The antioxidant enzymes system in the shoot of the Zea mays was checked in the laboratory. The seeds Soil culture of Zea mays (cv. African Tall-09) were purchased from the local market and sterilized properly. The growth was done for five days and treated by two NaCl (100mM and 200mM) levels, two kinetin (2µM and 5µM) levels and their respective combinations. The enzymatic assays of key antioxidant enzymes i.e. catalase, guaiacol peroxidase, superoxide dismutase, guaiacol peroxid, superoxide dismutase and glutathione reductase (GR) in maize leaves were done. The assessment of important metabolic parameters i.e. lipid peroxidation, non-protein thiol levels and proline content in maize leaves was also performed. The activity of catalase was very strongly responsive for kinetin and increased its activity at both the levels. The activity of GR was also inhibited in case of salt and kinetin grouping of lower concentration level. The GPX activity was inhibited by 100 and 200mM of NaCl levels in external medium but stimulated by 5µM Kinetin level alone as well as in high amount salt combination levels while superoxide dismutase of Zea mays shoots was stimulated and showed noteworthy gain were shown in response to 200mM NaCl level as well as kinetin levels. The lower combination of both salt and kinetin suppressed the activity of ascorbate peroxide while the lipid peroxidation and proline content were increased by both the salt treatments.

**Keywords:** Kinetin, guaiacol peroxidase, catalase, superoxide dismutase, guaiacol peroxidase, ascorbate peroxidase and lipid peroxidation

**INTRODUCTION**

Salinity reduces the ability of plants to take up water and this quickly causes reductions in growth rate. The effect of short-term salt stress and recovery on growth, water relations and the activity of some antioxidant enzymes was studied in pea. Salinity can damage the plant through its osmotic effect, which is equivalent to a decrease in water activity, through specific toxic effects of ions and by disturbing the uptake of essential nutrients. Generally, enzymes and metabolic activities in plants are highly influenced by both amount and type of salts. The NaCl stress is also expected to generate ROS. The antioxidant enzymes get stimulated, by endogenous or exogenous factors, to counteract the detrimental effects of ROS. This has been reported in no. of plants like Lycopersicon pennellii, Cassia angustifolia, Maize, Hordeum vulgare, Kandelia candel & Bruguiera gymnorrhiza, Orzya sativa, Brassica oleracea, Daucus carota, Lablab purpureus, Phasolus vulgaris, Vigna unguiculata, Arachis hypogea, Pisiwm sativum, chickpea, Gynotus abyssinica and Brassica juncea.

Kinetin is the synthetic cytokinin which is a plant hormone that plays a major role in cell division and cell differentiation. Kinetin stimulates an increased composition of haploid ascospores in Saccharomyces cerevisiae. Kinetin inhibits auxin induced rooting of mung bean cuttings effectively as the intercalating DNA chemicals proflavine, acridine and ethidium bromide. Generally, kinetin is used in plant tissue culture for the formation of callus and to regenerate shoot tissue from callus which has the lower concentration of auxin, but it is used for exogenous application also. Cytokins are also involved in enhancing abiotic stress tolerance in plants exposed to against high salinity and temperature stresses.

**MATERIALS AND METHODS**

**Source of biological material**

The seeds of Zea mays (African Tall-09) were purchased from the local market.

**Growth and collection of plant material**

Seeds of Zea mays (African Tall-09) were soaked overnight in minimum DW and surface sterilized next day in 0.1% HgCl2. The pre-soaked and sterilized seeds were then regerminated hydroponically in nutrient solution containing 8mM KNO3, 2mM Ca(NO3)2, 1mM KH2PO4, 1mM MgSO4, and micronutrients: 50µM H3BO3, 5µM MnSO4, 1µM CuSO4, 1µM (NH4)6Mo7O24 and 1µM ZnSO4. After 3 days of normal Hoagland treatment, the solutions with 2µM and 5µM kinetin were used to irrigate two sets of plants besides a third set of plants without kinetin as control. Two more sets of plants were treated by 100mM and 200mM NaCl-containing nutrient solution. Final two sets of plants were treated with 100mM and 200mM NaCl added to 2µM and 5µM kinetin respectively. The seedlings were collected for leaves from all the seven sets after 7 days of treatment.

**Preparation of crude extract**

Fresh leaf tissue was homogenized (1:5, w/v) in ice-cold 0.1M potassium phosphate buffer of pH 7.5 and containing 3mM dithiothreitol, 1mM EDTA and 1% PVP. The mixture was...
centrifuged at 12000 rpm for 15 min at 4°C and supernatant used for enzyme assays.

Assays of antioxidant enzymes

Assay of Catalase (CAT) - It was estimated by the method of Vitória et al (2001). The assay mixture was 0.1 ml extract added to 1.9 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 10 mM H₂O₂. The decrease in absorption was noted at 240 nm and quantified from extinction coefficient of 0.036 mM⁻¹ cm⁻¹ and activity expressed as μmol H₂O₂ reduced min⁻¹ g⁻¹ fw.

Assay of Guaiacol Peroxidase (GPX) - It was estimated with guaiacol as substrate according to the method of Vitória et al (2001). The assay mixture was 0.1 ml extract added to 1.9 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 10 mM H₂O₂ and 9 mM guaiacol. The formation of tetraguaiacol was monitored by noting increase in absorbance at 470 nm and the activity was expressed as μmol guaiacol oxidized min⁻¹ g⁻¹ fw.

Assay of Superoxide Dismutase (SOD) - Activity of SOD was done according to the modified method of Beauchamp and Fridovich (1971) by monitoring inhibition of photochemical reduction of nitroblue tetrazolium (NBT) to purple formazan at 560 nm. Illumination of riboflavin in the presence of O₂ and the electron donor methionine generates superoxide anions which reduce the NBT to purple formazan.

The 3 ml reaction mixture consisted of 2.9 ml 50 mM K phosphate buffer (pH 7.8) containing 10 mM methionine, 16.5 µM NBT, 0.025 % Triton X-100 and 1.17 µM riboflavin, and 0.1 ml enzyme. The assay was carried out by placing the test-tubes in yellow light for 15 min. The amount of methionine-mediated formazan formed (A₀) was compared with amount of formazan formed in the absence of enzyme (Aₐ). Since one unit of SOD is defined as the enzyme causing 50% inhibition of formazan formation, the activity of SOD was calculated as follows:

\[
\text{Activity (Units/ml)} = \frac{A_c - A_t}{A_c \times 0.5}
\]

This activity was finally reported as Units per min per mg FW.

Assay of Ascorbate Peroxidase - It was estimated by the method of Mishra et al (2003) by monitoring the rate of ascorbate oxidation (ΔA = 2.8 mM⁻¹ cm⁻¹). The assay mixture was 0.1 ml extract added to 1.9 ml 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM H₂O₂, 0.5 mM ascorbate and 0.1 mM EDTA. The change in absorbance was monitored at 290 nm and activity expressed as μmol ascorbate oxidized min⁻¹ g⁻¹ fw.

Assay of Glutathione Reductase - GR was assayed by noting decrease in absorbance at 340 nm due to NADPH oxidation (ΔA = 6.2 mM⁻¹ cm⁻¹). The reaction mixture was 1.9 ml 50 mM potassium phosphate buffer (pH 7.6) containing 1 mM EDTA, 0.5 mM GSSG, 0.15 mM NADPH and 3 mM MgCl₂ with 0.2 ml enzyme source.

Other estimations

Lipid Peroxidation - The level of lipid peroxidation in sorghum leaves was determined as the amount of 2-thiobarbituric acid-reactive substances (TBARS) mainly malondialdehyde (MDA) content formed as described. 1g leaves were homogenized in 5 ml 0.1% trichloroacetic acid (TCA) and centrifuged at 10,000 rpm for 10 min at 4°C. To 2 ml supernatant, 2 ml of 20% TCA containing 0.67% TBA was added. The mixture was heated at 90°C for 30 min for formation of pink-colored 1:2 adduct between MDA & TBA and then quickly cooled on ice. After centrifugation at 10,000 rpm for 10 min, the absorbance of the supernatant at 532 nm was read, and the value for the non-specific absorption at 600 nm was subtracted. The concentration of MDA was calculated using an extinction coefficient (ε) of 155 mM⁻¹ cm⁻¹ and expressed as nmol g⁻¹ fw.

Non-protein thiol contents - The contents of non-protein thiols (NPT) were estimated as described. First total thiols was estimated by reaction with DTNB [5,5'-dithio-bis(2-nitrobenzoic acid)] which gets reduced to NTB [2-nitro-5-thiobenzoic acid] having absorption maximum at 412 nm.

500 mg leaves were homogenized in 5 ml 50 mM Tris-HCl buffer (pH 7.5) containing 1 mM EDTA and 0.2% Triton X-100. After centrifugation at 12000 rpm for 20 min, 1 ml supernatant was mixed with 1 ml 12% TCA and recentrifuged at 12000 rpm for 15 min. To 1 ml of this supernatant were added 1.9 ml methanol and 0.1 ml 0.01 M DTNB in methanol. The absorbance was taken at 412 nm against 0.5 ml 12% TCA, 0.5 ml DW and 1.9 ml methanol containing 0.1 ml 0.01 M DTNB. For calculation, extinction coefficient (ε) 14.15 mM⁻¹ cm⁻¹ was used and thiols were reported as μmols per g FW.

Proline Estimation - The proline in dried shoot tissues were estimated by the method of Bates et al (1973). First, acid-ninhydrin was prepared by warming 1.25 g ninhydrin in 30 ml acetic acid and 20 ml 6M orthophosphoric acid. About 0.5 g plant sample was homogenized in 10 ml 3% w/v sulphosalicylic acid and the homogenate was filtered. Then, 2 ml filtrate was treated with 2 ml each of glacial acetic acid and acid ninhydrin, boiled for 60 min in water bath, and the reaction was stopped by using ice bath. The mixture was extracted with 4 ml toluene, and the absorbance of fraction with toluene aspirated from liquid phase was read at 520 nm. Proline concentration was determined using calibration curve and calculated as μg g⁻¹ dry wt.

RESULTS AND DISCUSSION

Kinetin is one of the cytokinins known to significantly improve the growth of crop plant grown under salinity and soil acidity. Cytokinins are also known to enhance abiotic stress tolerance in plants against high salinity and temperature stresses. Cytokinin-mediated reduction of salinity-induced growth inhibition is known and so this hormone has potential to be used exogenously to examine response of a salt stressed plant. So, kinetin was selected in present study for exogenous application. This study was conducted to carry out brief in vivo analysis of antioxidant enzymes behaviour in shoots of Zea mays plants. For this, key antioxidant viz. catalase, ascorbic peroxidase, guaiacol peroxidase and superoxide dismutase were analyzed in plants grown in 0 to NaCl (100 mM and 200 mM) and two kinetin (2 µM and 5 µM) levels with their combinations for 7 days.

Assays of enzymes

Catalase - This enzyme from Zea mays shoots was suppressed with increase in exogenous NaCl levels but stimulated by exogenous kinetin levels alone as well as in both combination levels (Figure 1). So, catalase was very strongly responsive to kinetin. NaCl mediated decrease catalase have been reported in other plants also. Dogan (2012) exposed sand grown plants to 0 to 150 mg NaCl for 7 days and found decrease in leaf catalase activity of Lycopersicon esculentum. Kachout et al (2013)
studied the influence of salt stress on two varieties of Atriplex hortensis by giving up to 260mM NaCl and found decrease in catalase. Carrasco-Rios and Pinto (2014) grew the maize plants in 50 and 100mM NaCl stress and reported decreased catalase activity in plant shoots at 100mM NaCl stress. Kavas et al (2015) treated two 12-day old peanut cultivars with 0/100/300mM NaCl for 16 days and found decreased catalase activity in both. Naik et al (2016) treated 10-day old niger plants with 0-500mM NaCl for 24/48/72 hours and reported reduction in catalase activity.

Glutathione reductase- Enzyme glutathione reductase in Zea mays plant was inhibited by NaCl salt treatment of 100mM and 200mM levels. GR was also inhibited in case of NaCl and kinetin combination of lower concentration level (Figure 2). It was stimulated by the 5 µM Kinetin treatment alone as well as in presence of 200mM NaCl. So, GR was significantly responsive to 5 µM Kinetin and restored to almost control level even in presence of 200mM NaCl. There were two other reports of NaCl mediated inhibition. Neto et al (2005) grew the maize plants in 100mM salt stress and reported decreased reduced GR activity in roots of salt-sensitive genotype and no change in roots of salt-tolerant genotype. Dogan (2012) sand-cultured tomato in 0-150mM NaCl for 7 days and noted reduction in leaf GR with increase in NaCl treatment at 75mM and more. However, majority of reports are of increase.
Salt stimulated GR increase has also been reported in a number of plants like *Gossypium hirsutum* (0-150mM NaCl for 30 days)\(^{(34)}\), *Phaseolus vulgaris* (5-day old plants with 400mM NaCl for 48 hours)\(^{(35)}\), *Atriplex hortensis* (260mM NaCl for 3 months)\(^{(32)}\), *Zea mays* (21 day old two maize varieties in 0/50mM/100mM NaCl for 15 days)\(^{(35)}\), *Arachis hypogea* (two 12-day old peanut cultivars with 0/100/300mM NaCl for 16 days)\(^{(13)}\), chickpea (0-200 mg L\(^{-1}\) NaCl)\(^{(15)}\), *Brassica juncea* (250mM NaCl stress for 6 hours)\(^{(17)}\) and *Guizotia abyssinica* (0-500mM NaCl for 24/48/72 hours)\(^{(16)}\).

**Guaiacol peroxidase (GPX)-** This enzyme from *Zea mays* shoots was inhibited by 100 and 200mM of NaCl levels in external medium but stimulated by 5µM Kinetin level alone as well as in high amount salt combination levels. In both combinations of NaCl and kinetin concentration, GPX activity was induced highly significantly (Figure 3). So, kinetin was able to decisively increase activity of this enzyme in salinity stress. NaCl mediated GPX inhibition has been reported in few other plants also. Carrasco-Rios and Pinto (2014)\(^{(33)}\) grew the maize plants in 50 and 100mM NaCl stress and reported decreased guaiacol peroxidase activity in Lluteno cultivar but increased activity in Jubilee cultivar. Bano et al (2012)\(^{9}\) cultured 10-days’ old carrot plants in 0, 50, 100 and 150 mM NaCl for 4 weeks, salt concentration was increase in a stepwise manner with 50 mM NaCl per day and found decreased peroxidase activity in plant leaves in both DC-4 and T-29 cultivars of *Daucus carota*. Ramachandran and Vincent (2013)\(^{36}\) treated pot cultured *Capsicum annum* plants with 10mM, 20mM, 30mM, 40mM and 50mM NaCl: and found significant decrease in leaves SOD activity till 30 mM NaCl.

**Superoxide dismutase-** The superoxide dismutase of *Zea mays* shoots was stimulated and significant increases were shown in response to 200mM NaCl level as well as kinetin levels. Kinetin had shown the augmenting effect of NaCl on the stimulation of SOD activity in both combinations (Figure 4). The present finding in NaCl stress is similar to a number of earlier reports of NaCl-mediated stimulation of this enzyme. Mittova et al (2004)\(^{(1)}\) exposed hydroponically grown plants to 100mM NaCl and found increased superoxide dismutase activity in plant leaves of *Lycopersicon pennelli*. Agarwal and Pandey (2004)\(^{(2)}\) exposed plants to 0, 20, 50 and 100 mM NaCl for 7 days and observed increase in the activity of superoxide dismutase in plant shoots *Cassia angustifolia* of till 50mM NaCl but decrease at 100mM NaCl. Zhang et al (2006)\(^{(6)}\) treated both the plants with 10% NaCl stress and found increased superoxide dismutase activity of *Kandelia candel* and *Bruguiera gymnorhiza*. Khosravinejad et al (2008)\(^{(37)}\) exposed barley seedlings to 0 to 400mM NaCl for 3 days and found increase in superoxide dismutase activity in leaves of plant in both tested varieties of *Hordeum vulgare*. Bayram et al (2014)\(^{(1)}\) exposed 21 days old plants to 150 and 300 mM NaCl found increase in leaf superoxide dismutase activity of *Phaseolus vulgaris*. Yasar et al (2016)\(^{(38)}\) treated 7 pea cultivars of 4th leaf stage with 75mM NaCl for 14 days and reported increased leaf SOD activity in all of them.

![Figure 3: Changes in shoot guaiacol peroxidase activity (µmoles tetraguaiacol formed/min g fw) formed of *Zea mays* (African Tall-09) seedlings under various regimes of NaCl and kinetin.](image-url)
However, Bano et al. (2014) cultured 10-days’ old carrot plants in 0, 50, 100 and 150 mM NaCl for 4 weeks, salt concentration was increase in a stepwise manner with 50 mM NaCl per day and found decreased superoxide dismutase activity in plant leaves in DC-4 cultivar but no change in T-29 cultivar of Daucus carota. Carrasco-Rios and Pinto (2014) grew the maize plants in 50 and 100mM NaCl stress and reported decreased superoxide dismutase activity in plant leaves.

**Ascorbate peroxidase**- The ascorbate peroxidase activity was increased continuously with increasing concentration of exogenous NaCl and kinetin levels, so both are same in promoting this enzyme (Figure 5). It may be suggested that plant tolerance mechanism involve a system that remove free radicals, preventing the accumulation of O$_2^-$, and therefore increasing the requirement of antioxidative peroxidases viz. ascorbate peroxidase. It is also proposed that increasing NaCl stress lead to increased H$_2$O$_2$ production and level of ascorbate peroxidase increased to detoxify H$_2$O$_2$ which also indicating their role in detoxification of H$_2$O$_2$ under high salinity stress. NaCl mediated ascorbate peroxidase increase has been reported in leaves of Lycopersicon pennelli (100mM NaCl), leaves of barley (0 to 400mM NaCl for 3 days), Phaseolus vulgaris (400mM NaCl for 48 hours), nodal segments of chickpea (0-200mg/L NaCl), pea leaf of 7 cultivars (75mM NaCl for 14 days), Yasar et al 2016 and 10-day old niger plants (0-500mM NaCl). On the contrary, NaCl mediated inhibition of APX has been reported in Lycopersicon esculentum (0 to 150mg NaCl for 7 days), Zea mays (50 and 100mM salt stress) and Daucus carota (0-150mM NaCl for 90 days).

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**Figure 4:** Changing in shoot superoxide dismutase activity (Units/min/g fw) of *Zea Mays* (African Tall-09) seedlings under various regimes of NaCl and kinetin (highly significant) [\( p<0.001 \) (highly significant); \( p<0.001 \) (highly significant from 100mM NaCl); \( p<0.05 \) (definitely significant from 200mM NaCl)].

**Figure 5:** Changes in shoot ascorbate peroxidase activity of *Zea mays* (African Tall-09) seedlings under various regimes of NaCl [\( p<0.05 \) (probably significant); \( p<0.01 \) (definitely significant); \( p<0.001 \) (highly significant); \( p<0.001 \) (highly significant from 200mM NaCl)].
Lipid Peroxidation

In present study, level of lipid peroxidation continuously increased strongly by both NaCl and 2µM kinetin also caused small increase in peroxidation. But combined treatment showed that kinetin managed to keep peroxidation much lower than in individual salt treated plants (Figure 6). So, both compounds increased peroxidation but kinetin was stronger stimulant. According to the previous studies whose result resemble with present study, it can be suggested that lipid peroxidation was due to high MDA level in plant which itself is produced due to accumulation of H₂O₂ and lipid peroxidation can also be induced via an enzymatic pathway by the activity of lipoxygenases, which have been observed to be induced by salt stress39.3.

NaCl mediated increase lipid peroxidation is universal and reported in many plants like Lycopersicon pennellii, Cassia augustifolia, Hordeum vulgare, Phaseolus vulgaris, Arachis hypogea, chickpea, Pisum sativum and Guizotia abyssinica.

Non-protein thiols

The non-protein thiols like glutathione are critical to plant for supply of reducing power to antioxidant enzymes for combating stress and so their increase in is an important step in stress resistance. In the present study too, non-protein thiols of maize increased at 200mM NaCl but increase by kinetin was much stronger (Figure 7).
Proline Estimation

Both the NaCl stresses increased proline as compared to control and indicative of the effort put on by plant to tolerate salt stress. But, kinetin decreased the proline contents at both levels (2μM and 5μM) given individually and also in presence of salt as compared to individual salt treatments (Figure 8). So, kinetin probably mitigated the stress effect to offset the proline production in plant shoot.

The NaCl caused proline accumulation has been reported in a number of plants like Opuntia streptacantha, Brassica napus, Nicotiana tabacum, Halianthus tuberosus, Medicago sativa, Excoecaria agallocha, Arachis hypogea, Oryza sativa, 4 chickpea cultivars and Guizotia abyssinica.

CONCLUSION

For this study, plants were grown in soil irrigated with 100 and 200mM sodium chloride, 2μM and 5μM kinetin and their combinations. Leaves were taken from soil culture at 7 days of growth for estimation of various antioxidant enzymes: catalase, guaiacol peroxidase, superoxide dismutase and ascorbate peroxidase. These studies were conducted in seedling stage of Zea mays plants due to this stage being the most sensitive stage of plant to damage by NaCl stress (100mM & 200mM) and kinetin stress (2μM & 5μM) as well as their combination also.

Leaf catalase was suppressed by NaCl stress but strongly stimulated by kinetin even in presence of NaCl. Leaf glutathione reductase and guaiacol peroxidase were also inhibited but restored only by 5μM kinetin. Leaf superoxide dismutase and ascorbate peroxidase behaved differently by getting increased to 200mM NaCl and kinetin as well. Maize leaf also showed increased peroxidation by NaCl stresses but combination treatments showed that kinetin treatments reduced the same probably by promoting non-protein thiols content which was also promoted by the hormone. As the typical salt stress response, proline content also accumulated in response but the same was lowered in presence of kinetin, indicating the lower stress felt by plant. Briefly, NaCl was toxic to a limit to shoot system of Zea mays plants as indicated by consistent increase in peroxidation and suppression of catalase, guaiacol peroxidase and glutathione reductase enzyme but kinetin increased tolerance by promoting all the enzymes specially at 5μM level and restricting lipid peroxidation and proline accumulation while increasing non-protein thiols content.

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