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Plant growth, metabolism and adaptation in relation to stress conditions. XXIV. Salinity-biofertility interactive effects on proline, glycine and various antioxidants in *Lactuca sativa*

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Abstract

Proline and glycine contents in lettuce plants appeared to show additional significant increments, in response to treatment with phosphorein biofertilizer, above those increments maintained in response to salinization. Administration of nitrobein biofertilizer to the NaCl media led to significant increases in proline and glycine contents above the water control levels, but the amino acid content of NaCl-treated plants appeared consistently higher than that content in NaCl + nitrobein-treated plants. Supplemental addition of phosphorein to the salinized culture media induced significant increases in the contents of antioxidant compounds, throughout the experimental period. As compared with the saline control values, total ascorbate (ASA + DASA) and total glutathione (GSSG + GSH) contents were found either to decrease (with 4 & 6 mmhos NaCl) or to increase (with 8 & 10 mmhos NaCl) significantly in response to addition of nitrobein to the saline culture media. The activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APO) and glutathione reductase (GR) in the salinized lettuce plants fortified with the recommended dose of phosphorein or nitrobein were, in general, significantly up-regulated above the salinized control levels; the magnitude of up-regulation being dependent on the concentration of NaCl, the stage of growth and on the enzyme under investigation. With lettuce plants, the present results are discussed in relation to applicability of two biofertilizers to sodic salty soils in Egypt.

Keywords: Lettuce, salinity, biofertilizers, amino acids, antioxidant compounds.

Introduction

It is now well known that water stress induced by salinity affects every aspect of plant growth and metabolism. Plant responses to water stress appeared to depend upon various factors such as duration and degree of stress, growth stage and time of stress exposure (Gupta and Sheoran, 1983; Younis *et al.,* 2008). Recent research has focused on elucidate-ing the effect of stresses on plant nutrients level and/or on the role that plant nutrients have on stress resistance mechanisms (Lorenzo *et al.,* 2001; Andrews, 2002). Nitrogen and/or phosphorus fertilization as a technique to alleviate salinity effects has received much attention due to their being required in large amounts for growth, as well as for their osmoregularity effects (Lorenzo *et al.,* 2001).

A number of nitrogen containing compounds has been shown to accumulate in a number of plants exposed to saline stress condition; however, the magnitude of accumulation of these compounds varied with plant species (Stewart and Rhodes, 1978). Among N containing compounds proline has been suggested to function either as a compatible osmotic solute (Stewart and Lee, 1974) and/or as protectants which stabilize nucleic acids, proteins and membranes (Tester and Davenport, 2003; Apel and Hirt, 2004) and hence play a significant role in salt-tolerance in plants.

In addition to suppression of growth and productivity, salt stress can lead to oxidative stress in plant tissues through the increase in the highly reactive oxygen species (ROS), that may cause cellular damage through oxidation of lipids, proteins and nucleic acids (Apel and Hirt, 2004). To minimize the effects of oxidative stress, plant cells have evolved a complex antioxidant system, which includes three general classes: (1) lipid soluble,

membrane-associated antioxidants (e.g. α-tocopherol and β-carotene); (2) water soluble reductants (e.g. glutathione and ascorbate); and (3) enzymatic antioxidants e.g. SOD, CAT, APO, POX (Apel and Hirt, 2004).

In our earlier communications (Younis *et al.,* 2008; Hasaneen *et al.,* 2008 a and b), using lettuce plants, urea, as a foliar spray, as well as phosphorein and nitrobein biofertilizers were used to alleviate the damage effects induced by different levels of salinity. Foliar spray with urea appeared beneficial to growth and metabolic activities of lettuce plants. Furthermore, application of phosphorein at a recommended dose, to NaCl-culture media appeared beneficial to growth and physiological processes of the salinized lettuce plants. On the other hand, application of nitrobein appeared to induce slight, if any beneficial changes, in growth and development of the salinized lettuce plants.

The present work aimed to investigate the effect of phosphorein and nitrobein on the response of proline and glycine contents, and the major components of enzymatic antioxidant system in saline-treated lettuce plants.

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

Of the Egyptian biofertilizers 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.

Estimation of amino acids

Glycine was estimated following the method of Muting and Kaiser (1963). The extract was deproteinized with ethanol/acetone mixture and the glycine was then determined colourimetrically with ninhydrin. Proline concentration was determined by means of a rapid colourimetric method developed for plant tissues by Bates *et al.* (1973).

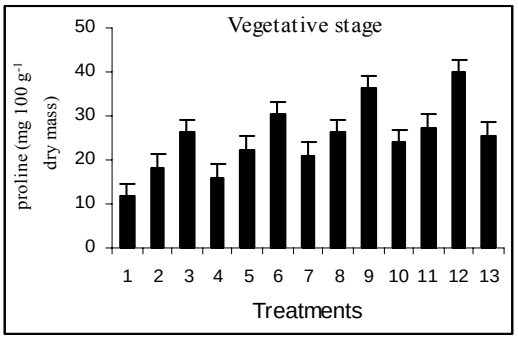
Estimation of antioxidant compounds

a- Total glutathione was determined by the method of Gossett *et al.* (1994). Over ice, 1 g of frozen tissue was ground with sterilized sand and 5 cm^3 of ice-cold 6% (v/v) phosphoric acid (pH 2.8) containing 1 mM EDTA in a mortar. The homogenate was centrifuged at 22000 g for 15 min and the supernatant was removed and then filtered through ultrafilter. Total glutathione was measured in a reaction mixture consisting of 0.4 cm^3 of solution A [1 mM Na_2HPO_4, 4 mM NaH_2PO_4, 0.15 mM EDTA, 0.14 mM 5,5-dithiobis-(2-nitrobenzoic acid) and 0.05 cm^3 of bovine-serum albumin (BSA)], 0.32 cm^3 of solution B [0.05 mM EDTA, 12.5 mM imidazole, 0.05 cm^3 BSA, 5 U mg^{-1} (protein) GR, 0.4 cm^3 of the plant extract diluted 1:50 in 5% (w/v) Na_2HPO_4 (pH 7.5), and 0.8 mM NADPH]. The reaction was measured spectrophotometrically following the change in absorbance at 412 nm for 10 min.

b- Total ascorbate was determined by a modified method of Gossett *et al.* (1994). Over ice, 1 g of frozen tissue was ground with sterilized sand and 10 cm^3 5% (v/v) phosphoric acid (pH 2.8) in a mortar. The homogenate was centrifuged at 22000 g for 15 min. Total ascorbate was determined in a reaction mixture consisting of 0.2 cm^3 of supernatant, 7.5 mM KH_2PO_4 buffer (pH 7.4) containing 5 mM EDTA and 0.1 mM dithiothreitol (DTT) to reduce DASA to ASA. After 10 min at room temperature, 0.05% (w/v) N-ethylmaleimide was added to remove excess DTT. Colour was developed in the reaction mixture with the addition of 0.4 cm^3 of 10 % (w/v) trichloroacetic acid, 0.4 cm^3 of 4.4 % (v/v) o-phosphoric acid, 0.4 cm^3 of dipyridyl in 70 % (v/v) ethanol and 0.2 cm^3 of 3 % $FeCl_3$. The reaction mixture was incubated at 40 ^0C for 1 hr and quantified spectrophotometrically at 525 nm.

Determination of antioxidant enzyme activities

The extraction for the estimation of antioxidant enzymes was done following the method of El-Saht (1998). Fresh plant tissues (5 g) were homogenized to a fine powder in a mortar under temperature of -5 ^0C. Subsequently soluble proteins were extracted by grinding the powder with a small amount of sterilized sand on ice, in 5 cm^3 of 50 mM Tris-HCl, pH 7.0, containing 20 % (v/v) glycerol, 1 mM ascorbate, 1 mM EDTA, 1 mM GSH, 5 mM $MgCl_2$, 1mM DTT. After two centrifugation steps (6 min at 12000 g and 16 min at 22000 g), the supernatant was stored in



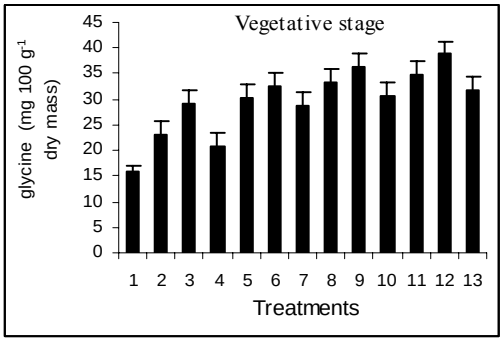


Fig 1. The effects of increasing concentrations of NaCl either alone or in combination with phosphorein or nitrobein biofertilizer on amino acids of lettuce plants. Vertical bars represent the L.S.D at 5% level.

In this figure treatments were as follows:

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

liquid nitrogen for determination of the activities of SOD, CAT, APO and GR.

a - Superoxide dismutase activity

Superoxide dismutase activity was determined according to the method of Giannopolitis and Ries (1977). One enzyme unit of SOD activity is defined as the amount of enzyme required to cause 50 % inhibition of the rate of nitrobluetetrazolium (NBT) reduction measured at 650 nm. The reaction mixture contained 0.1 cm^3 of 1.3 µM riboflavin, 0.1 cm^3 of 13 mM methionine, 0.1 cm^3 of 63 µM NBT in 0.1 M phosphate buffer (pH 7.8), and 0.05-0.1 cm^3 of enzyme extract in a final volume of 3 cm^3.

b - Catalase activity

Catalase activity was determined according to Aebi (1983) by measuring changes in absorbance at 240 nm corresponding to the decomposition of H_2O_2 in a reaction mixture containing 0.1 cm^3 of 50 mM KH_2PO_4, pH 7.0, 0.1 cm^3 of 10 mM H_2O_2 and 0.1 cm^3 of enzyme extract in a final volume of 1 cm^3 at 25 ^{0}C.

c - Ascorbate peroxidase activity

Ascorbate peroxidase was assayed, as the decrease in absorbance at 290 nm due to ascorbate oxidation, by the method of Nakano and Asada (1981). The reaction mixture contained 0.1 cm^3 of 50 mM KH_2PO_4, pH 7.0, 0.1 cm^3 of 1 mM sodium ascorbate, 0.1 cm^3 of 2.5 mM H_2O_2 and 0.1 cm^3 of enzyme extract in a final volume of 1 cm^3 at 25 ^{0}C.

d - Glutathione reductase activity

Glutathione reductase activity was assayed as the increase of absorbance at 340 nm due to the connection of GSSG to 1-chloro-2,4-dinitrobenzene (CDNB) as described by Drotar *et al.* (1985). The reaction mixture contained 0.1 cm^3 of 100 mM KH_2PO_4, pH 7.0, 0.1 cm^3 of 2 mM CDNB, 0.1 cm^3 of 2 mM GSSG and 0.5 cm^3 of enzyme extract in a final volume of 2 cm^3.

Time course experiment

During the period from August to December 2005, a large-scale outdoor experiment was carried out under normal day and light conditions, 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. A total of 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

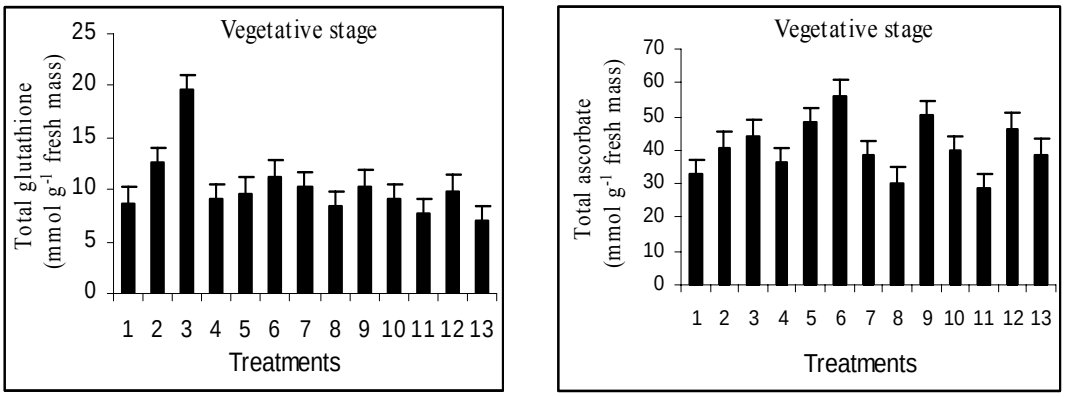


Fig 2. The effects of increasing concentrations of NaCl either alone or in combination with phosphorein or nitrobein biofertilizer on the antioxidant compounds in lettuce plants. Vertical bars represent the L.S.D at 5% level. Treatments 1-13 as in Fig 1.

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. Uniform 25-d-old lettuce (*lactuca sativa* cv. Baladi) transplants were selected, thoroughly washed with tap water and then transplanted in a mixture of clay-loamy soil (2:1, v/v) in pots (30×28×26 cm). 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. After thinning, 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. Every three days, all pots were irrigated with tap water, to maintain the soil at the field capacity throughout the experiment.

After 25, 122, and 150 days from the date of transplantation, samples were collected to represent 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 proline and glycine contents as well as for determination of different non-enzymic and enzymic components of the antioxidant system.

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

Irrespective of the stage of growth of the lettuce plant, the pattern of changes in the proline and glycine contents as well as in the various detected components of the antioxidant system, in response to NaCl when used either alone or in combination with phosphorein or nitrobein biofertilizer, were found to be comparable. Therefore, for convenience of expression of our results, the data obtained at the vegetative stage are only presented in the respective figures.

Changes in amino acid contents

In summary, the following points were typical of the present study:

a- The contents of proline and glycine in the control and in the variously treated plants appeared to increase with progress in the duration of the experimental period. A progressively greater significant increase in the contents of both proline and glycine in the salinized lettuce plants above the water control levels was maintained throughout the three successive growth stages. Of interest, the magnitude of increase appeared more or less comparable for both proline and glycine (Fig 1).

b- Proline and glycine contents in lettuce plants appeared to show additional significant increments, in response to treatment with phosphorein, above those increments maintained in response to salinization, throughout the entire period of the experiment. The magnitude of response, however, appeared markedly higher for proline than for glycine (Fig 1).

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

Amino acids			
Parameters / Treatments		Proline	Glycine
Inhibition	4 mmhos NaCl	56.9	47.1
	6 mmhos NaCl	92.2	92.4
	8 mmhos NaCl	125.9	112.7
	10 mmhos NaCl	136.2	122.3
Recovery	4 mmhos NaCl+phosphorein	69.0	38.9
	4 mmhos NaCl+nitrobein	-19.0	-14.0
	6 mmhos NaCl+phosphorein	69.0	14.0
	6 mmhos NaCl+nitrobein	-11.2	-8.3
	8 mmhos NaCl+phosphorein	86.2	17.9
	8 mmhos NaCl+nitrobein	-19.9	-17.8
	10 mmhos NaCl+phosphorein	107.8	24.8
	10 mmhos NaCl+nitrobein	-15.5	-19.1
Antioxidant compounds			
Parameters / Treatments		Total glutathione	Total ascorbate
Inhibition	4 mmhos NaCl	44.8	25.2
	6 mmhos NaCl	10.3	47.5
	8 mmhos NaCl	-4.6	-7.4
	10 mmhos NaCl	-12.6	-12.3
Recovery	4 mmhos NaCl+phosphorein	80.5	10.4
	4 mmhos NaCl+nitrobein	-41.4	-13.9
	6 mmhos NaCl+phosphorein	19.6	24.9
	6 mmhos NaCl+nitrobein	6.9	-29.7
	8 mmhos NaCl+phosphorein	23.0	61.1
	8 mmhos NaCl+nitrobein	9.2	28.9
	10 mmhos NaCl+phosphorein	26.4	54.3
	10 mmhos NaCl+nitrobein	-6.9	30.7
Antioxidant enzymes					
Parameter / Treatments		SOD	APO	CAT	GR
Inhibition	4 mmhos NaCl	44.8	17.7	22.4	43.1
	6 mmhos NaCl	78.3	35.5	36.9	68.6
	8 mmhos NaCl	87.7	38.7	46.1	74.5
	10 mmhos NaCl	92.1	38.7	48.8	78.4
Recovery	4 mmhos NaCl+phosphorein	18.3	24.2	15.8	15.7
	4 mmhos NaCl+nitrobein	-15.2	-24.2	-11.6	-3.9
	6 mmhos NaCl+phosphorein	31.6	4.8	9.2	11.8
	6 mmhos NaCl+nitrobein	-11.3	-6.5	-9.8	-1.9
	8 mmhos NaCl+phosphorein	35.5	1.6	2.2	19.6
	8 mmhos NaCl+nitrobein	-10.4	-21.0	-10.9	-5.9
	10 mmhos NaCl+phosphorein	34.0	-8.4	5.4	37.3
	10 mmhos NaCl+nitrobein	-10.3	-22.6	-12.8	-5.9

c- Administration of nitrobein to the NaCl media led to significant increases in proline and glycine contents above the water control levels. The magnitude of response was most pronounced with the highest concentration of NaCl. Moreover, the amino acid content of NaCl-treated plants appeared consistently higher than that content in NaCl + nitrobein-treated plants (Fig 1).

In varied plant species, it has been shown that salt tolerance associates with the capacity of a species to accumulate proline which acts as a compatible solute involved in osmotic adjustment at the plant cell level (Younis *et al.,* 1989; Delauney and Verma, 1990; Younis *et al.,* 1991; Younis *et al.,* 1994). Furthermore, Hasaneen *et al*. (2008a) observed a marked increase in proline and glycine contents in lettuce

plants salinized with various levels of NaCl. Foliar application of urea fertilizer to the variously salinized lettuce plants induced significant progressive increases in the contents of both proline and glycine with an increase in urea concentrations. The higher was the salt concentration used in combination with urea, the higher was the accumulation of proline and glycine contents. Hasaneen *et al*. (2008a) correlated the increased proline and glycine contents with NaCl tolerance in the lettuce plant. Accepting that salinized plants possess some system for excluding salt from the site of metabolism and/or for internal redistribution of ions, there is then a necessity for some form of osmotic adjustment to take place within the cell. A variety of compounds has been suggested as having a function in osmotic adjustment including carbohydrates, organic acids, sugar alcohols and amino acids (Stewart and Lee, 1974; Stewart and Rhodes, 1978). In addition to proline, amino acids such as taurine, glycine, alanine, glutamate, arginine and ornithine increase under saline treatment (Stewart and Lee, 1974). The role in osmotic balance cannot be assigned to any single solute. However, it seems that when one of the principal solutes decreased, its role was replaced by appearance of others.

Thus, the observed accumulation of proline and glycine contents in lettuce plants treated with NaCl either alone or in combination with biofertilizers, can be considered to act as (1) a protective mechanism, to act as (2) an osmoticum in the cell against stress conditions for the maintenance of other normal metabolic processes and/or to act as (3) storage compounds for nitrogen (Stewart and Lee, 1974; Younis *et al*., 1989; Younis *et al*., 1991).These possible explanations may be a part of many adjustments of the plant regulatory mechanisms following stress. Thus, proline action has been suggested to involve effects on the hydration layer surrounding phospholipids and possibly also its intercalation between phospholipid head groups (Rudolph *et al.,* 1986). Furthermore, it is striking that many of the solutes which accumulate in stressed plants and which have protective properties are also reported to reduce free radical activity. In this regard it has been indicated that proline can also detoxify free radicals by forming long-lived adducts with them (Floyd and Zs Nagy, 1984).

Although in the past, most attention has been concerned with the role of proline in stress tolerance, yet the role of glycine has received far less attention. Glycine seems to meet the requirements of a compound that can have a role in stress tolerance (see Waditee *et al.,* 2005): it is the major amino acid in all living organisms and can be synthesized *in vivo* , considerable amounts of glycine may be used for betaine synthesis under salt stress conditions, the demonstration of the usefulness of glycine for the improvement of abiotic stress tolerance in crop plants and finally Waditee *et al.* (2005) obtained results indicating that glycine is limiting for maximal salt-stress tolerance in plants. All the above mentioned parameters in addition to the present comparable patterns of changes in proline and glycine contents point to the fact that the role of glycine in salt tolerance cannot be ignored.

Changes in antioxidant compounds

From the results shown up in figure 2, the contents of total ascorbate and total glutathione in lettuce plants, variously treated with different concentrations of NaCl either alone or in combination with phosphorein or nitrobein, showed a progressive, if any increase, throughout the entire period of the experiment, As compared with water control levels, a significant increase in both ascorbate and glutathione contents was observed in lettuce plants treated with 4 and 6 mmhos NaCl throughout the entire experimental period, except at the flowering and fruiting stages, when a significant decrease below the control level was apparent for the total glutathione content. On the other hand, with 8 and 10 mmhos NaCl, a significant decrease in both ascorbate and glutathione contents of the treated plants was apparent below the control levels, throughout the duration of the experiment.

Supplemental addition of a recommended dose of phosphorein induced significant increases in the contents of ASA + DASA and GSH + GSSG, throughout the experimental period. The magnitude of response was most pronounced with 4 mmhos NaCl + phosphorein and with 6 mmhos NaCl + phosphorein for total glutathione and total ascorbate, respectively (Fig 2).

As compared with the respective saline control values, ascorbate and glutathione contents were found, in general, either to decrease significantly in response to treatment with 4 mmhos NaCl + nitrobein and 6 mmhos NaCl + nitrobein, or to increase significantly in response to treatment with 8 mmhos NaCl + nitrobein and with 10 mmhos NaCl + nitrobein (Fig 2).

Changes in antioxidant enzymes

The changes in the activities of the various antioxidant enzymes in response to treatment with NaCl either alone or in combination with each of the two biofertilizers are illustrated graphically in figure 3. Careful examination of the figure revealed the following main points:

a- With an increase in concentration of NaCl used, a progressively greater significant increase in the

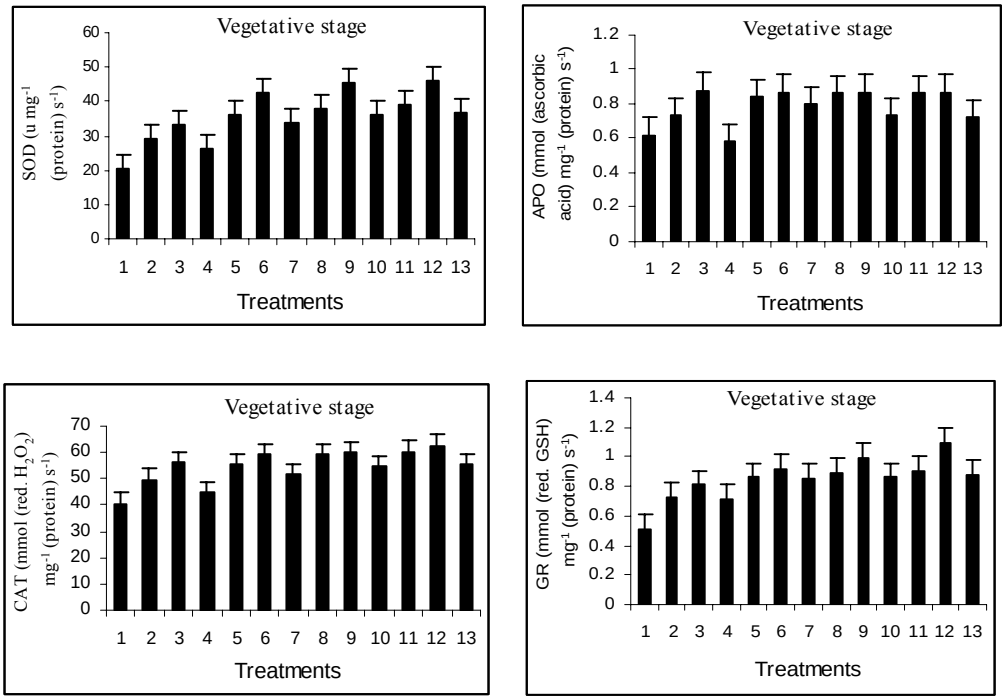


Fig 3. The effects of increasing concentrations of NaCl either alone or in combination with phosphorein or nitrobein biofertilizer on the activities of the antioxidant enzymes in lettuce plants. Vertical bars represent the L.S.D at 5% level. Treatments 1-13 as in Fig 1.

activities of the various antioxidant enzymes detected was observed throughout the experimental period.

b- The activities of SOD, CAT, APO and GR in the salinized lettuce plants fortified with the recommended dose of phosphorein were, in general, signifycantly increased above the respective salinized control levels, the magnitude of increase was most pronounced with the highest concentration of NaCl.

c- The activities of SOD, CAT, APO and GR in the salinized lettuce plants fortified with the recommended dose of nitrobein showed, in general, significant increases; the magnitude of increase being dependent on the concentration of NaCl, the stage of growth and on the enzyme under investigation.

Our results lend a strong support to those of Chaparzadeh *et al.* (2004) who reported that, under high salinity stress, a decrease in total glutathione and an increase in total ascorbate accompanied by enhanced GR and APO activities were observed in leaves of *Calendula officinalis* L. Comba *et al.* (2004) reported that under mild saline stress, the elevated levels of the antioxidant enzymes and reduced glutathione protect nodules of *Glycine max* L. against the ROS, thus avoiding lipid and protein peroxidation, and leghaemoglobin breakdown. After returning the salinized plants to a non-saline environment (recovery), the enzymatic activities returned to the initial values. Furthermore, Zhang and Kirkham (1994) stated that in different species, tissues, and at developmental stages tolerant to water stress, reduced membrane damage has been linked to increased enzymatic defences against oxygen radicals, together with synthesis of free radical scavengers.

When plants are subjected to stress, the balance between the production of ROS and the quenching activity of the antioxidant is upset, often resulting in antioxidative stress (Junklang, 2005). Plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater resistance to this oxidative damage (Bor *et al.,* 2003 and Ducic *et al.,* 2003) by regulating the redox equilibrium in the cell.

In the present study, a clear explanation for the involvement of APO, SOD, CAT and GR in the salt-stress response might be that exposure of lettuce plants to NaCl during growth reduces water availability and therefore has a similar effect as drought or cold, i.e. the production of ROS due to the electron leakage from electron transport chains in chloroplasts and mitochondria (Lopez *et al.,* 1996). Because different environmental stress conditions (drought, cold, salt, etc.) are likely to affect the same physiological parameters, it is not really surprising to

find that the present state of salt stress triggers the expression of antioxidative defense systems.

This is evident from the fact that non-enzymatic and enzymatic antioxidants showed, in general, an increase with the increasing salt stress indicating a cellular capacity to overcome the stress. This conclusion can be substantiated if we consider the calculated percentages of recovery in total glutathione and total ascorbate contents as well as in SOD, APO, CAT and GR activities in the differently salinized lettuce plants treated with a recommended dose of either phosphorein or nitrobein (Table 1). The significant positive results maintained in response to phosphorein treatment indicate varied partial nullification of salinity by phosphorein application. Nitrobein treatment, on the other hand, appeared much less beneficial.

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