

## Responses of *Zea mays* seedlings to salinity stress and exogenous nitrogen supply.

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**Abstract:** The present study investigated the physiological responses of *Zea mays* seedlings to short-term salinity stress, foliar supply of nitrogen and their interaction. Seedlings were grouped into four categories, each representing a treatment and replicated 6 times. Category 1 (W) which served as the control received 350 ml of water every 3 days throughout the experiment period; category 2 (N) received 350 ml of water every 3 days and a weekly foliar spray of 100 ml 0.1 M ammonium nitrate solution throughout the study period; category 3 (S) received 350 ml of 0.1 M NaCl solution every 3 days throughout; while the 4<sup>th</sup> category (S+N) received 350 ml of 0.1 M NaCl solution every 3 days and a weekly foliar spray of 100 ml 0.1 M ammonium nitrate. Growth and physiological parameters were evaluated after the treatments. The results showed that salinity caused a decrease in plant biomass, relative growth rate, relative water content (RWC), protein and a significant increase in lipid peroxidation, and activities of catalase enzyme. It was observed that foliar application of nitrogen significantly increased the growth parameters, protein content and nitrate reductase activity. The data presented in this work underscored the positive effects of foliar nitrogen supply on *Z. mays* exposed to salinity stress.

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### 1. Introduction

Salinity is a major environmental stress that hinders plant growth and yield through ionic toxicity and osmotic stress (Hu and Schmidhalter, 2005). High concentration of salt in the soil, most commonly sodium chloride (NaCl) has detrimental effects on plant growth and productivity (Zilli, et al., 2008). Among the various sources of soil salinity, irrigation combined with poor drainage is the most serious, because it represents losses of once productive agricultural land. The reason for this is that water will evaporate leaving the salts to remain and accumulate in the soil. The stresses created by a high salt concentration in the soil solution are attributed to ionic toxicity and osmotic pressure that makes water absorption difficult for plants. Sodium ions are toxic to most plants, and some plants are also inhibited by high concentrations of chloride ions. Soils contain extreme ratios of  $\text{Na}^+ : \text{Ca}^{2+}$ ,  $\text{Na}^+ : \text{K}^+$ ,  $\text{Ca}^{2+} : \text{Mg}^{2+}$ , and  $\text{Cl}^- : \text{NO}_3^-$  under saline conditions, which cause reduced plant growth due to specific ion toxicities and ionic imbalances acting on biophysical and/or metabolic components of plant growth (Grattan and Grieve, 1999).

High salt (NaCl) uptake competes with the uptake of other nutrients ions, such as  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , N, P resulting in nutritional disorders and eventually, reduced yield and quality (Grattan and Grieve, 1999). Entry of sodium and chloride ions in large amount in chloroplasts leads to several disorders. According to Munns and Tester (2008), induced nutritional

disorders are one of the noticeable effects of salinity stress in plants. The normal absorption of mineral nutrition is affected by salinity because the distribution of essential nutrients is mainly influenced by NaCl.

Nitrogen accounts for about 80% of total mineral nutrients absorbed by plants (Marschner, 1995). Nitrogen deficiency is one of the major yield limiting factors for cereals (Shah et al., 2003), hence nitrogen fertilizer application is an essential input for crop productivity in most areas of the world (Idris and Mohammad, 2001). Nitrate and ammonium ions are the most abundant nitrogen sources for higher plants and their availability for plant absorption constitutes a limiting factor for the normal growth of plants (Causin and Barneix, 1993). Saline conditions can influence the different steps of nitrogen metabolisms such as uptake, reduction and protein synthesis that may be responsible for the reduction in the plant growth (Frechill et al., 2001).

There is a dangerous trend of a 10% per year increase in the saline area throughout the world (Pannamieruma, 1984), and this portend danger for agriculture because only few crop species are adapted to saline conditions. Irrigated land has at least twice the productivity of rain-fed land, and may therefore produce one-third of the world's food (Ghassemi et al., 1995). Considering the effects of salinity on crop productivity, It will be worthwhile to find means of reducing its impact on plant growth and yield. This could be achieved through gene manipulation or by application of additives to the soil concerned. It has

been shown that nitrogen is one of the most important mineral elements that plants require in large amount and is a constituent of many plant cell components (Hu and Schmidhalter, 2005). It has also been established that nitrogen availability is one of the primary environmental factors limiting plant biomass production in saline environments (Liu et al., 2004). It is therefore logical that exogenous application of nitrogen to plants might alleviate the salinity effects on plant growth. In the present study, the author investigated the single and interactive effects of salinity stress and nitrogen supply on *Zea mays*. The hypothesis was that the negative effects of salinity stress on *Z. mays* would be alleviated by exogenous application of nitrogen. The objective of this study was to evaluate the growth and physiological responses of *Z. mays* seedlings to short-term salinity stress, nitrogen supply and their combination.

## 2. Materials and methods

### 2.1 Plant growth and treatments

Maize grains were planted in polythene bags containing sandy loam soil to achieve 3 seeds per bag. After germination, the seedlings were thinned out to 1 seedling per nursery bag, and were arranged in a randomized block design. Seedlings were watered and allowed to grow for 1 week before treatments. Plants were grouped into four categories, each representing a treatment and replicated 6 times. Category 1 (W) served as the control and received 350 ml of water every 3 days throughout the experiment period; category 2 (N) received 350 ml of water every 3 days and a weekly foliar spray of 100 ml 0.1 M ammonium nitrate solution throughout the study period; category 3 (S) received 350 ml of 0.1 M NaCl solution every 3 days throughout; while the 4<sup>th</sup> category (S + N) received 350 ml of 0.1 M NaCl solution every 3 days and a weekly foliar spray of 100 ml 0.1 M ammonium nitrate.

### 2.2 Biomass determination

Harvested plants were washed thoroughly in running tap water to remove attached soil particles and rinsed twice with distilled water. They were then placed in labeled paper bags and weighed after oven-dried at 65°C for 72 h.

### 2.3 Relative growth rate (RGR)

The relative growth rate of the plants was determined according to methods described by Causton (1994) using the formula:

$$RGR = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \quad \text{g g}^{-1} \text{week}^{-1}$$

Where  $W_2$  and  $W_1$  are plant dry weights at  $t_2$  and  $t_1$  (4th and 2nd week) respectively.

### 2.4 Relative water content (RWC)

The fourth leaf from top (fully expanded young leaf) of the plants representing each treatment were harvested and weighed to determine their fresh

weight (FW). The leaves were submerged separately in distilled water for 24 h in the dark. They were removed from the water after this period, mopped dry using an absorbent and weighed to determine their saturated weight (SW). The leaves were then placed in paper bags and dried in an oven at 65 °C for 72 h, the weights were then taken to get the dry weight (DW). The relative water content was calculated according to Turner (1981) using the formula:  $RWC (\%) = [(fresh\ weight - dry\ weight)/(saturated\ weight - dry\ weight)] \times 100$ .

### 2.5 Nitrate reductase activity

The extraction and assay of nitrate reductase activity (NRA) was estimated by a modified method of Fan *et al.*, (2002). Fresh leaves (0.5g) was ground and extracted in 10 ml of distilled water, centrifuged at 10000 g for 5 min. About 2 ml of the crude extract was then incubated at 25 °C in the dark for 1h in 5 ml of the substrate assay solution in a test tube. The substrate assay solution contained 1 ml each of 0.1M  $KNO_3$ , 15ml  $l^{-1}$  propan-1-ol and 0.1M potassium phosphate buffer (pH 7.5). 1ml of the solution was transferred using a pipette after the incubation period into a clean test tube. 1ml each of 1% sulphanic acid and 0.02% naphthylenediamine (NED) was added, the mixture was thoroughly shaken and left to stand for 1 h to allow full colour development. A blank was prepared by mixing 1ml of substrate assay solution, 1ml of 1% sulphanic acid and 1ml of 0.02 % naphthylenediamine in a test tube. The mixture was allowed to stand for 1 h for colour development. Colour development due to nitrite was then measured spectrophotometrically at 540nm. The values were compared to a standard curve generated using solutions of  $NaNO_2$ .

### 2.6 Lipid peroxidation measurement

Lipid peroxidation was measured by estimation of the malondialdehyde (MDA) content following a modified procedure of Wang and Jin (2005). Fresh leaves (0.5 g) were homogenized in 5 ml 20% trichloroacetic acid (TCA). The homogenate was centrifuged at 10000 g for 5 min. The supernatant (1ml) was mixed with equal volume of 0.6% (w/v) thiobarbituric acid solution comprising 10% TCA. The mixture was incubated for 30 min in a boiling water bath and cooled quickly on ice bath. The absorbance of the mixture was read at 450, 532 and 600 nm. The concentration of MDA was calculated as  $6.45 (A_{532} - A_{600}) - 0.56 A_{450}$ .

### 2.7 Protein determination

The protein level was determined using the micro-Kjeldahl method for the estimation of total organic nitrogen in the oven dried leaves as previously described by Piorreck *et al.*, (1984). The total nitrogen content was multiplied by 6.25 to obtain the amount of protein.

## 2.8 Determination of catalase activity

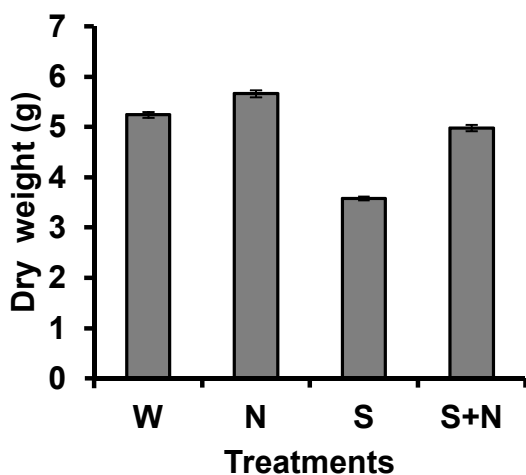
Catalase (CAT) activity was determined according to Aebi (1984). The activity was assayed by monitoring the decrease in the absorbance at 240nm as a consequence of H<sub>2</sub>O<sub>2</sub> disappearance.

## 2.9 Statistical analysis

Means of three replicates as well as their standard errors (SE) were determined. The test of significance between the treatments was done using a one way analysis of variance (ANOVA).

## 3. Results

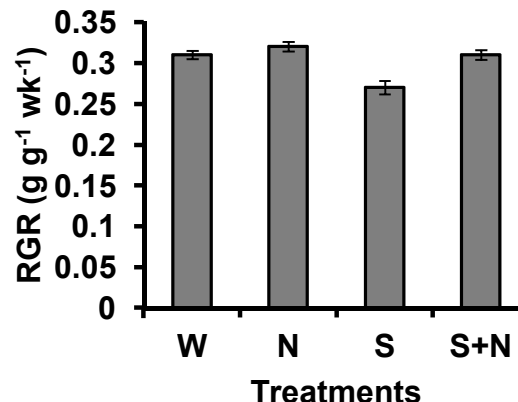
Data showing the effects of salinity and exogenous nitrogen supply on biomass accumulation of *Z.mays* is represented in figure 1. Plants grown in the non-saline soils had higher dry matter compared to the plants grown in saline soils. It was observed that exogenous supply of nitrogen had a significant effect on plant growth as it consistently increased the biomass of the plants when grown under saline or non-saline conditions. While plants that received only water had a mean dry weight of  $5.24 \pm 0.06$ , those that received water and nitrogen, salinity, nitrogen in addition to salinity respectively had  $5.66 \pm 0.07$ ,  $3.58 \pm 0.04$  and  $4.98 \pm 0.06$  g.



**Figure 1.** Biomass accumulation in *Z. mays* under well watered (W), well watered and nitrogen (N), salinity (S), and salinity and nitrogen (S+N) conditions. Means and SE of 3 replicates are presented.

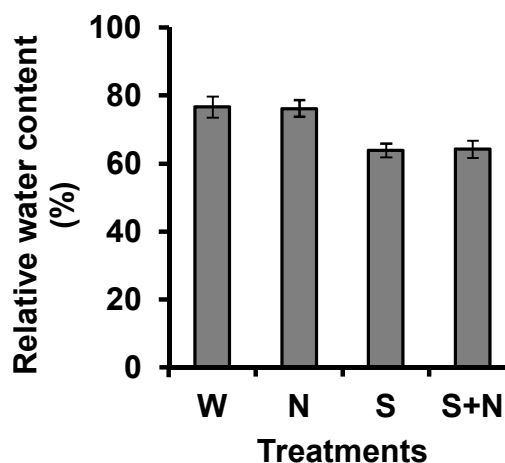
The effects of salinity and foliar supply of nitrogen on the relative growth rate (RGR) *Z. mays* is depicted in figure 2. It was evident in the study that salinity greatly lowered the relative growth rate of the experimental plant. The plant had the least RGR value of  $0.27 \pm 0.008$  g g<sup>-1</sup> wk<sup>-1</sup> when grown under salinity stress. It was observed that the foliar supply of

nitrogen to the plant increased the RGR value to  $0.31 \pm 0.006$  g g<sup>-1</sup> wk<sup>-1</sup> under saline condition.



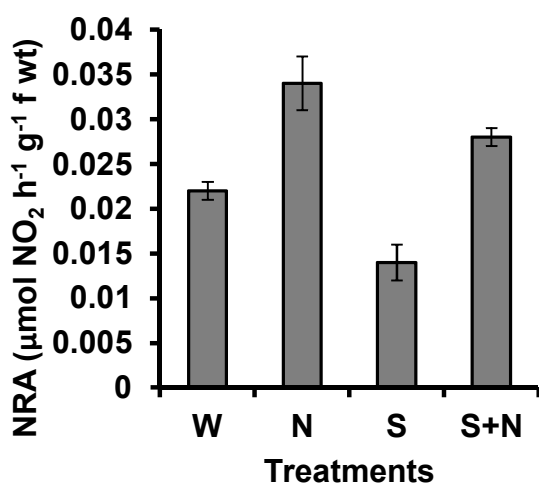
**Figure 2.** Relative growth rate of *Z. mays* grown under well watered (W), well watered and nitrogen (N), salinity (S), and salinity and nitrogen (S+N) conditions. Means and SE of 3 replicates are presented.

Figure 3 showed the effects of salinity and nitrogen supply on the relative water content (RWC) of *Z. mays*. Generally, salinity consistently led to low levels of RWC in the leaves of *Z. mays*. Plants grown under well watered condition had RWC value of  $76.6 \pm 3.11$  while those that received foliar spray of nitrogen supply had a mean value of  $76.2 \pm 2.45$  %. It was observed in this study that exogenous supply of nitrogen to *Z. mays* had no significant effect on the relative water content of the plant.



**Figure 3.** Relative water content (RWC) of *Z. mays* grown under well watered (W), well watered and nitrogen (N), salinity (S), and salinity and nitrogen (S+N) conditions. Means and SE of 3 replicates are presented.

In this study, it was observed that the nitrate reductase activity (NRA) of *Z. mays* that were not supplied with exogenous nitrogen was consistently low, compared to the values observed for those that received nitrogen (figure 4). There was a dramatic increase in NRA when there was nitrogen supply. Although the salinity effect on NRA was significant in plants that were not supplied with exogenous nitrogen, the situation was different when nitrogen was supplied even under saline condition. Plants that received water only had a mean value of  $0.022 \pm 0.001 \mu\text{mol NO}_2 \text{ h}^{-1} \text{ g}^{-1} \text{ f wt}$ , while those that received nitrogen in addition to water had a significantly higher NRA value of  $0.034 \pm 0.03 \mu\text{mol NO}_2 \text{ h}^{-1} \text{ g}^{-1} \text{ f wt}$ .

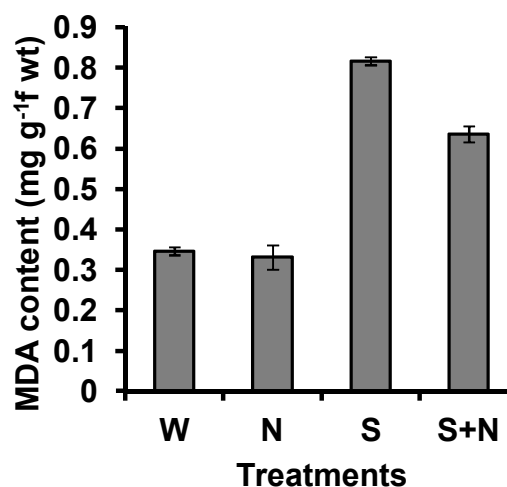


**Figure 4.** Nitrate reductase activity (NRA) of *Z. mays* grown under well watered (W), well watered and nitrogen (N), salinity (S), and salinity and nitrogen (S+N) conditions. Means and SE of 3 replicates are presented.

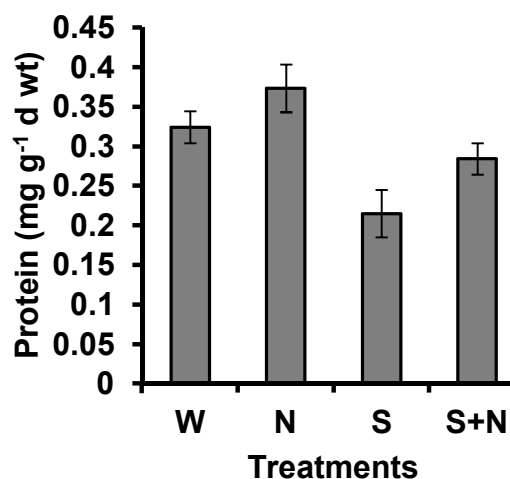
To assess the effects of salinity and nitrogen supply on lipid peroxidation, malondialdehyde (MDA) content in the leaves of *Z. mays* was measured. It was observed in this study that salinity led to more than 100% increase in MDA contents when plants were grown with or without foliar spray of nitrogen (figure 5). Under non-saline condition, nitrogen supply had no significant influence on the MDA content. There was a significant reduction in the level of MDA due to nitrogen supply when the plants were grown under salinity stress. While the MDA value observed for plants that were treated with 0.1 M NaCl with nitrogen supply had  $0.817 \pm 0.01 \text{ mg g}^{-1} \text{ f wt}$ , those that received nitrogen in addition to salinity treatment had  $0.636 \pm 0.02 \text{ mg g}^{-1} \text{ f wt}$ .

Figure 6 depicts the protein content in *Z. mays* leaves under salinity and nitrogen treatments. As expected, application of nitrogen significantly

increased the protein content in *Z. mays* under saline of non-saline conditions. It was observed in this study that salinity generally reduced the level of protein in the plant, the positive influence of nitrogen supply was however significant. Mean values of  $0.324 \pm 0.02$ ,  $0.373 \pm 0.03$ ,  $0.215 \pm 0.03$  and  $0.284 \pm 0.02$  were respectively observed as protein contents in *Z. mays* when grown under well watered (W), well watered and nitrogen (N), salinity (S), and salinity and nitrogen (S+N) conditions.

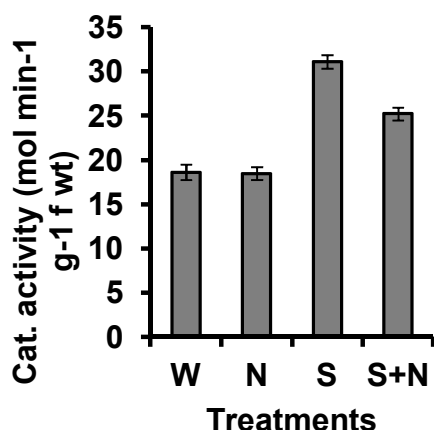


**Figure 5.** Malondialdehyde (MDA) in leaves of *Zea mays* under well watered (W), well watered and nitrogen (N), salinity (S), and salinity and nitrogen (S+N) conditions. Means and SE of 3 replicates are presented.



**Figure 6.** The amount of protein in leaves of *Zea mays* under well watered (W), well watered and nitrogen (N), salinity (S), and salinity and nitrogen (S+N) conditions. Means and SE of 3 replicates are presented.

Catalase activity was evaluated as a representative enzyme involved in antioxidant metabolism. The mean values observed in *Z. mays* grown under different growth conditions are as shown in figure 7. In this study, it was observed that foliar nitrogen supply had no influence on catalase activity under non-saline condition. It was however observed that salinity significantly increased catalase activity and the effect of foliar application of nitrogen was obvious. Plants exposed to salinity without foliar application of nitrogen had the highest catalase activity of  $31.09 \pm 0.78 \mu\text{mol}/\text{min}/\text{g f wt}$ , while plants that received nitrogen in addition to water supply had the least value of  $18.48 \pm 0.72 \mu\text{mol}/\text{min}/\text{g f wt}$ .



**Figure 7.** Catalase activity in leaves of *Zea mays* under well watered (W), well watered and nitrogen (N), salinity (S), and salinity and nitrogen (S+N) conditions. Means and SE of 3replicates are presented.

#### 4.0 Discussion

Salinity results in a considerable decrease in whole plant dry weight as well as the relative growth rates of the plant. These effects could be attributed to the difficulty in water and mineral absorption encountered by the roots due to high osmotic pressure generated by the saline condition. Increase in biomass and relative growth rate observed in this study as a result of nitrogen application could be attributed to increased photosynthetic capacity of the plants which could either result from increased stomatal conductance or from increased carboxylation capability. This finding agreed with the reports of Brown et al. 1996, Chen et al. 2005, Lu et al. 2003. The decrease in the relative water content (RWC) under saline condition could be related to the low water potential of the external solution due to high salt content. This directly affects water absorption by plant roots.

Nitrate taken up by plants is reduced to nitrite by nitrate reductase (Cao et al. 2008; Rosales et al.

2011). This enzyme catalyzes the reduction of nitrate to nitrite with pyridine nucleotide in N assimilation in higher plants (Ahmad and Abdin 1999). Salinity resulted in a reduction in NR activity. This observation was in agreement with the previous findings, indicating a similar inhibition in NRA in plants due to salinity (Gouia et al 1994, Rao and Gnanam 1990, Silveira et al 2001). The inhibition of NRA by salinity could possibly due to inactivation of the enzyme by osmotic shock as well as non-availability of cofactors required for the activation of the enzyme in plant tissues. Nitrogen application induced a significant increase NRA. This was expected, as there was more substrate available for the enzyme to convert to nitrite. This result also agreed with the findings reported by Aslam and Oaks, 1976; Lips et al 1990.

Salinity induced considerable damage on the cellular membrane of *Z. mays* leaves, as assessed by lipid peroxidation. The capacity to avoid membrane damage during dehydration process is crucial for the maintenance of membrane integrity. Reactive oxygen species (ROS) are major mediators of salt-induced cell damage in plants. In several plant species, salinity stimulated the activity of antioxidative enzymes, which suggests a role of salt stress in ROS formation (Wang et al. 2005). On the basis of inhibitor studies and ROS production measurement, it has been shown that a plasma membrane-bound NADPH oxidase is involved in the generation of ROS under saline conditions (Kawano et al. 2002; Aktas et al. 2005). It was observed in this study that application of nitrogen reduced the ROS effect on lipid peroxidation. This observation could be related to the role of nitrogen in utilization of absorbed light energy and photosynthetic carbon metabolism (Kato et al. 2003).

The decrease in the protein content in plants grown under saline condition could be as a result of physiological imbalance generated by potassium exclusion from the plant. One of the characteristics of salinity stress is the removal of potassium ions from the plant via its roots. Since potassium is necessary for protein synthesis, it would consequently result to the decline in level of protein in the plant (Caplan et al. 1990). Salt stress is complex and imposes a water deficit because of osmotic effects on a wide variety of metabolic activities (Cheeseman, 1988). This water deficit leads to the formation of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl radical, and singlet oxygen (Elstner, 1987). These cytotoxic activated oxygen species can seriously disrupt normal metabolism through oxidative damage to lipids (Wise and Naylor, 1987) and to protein and nucleic acids (Fridovich, 1986). Plants possess a number of antioxidants that protect against the potentially cytotoxic species of activated oxygen. The metalloenzyme superoxide dismutase converts

superoxide to hydrogen peroxide. Catalase and a variety of peroxidases (Chang et al., 1984) catalyze the breakdown of hydrogen peroxide. When plants are subjected to environmental stress conditions such as high light intensity, temperature extremes, drought, high salinity, herbicide treatment, or mineral deficiencies, the balance between the production of reactive oxygen species and the quenching activity of the antioxidants is upset, often resulting in oxidative damage (Spsychalla and Desborough, 1990). Plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater resistance to this oxidative damage (Wise and Naylor, 1987; Spsychalla and Desborough, 1990). The activities of the antioxidative enzymes such as catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (POD), glutathione reductase (GR), and superoxide dismutase increase under salt stress in plants and a correlation of these enzyme levels and salt tolerance exists (Lee et al., 2001; Mittova et al., 2003). It was observed in this study that the negative effects of salinity stress were significantly ameliorated by foliar supply of nitrogen. It could be concluded that the yield of crop plants under saline condition could be increased by proper nitrogen supply. The data presented in this work underscored the positive effects of foliar nitrogen supply on *Z. mays* exposed to salinity stress.

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