Plant Omics Journal
2(2):60-69 (2009)
ISSN: 1836-3644

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Plant growth, metabolism and adaptation in relation to stress conditions XXIII. Salinity-biofertility interactive effects on growth, carbohydrates and photosynthetic efficiency of *lactuca sativa*

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Abstract

The interactive effects of different levels of NaCl and two biofertilizers on certain aspects of growth and metabolism of lettuce plants were investigated. The addition of a recommended dose of phosphorein biofertilizer to salinized soil, induced significant increases in all growth and reproductive parameters determined in growing lettuce plants. On the other hand, fertigation of such sodic salty soil with a recommended dose of nitrobein biofertilizer, induced slight decreases in the growth and reproductive parameters. The carbohydrate as well as pigment components and the activity of PS II of the salinized lettuce plants fertigated with phosphorein, were increased throughout the three successive growth stages, above the control levels. On the other hand, nitrobein did slight changes in all the metabolites determined, throughout the entire period of the experiment. The results are discussed in relation to applicability of the biofertilizers to sodic salty soil.

Keywords: lettuce; salinity; biofertilizers; growth; carbohydrates; pigments; PS II activity.

Introduction

Various crops show different sensitivities to different salinity levels. Plants are generally divided into four salinity rating groups: sensitive, moderately sensitive (lettuce), moderately tolerant, and tolerant (Jungklang, 2005). Lettuce is an important leafy vegetable crop in Egypt. It is considered as an excellent nutritive source of minerals and vitamins as it is consumed as fresh green salad. Crop with such promising potentialities for local markets, would necessitate much research for improving its production quantity and quality.

In Egypt, soil fertility is diminishing gradually due to soil erosion, loss of nutrients, accumulation of salts and other toxic elements, water logging and unbalanced nutrient compensation (Shehata and El-Khawas, 2003). Biofertilizers are considered as an important part of environment friendly sustainable agricultural practices (Shehata and El- Khawas, 2003). The biofertilizers include mainly the nitrogen fixing, phosphate solubilizing and plant growth-promoting microorganisms (Goel *et al.,* 1999).

Applying phosphorein to the soil increased soluble phosphate, plant growth, dry matter, protein and N and P contents of maize plant (El- Sawah *et al*., 1995). Nitrogen supply is a key-limiting ingredient in crop production in many African countries. The beneficial effect of bio-N-fertilizers application is the improvement of nitrogen contents, as well as the improvement of the physical and chemical properties of the yield (Shehata and El-Khawas, 2003). In this work, the possibility of nullification of salt stress damage effects by phosphorein and nitrobein biofertilizers was investigated.

Materials and methods

Plant material

Pure strain of *Lactuca sativa* cv. baladi transplants (25-d-old) were kindly supplied by the Horticulture Research Centre, Ministry of Agriculture, Giza, Egypt.

Chemicals

Egyptian biofertilizers are commonly used with vegetable crops, for increasing production and improvement of quality, only two were chosen, namely: phosphorein (containing P dissolving bacteria; *Bacillus megatherium* var. phosphaticum) and nitrobein (containing N fixing bacteria; *Azospirillum sp.* and *Azotabacter sp.*). These were kindly supplied by Soil Fertility Sector at Mansoura, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. Analytical grade chemicals were used throughout this investigation.

Measurement of growth parameters

The length of roots and shoots was measured to the nearest mm. Immediately after sampling, the fresh weight of samples was recorded before being dried at 80^{o}C in an aerated oven to constant dry weight. The leaf area was measured using a recording planimeter.

Estimation of carbohydrates

The method of extraction of carbohydrate fractions was patterned after those adopted by Younis (1963) and Handel (1968). Glucose was estimated in the ethanolic extract using the o-toluidine procedure of Fertris (1965). Sucrose was determined by first degrading reactive sucrose present in 0.1 cm^3 extract with 0.1 cm^3 5.4 N KOH at 97 ^{0}C for 10 min. Three cm^3 of freshly prepared anthrone reagent [150 mg anthrone + 100 cm^3 72% (w/w) H_2SO_4] were then added to the cooled reaction products and the mixture was heated at 97 ^{0}C for 5 min, cooled and the developed colour was read at 620 nm, using spectrophotometer. Polysaccharides were determined by the method of Thayermanavan and Sadasivam (1984).

Determination of pigments

Chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids (Car) were determined following the method of Metzner *et al.* (1965).

Estimation of photosynthetic activity (PS II)

An assay that measures the change in absorbance of a reaction mixture containing dichlorophenolindo-phenol (DCPIP) can demonstrate the activities of photosynthesis (Arnon, 1949 and Trebest, 1972). In this assay, the degree of reduction of DCPIP is determined by measuring the change in absorbance of light at 600 nm with a spectrophotometer. The assay reaction mixture for determination of PS II activity contained 200 mM-Na phosphate (pH 7.2), 2 mM-$MgCl_2$ and 0.5 mM 2,6-DCPIP. A calibration curve in terms of micromoles of dye reduced (Dean and Miskiewicz, 2003) was made using 2, 6-DCPIP range between 10-50 µM in the reaction mixture (4 cm^3).

Time course experiment

A large-scale experiment, carried outdoor, under normal day and light conditions, was designed so as to study the effects of four different levels of NaCl, namely: 4 mmhos[*], 6 mmhos, 8 mmhos and 10 mmhos; each level being used either alone or in combination with a recommended dose of each of the attempted biofertilizers. Thus, 65 pots, divided into 13 groups (each of five pots) were used. One of these groups was left without treatment to serve as water control and the other 12 groups were separately treated with each of the four NaCl levels either alone or in combination with the recommended dose of one of the biofertilizers. Thus, a total of 13 treatments representing all planned possible combinations of salinity levels and biofertilizers were penta-replicated in a completely randomized design.

Uniformly-sized groups of 25-d-old lettuce (*lactuca sativa* cv. Baladi) transplants were selected, washed thoroughly with tap water and then transplanted in a mixture of clay-loamy soil (2:1, v/v) in pots (30×28×26 cm). The soil, obtained from the Agriculture Research Station of Mansoura, Dakahlia Governorate, was taken from the upper 30 cm arable layer. All pots contained equal amounts of homogeneous soil (8 Kg) in which eight to nine lettuce transplants were planted and given one week for establishment in the soil. That was followed by thinning and only five plants/pot were left for experimentation.

Treatment of lettuce transplants with NaCl and the biofertilizers was carried out after one week from the date of transplantation. The appropriate amounts of NaCl and the recommended dose for each of the biofertilizers used were calculated and added to each pot with irrigation water.

[*] 1 mmhos/cm = 1ds/m = 10 meq/l = 640 ppm approx.

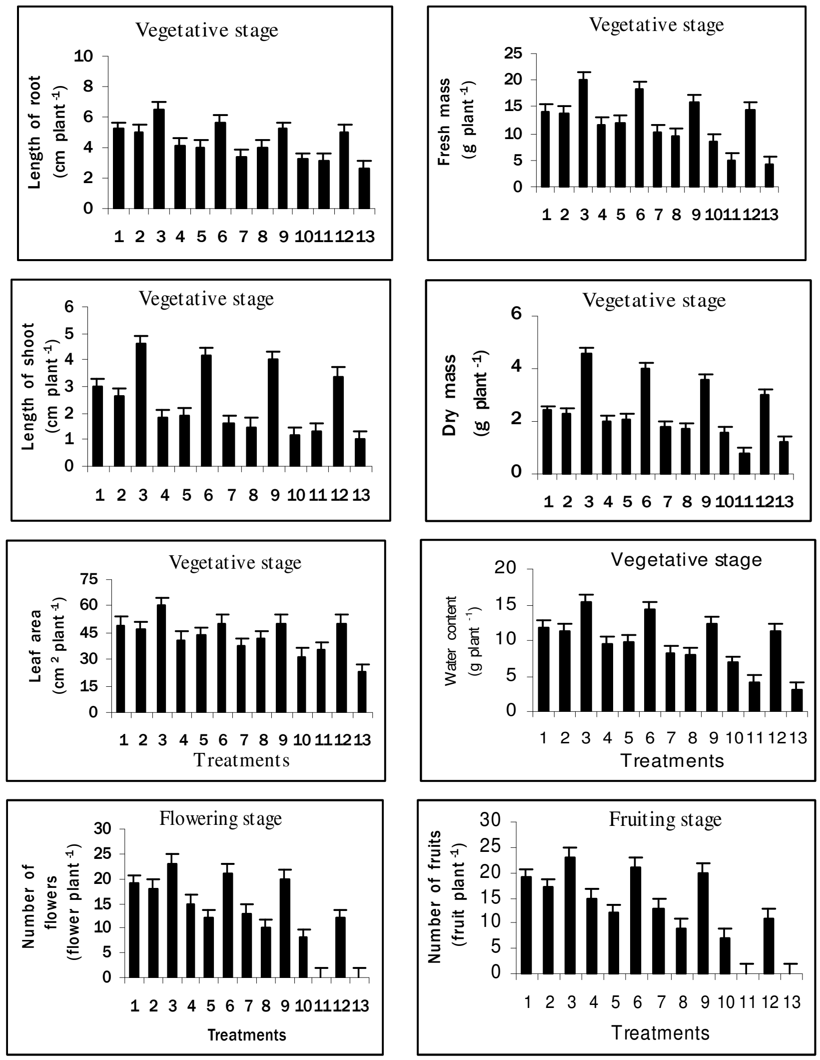

Fig 1. The effects of increasing concentrations of NaCl either alone or in combination with phosphorein or nitrobein biofertilizers on growth and reproductive parameters of lettuce plants. Vertical bars above each panel represent the L.S.D at 5% level.

In this figure treatments were as follows:

1) Control (H_2O)
2) 4 mmhos NaCl
3) 4 NaCl + phosphorein
4) 4 NaCl + nitrobein
5) 6 mmhos NaCl
6) 6 NaCl + phosphorein
7) 6 NaCl + nitrobein
8) 8 mmhos NaCl
9) 8 NaCl + phosphorein
10) 8 NaCl + nitrobein
11) 10 mmhos NaCl
12) 10 NaCl + phosphorein
13) 10 NaCl + nitrobein

All pots were irrigated with tap water, every three days, to maintain the soil at the field capacity throughout the experiment. Samples were taken after 25, 122, and 150 days from the date of transplantation; representing vegetative, flowering and fruiting stages, respectively. Sampling was made in a way so as to include all plants allotted for each treatment in the five pots. Samples were used for determination of growth parameters as well as carbohydrate and photosynthetic components. The data presented were statistically analyzed; an analysis of variance was performed on the data using the F-ratio test. Comparisons among means from duplicate determinations and quadruplicate samples, were carried out by calculating the least significant difference (L.S.D.) at 5% level.

Results and discussion

Throughout the present investigation, the pattern of changes in growth components including the total amount and the relative composition of the pigment and carbohydrate pools were compared at all the three growth and reproductive stages of the lettuce plants. This indicated the similarity of plant growth as well as pigment and carbohydrate compositional responses to NaCl when used either alone or in combination with phosphorein or nitrobein biofertilizer, irrespective of the stage of growing lettuce plants. Therefore, for convenience of expression of our results, the data obtained from only the vegetative stage are mainly presented in the respective figures.

Changes in growth parameters

The values of growth and reproductive parameters appeared to decrease progressively and significantly with an increase in concentration of the administered NaCl, as compared with control levels (Fig 1). Addition of each of the two biofertilizers to the variously salinized lettuce plants induced the following changes:

(a) Phosphorein significantly increased all growth and reproductive parameters; the magnitude of response being most pronounced with the lowest concentration (4 mmhos NaCl) of salinity (Fig 1).

(b) Addition of nitrobein appeared without any beneficial effect on all growth and reproductive parameters determined. Thus, the obtained values of NaCl + nitrobein-treated plants appeared consistently lower than the comparable values for the control salinized plants. The magnitude of decrease appeared to be progressive with the increase in concentration of NaCl applied to soil in combination with nitrobein (Fig 1).

Greenway (1973) reported that the inhibited vegetative growth in highly saline media might be due to reduced cell division, cell enlargement and cell wall expansion. The growth of plants may be reduced under salt stress because of (a) an osmotic stress due to a lowering of the external water potential, or (b) effects of specific ions on metabolic processes ranging from the absorption of nutrients to enzyme activation or inhibition. Lettuce plant performance under different treatments of NaCl appeared to be affected both by ionic and osmotic effects (Younis *et al.,* 2008).

The interaction between salinity and P and N nutrition of plants is highly dependent upon the plant species (or cultivar), plant development age (Zhukovskaya, 1973), the composition and level of salinity, and the concentration of P and N in the substrate. Therefore, depending upon plants selected and conditions of the experiment, different results can be obtained (Zhukovskaya, 1973). In support of the present results, addition of bio-phosphorus fertilizers resulted in significant increases in vegetative growth , plant dry weight as well as in total marketable yield and its components of a variety of crop plants (Srivastava *et al.,* 1998; Abdalla, 2002; Hassan *et al*., 2005).

The promoting effect of phosphorein biofertilizer on growth and development of the variously salinized lettuce plants may be due to the active bacteria in phosphorein which, as stated by Sherif *et al*. (1997), allow to convert insoluble phosphate to soluble forms; or secreting organic acids such as formic, acetic and lactic acids. Such acids lower the pH and bring about the dissolution of bound forms of phosphate and render them available for growing plants.

The response of plants to bio-N-fertilizers was studied by Fisinin *et al*. (1999), Rizk and Shafeek (2000) and Adam (2002) who reported that bio-N-fertilizer has a great number of bacteria which are responsible for nitrogen fixation by atmosphere. The stimulatory effects of nitrobein biofertilizer might be attributed to the activation of the growth of microflora including production of many plant growth stimulators.

However, the reduction in growth as well as in reproductive parameters of salinized lettuce plants treated with nitrobein (see Fig 1) lend a strong support to the findings of El-Saht *et al.* (2000 and 2001). They stated that in certain plants, the reduced form of nitrogen directly available for

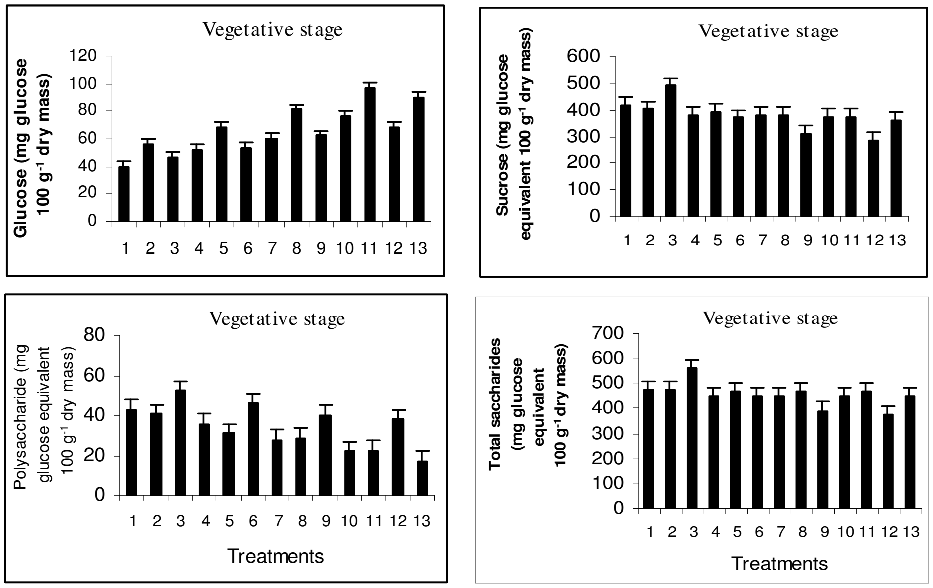

Fig 2. The effects of increasing concentrations of NaCl either alone or in combination with phosphorein or nitrobein biofertilizers on the carbohydrate content of lettuce plants. Vertical bars represent the L.S.D at 5% level. Treatments 1-13 as in Fig 1.

direct assimilation is NH_4^+ from symbiotic N_2 fixation (nitrobein), urea application or photorespiration. Since NH_4^+ is generally toxic to plant cells at high concentrations (Vance *et al.,* 1988), this could be the reason that salinized lettuce plants did not respond positively to the recommended dose of nitrobein biofertilizer. So, the present observed decreases in all growth and reproductive parameters appeared to be a function of N pools maintained with the recommended doses of nitrobein biofertilizer (Fig 1).

Changes in Carbohydrate content

As presented in Fig 2, we observed a sharp progressive significant increase in glucose content with increased concentration of NaCl applied; an opposite situation being apparent for sucrose and polysaccharide contents. Total saccharides either did not change (at the vegetative and fruiting stages) or decreased (at the flowering stage), below the control content.

Inclusion of phosphorein into the media of the salinized lettuce plants either significantly decreased (at the vegetative stage) or significantly increased (at the flowering and fruiting stages) the contents of glucose and sucrose. The polysaccharide contents appeared to increase throughout the entire period of the experiment. On the other hand, for the total saccharides, their content appeared either to decrease significantly (at the vegetative stage) or to increase at the latter flowering and fruiting stages (Fig 2). Administration of nitrobein to NaCl-treated plants appeared, in general, to decrease the contents of glucose, sucrose, polysaccharides and consequently the total saccharides content (Fig 2).

In support to the present results, Kafiet *et al.* (2003) and Younis *et al.* (2008) observed a significant decrease in soluble and hydrolysable sugars content of wheat and lettuce plants treated with NaCl. Fernandes *et al.* (2004) reported that glucose content decreased with salt stress, sucrose content was almost three times higher in *Lupinus albus* plants treated with 150 mM NaCl and fructose content did not change significantly. The most significant response of lupin plants to excess NaCl is the increase of sucrose content in leaves, partially due to increased sucrose synthase activity under salinity. Furthermore, Timpa *et al.* (1986) found that the salt-stressed cotton plants showed two to three times greater amounts of carbohydrates (glucose and sucrose) over the values determined for the control samples. These carbohydrate changes are of particular importance because of their direct relationship with such physiological processes as photosynthesis, translocation and

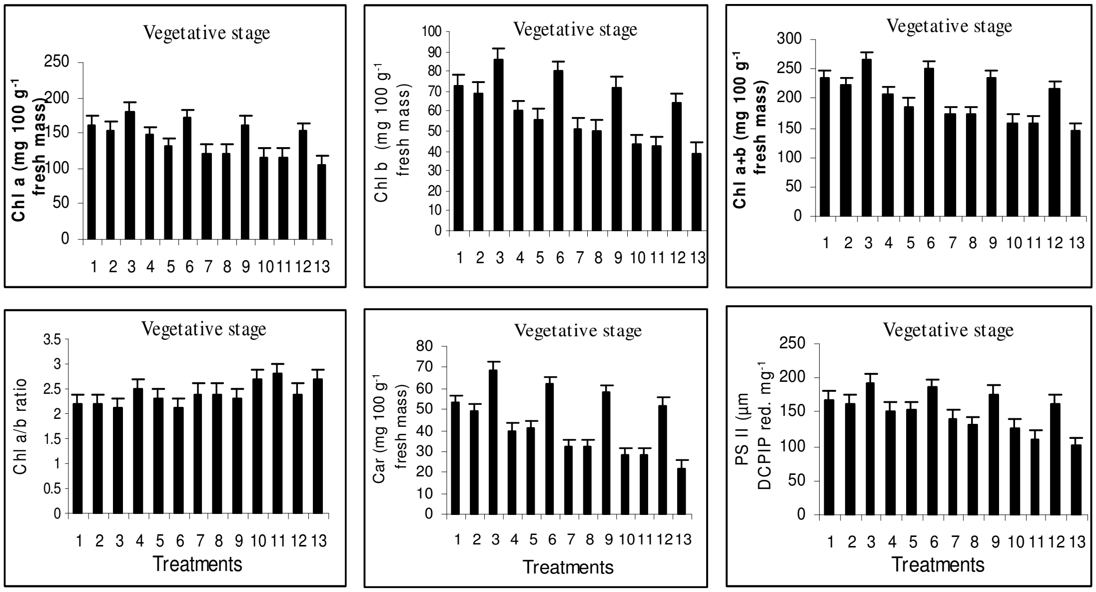

Fig 3. The effects of increasing concentrations of NaCl either alone or in combination with phosphorein or nitrobein biofertilizers on the photosynthetic parameters and on the photosynthetic activity (PS II activity) in lettuce plants. Vertical bars represent the L.S.D at 5% level. Treatments 1-13 as in Fig 1.

respiration. Among the soluble carbohydrates, Kerepesi and Galiba (2000) stated that sucrose and fructans have a potential role in adaptation to stress conditions. A possible explanation for the increased total saccharides content of salt-treated lettuce plants fortified with phosphorein biofertilizer at flowering and fruiting stages could be due to the fact that phosphorein biofertilizer plays a fundamental role in converting P fixed form to be soluble form more easily used for plant nutrition by plants. Similar results were reported by Zayed (1998) who found that soil microorganisms, known as phosphate solubilizing bacteria, play a fundamental role in converting P fixed form to be available for plant nutrition.

In response to nitrobein application, the observed decreases in carbohydrate constituents throughout the experimental period appeared to coincide with all the changes in growth and developmental parameters as well as photosynthetic parameters (Fig 1, 2 and 3). These correlated changes appear to be a function of N pools maintained, in salinized lettuce plants, with the recommended dose of nitrobein used in the present study.

Changes in photosynthetic parameters

In relation to water control levels, the contents of photosynthetic pigments in lettuce plants treated with increasing concentrations of NaCl, showed a progressive significant decrease with an increase in the concentration of NaCl throughout the three growth stages. With increased concentration of NaCl used, a decrease in the total content of both chlorophylls (a+b) was observed and this change was associated with a progressive increase in the values of Chl a/b maintained throughout the experimental period (Fig 3).

Supplemental addition of phosphorein biofertilizer to the salinized lettuce plants, induced significant increase in Chl a, Chl b and Car contents. The magnitude of response was most pronounced with 4 mmhos NaCl + phosphorein. On the other hand, administration of nitrobein to the salinized media maintaining lettuce plants, induced additive decreases in Chl a, Chl b and Car below those levels detected in the control salinized plants. The magnitude of response was most pronounced with 10 mmhos NaCl + nitrobein (Fig 3).

Again, the pattern of changes in PS II activity of the salinized lettuce plants, showed a progressive significant decrease with an increase in concentration of NaCl. On the other hand, PS II activity in the salinized lettuce plants fortified with the optimum dose of phosphorein, appeared to increase significantly with an increase in salinization (Fig 3). For nitrobein, the pattern of changes in PS II activities of the salinized and salinized + biofertilizer-treated plants were essenti-

Table 1. The percentage inhibitory effects of increasing concentrations of NaCl and the optimum percentage recovery (improvement) induced by application of biofertilizers, for the various growth, carbohydrate and photosynthetic components of lettuce plants at the vegetative growth stage.

	Growth components						
	Treatments \ parameters	Length of root	Length of shoot	Leaf area	Fresh mass	Dry mass	Water content
Inhibition	4 mmhos NaCl	-3.8	-13.3	-5.7	-4.2	-4.2	-4.2
	6 mmhos NaCl	-23.1	-36.7	-11.6	-16.2	-12.5	-16.9
	8 mmhos NaCl	-23.1	-50.0	-15.9	-32.4	-29.2	-33.1
	10 mmhos NaCl	-40.4	-56.7	-29.1	-65.5	-66.3	-65.3
Recovery	4 mmhos NaCl+phosphorein	28.8	66.6	28.1	45.7	95.9	35.6
	4 mmhos NaCl+nitrobein	-17.4	-26.7	-11.0	-14.1	-12.5	-14.4
	6 mmhos NaCl+phosphorein	30.8	76.7	13.2	45.1	79.2	38.2
	6 mmhos NaCl+nitrobein	-11.5	-10.0	-13.2	-12.7	-12.5	-12.8
	8 mmhos NaCl+phosphorein	24.0	83.3	17.9	45.1	79.2	38.2
	8 mmhos NaCl+nitrobein	-15.4	-10.0	-20.5	-8.4	-4.1	-9.3
	10 mmhos NaCl+phosphorein	36.6	70.0	31.1	66.2	91.3	61.1
	10 mmhos NaCl+nitrobein	-9.6	-10.0	-24.8	-4.9	16.3	-9.3

	Carbohydrate components				
	Treatments \ parameters	Glucose	Sucrose	Polysaccharide	Total saccharides
Inhibition	4 mmhos NaCl	43.1	-3.6	-5.8	0.1
	6 mmhos NaCl	75.3	-6.0	-27.4	-1.0
	8 mmhos NaCl	107.1	-8.2	-33.2	-0.7
	10 mmhos NaCl	146.4	-10.8	-47.6	-0.8
Recovery	4 mmhos NaCl+phosphorein	-25.0	21.4	27.4	18.0
	4 mmhos NaCl+nitrobein	-10.4	-4.8	-10.7	-5.8
	6 mmhos NaCl+phosphorein	-39.8	-5.1	34.8	-4.7
	6 mmhos NaCl+nitrobein	-22.2	-2.7	-7.6	-4.7
	8 mmhos NaCl+phosphorein	-48.7	-17.3	26.5	-16.3
	8 mmhos NaCl+nitrobein	-13.3	-2.1	-15.8	-4.2
	10 mmhos NaCl+phosphorein	-72.7	-20.5	36.0	-20.1
	10 mmhos NaCl+nitrobein	-16.8	-2.7	-13.0	-4.7

	Photosynthetic components				
	Treatments \ Parameters	Chl a	Chl b	Car	Total pigments
Inhibition	4 mmhos NaCl	-5.0	-5.6	-7.5	-5.6
	6 mmhos NaCl	-18.6	-23.4	-22.6	-20.6
	8 mmhos NaCl	-24.2	-31.6	-39.6	-28.9
	10 mmhos NaCl	-28.0	-42.5	-47.2	-35.2
Recovery	4 mmhos NaCl+phosphorein	16.8	23.2	37.7	22.3
	4 mmhos NaCl+nitrobein	-3.7	-12.3	-17.0	-8.4
	6 mmhos NaCl+phosphorein	24.8	32.8	39.6	29.6
	6 mmhos NaCl+nitrobein	-6.2	-6.6	-17.0	-8.3
	8 mmhos NaCl+phosphorein	24.8	30.1	49.0	30.6
	8 mmhos NaCl+nitrobein	-3.8	-9.6	-7.6	-6.0
	10 mmhos NaCl+phosphorein	22.4	30.1	45.3	28.5
	10 mmhos NaCl+nitrobein	-6.8	-4.1	-11.3	-7.0

ally comparable to the changes in the photosynthetic pigments (Fig 3).

Our results are supported by the previous studies showing salinity as well as many other stress factors causing changes in physiological processes in plants (Younis *et al.,* 2008). Of utmost importance, photosynthesis of many plants decreased drastically as a result of NaCl salinity (Greenway and Munns, 1980). Data relating to the effect of salinity on the primary photochemical reactions under *in vivo* conditions, however, are limited and conflicting (Maslenkova *et al*., 1991). Salinity-caused reduction in net photosynthetic rate has been attributed to reduced stomatal conductance and/or to reduction in capacity of photosynthetic machinery (Seeman and Sharkey, 1986).

Abu-Hussein *et al.* (2002) showed that the micro-organisms in phosphorus biofertilizers produce growth promoting substances which increase the plant growth leading to an increased assimilation rates. The present results concerning the positive effects of phosphorein application to salinized lettuce plants upon the photosynthetic components (pigment content and PS II activity), appeared to coincide with those positive effects on the dry mass accumulation, carbohydrates and yield in lettuce plants. In both cases the values maintained appeared to increase positively and significantly. This gives us a reason to admit that one of the factors providing higher dry mass accumulation and higher yield in treated lettuce plants was the increased capacity for CO_2 assimilation (Jungklang, 2005; Younis *et al.,* 2008).

The influence of nitrogen on plant growth and development is often connected with the process of photosynthesis because the quantity of nitrogen, in the highest degree, determines the formation and the functional state of assimilation apparatus of plants (Ivanova and Vassilev, 2003). It is well known that nitrogen influences the content of photosynthetic pigments, the synthesis of the enzymes taking part in the carbon reduction and the formation of the membrane system of chloroplasts (Lishtenthaler and Wellburn, 1983).

In the present study, the decreasing tendency of Chl content and Chl fluorescence appeared to coincide with the biomass reduction in salinized lettuce plants fortified with nitrobein throughout the entire period of the experiment (Fig 3). The decreased chlorophyll content can be attributed to the fact that NaCl stress decreases total chlorophyll content by increasing the activity of cell degrading chlorophyllase (El-Saht, 2001; Younis *et al*., 2008) and inducing the destruction of chloroplast structure and the instability of pigment protein complex (Singh and Dubey, 1995). Moreover, we can point out that the decrease in Chl a+b content is mainly attributed to the distribution of Chl b, which appears to be more sensitive to salinity than Chl a. Jungklang (2005) also reported that the decrease in Chl a+b content in *Sesbania rostrata* and *Phaseolus vulgaris* was mainly due to the distribution of Chl b which is more sensitive to salinity than Chl a.

As a final remark, the data summarized in table 1 show the inhibitory effects of the different levels of salinity used at the vegetative stage. As adopted by Younis *et al.* (2008), the calculated optimum percent recoveries, for growth, carbohydrate and photosynthetic components determined, in response to application of the recommended dose of either phosphorein or nitrobein biofertilizers to the variously salinized lettuce plants, are also included. These data in addition to careful examination of the figures would facilitate the reading and comprehe-nsion of the various above mentioned conclusions.

Thus, from our present results, we can conclude that application of phosphorein biofertilizer to NaCl-culture media appeared to be beneficial to growth and development as well as to the physiological processes of the salinized lettuce plants; the magnitude of amelioration of the adverse effects of NaCl being most pronounced with 4 mmhos NaCl. On the other hand, application of nitrobein biofertilizer to NaCl-culture media appeared to induce slight, if any beneficial changes, in growth and development of salinized lettuce plants, throughout the three successive stages. We can thus suggest that biofertilizers, if appropriately used, can lower the amount of added chemical fertilizer to the saline soil and consequently mitigation of pollution.

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