

## **Plant growth, metabolism and adaptation in relation to stress conditions. XXI. Reversal of harmful NaCl-effects in lettuce plants by foliar application with urea**

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### **Abstract**

In relation to water control levels, administration of NaCl at low (3 dSm<sup>-1</sup>), medium (5 dSm<sup>-1</sup>) or high (7 dSm<sup>-1</sup>) concentration, in the growth medium of the lettuce plants, induced significant decreases in growth components as well as in the metabolites and enzymes determined, at vegetative and adult growth stages. On the other hand, significant increases in all growth and photosynthetic components determined, as well as in carbohydrate contents and in the activities of the antioxidant enzymes were obtained, above the water control levels, in response of treatment of lettuce plants with urea fertilizer up to 4 %, above which urea at 5% and 6% induced significant decreases in all the above mentioned components, throughout the entire periods of the experiments. Foliar application of urea up to 5 % to the variously salinized lettuce plants induced significant increases in all growth components as well as in all metabolites determined as compared with values of control-salinized plants. At 6% urea, significant decreases in growth parameters and in metabolites determined for the variously salinized plants were apparent. Thus, foliar application of urea can, at least, partially alleviate the inhibitory effects of salinity on growth and metabolism of lettuce plants; the magnitude of response being most pronounced with 3-4% urea.

**Key words:** antioxidant enzymes, carbohydrates, growth, *Lactuca sativa*, NaCl, photosynthetic efficiency, urea

### **Introduction**

Lettuce (*Lactuca sativa*) for fresh consumption is an important field vegetable crop. In Egypt, lettuce is commonly grown on the clay-loam and clay soils under irrigated conditions. Knowledge of the water consumptive use and the influence of different water regimes on the yield and nitrogen (N) uptake by lettuce are still insufficient (Karam et al., 2002). Power and Schepers (1989) showed that vegetables require a greater degree of management and utilize a larger N input than most agronomic cropping

systems. Efficient recycling of reduced N present in the form of urea is important for plant growth since urea contains a significant amount of this element (Polacco and Holland, 1993). In addition to internally generated urea, externally applied urea, in particular as foliar spray, can be rapidly absorbed and utilized by plants. Urea is a widely used fertilizer because of its low cost, ease in handling and high N content. The response of plants to excess NaCl is complex and involves changes in their morphology, physiology

**Table 1.** The percent inhibitory effects of low, medium and high concentrations of NaCl and the optimum percentage recovery (improvement), induced by urea as a foliar spray, for the various growth parameters of lettuce plants at the vegetative (V) and adult (A) growth stages.

Parameters		Length of root	Length of shoot	Leaf area	Fresh mass	Dry mass	Water content	
								Treatments
Inhibition	3 dSm <sup>-1</sup> NaCl	V	-25.0	-20.0	-4.4	-22.3	-18.8	-22.8
		A	-21.7	-11.1	-14.6	-25.7	-46.0	-22.2
	5 dSm <sup>-1</sup> NaCl	V	-35.0	-48.0	-27.6	-29.7	-34.4	-29.1
		A	-30.4	-26.7	-29.7	-53.0	-60.0	-51.8
	7 dSm <sup>-1</sup> NaCl	V	-45.0	-56.0	-40.0	-39.0	-46.9	-38.0
		A	-39.1	-31.1	-38.7	-62.2	-69.0	-61.0
Recovery	3% urea+3 dSm <sup>-1</sup> NaCl	V	25.0	32.0	19.8	46.5	37.5	47.7
		A	21.7	17.8	18.9	34.7	77.0	27.5
	3% urea+ 5 dSm <sup>-1</sup> NaCl	V	27.5	28.0	23.3	43.5	34.4	44.7
		A	23.9	15.6	30.7	29.3	67.0	22.9
	3% urea+ 7 dSm <sup>-1</sup> NaCl	V	20.0	32.0	31.0	38.3	34.4	38.8
		A	17.4	17.8	28.2	24.8	45.0	21.4

and metabolism (Mass and Hoffman, 1977; Tarakcioglu and Inal, 2002). Of importance, salt stress is the major environmental factor that limits the efficiency of photosynthesis (Allakhverdiev et al., 2002 and Liska et al., 2004) in most plants. Thus, Younis et al., (1993) and El-Saht et al., (1994), working with broad bean, castor bean and maize plants, found that salinity decreased the content of chlorophylls (Chl) a and b in leaves of the salinized plants and these changes are, in general, accompanied by a marked increase in the content of carotenoids (Cars) throughout all stages of growth.

Salt stress is also now known to cause several physiological changes including oxidative stress (Lee et al., 2001; Panda and Upadhyay, 2003). Production of several active oxygen species (AOS) increases in the presence of NaCl and has been stated to damage almost every macromolecule (Panda, 2002). In plant cells both enzymatic (superoxide dismutase; SOD, catalase; CAT, ascorbate peroxidase; APO, guaiacol peroxidase; GPO and glutathione reductase; GR) and non-enzymatic (ascorbate, glutathione and  $\alpha$ -tocopherol) antioxidant defence systems exist, which help in detoxifying the AOS (Lee et al., 2001; Malenčić et al., 2003).

Consequently, this study was designed to investigate and correlate the effects of urea foliar application on growth, photosynthetic efficiency, carbohydrate content and on the activities of different antioxidant enzymes, in normal as well as in stressed lettuce plants.

## Materials and methods

### Time course experiments

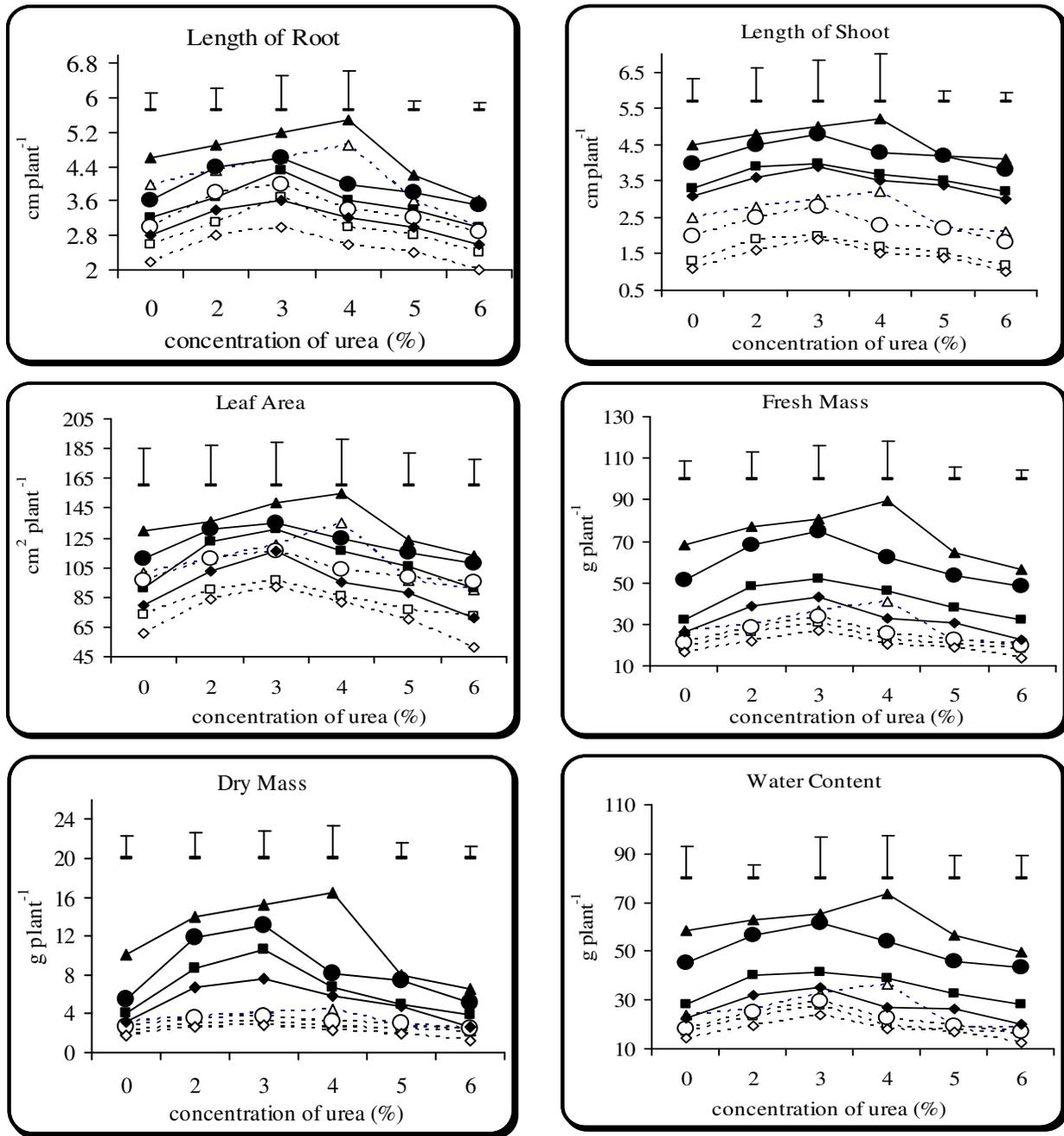
Homogeneous transplants of lettuce (*Lactuca sativa* L. cv. Baladi) were used. The details of the experimental set-up as well as of culturing and growing of transplants were essentially those described by El-Bialy (2005). The transplants (25-days old) were washed thoroughly with tap water and then transplanted in a mixture of clay-loamy soil (2: 1, v/v) in pots (30 × 28 × 26 cm). All pots contained equal amounts of homogeneous soil (8 kg). The experiments were carried out outdoor under normal day and light conditions. In all cases, treatment of lettuce transplants with urea and/or NaCl was carried out after one week from the date of transplantation. All pots were irrigated with tap water, at three-days

**Table 2.** Pearson's Correlation coefficients between the changes in growth parameters at vegetative (V) and adult (A) growth stages and the associated changes in total saccharides, total pigments, SOD and APO activities. \*correlation is significant at the 0.05 level (2-tailed).

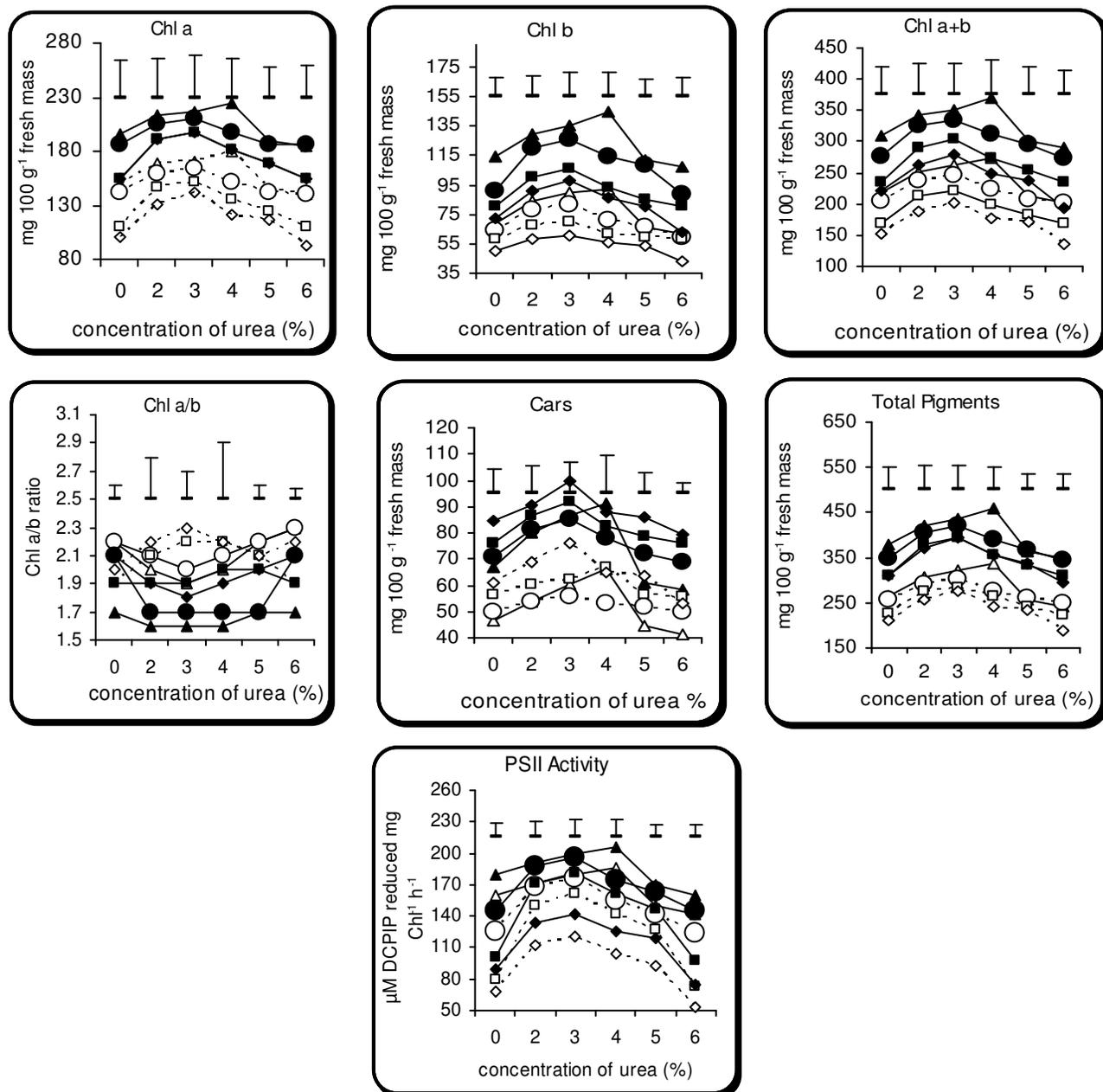
Treatments	Growth parameter		Total saccharides	Total pigments	Antioxidant enzymes	
					SOD	APO
Urea only	Length of root	V	0.938*	0.956*	0.832*	0.807*
		A	0.927*	0.959*	0.849*	0.783*
	Length of shoot	V	0.938*	0.956*	0.832*	0.807*
		A	0.927	0.959*	0.837*	0.783*
	Leaf area	V	0.975*	0.979*	0.959*	0.929*
		A	0.938*	0.974*	0.913*	0.847*
	Water content	V	0.965*	0.967*	0.934*	0.918*
		A	0.902*	0.962*	0.901*	0.874*
Urea + 3 dSm <sup>-1</sup> NaCl	Length of root	V	0.982*	0.999*	0.436	0.508
		A	0.982*	0.989*	0.597	0.604
	Length of shoot	V	0.982*	0.994*	0.436	0.508
		A	0.982*	0.989*	0.597	0.604
	Leaf area	V	0.965*	0.994*	0.451	0.493
		A	0.982*	0.995*	0.710	0.723
	Water content	V	0.948*	0.976*	0.403	0.437
		A	0.971*	0.980*	0.806*	0.813*
Urea + 5 dSm <sup>-1</sup> NaCl	Length of root	V	0.851*	0.933*	0.624	0.657
		A	0.944*	0.952*	0.703	0.553
	Length of shoot	V	0.883*	0.991*	0.615	0.635
		A	0.984*	0.993*	0.724	0.547
	Leaf area	V	0.912*	0.992*	0.648	0.684
		A	0.995*	0.992*	0.778*	0.601
	Water content	V	0.904*	0.925*	0.480	0.521
		A	0.966*	0.974*	0.838*	0.685
Urea + 7 dSm <sup>-1</sup> NaCl	Length of root	V	0.810*	0.823*	0.708	0.686
		A	0.881*	0.806*	0.708	0.686
	Length of shoot	V	0.992*	0.985*	0.800*	0.772*
		A	0.973*	0.992*	-0.427	-0.386
	Leaf area	V	0.992*	0.985*	0.800*	0.772*
		A	0.973*	0.992*	-0.427	-0.386
	Water content	V	0.950*	0.982*	0.694	0.661
		A	0.963*	0.984*	-0.457	-0.420

intervals, to maintain the soil at the field capacity throughout the experiment. A total of 48 treatments representing all planned possible combinations of urea and salinity levels were replicated twice in a completely randomized design (CRD). An analysis of

variance (ANOVA) was performed on the data using the F-ratio test. Comparison among means, from duplicate determinations and quadruplicate samples, was carried out by calculating the least significance difference (LSD) at the 5% probability level.



**Fig 1.** The effects of increasing concentrations of urea fertilizer either alone or in combination with low, medium or high concentrations of NaCl on growth parameters of lettuce plants, at vegetative (V) and adult (A) growth stages. Vertical bars represent the LSD at 5 % level. Dotted lines represent the vegetative growth stage and solid lines represent the adult growth stage [---Δ--- urea only (V); —▲— urea only (A); ---○--- urea + 3 dSm<sup>-1</sup> NaCl (V); —●— urea + 3 dSm<sup>-1</sup> NaCl (A); ---□--- urea + 5 dSm<sup>-1</sup> NaCl (V); —■— urea + 5 dSm<sup>-1</sup> NaCl (A); ---◇--- urea + 7 dSm<sup>-1</sup> NaCl (V); —◆— urea + 7 dSm<sup>-1</sup> NaCl (A)].



**Fig 2.** The effects of increasing concentrations of urea fertilizer either alone or in combination with low, medium or high concentrations of NaCl on pigment constituents and PSII activity of lettuce plants, at vegetative (V) and adult (A) growth stages. Vertical bars represent the LSD at 5 % level. Dotted lines represent the vegetative growth stage and solid lines represent the adult growth stage [--- $\Delta$ --- urea only (V); — $\blacktriangle$ — urea only (A); --- $\circ$ --- urea + 3 dSm<sup>-1</sup> NaCl (V); — $\bullet$ — urea + 3 dSm<sup>-1</sup> NaCl (A); --- $\square$ --- urea + 5 dSm<sup>-1</sup> NaCl (V); — $\blacksquare$ — urea + 5 dSm<sup>-1</sup> NaCl (A); --- $\diamond$ --- urea + 7 dSm<sup>-1</sup> NaCl (V); — $\blacklozenge$ — urea + 7 dSm<sup>-1</sup> NaCl (A)].

Also, correlation coefficients between growth parameters and various appropriate metabolites were also carried out (Table 2).

Samples for determination of growth, photosynthetic and carbohydrate components as well as enzyme activities were taken from plants after 20 and 35 days from the date of transplantation. Thus the vegetative and adult growth stages were respectively presented. Leaf area was measured by square-paper method (Hasaneen et al., 1994). Fresh and dry weights, after drying fresh samples in an oven at 70 °C to constant mass, were also measured.

#### ***Determination of photosynthetic components***

Photosynthetic pigments (Chl a, Chl b and Cars) were determined in the fresh tissues after extraction with 85% acetone using the spectrophotometric method as described by Metzner et al., (1965). Photosystem II activity, as indicated by the rate of 2,6-dichlorophenol indophenol (DCPIP) photoreduction, was monitored at 600 nm using a spectrophotometer. As described by Arnon (1949), 4 g of detached leaves were used for preparation of chloroplast pellets that were suspended in 1 mM Na-Tricine (pH 7.8), 10 mM NaCl and 10 mM MgCl<sub>2</sub> and then kept at 0-4 °C until required.

#### ***Determination of saccharides***

The method of extraction of the different saccharide fractions was essentially that adopted by Handel, (1968). Glucose was determined in ethanolic extract using the o-toluidine procedure (Fertris, 1965). The sucrose content was determined after Handel (1968). Polysaccharides, being considered mainly as starch, was determined by the method of Thayumanavan and Sadasivam, (1984).

#### ***Extraction and assay of enzymes***

Plant tissues (5 g) were homogenized in a mortar under temperature of -5 °C. Subsequently soluble proteins were extracted by grinding the macerate with a small amount of sterilized sand on ice, in 5 cm<sup>3</sup> of 50 mM Tris-HCl, pH 7.5, containing 20 % (v/v) glycerol, 1 mM ascorbate, 1 mM EDTA, 1 mM GSH, 5 mM MgCl<sub>2</sub> and 1 mM DTT (dithiothreitol). After two centrifugation steps (6 min at 12000 g and 16 min at 22000 g), the supernatant was stored in liquid

nitrogen for determination of the activities of SOD, APO, CAT and GR (El-Saht, 1998).

Superoxide dismutase activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitrobluetetrazolium (NBT) (Giannopolitis and Ries, 1977; El-Saht, 1998); one unit of SOD activity being defined as the amount of enzyme required to cause 50 % inhibition of the rate of NBT reduction at 650 nm. The reaction mixture contained 0.1 cm<sup>3</sup> of 1.3 μM riboflavin, 0.1 cm<sup>3</sup> of 13 mM methionine, 0.1 cm<sup>3</sup> of 63 μM NBT in 0.1 M phosphate buffer (pH 7.8), and 0.05-0.1 cm<sup>3</sup> of enzyme extract in a final volume of 3 cm<sup>3</sup>.

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<sup>3</sup> of 50 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.0, 0.1 cm<sup>3</sup> of 1 mM sodium ascorbate, 0.1 cm<sup>3</sup> of 2.5 mM H<sub>2</sub>O<sub>2</sub> and 0.1 cm<sup>3</sup> of enzyme extract in a final volume of 1 cm<sup>3</sup> at 25 °C.

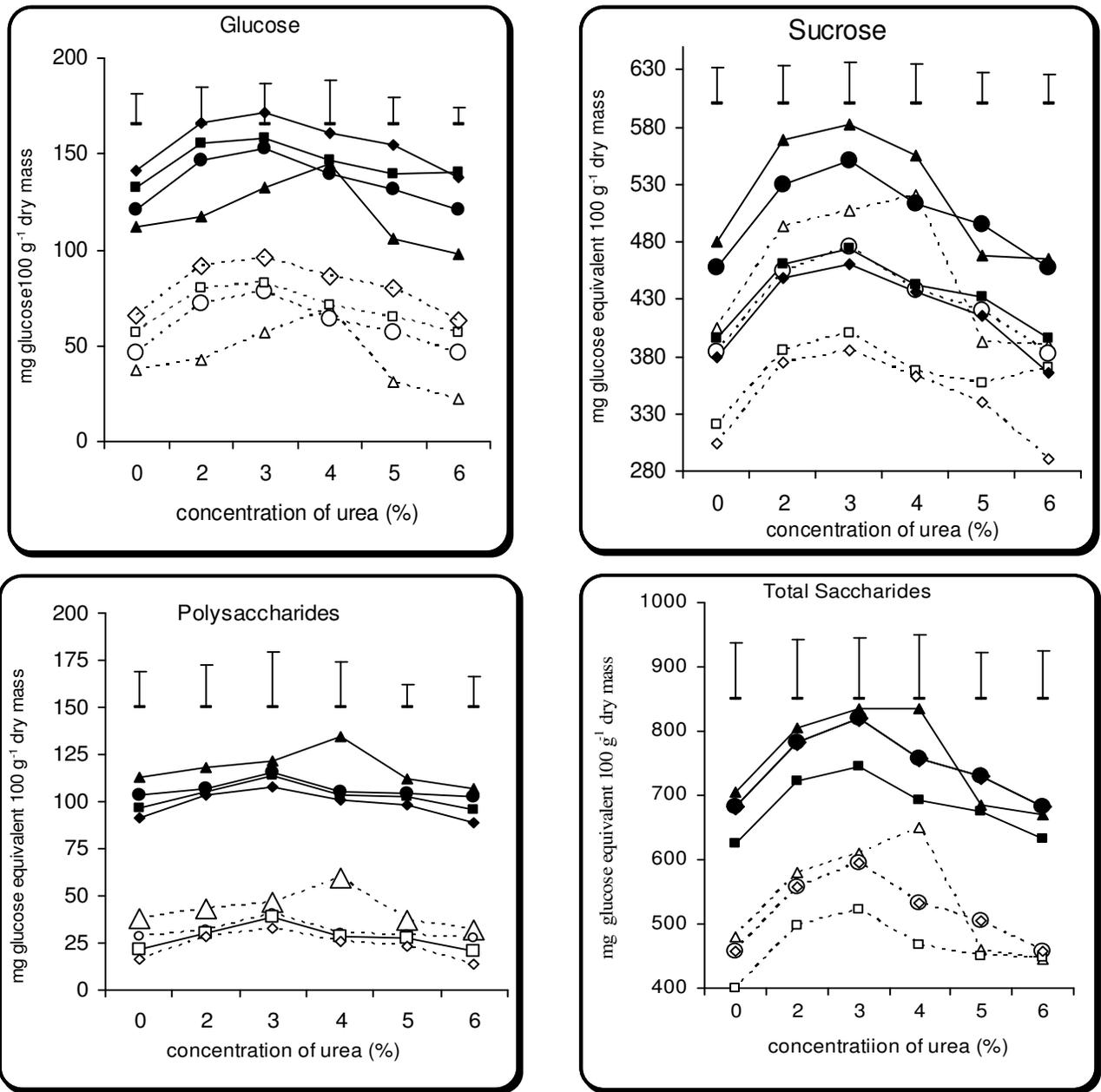
Catalase activity was determined by measuring changes in absorbance at 240 nm corresponding to the decomposition of H<sub>2</sub>O<sub>2</sub> in a reaction mixture containing 0.1 cm<sup>3</sup> of 50 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.0, 0.1 cm<sup>3</sup> of 10 mM H<sub>2</sub>O<sub>2</sub> and 0.1 cm<sup>3</sup> of enzyme extract in a final volume of 1 cm<sup>3</sup> at 25 °C (Aebi, 1983).

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<sup>3</sup> of 100 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.0, 0.1 cm<sup>3</sup> of 2 mM CDNB, 0.1 cm<sup>3</sup> of 2 mM GSSG and 0.5 cm<sup>3</sup> of enzyme extract in a final volume of 2 cm<sup>3</sup>.

## **Results and Discussion**

### ***Changes in growth components***

During the experimental period, all growth components appeared to increase significantly with an increase in the concentration of urea fertilizer up to 4%. At higher concentrations (5% and 6%) of urea, a significant reduction in growth was obtained in relation to control (Fig. 1). Foliar administration of urea to lettuce plants treated with low (3 dSm<sup>-1</sup>), medium (5 dSm<sup>-1</sup>) and high (7 dSm<sup>-1</sup>) NaCl, induced significant increases in all the measured components of growth as compared with control plants treated with NaCl alone (Fig. 1); indicating counteraction of the injurious effects of salinity on the different growth



**Fig 3.** The effects of increasing concentrations of urea fertilizer either alone or in combination with low, medium or high concentrations of NaCl on carbohydrate content of lettuce plants, at vegetative (V) and adult (A) growth stages. Vertical bars represent the LSD at 5% level. Dotted lines represent the vegetative growth stage and solid lines represent the adult growth stage [---Δ--- urea only (V); —▲— urea only (A); ---○--- urea + 3 dSm<sup>-1</sup> NaCl (V); —●— urea + 3 dSm<sup>-1</sup> NaCl (A); ---□--- urea + 5 dSm<sup>-1</sup> NaCl (V); —■— urea + 5 dSm<sup>-1</sup> NaCl (A); ---◇--- urea + 7 dSm<sup>-1</sup> NaCl (V); —◆— urea + 7 dSm<sup>-1</sup> NaCl (A)].

components. The magnitude of counteraction appeared to be a function of the urea and the NaCl concentration. This is also evident from data depicted in Table 1 showing calculated percent improvement for the dry mass parameter. For all the other growth components, comparable patterns of changes in the calculated percent improvement were, in general, obtained.

For clarity, we found necessarily to denote how the percentage of recovery (improvement) for each of the growth and metabolite components was calculated (Liu and Dickmann, 1996) throughout this investigation:

1. Percent change (increase or decrease) due to treatment with each specified concentration of NaCl:  $[(\text{salt treated parameter level} - \text{water control parameter level}) / \text{water control parameter level}] \times 100$ .
2. Percent change due to treatment with each specified concentration of NaCl in combination with each specified concentration of urea:  $[(\text{salt} + \text{urea-treated parameter level} - \text{water control parameter level}) / \text{water control parameter level}] \times 100$ .
3. Percent recovery (improvement) was obtained by subtracting "1" from "2".

In support of the present observations, Puttanna et al. (2001) demonstrated that foliar application of urea fertilizer significantly enhanced the growth and yield of citronella plants. High rates of urea fertilizer can produce salt levels that are high enough to damage plants and reduce growth and yield. Furthermore, urea can produce ammonia gas which can be highly toxic to plants (Halverson, 1989). Thus Pew et al. (1984) recorded reduced head lettuce growth and yield when  $198 \text{ Kg N ha}^{-1}$  as urea was applied.

Miceli et al. (2003) showed that an increase in salinity of nutrient solution of lettuce plants was associated with a reduction of marketable growth and yield, average plant fresh weight and leaf number per plant. Recently, Burman et al. (2004), studying the effects of urea fertilization on clusterbean plants subjected to water stress, found that water stress significantly decreased shoot water potential, fresh and dry mass and maintained a reduction of water content. Application of urea increased most of these parameters. These results revealed synergistic effects of urea in enhancing leaf area and growth rate, leading to significant improvement in plant growth and yield under stress conditions and lend a strong

support to our present investigation with the moderately-saline-sensitive lettuce plants.

### *Changes in photosynthetic components*

Foliar application of urea at 2%, 3% and 4% levels induced significant progressive increases in Chl a, Chl b, Chl a + b, Cars and consequently in total pigment contents, throughout the duration of the experiment, in relation to control levels. Variable significant changes in Chl a/b ratios of the differently treated lettuce plants were apparent. On the other hand, at high concentrations (5% and 6%) of urea fertilizer, the pigment fraction contents were found either not to change or to decrease significantly, at vegetative and adult stages of lettuce growth, as compared with control plants (Fig. 2). The influence of N on plant growth and development is often connected with the process of photosynthesis, because the quantity of N, in the highest degree, determines the formation and the functional state of assimilation apparatus of plants including 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 (Stanev, 1984; Ivanova and Vassilev, 2003).

The content of the individual pigment fractions of the variously salinized lettuce plants that were fertilized with increasing concentrations of urea up to 5%, showed significant increases above those levels in control salinized plants. At 6% urea, all the pigment contents were found either to decrease significantly (with  $7 \text{ dSm}^{-1}$  NaCl) or to show slight, if any change (with  $3 \text{ dSm}^{-1}$  and  $5 \text{ dSm}^{-1}$  NaCl), from the control levels (Fig. 2). The decreasing tendency of chlorophyll content can be attributed to the fact that NaCl stress decreases total chlorophyll content by increasing the activity of Chl degrading chlorophyllase (El-Saht, 2001), inducing the destruction of chloroplast structure and the instability of pigment protein complex (Singh and Dubey, 1995).

In relation to control levels, the pattern of changes in PS II activity of the differently treated lettuce plants, at vegetative and adult growth stages, appeared variable; either increased or decreased, depending upon the concentration of urea used either alone or in combination with each of the different levels of NaCl (Fig. 2). As compared with control salinized lettuce plants, the calculated percent improvement, in the

various photosynthetic pigment contents as well as in the photosynthetic activity determined in the salinized lettuce plants, in response to foliar application of urea fertilizer throughout the two growth stages, showed significant positive values; indicating that urea has induced partial nullification of the adverse effects of NaCl on the pigment content as well as on the photosynthetic rate.

The present results, concerning the positive effects of foliar urea application upon the photosynthetic machinery (pigment content and PS II activity), appeared to coincide with those positive effects on the dry mass accumulation 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 in treated plants was the increased capacity for CO<sub>2</sub> assimilation. This conclusion can be further substantiated when Pearson's correlation coefficients were carried out between the changes in growth parameters and the changes in total pigments content; positive significant correlation being obtained (Table 2).

#### ***Changes in carbohydrate content***

Throughout the experimental period, foliar application of urea fertilizer at 2%, 3% and 4% levels, induced significant progressive increases in glucose, sucrose, polysaccharides and in total saccharides content; the magnitude of increase was most pronounced with 4%, whereas at 5% and 6% levels of urea, a significant progressive decrease in the saccharide fractions determined as well as in the total saccharides content was obtained (Fig. 3). El-Saht (1995) observed a greater increase in reducing sugars associated with progressively greater decreases in the content of sucrose, polysaccharides and total saccharides in soybean plants with the increase in concentration of urea fertilizer. An opposite pattern of changes was, however, observed for the different saccharide components in maize plants with increasing concentrations of urea.

Lettuce plants salinized with low, medium or high concentration of NaCl showed a significant increase in glucose content and significant decreases in sucrose, polysaccharides and in total saccharide contents at vegetative and adult growth stages, as compared with water control plants (Fig. 3).

Foliar application of urea at the levels of 2%, 3%, 4% and 5% to the variously salinized lettuce plants induced significant increases in glucose, sucrose,

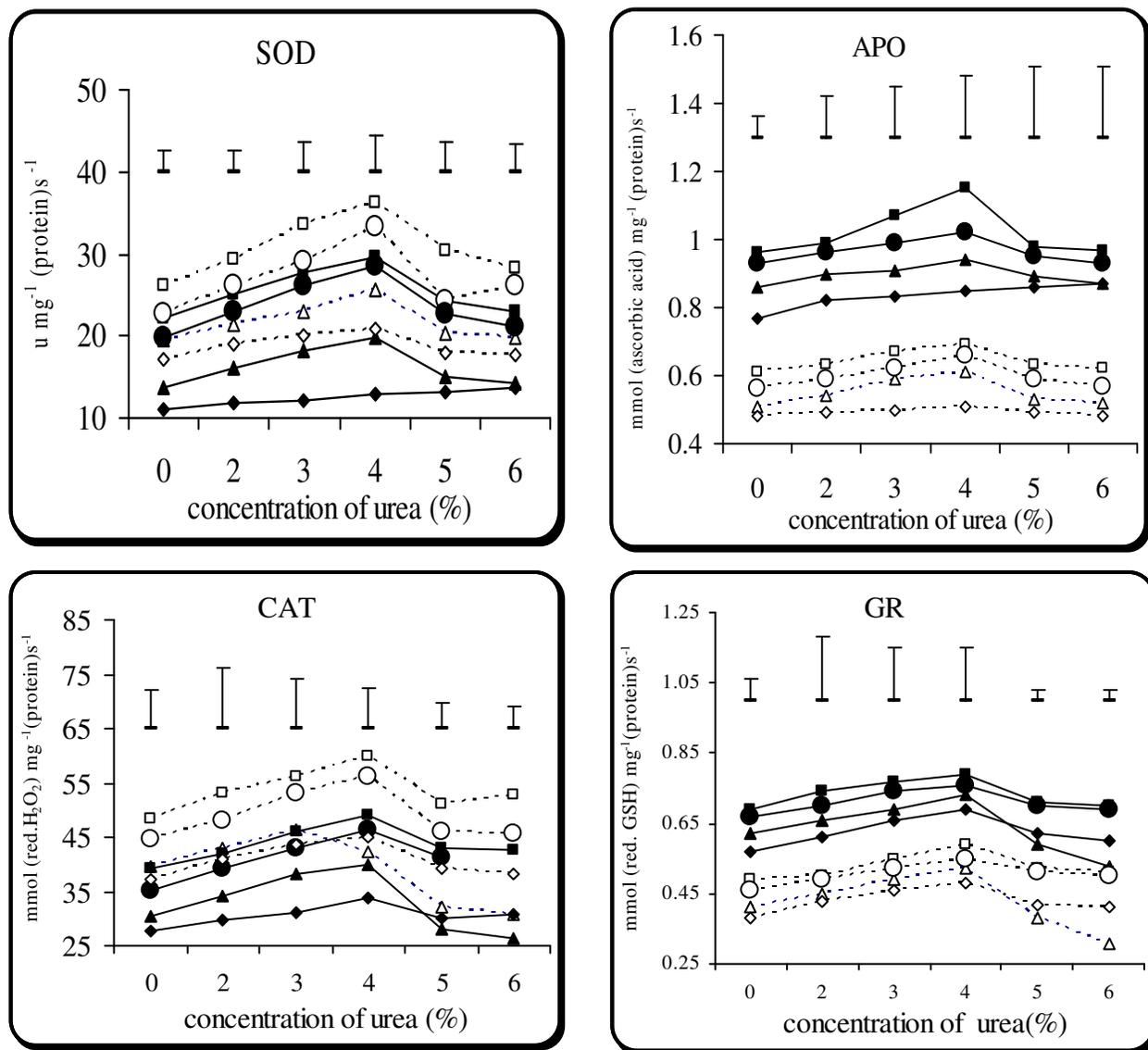
polysaccharides and in total saccharide contents at vegetative and adult stages. The magnitude of increase appeared most pronounced with 3% urea. On the other hand, at 6% urea either a non-significant change or a significant decrease in these saccharide fractions was obtained (Fig. 3). Badawy (1989) stated that fertilization with urea, generally, did not induce significant changes in the values of reducing sugars and polysaccharides in the salt-treated *Vicia faba* plants. On the other hand, values of sucrose appeared to be decreased by fertilization with urea, especially in the plants treated with the higher salt concentrations.

As apparent from Fig. 1 and 3, the changes in saccharide contents (increase or decrease) appeared to coincide with the changes (increase or decrease) in growth parameters, respectively. Thus, positive significant Pearson's correlation coefficients appeared to exist between growth parameters and total saccharide contents (Table 2).

In the present work the calculated percent improvement of total saccharide contents, e.g. in 3 dSm<sup>-1</sup> NaCl-treated lettuce plants due to foliar application of urea fertilizer throughout the two growth stages, were as follows: 20.9% for 2% urea, 28.4% for 3% urea, 15.5 % for 4% urea, 9.9% for 5% urea and -0.3 % for 6% urea, at the vegetative growth stage and 14.2% for 2% urea, 19.4% for 3% urea, 10.6 % for 4% urea, 6.7% for 5% urea and -0.2 % for 6% urea, at the adult growth stage; in relation to the control values in salinized lettuce plants. With medium and high concentrations of NaCl, comparable values of percentages of improvement were obtained. This apparently indicate partial nullification of the damage maintained by different levels of NaCl, in response to treatment with urea; the magnitude of nullification of the damage being most pronounced with 3-4% urea used as a foliar spray.

#### ***Changes in activities of antioxidant enzymes***

The foliar application of urea fertilizer to lettuce plants, at vegetative and adult growth stages, appeared to affect the activities of enzymes known to participate in the H<sub>2</sub>O<sub>2</sub>-scavenging ascorbate-glutathione cycle. In general, significant increases in SOD and APO activities due to fertilization with urea were apparent as compared with controls; the magnitude of increase being most pronounced with 4% urea (Fig. 4). On the other hand, CAT and GR activities were found either to increase significantly



**Fig 4.** The effects of increasing concentrations of urea fertilizer either alone or in combination with low, medium or high concentrations of NaCl on antioxidant enzyme activities of lettuce plants, at vegetative (V) and adult (A) growth stages. Vertical bars represent the LSD at 5 % level. Dotted lines represent the vegetative growth stage and solid lines represent the adult growth stage [--- $\Delta$ --- urea only (V); --- $\blacktriangle$ --- urea only (A); --- $\circ$ --- urea + 3  $\text{dSm}^{-1}$  NaCl (V); --- $\bullet$ --- urea + 3  $\text{dSm}^{-1}$  NaCl (A); --- $\square$ --- urea + 5  $\text{dSm}^{-1}$  NaCl (V); --- $\blacksquare$ --- urea + 5  $\text{dSm}^{-1}$  NaCl (A); --- $\diamond$ --- urea + 7  $\text{dSm}^{-1}$  NaCl (V); --- $\blacklozenge$ --- urea + 7  $\text{dSm}^{-1}$  NaCl (A)].

(with 2%, 3% and 4% urea) or to decrease significantly with (5% and 6% urea), at both stages of growth, in relation to control activities (Fig. 4). Salinization of lettuce plants with low and medium salt concentrations, at vegetative and adult stages, induced significant increases in SOD, APO, CAT and GR activities. On the other hand, the high concentration of NaCl led to a significant decrease in the activities of all antioxidant enzymes, as compared with controls (Fig. 4). In general, foliar application of urea fertilizer to the variously salinized lettuce plants induced significant increases in the activities of all the antioxidant enzymes as compared with those activities maintained in control salinized plants (Fig. 4).

The observed increases as well as decreases in the activities of SOD, APO, CAT and GR due to foliar application of urea fertilizer, either alone or in combination with NaCl, to lettuce plants throughout the entire period of experiments, may suggest that under low and medium saline stress, the elevated levels of the antioxidant enzymes appear to overcome the oxidation stress induced either by high urea concentration (5 % and 6 %) or by salt stress; thus avoiding lipid and protein peroxidation as recently reported by Comba et al. (2004). The calculated percent recovery in SOD, APO, CAT and GR activities in the variously salinized lettuce plants, sprayed with increasing concentrations of urea fertilizer, showed, in general, significant positive results indicating, at least, varied partial nullification of the adverse effects of salinity by urea fertilization. Moreover, the calculated significant positive correlation coefficients that appeared to exist between the changes in growth components and the changes maintained in the activities of SOD and APO lend a strong support to this conclusion.

In support of these suggestions, Panda and Upadhyay (2003) showed that the lipid peroxidation in *Lemna minor* L. roots treated with NaCl, was caused due to interaction of NaCl with the root surface. Both the non-enzymatic (ascorbate and glutathione) and enzymatic (SOD, CAT, APO and GR) antioxidants showed an increase with the increasing salt stress indicating a cellular capacity to overcome the stress (El-Saht, 1998; Lee et al., 2001).

Increasing evidence suggests that many damaging environmental stresses have their effects directly or indirectly through the formation of AOS following impairment of electron transport systems (Elstner, 1982; Smirnoff, 1993). The balance between the

formation and detoxification of AOS is critical to cell survival during periods of water stress. In the hydrated tissues, free radical production normally is regulated through the antioxidant system (Zhang and Kirkham, 1994).

In conclusion, our data summarized in Table 1 and shown up graphically in Fig. 1-4 indicate the different magnitudes of inhibition, induced by the different levels of salinity used, as well as the optimum percentage recoveries maintained for all growth components determined, in response to application of 3% (possibly 4%) urea as a foliar spray, to the variously salinized lettuce plants. That exogenous application of urea to NaCl-stressed lettuce plants can, at least, partially counteract the stress-induced damage is also substantiated by the detailed changes already presented in this study for the contents of a wide array of metabolites including antioxidant enzymes.

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