentration 120 mM.

Comparing the achieved values for fresh weight with the values of control plants, more closely, it is obvious that the differences were significant in plants that were treated with the minimum concentration, 60 mM, at the first measurement. Also, using the concentration 120 mM showed a significant increase at the second measurement. The differences were not significant using the other concentrations in any at the two measurement periods. Also, the significant differences disappeared for dry weight.

These results for fresh and dry weights for the shoot system in this study, agree with the results presented by Andriolo et al. (2005) in their study on lettuce (*Lactuca sativa* L.), where they reported that the treatment with salt increased the fresh weight by about 28%, and also agree with the results of a study by Dantus et al. (2005) on cowpea (*Vigna unguiculata* L.), where they report that using 10 mM of sodium chloride increased fresh and dry weights of the shoot system of their seedlings. Other supporting results include that of Niaz et al. (2005), who report that the treatment of fodder beet (*Beta vulgaris* L.) and sea beet (*Beta maritime* L.) with 200 mM of sodium chloride increases the fresh weight of the shoot system, which was significant for both kinds. Also, the study made by Orak and Ates (2005) on common vetch (*Vicia sativa* L.), and Nedjimi et al. (2006), on (*Atriplex halimus* L.) where they report an increase in the fresh and dry weight for root and shoot systems of the plants with concentrations of NaCl.

In spite of the fact that many studies have pointed to the positive effect of sodium chloride on fresh and dry weight, there are contrary results, as well, pointing to the negative effect of salt stress on fresh and dry weight. These include a study by Jamil et al. (2007) on radish plants *Raphanus sativus* L., a study by Ha et al. (2008) on *Kyllinigia peruviana* L., a study by Rui et al. (2009) on *Bruguiera gymnorrhiza* L., and finally a study by Memon et al. (2010) on *Brassica campestris* L.

The increase in fresh weight of the shoot system may be due to the ability of the plant to increase the size of its sap vacuoles, which allows for the collection of a lot of water, and this in turn dissolves salt ions that have accumulated and leads to the subsequent increase in fresh weight (Munns, 2002).

3.2. Effect of salt stress on internal water relations

3.2.1. Osmotic potential of the leaf sap

Results presented in Table 6 showed that increasing salinity decreased the osmotic potential of the plant for both measurement periods. This change is considered one of the defensive means by which plants tolerate stress, as this increases its ability to absorb water. The values detected show an inverse relationship between salt stress and osmotic potential for the sap of bean leaves, where the reduction of osmotic potential increases with the increase of sodium chloride added to the nutri-

Table 4 Effect of treatment with different concentrations of (NaCl) On fresh weight (g) of bean plants.

Number of days after treatment Concentration (mM)	10 days after treatment Mean ± Std. error	At the death of 40% of plants Mean ± Std. error
Zero	3.862 ± 0.661	7.236 ± 0.497
60	6.911 ± 0.664*	8.279 ± 1.231
120	5.396 ± 0.961	10.459 ± 0.975*
240	5.168 ± 0.582	8.141 ± 0.178
F	2.919	2.724
P value	0.100	0.114

Values are means of three replicates with three plants per replicate.
* $P < 0.05$.

Table 5 Effect of treatment with different concentrations of (NaCl) on dry weight (g) of bean plants.

Number of days after treatment Concentration (mM)	10 days after treatment Mean ± Std. error	At the death of 40% of plants Mean ± Std. error
Zero	0.461 ± 0.056	0.790 ± 0.594
60	0.613 ± 0.077	0.890 ± 0.182
120	0.600 ± 0.057	1.035 ± 0.196
240	0.571 ± 0.067	0.867 ± 0.266
F	1.139	0.554
P value	0.390	0.660

Values are means of three replicates with three plants per replicate.

Table 6 Effect of treatment with different concentrations of (NaCl) on the osmotic potential (Bars) of bean plants.

Number of days after treatment Concentration (mM)	10 days after treatment Mean ± Std. error	At the death of 40% of plants Mean ± Std. error
Zero	−1.667 ± 0.333	−1.800 ± 0.058
60	−1.733 ± 0.088	−2.500 ± 0.116*
120	−2.300 ± 0.116*	−2.767 ± 0.033*
240	−2.700 ± 0.058*	−3.200 ± 0.153*
F	37.681	33.514
P value	0.000	0.000

Values are a means of three replicates with three plants per replicate.
* $P < 0.05$.

ent solution, as well as with the increase in the length of stress exposure, as this was noticed in both measurement periods.

The statistical analysis demonstrated, that the reduction in osmotic potential was significant with all concentrations in both measurement periods, except the minimum concentration 60 mM after 10 days of the treatment.

The results registered by many researches in this field, correspond to the results we obtained. For example, Yagmur et al. (2006) recorded a significant decrease in the osmotic potential of barley plant leaves (*Hordeum vulgare* L.), that were treated with the concentrations of zero, 200, 400, and 600 mgk kg^{-1} of potassium sulfate. Also a notable decrease in osmotic potential of bean seedlings (*Phaseolus vulgaris* L.), which were treated with sodium chloride was reported (Gama et al., 2007, 2009; Stoeva and kaymakanova, 2008). Qin et al. (2009) registered a significant reduction in osmotic potential for leaf sap of seabukthorr (*Hippophae rhamoides* L.) when treated with the concentrations zero, 200, 400, and 600 mM of NaCl.

It can be said that, the ability of the plant to maintain its osmotic potential at levels below that of the osmotic potential of the soil surrounding the plant (Zhang et al., 1999; Zhu, 2001) is a means by which it tolerates the harmful effect of the accumulation of salt inside its cells during salt stress (Cachorro et al., 1995).

3.3. Effect of salt stress on chemical content

3.3.1. Photosynthetic pigments

Table 7 demonstrates the effect of salt stress, using different concentrations of sodium chloride, on the chlorophyll content of the bean plants under study, including chlorophyll 'a', 'b' and total chlorophyll. The results show an inverse relationship between salt concentration and chl. 'a' content. Whenever the concentration increased, chlorophyll 'a' content decreased, reaching its lowest content, 0.270 and 0.426 mg/g fresh weight, at 240 mM, for both periods of measurement, compared to control plant fresh weights, 0.528 and 0.532 mg/g, respectively.

A statistical analysis indicated that the observed differences were significant, except for the minimum concentration, 60 mM, when 40% of plants had died.

On the other hand, it was noticed from the results that the relationship between chlorophyll 'b' and total chlorophyll and the same concentrations of sodium chloride had followed a different path for the two measurement periods. The general trend for the treatment at the first measurement period (after 10 days of the treatment) reflects a gradual reduction in the content. This reduction increased by increasing salt concentrations. Whereas, this reversed in the second measurement period where salt stress led to the increase in chlorophyll 'b' content and total chlorophyll, and this increase was proportional to the increase in salt concentration.

The reduction was significant in the first measurement period for all used concentrations. Also, the "increase" found during the second measurement period, was also significant, except for the concentration of 60 mM.

Our results regarding a decrease in chlorophyll 'a', 'b', and total chl., agree with what Tort and Turkyilmaz (2004) reported, that the exposure of barley (*Hordeum vulgare* L.) to zero, 120, and 240 mM of sodium chloride led to the decrease in chlorophyll 'a', chlorophyll 'b' and total chlorophyll content. Also Lee et al. (2004) in their study on *Paspalum vaginatum* (L.)

Table 7 Effect of treatment with different concentrations of (NaCl) on chlorophyll content (mg/g Fw) of bean plants.

Type of chlorophyll	Chlorophyll a		Chlorophyll b		Total chlorophyll	
Number of days after treatment	10 days after treatment	At the death of 40% of plants	10 days after treatment	At the death of 40% of plants	10 days after treatment	At the death of 40% of plants
Concentration (mM)	Mean ± Std. error	Mean ± Std. error	Mean ± Std. error	Mean ± Std. error	Mean ± Std. error	Mean ± Std. error
Zero	0.528 ± 0.006	0.532 ± 0.009	0.291 ± 0.044	0.444 ± 0.007	0.819 ± 0.039	0.976 ± 0.005
60	0.463 ± 0.021*	0.521 ± 0.008	0.195 ± 0.011*	0.447 ± 0.007	0.659 ± 0.011*	0.976 ± 0.012
120	0.311 ± 0.005*	0.492 ± 0.008*	0.129 ± 0.013*	0.575 ± 0.008*	0.440 ± 0.008*	1. 071 ± 0.004*
240	0.270 ± 0.007*	0.0426 ± 0.014*	0.060 ± 0.006*	0.602 ± 0.003*	0.330 ± 0.001*	1. 024 ± 0.012*
F	107. 274	22. 888	16. 705	160.784	110.871	28. 237
P value	0.000	0.000	0.001	0.000	0.000	0.000

Values are mean of three replicates.
* $P < 0.05$.

Table 8 Effect of treatment with different concentrations of (NaCl) on carotenoid content (mg/g Fw) of bean plants.

Number of days after treatment Concentration (mM)	10 days after treatment Mean ± Std. error	At the death of 40% of plants Mean ± Std. error
Zero	0.148 ± 0.002	0.135 ± 0.001
60	0.127 ± 0.006*	0.135 ± 0.001
120	0.114 ± 0.006*	0.145 ± 0.001*
240	0.110 ± 0.001*	0.136 ± 0.001
F	15.568	25.568
P value	0.001	0.001

Values are mean of three replicates.
* $P < 0.05$.

Table 9 Effect of treatment with different concentrations of (NaCl) on protein content (mg/g Dw) of bean plants.

Number of days after treatment Concentration (mM)	10 days after treatment Mean ± Std. error	At the death of 40% of plants Mean ± Std. error
Zero	251.667 ± 22.482	245.667 ± 2.603
60	252.667 ± 22.183	256.333 ± 21.458
120	255.667 ± 16.756	253.667 ± 17.023
240	260.000 ± 19.035	251.33 ± 18.022
F	0.022	0. 076
P value	0.995	0.971

Values are means of three replicates.

and Siler et al. (2007) in their study on *Centaurium erythraea* (L.) reported that chlorophyll 'a', 'b' and total chlorophyll decreased with the increase of salt concentrations. Supporting results also include what Turan et al. (2007), on bean plant *Phaseolus vulgaris* (L.), Cheruth et al. (2008), on *Catharanthus roseus* (L.), Taffouo et al. (2009), on cowpea (*Vigna unguiculata* L.) and Taffouo et al. (2010), on *Vigna subterranean* (L.). Demonstrate that salt stress of sodium chloride caused a decrease in total chlorophyll content.

The results regarding the "increase" in chlorophyll content with the increase of salt concentrations agree with results reported by Misra et al. (1997). They indicated that stressing rice seedlings *Oryza sativa* L. with sodium chloride increased significantly the chlorophyll content of seedlings, which were 15 days old. Also, it was mentioned by Jamil et al. (2007) that increased concentrations of sodium chloride (zero, 50, 150 mM) increased the total chlorophyll content of sugar cane leaves (*Beta vulgaris* L.), and that it was a significant increase.

By following carotene content during exposure of the bean plants to salt stress, it appears from Table 8 that salt stress was an inhibiting factor for the formation of carotenes inside the stressed plants at the first stage of measurement (after 10 days of treatment), where the carotene content decreased. There was an inverse relationship between salt concentration and carotene content, as it is obvious from the data in the indicated table that the least carotene content appeared with the concentration 240 mM, where it reached to 0.110 mg/g fresh weight, while the value for control plants was 0.148 mg/g fresh weight.

By studying the effect of the salt stress at the second period of measurement, it appears from the data in the same table that there was no effect using the treatment 60 mM, while 120 mM caused an increase, then a decreases in carotene content was noticed using the 240 mM concentration.

The differences according to the statistical analysis were significant at the first measurement period with all the used concentrations. Whereas, there were no significant differences at the second measurement period except for the concentration of 120 mM.

The results obtained, regarding the reduction of carotene content, agree with those registered by Tort and Turkyilmaz (2004), where they observed that the stressing of barley seedlings (*Hordeum vulgare* L.) with zero, 120, 180, and 240 mM of sodium chloride, reduced leaf content of carotenes. Also, Mustard and Renault (2006) registered a reduction of carotene content in seedlings of dogwood (*Cornus sericea* L.), which were stressed with salt. On the other hand, our results regarding the increase in carotene content using salt stress, agree with those obtained by Misra et al. (1997) who found that submitting rice seedlings *Oryza sativa* L. to salt stress by sodium chloride, led to a significant increase in carotene content for 15 day old plants.

3.3.2. Protein content

Table 9 results indicate a positive effect for sodium chloride using various concentrations on total protein of bean plant shoots after 10 days. It appears from the data that there was a general increase in protein content that corresponded with the increase in salt concentrations, whereas there was a general reverse in protein content, when 40% of the plant had died. There appeared to be an inverse relationship between salt concentrations and protein content, although protein content was still higher than the controls. Statistical analysis did not show a significant difference during the two measurement periods.

The results obtained, in general agree with what Chao et al. (1999) had presented. They noticed an increase of protein content of the tomato plant *Lycopersicon esculentum* (L.) in response to salt treatment. Again, Sibole et al. (2003), reported that the treatment of clover plant (*Medicago citrna* L.) for 30 days with concentrations of zero, 1, 50, 100, 200 mM. of NaCl increased soluble protein content in the seedlings, compared with control plants. Also, Tort and Turkyilmaz (2004) recorded a big increase in protein content when treating barley plant (*Hordeum vulgare* L.) with 120 mM of sodium chloride. Results of a recent study by Kapoor and Srivastava (2010) on *Vigna mungo* (L.) support the previous results. They observed an increase in protein content when increasing salt concentration.

Despite results of many researchers indicating a positive effect for salt stress on protein content, there are people who presented contrary results as well, indicating a negative effect for salt stress. For example, Chen et al. (2007) found that exposing *Vigna unguiculata* (L.) plants, at the age of 14 days, to salt treatment using 75 mM of sodium chloride, reduced soluble protein content in the plant.

The results of the previous study were confirmed by Cheruth et al. (2008), with their study on *Catharnathus roseus* (L.) and Khosravinejad et al. (2009) with their study on barley *Hordeum vulgare* L. They observed that the treatment with sodium chloride reduced protein content in the plant seedlings.

References

Andriolo, J.L., Gean, L.D., Maiquel, H.W., Rodrigo, D.S.G., Gis, O.C.B., 2005. Growth and yield of lettuce plants under salinity. Hortic. Bras. 23 (4), 931–934.

Bayuelo Jimenez, J.S., Debouk, D.G., Lynch, J.P., 2002. Salinity tolerance in phaseolus species during early vegetative growth. Crop Si. 42, 2184–2192.

Beltagi, M.S., Ismail, M.A., Mohamed, F.H., 2006. Induced salt tolerance in common bean (*Phaseolus vulgaris* L.) by gamma irradiation. Pak. J. Biol. Sci. 6, 1143–1148.

Cachorro, P., Martinez, R., Ortiz, A., Cerda, A., 1995. Abscisic acid and osmotic relations in (*Phaseolus vulgaris* L.) under saline conditions. Plant Sci. 95, 29–32.

Chao, W.S., Gu, Y.Q., Pautot, V., Bray, E.A., Walling, L.L., 1999. Leucine aminopeptidase RNAs, proteins, and activities increase in response to water deficit, salinity, and the wound signals systemin, methyl jasmonate, and abscisic acid. Plant Physiol. 120, 979–992.

Chen, C., Tao, C., Peng, H., Ding, Y., 2007. Genetic analysis of salt stress responses in asparagus bean (*Vigna unguiculata* L. ssp. Sesquipedalis verdc.). J. Hered. 98 (7), 655–665.

Cheruth, J., Ragupathi, G., Ashot, K., Paramasivam, M., Beemarao, S., Rajaram, P., 2008. Interactive effects of triadimefon and salt stress on antioxidative status and ajmalicine accumulation in *Catharanthus roseus*. Acta Physiol. Plant. 30 (3), 287–292.

Dantus, B.F., Ribeiro, L., Aragao, C.A., 2005. Physiological response of cowpea seeds to salinity stress. Rev. Bras. Sementes. 27 (1), 144–148.

Day, A.D., Thompson, P.K., 1975. Effect of soil moisture regimes on growth of barely. Agron. J. 67, 430–433.

Etherton, B., 1963. Relationship of cell transmembrane electropotential to potassium and sodium accumulation ratios in oat and pea seedlings. Plant Physiol. 38, 581–585.

Fisher, R.A., Turner, N.C., 1978. Plant productivity, in arid and semi arid zones. Ann. Rev. Plant Physiol. 29, 897–912.

Gama, P.B.S., Inanaga, S., Tanaka, K., Nakazawa, R., 2007. Physiological response of common bean (*Phaseolus vulgaris* L.) seedlings to salinity stress. Afr. J. Biotechnol. 6 (2), 79–88.

Gama, P.B.S., Tanaka, K., Eneji, A., Eltayeb, A.E., Elsiddig, K., 2009. Salt induced stress effects on biomass, photosynthetic rate and reactive oxygen species scavenging enzyme accumulation in common bean. J. Plant Nutr. 32 (5), 837–854.

Gerber, Susan B., Kristin, E. Voelki, Andrson, T.W., Finn, Jenemy D., 1997. SPSS Guide to New Statistical Analysis of Data. Springer, New York.

Gusev, N.A., 1960. Some methods in studying plant water relation. Leningrad Acad. Of science, U.S.S.R.

Ha, E., Ikhajiagba, B., Bamidele, J.F., Ogic-odia, E., 2008. Salinity effects on young healthy seedling of kyllingia peruviana collected from escravos, Delta state. Global J. Environ. Res. 2 (2), 74–88.

Hamada, A.M., 1995. Alleviation of the adverse effects of NaCl on germination, seedling, growth and metabolic activities of maize plants by calcium salts. Bull. Fac. Sci. Assiut Univ. 24, 211–220.

Hasegewa, P.M., Bressan, R.A., Zhu, J.K., Bohnert, H.J., 2000. Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Physiol. Mol. Biol. 51, 463–499.

Houimli, S.I.M., Denden, M., Elhadj, S.B., 2008. Induction of salt tolerance in pepper (B′ or Hbt 1′mmtt1) by 24-epibrassinolide. Eurasia J. Biol. Sci. 2, 83–90.

Jamil, M., Lee, C.C., Rehman, S.U., Lee, D.B., Ashraf, M., Rha, E.S., 2005. Salinity (NaCl) tolerance of brassica species at germination and early seedling growth. Electronic J. Environ. Agric. Food Chem., ISSN: 1579–4377.

Jamil, M., Rehman, S., Rha, E.S., 2007a. Salinity effect on plant growth, ps11 photochemistry and chlorophyll content in sugar beet (*Beta vulgaris* L.) and cabbage (*Brassica oleracea capitata* L.). Pak. J. Bot. 39 (3), 753–760.

Jamil, M., Rehman, S.U., Lee, K.J., Kim, J.M., Rha, H.K., 2007b. Salinity reduced growth ps2 photochemistry and chlorophyll content in radish. Sci. Agric. (Piracicaba, Braz.) 64 (2), 111–118.

Kapoor, K., Srivastava, A., 2010. Assessment of salinity tolerance of *Vinga mungo* var. Pu-19 using ex vitro and in vitro methods. Asian J. Biotechnol. 2 (2), 73–85.

Karen, W., Anthony, R.Y., Timothy, J.F., 2002. Effects of salinity and ozone, individually and in combination on growth and ion contents of two chickpea (*Cicer aritinum* L.) varieties. Environ. Pollut. 120 (2), 397–403.

Kaymakanova, M., Stoeva, N., 2008. Physiological reaction of bean plants (*Phaseolus vulgares* L.) to salinity stress. Gen. Appl. Plant Physiol. 34 (3–4), 177–188.

Kaymakanova, M., Stoeva, N., Mincheva, T., 2008. Salinity and its effect on physiological response of bean (*Phaseolus vulgares* L.). central. Eur. Agric. 9 (4), 749–756.

Khosravinejad, F., Heydari, R., Farboodnia, T., 2009. Effect of salinity on organic solutes contents in barley. Pak. J. Biol. Sci. 12 (2), 158–162.

Lee, G., Carrow, R.N., Duncan, R.R., 2004. Photosynthetic responses to salinity stress of halophytic seashore *paspalum ecotypes*. Plant Sci. 166 (6), 1417–1425.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Mathur, N., Singh, J., Bohra, S., Bohra, A., Vyas, A., 2006. Biomass production, productivity and physiological changes in moth bean genotypes at different salinity levels. Am. J. Plant Physiol. 1 (2), 210–213.

Mazher, A.M.A., El-Quesni, E.M.F., Farahat, M.M., 2007. Responses of ornamental and woody trees to salinity. World J. Agric. Sci. 3 (3), 386–395.

Memon, S.A., Hou, X., Wang, L.J., 2010. Morphological analysis of salt stress response of pak Choi. EJEAFChe 9 (1), 248–254.

Misra, A., Sahu, A.n., Misra, M., Singh, P., Meera, I., Das, N., Kar, M., Sahu, P., 1997. Sodium chloride induced changes in leaf growth, and pigment and protein contents in two rice cultivars. Biol. Plantarum 39 (2), 257–262.

Misra, A.N., Latowski, D., Stirzalka, K., 2006. The xanthophyll cycle activity in kidney bean and cabbage leaves under salinity stress. Biomed. Life Sci. 53 (1), 102–109.

Munns, R., 1993. Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant Cell Environ. 16, 15–24.

Munns, R., 2002. Comparative physiology of salt and water stress. Plant Cell Environ. 25, 239–250.

Murillo-Amador, B., Yamada, S., Yamaguch, T., Puente, E.R., Serrano, N.A., Hernandez, L.G., Aguilar, R.L., Dieguez, E.T., Garibay, A.N., 2007. Salinity toxicity influence of calcium silicate on growth physiological parameters and mineral nutrition in two legume species under salt stress. J. Agron. Crop Sci. 193 (6), 413–421.

Mustard, J., Renault, S., 2006. Response of red-osier dogwood (*Cornus sericea*) seedling to NaCl during the onset of bud break. Can. J. Bot. 84 (5), 844–851.

Nedjimi, B., Daoud, Y., Touati, M., 2006. Growth, water relations, proline and ion content of invitro cultured *Atriplex halimus* sub sp. Schweinfurthii as affected by $CaCl_2$. Commun. Biometry Crop Sci. 1 (2), 79–89.

Netondo, G.W., Onyango, J.C., Beck, E., 2004. Crop physiology and metabolism Sorghum and salinity II – gas exchange and chlorophyll fluorescence of sorghum under salt stress. Crop Sci. 44 (3), 806–811.

Neumann, P., 1997. Salinity resistance and plant growth revisited. Plant Cell Environ. 20, 1193–1198.

Niaz, B.H., Athar, M., Salim, M., Rozema, J., 2005. Growth and ionic relations of fodder beet and sea beet under saline. CEERS 2 (2), 113–120.

Orak, A., Ates, E., 2005. Resistance of salinity stress and available water levels at the seedling stage of the common vetch (*Vicia sativa* L.). Plant Soil Environ. 51 (2), 51–56.

Qin, J., Dong, W., He, K., Chen, J., Wang, A.Z., 2009. Short-term responses to salinity of seabuckthorn (*Hippohae rhamnoides* L.). seedlings in the extremely cold and saline Qinghai region of China. For. Stud. China 11 (4), 231–237.

Raul, L., Andres, O., Armado, L., Bernardo, M., Enrique, T., 2003. Response to salinity of three grain legumes for potential cultivation in arid areas (plant nutrition). Soil Sci. Plant Nutr. 49 (3), 329–336.

Rodriguez, P., Amico, J.D., Morales, D., Blanco, M.J.S., Alarcon, J.J., 1997. Effects of salinity on growth, shoot water relations and root hydraulic conductivity in tomato plants. J. Agric. Sci. 128, 439–444.

Rui, L., Wei, S., Mu-xiang, C., Cheng-jun, J., Min, W., Bo-ping, Y., 2009. Leaf anatomical changes of *Burguiera gymnorrhiza* seedlings under salt stress. J. Trop. Subtrop. Bot. 17 (2), 169–175.

Saffan, S.E., 2008. Effect of salinity and osmotic stresses on some economic plants. Res. J. Agric. Biol. Sci. 4 (2), 159–166.

Saqib, M., Zorb, C., Schubert, S., 2006. Salt resistant and salt-sensitive wheat genotypes show similar biochemical reaction at protein level in the first phase of salt stress. J. Plant Nutr. Soil Sci. 169, 542–548.

Schreiber, H.A., Stanberey, C.O., 1965. Barley production as influenced by timing of soil moisture and timing on Nappliciation. Agron. J. 57, 442–445.

Shanon, M.C., 1986. New insights in plant breeding efforts for improved salt tolerance. Hort. Technol. 6, 96–99.

Sibole, J.V., Cabot, C., Poschenreder, C., Barcelo, J., 2003. Efficient leaf ion partitioning, an overriding condition for abscisic acid-controlled stomatal and leaf growth responses to NaCl salinization in two legumes. J. Exp. Bot. 54 (390), 2111–2119.

Siler, B., Misic, D., Filipovic, B., Popovic, Z., Cvetic, T., Mijovic, A., 2007. Effects of salinity on in vitro growth and photosynthesis of common centaury (*Centaurium erythraea* Rafn.). Arch. Biol. Sci. 59 (2), 129–134.

Smith, J.H.C., Benitez, A., 1955. Chlorophylls analysis in plant materials. In: Peach, K., Tracey, M.V. (Eds.), . In: Modern Methods of Plant Analysis, vol. 4. Springer-Verlag, Berlin, pp. 142–196.

Steel, R.G., Torrie, J.H., 1981. Principles and Procedures of Statistics, second ed. McGraw Hill Book Comp., N.Y.

Stoeva, N., Kaymakanova, M., 2008. Effect of salt stress on the growth and photosynthesis rate of bean plants (*Phaseolus vulgares* L.). J. Cent. Eur. Agric. 9 (3), 385–392.

Sultana, N., Ikeda, T., Itoh, R., 2000. Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. Environ. Exp. Bot. 42 (3), 211–220.

Taffouo, V.D., Kouamou, J.K., Ngalangue, L.M.T., Ndjeudji, B.A.N., Akoa, A., 2009. Effects of salinity stress on growth, ions partitioning and yield of some cowpea (*Vigna ungiuculata* L., walp) cultivars. Int. J. Bot. 5 (2), 135–143.

Taffouo, V.D., Wamba, O.F., Yombi, E., Nono, G.V., Akoe, A., 2010. Growth, yield, water status and ionic distribution response of three bambara groundnut (*Vigna subterranean* (L.) verdc.) landraces grown under saline conditions. Int. J. Bot. 6 (1), 53–58.

Tort, N., Turkyilmaz, B., 2004. A physiological investigation on the mechanisms of salinity tolerance in some barley culture forms. J.F.S. 27, 1–16.

Turan, M.A., Kalkat, V., Taban, S., 2007. Salinity-induced stomatal resistance, proline, chlorophyll and Ion concentrations of bean. Int. J. Agric. Res. 2 (5), 483–488.

Yagmur, M., Kaydan, D., Okut, N., 2006. Potasyum Uygulamasinin Tuz Stresindeki arpanin fotosentetik pigment. Tarim Bilimeri Dergis 12 (2), 188–194.

Yao, A.R., 1998. Molecular biology of salt tolerance in the context of whole plant physiology. J. Exp. Bot. 49, 915–929.

Yilmaz, H., Kina, A., 2008. The influence of NaCl salinity on some vegetative and chemical changes of strawberries (*Fragaria x ananassa* L.). Afr. J. Biotechnol. 7 (18), 3299–3305.

Zhang, J., Nguyen, H.T., Blum, A., 1999. Genetic analysis of osmotic adjustment in crop plant. J. Exp. Bot. 50, 291–302.

Zhao, G.Q., Ma, B.L., Ren, C.z., 2007. Growth, gas exchange, chlorophyll fluorescence and ion content of naked oat in response to salinity. Crop Sci. 47 (1), 123–131.

Zhu, J.K., 2001. Plant salt tolerance. Trends Plant Sci. 6, 66–71.