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Research Article

MODULATION OF ANTIOXIDANT ENZYMES SYSTEM BY KINETIN IN SALT STRESSED SHOOTS OF *ZEA MAYS*

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ABSTRACT

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 (GPX), ascorbate peroxidase, 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 peroxidise 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*[1], *Cassia angustifolia*[2], Maize[3,4], *Hordeum vulgare*[5], *Kandelia candel* & *Bruguiera gymnorrhiza*[6], *Oryza sativa*[7], *Brassica oleracea*[8], *Daucus carota*[9], *Lublub purpureus*[10], *Phaseolus vulgaris*[11], *Vigna unguiculata*[12], *Arachis hypogea*[13], *Pisum sativum*[14], chickpea[15], *Guizotia abyssinica*[16] and *Brassica juncea*[17].

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*[18]. Kinetin inhibits auxin induced rooting of mung bean cuttings effectively as the intercalating DNA chemicals proflavine, acridine and ethidium bromide[19]. 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. Cytokinins 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% $HgCl_2$. The pre-soaked and sterilized seeds were then regerminated hydroponically in nutrient solution containing 8mM KNO_3, 2mM $Ca(NO_3)_2$, 1mM KH_2PO_4, 1mM $MgSO_4$ and micronutrients: 30µM H_3BO_4, 5µM $MnSO_4$, 1µM $CuSO_4$, 1µM $(NH_4)_6Mo_7O_{24}$ and 1µM $ZnSO_4$[20]. 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 12000rpm for 15min 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)[21]. The assay mixture was 0.1ml extract added to 1.9ml of 50mM potassium phosphate buffer (pH 7.0) containing 10mM H_2O_2. The decreases in absorption were noted at 240nm and quantified from extinction coefficient of 0.036 mM^{-1} cm^{-1} and activity expressed as µmol H_2O_2 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)[21]. The assay mixture was 0.1ml extract added to 1.9ml of 50mM potassium phosphate buffer (pH 7.0) containing 10mM H_2O_2 and 9mM guaiacol. The formation of tetraguaiacol was monitored by noting increase in absorbance at 470nm and quantified using the extinction coefficient 26.6 mM^{-1} cm^{-1} and activity 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)[22] by monitoring inhibition of photo-chemical reduction of nitroblue tetrazolium (NBT) to purple formazan at 560nm. Illumination of riboflavin in the presence of O_2 and the electron donor methionine generates superoxide anions which reduce the NBT to purple formazan.

The 3ml reaction mixture consisted of 2.9ml 50mM K phosphate buffer (pH 7.8) containing 10mM methionine, 168µM NBT, 0.025% Triton X-100 and 1.17µM riboflavin, and 0.1ml enzyme. The assay was carried out by placing the test-tubes in yellow light for 15 min. The amount of methionine-mediated formazan formed (A_t) was compared with amount of formazan formed in the absence of enzyme (A_c). 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* (2006)[23] by monitoring the rate of ascorbate oxidation (□=2.8 mM^{-1} cm^{-1}). The assay mixture was 0.1ml extract added to 1.9ml 50mM potassium phosphate buffer (pH 7.0) containing 0.1mM H_2O_2, 0.5mM ascorbate and 0.1mM EDTA. The change in absorbance was monitored at 290nm 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 340nm due to NADPH oxidation (□=6.2 mM^{-1} cm)[24]. The reaction mixture was 1.9ml 50mM phosphate buffer (pH 7.6) containing 1mM EDTA, 0.5mM GSSG, 0.15mM NADPH and 3mM $MgCl_2$ with 0.2ml 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[25]. 1g leaves were homogenized in 5ml 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 (□) 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[26]. 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 412nm.

500mg leaves were homogenized in 5ml 50mM Tris-HCl buffer (pH 7.5) containing 1mM EDTA and 0.2% Triton X-100. After centrifugation at 12000rpm for 20min, 1ml supernatant was mixed with 1ml 12% TCA and recentrifuged at 12000rpm for 10min. To 1ml of this supernatant were added 1.9ml methanol and 0.1ml 0.01 M DTNB in methanol. The absorbance was taken at 412nm against 0.5ml 12% TCA, 0.5ml DW and 1.9ml methanol containing 0.1ml 0.01M DTNB. For calculation, extinction coefficient (□) 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)[27]. First, acid-ninhydrin was prepared by warming 1.25g ninhydrin in 30ml acetic acid and 20ml 6M orthophosphoric acid. About 0.5g plant sample was homogenized in 10ml 3% w/v sulphosalicylic acid and the homogenate was filtered. Then, 2ml filtrate was treated with 2ml 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 4ml toluene, and the absorbance of fraction with toluene aspired 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[28] and soil acidity[29]. 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[30] 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 two NaCl (100mM and 200mM) and two kinetin (2µM and 5µM) 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)[31] exposed sand grown plants to 0 to 150mg NaCl for 7 days and found decrease in leaf catalase activity of *Lycopersicon esculentum.* Kachout *et al* (2013)[32]

studied the influence of salt stress on two varieties of Atriplex hortensis by giving upto 260mM NaCl and found decrease in catalase. Carrasco-Rios and Pinto (2014)[33] 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)[13] 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)[16] treated 10-day old niger plants with 0-500mM NaCl for 24/48/72 hours and reported reduction in catalase activity.

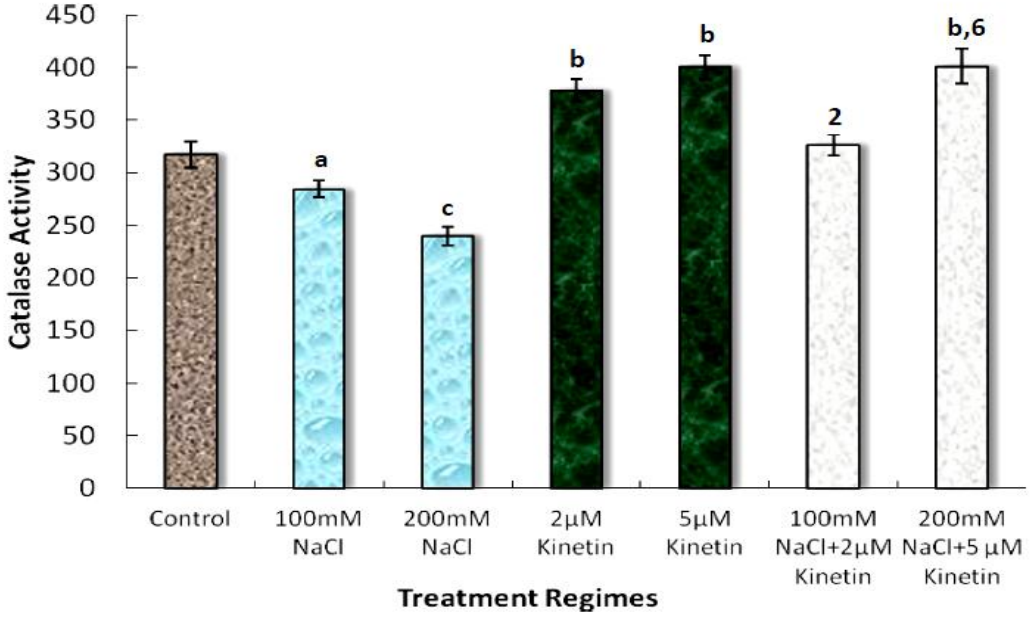

Figure 1: Changes in shoot catalase activity (nmoles/min/g fw) of 7-day old *Zea mays* (African Tall-09) seedlings under various regimes of NaCl and kinetin [$^{a}p<0.05$ (Probably significant); $^{b}p<0.01$ (Definitely significant); $^{c}p<0.001$ (Highly significant) $^{2}p<0.01$ (Definitely significant from 100mM NaCl); $^{6}p<0.001$ (Highly significant from 200mM NaCl)

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)[3] 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)[31] 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.

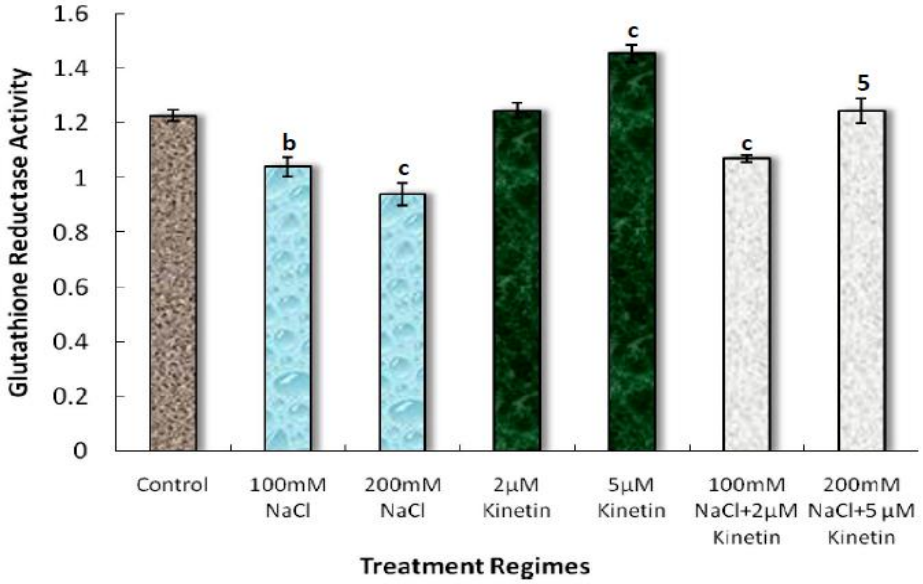

Figure 2: Changes in shoot glutathione reductase activity (µmoles NADPH oxidized/min/ g fw) of 7-day old *Zea mays* (African Tall-09) seedlings under various regimes of NaCl and kinetin [$^{b}p<0.01$ (Definitely significant); $^{c}p<0.001$ (Highly significant); $^{5}p<0.01$ (Definitely significant from 200mM NaCl)]

Salt stimulated GR increase has also been reported in a number of plants like *Gossypium hirsutam* (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 $NaCl_2$ and found significant decrease in leaves SOD activity till 30 mM NaCl.

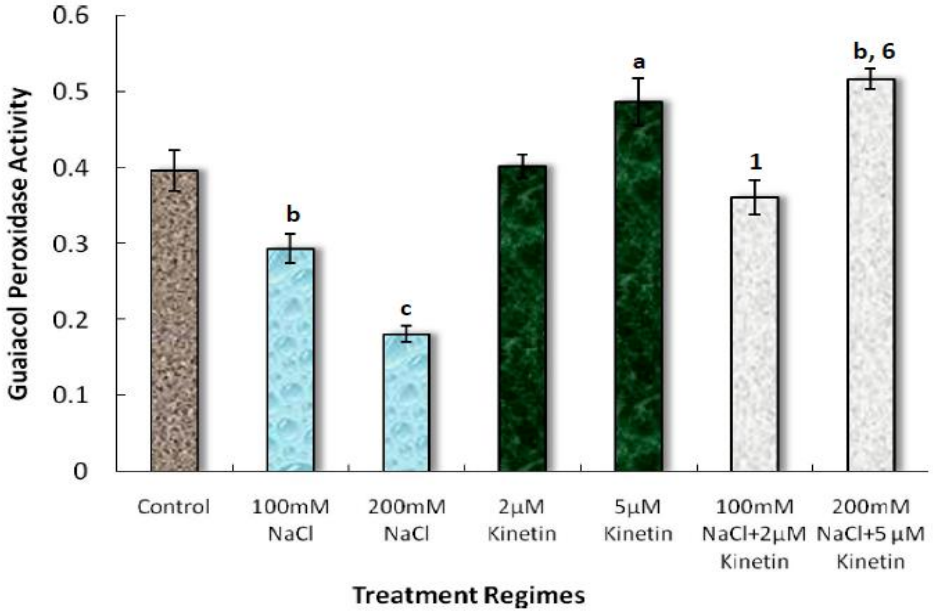

Figure 3: Changes in shoot guaiacol peroxidase activity (µmoles tetraguaiacol formed/min g fw) formed of *Zea mays* (African Tall-09) seedlings under various regimes of NaCl and kinetin. [$^{a}p<0.05$ (probably significant); $^{b}p<0.01$ (definitely significant); $^{c}p<0.001$ (highly significant); $^{1}p<0.05$ (probably significant from 100mM NaCl); $^{6}p<0.05$ (highly significant from 200mM 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 pennellii*. 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 gymnorrhiza*. 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)[11] 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)[14] treated 7 pea cultivars of 4th leaf stage with 75mM NaCl for 14 days and reported increased leaf SOD activity in all of them.

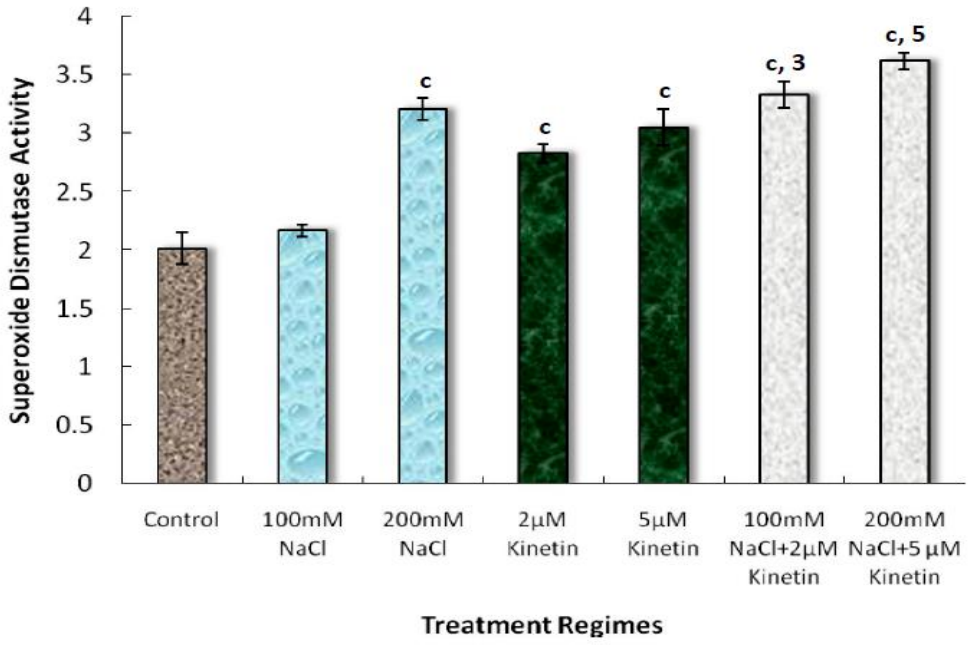

Figure 4: Changing in shoot superoxide dismutase activity (Units/min/g fw) of *Zea Mays* (African Tall-09) seedlings under various regimes of NaCl and kinetin (highly significant) [$^{c}p<0.001$ (highly significant); $^{3}p<0.001$ (highly significant from 100mM NaCl); $^{5}p<0.05$ (definitely significant from 200mM NaCl)].

However, Bano *et al* (2014)[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 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)[33] 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_2O_2 production and level of ascorbate peroxidase increased to detoxify H_2O_2 which also indicating their role in detoxification of H_2O_2 under high salinity stress[38]. NaCl mediated ascorbate peroxidase increase has been reported in leaves of *Lycopersicon pennelli* (100mM NaCl)[1], leaves of barley (0 to 400mM NaCl for 3 days)[37], *Phaseolus vulgaris* (400mM NaCl for 48 hours)[35], nodal segments of chickpea (0-200mg/L NaCl)[15], pea leaf of 7 cultivars (75mM NaCl for 14 days)[14]Yasar et al 2016 and 10-day old niger plants (0-500mM NaCl)[16]. On the contrary, NaCl mediated inhibition of APX has been reported in *Lycopersicon esculentum* (0 to 150mg NaCl for 7 days)[31], *Zea mays* (50 and 100mM salt stress)[33] and *Daucus carota* (0-150 mM NaCl for 90 days)[9].

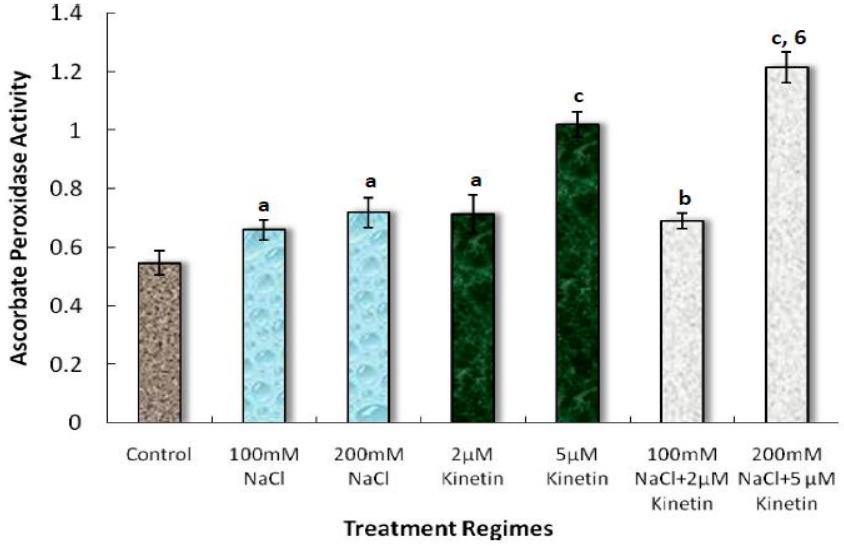

Figure 5: Changes in shoot ascorbate peroxidase activity of *Zea mays* (African Tall-09) seedlings under various regimes of NaCl [$^{a}p<0.05$ (probably significant); $^{b}p<0.01$ (definitely significant); $^{c}p<0.001$ (highly significant); $^{6}p<0.001$ (highly significant from 200mM NaCl)].

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 H_2O_2 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 stress[39,1].

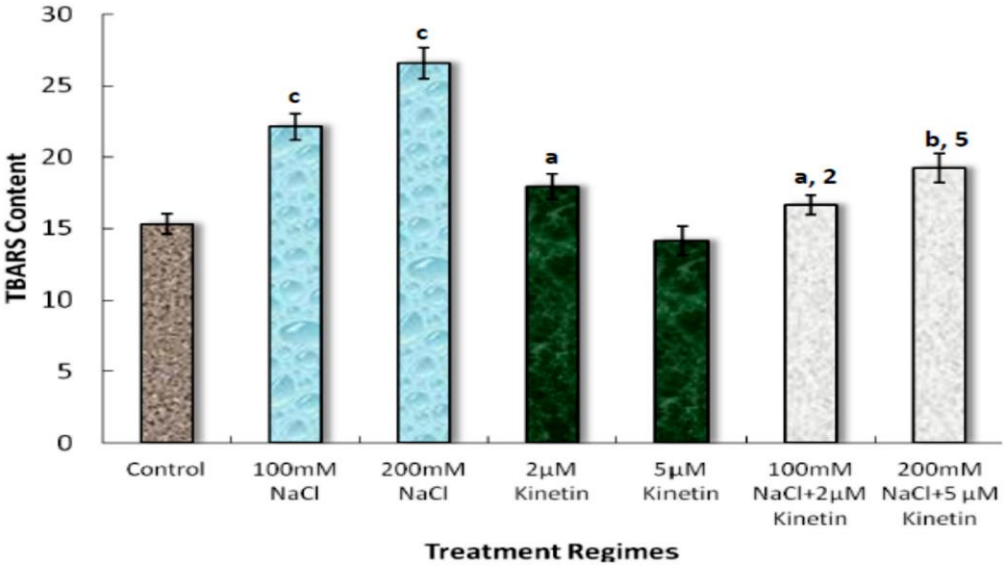

Figure 6: Changes in shoot lipid peroxidation (µmoles TBARS /g fw) in *Zea mays* (African Tall-09) seedlings under various regimes of NaCl [$^a p<0.05$ (probably significant); $^b p<0.01$ (definitely significant); $^c p<0.001$ (highly significant); $^2 p<0.01$ (definitely significant from 100mM NaCl); $^5 p<0.01$ (definitely significant from 200mM NaCl)].

NaCl mediated increase lipid peroxidation is universal and reported in many plants like *Lycopersicon pennellii*[1], *Cassia augustifolia*[2], *Hordeum vulgare*[37], *Phaseolus vulgaris*[11], *Arachis hypogea*[13], chickpea[15], *Pisum sativum*[14] and *Guizotia abyssinica*[16].

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).

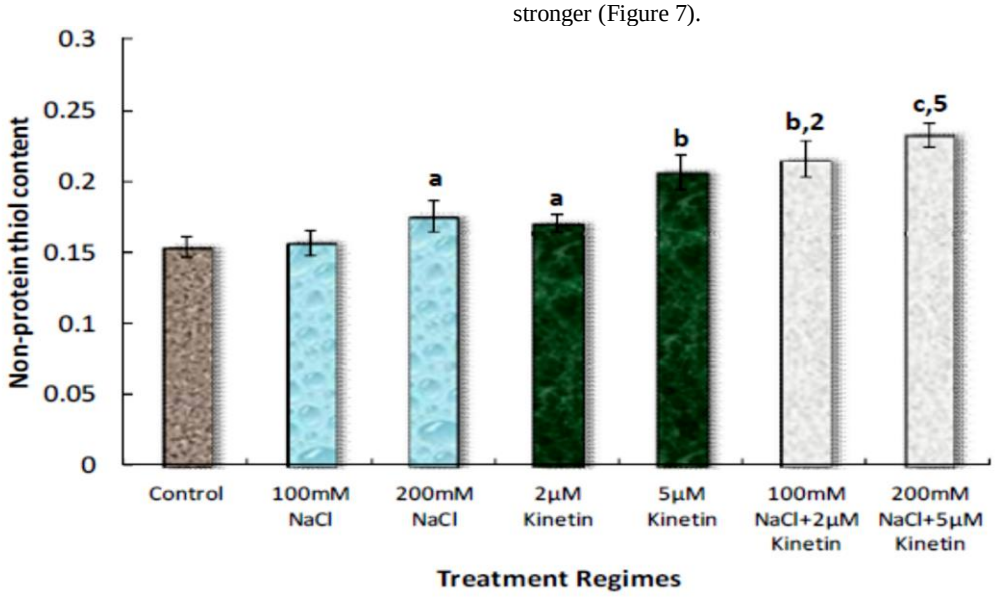

Figure 7: Changes in shoot non-protein thiol content (µmoles/g fw) in *Zea mays* (African Tall-09) seedlings under various regimes of NaCl [$^a p<0.05$ (probably significant); $^b p<0.01$ (definitely significant); $^c p<0.001$ (highly significant); $^2 p<0.01$ (definitely significant from 100mM NaCl); $^5 p<0.01$ (definitely significant from 200mM NaCl)].

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*[40], *Brassica napus*[41], *Nicotiana tabacum*[42], *Halianthus tuberosus*[43], *Medicago sativa*[44], *Excoecaria agallocha*[45], *Arachis hypogea*[13], *Oryza sativa*[46], 4 chickpea cultivars[47] and *Guizotia abyssinica*[16].

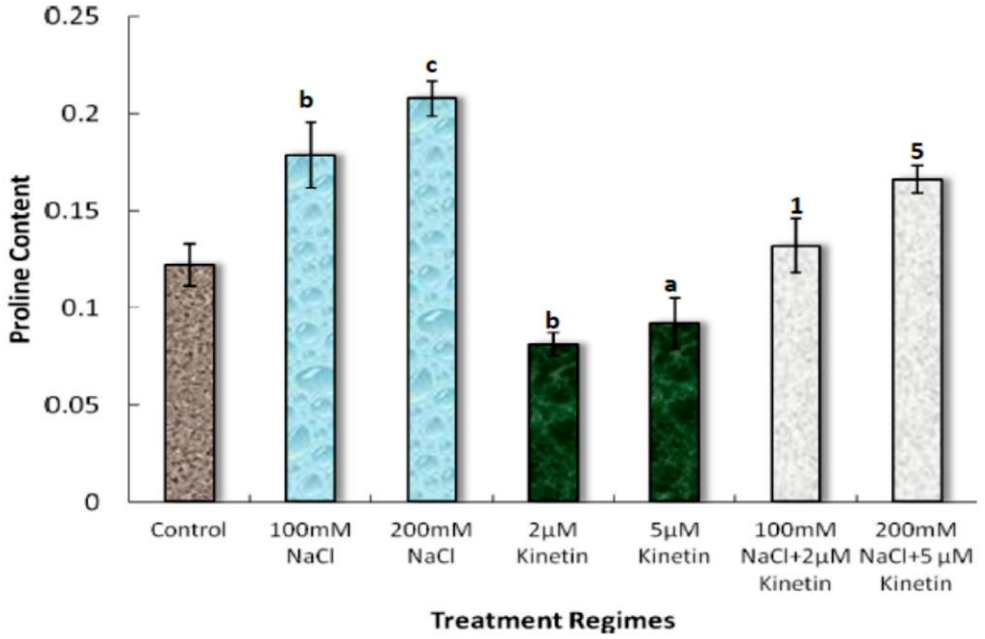

Figure 8: Proline content (mg/g fw) in *Zea mays* (African Tall-09) grown in various NaCl and kinetin regimes for 7 days as shown. [$^{a}p<0.05$ (change probably significant); $^{b}p<0.01$ (change definitly significant); $^{c}p<0.001$ (change highly significant); $^{1}p<0.05$ (change probably significant as compared to 100mM NaCl); $^{5}p<0.01$ (change definitely significant as compared to 200mM NaCl)]

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.

REFERENCES

1. Mittova V, Guy M, Tal M, Volokita M (2004). Salinity up-regulates the antioxidative system in root mitochondria and peroxisomes of the wild salt-tolerant tomato species Lycopersicon pennellii. J Exp Bot 2004; 55: 1105–13.
2. Agarwal S, Pandey V. Antioxidant enzyme response to NaCl stress in *Cassia angustifolia*. Biol Planta 2004; 48: 555-60.
3. Neto ADA, Prisco JT, Enéas-Filho J, Medeiros JR, Gomes Filho E (2006). Hydrogen peroxide pre-treatment induces salt stress acclimation in maize plants. J Plant Physiol 2006; 162: 1114-22.
4. Abbasi GH, Ijaz M, Akhtar J, Anwar-ul-haq M, Jamil M, Ali S, Ahmad R, Khan HN. Profiling of anti-oxidative enzymes and lipid peroxidation in leaves of salt tolerant and salt sensitive maize hybrids under NaCl and Cd stress. Sains Malaysiana 2016; 45: 177-84.
5. Kim SY, Lim JH, Park MR, Kim YJ, Park T, Seo YW, Choi KG, Yun SJ. Enhanced Antioxidant Enzymes are Associated with Reduced Hydrogen Peroxide in Barley Roots under Saline Stress. J Bio Mol Bio 2005; 38: 218-24.
6. Zhang J, Jia W, Yang J, Ismail AM. Role of ABA in integrating plant responses to drought and salt stressses. Field Crops Res 2006; 97: 111-9.
7. Khan MH, Panda SK. Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. Acta Physiol Plant 2008; 30: 81–89
8. Hernandez M, Olmos E, Fernandez-Garcia N, Lopez-Perez L (2009). Role of phi cells and the endodermis under salt stress in Brassica oleracea. New Phytol 2009; 181: 347-60.

9. Bano S, Ashraf M, Akram NA. Salt stress regulates enzymatic and nonenzymatic antioxidative defense system in the edible part of carrot (Daucus carota L.). J Plant Interac 2014; 9: 324-29.
10. Souza RD, Devaraj VR. Role of calcium in increasing tolerance of Hyacinth bean to salinity. J App Bio Biotech 2010; 1: 011-020.
11. Bayram D, Dinler BS, Tasci E. Differential Response of Bean (Phaseolus vulgaris L.) roots and leaves to salinity in soil and hydroponic culture. Not Bot Horti Agrobot Cluj-Napoca 2014; 42: 219-26.
12. Praxedes SC, Damatta FM, Claudivan Ldace, CFD Prisco JT, Filho EG. Salt stress tolerance in cowpea is poorly related to the ability to cope with oxidative stress. Acta Bot Croat 2014; 73: 51–62.
13. Kavas M, Akca OE, Akcay UC, Peksel B, Eroglu S, Oktem HA, Yucel M). Antioxidant responses of peanut (Arachis hypogea L.) seedlings to prolonged salt-induced stress. Arch Biol Sci 2015; 67: 1303-16.
14. Yasar F, Uzal O, Yasar O. Antioxidant enzyme activities and lipid peroxidation amount of pea varieties (Pisum sativum sp. Arvense L.) under salt stress. Fresen Environ Bull 2016; 25: 37-42.
15. Shankar V, Kumar D, Agrawal V. Assessment of antioxidant enzyme activity and mineral nutrients in response to NaCl stress and its amelioration through glutathione in chickpea. Appl Biochem Biotechol 2016; 178: 267-84.
16. Naik H, Naik K, Devaraj VR. Effect of salinity stress on antioxidant defense system of niger (Guizotia abyssinica Cass.). Am J Plant Sci 2016; 7: 980-90.
17. Singh LR, Pandey M and Suprasanna P (2016). Early osmotic, antioxidant, ionic, and redox responses to salinity in leaves and roots of Indian mustard (*Brassica juncea* L.). Protoplasma 2016; 253: 101-10.
18. Barciszewski J, Siboska G, Rattan SIS, Clark BFC. Occurrence, biosynthesis and properties of kinetin (N^6-furfuryladenine). Plant Growth Regul 2000; 32: 257–65.
19. Chen J, Witham FH, Heuser CW. Inhibition of NAA-induced Adventitious Roots in Mung Bean Cuttings by Kinetin, Zeatin, Ethidium Bromide and Other DNA Intercalators. World Wide Web J Biol 1996; 1:1-8.
20. Dguimi HM, Ghorbel MH, Gouia H. Tissue-specific cadmium accumulation and its effects on nitrogen metabolism in tobacco (*Nicotiana tabaccum, Bureley v*. Fb9). CR Biologies 2009; 332: 58-68.
21. Vitoria AP, Lea PJ, Azevedo RA. Antioxidant enzymes responses to cadmium in radish tissues. Phytochem 2001; 57:701-10.
22. Beauchamp C, Fridovich I (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 1971; 44: 276-87.
23. Mishra S, Srivastava S, Tripathi RD, Govindarajan R, Kuriakose SV, Prasad MNV. Phytochelatin synthesis and response of antioxidants during cadmium stress in *Bacopa monnieri* L. Plant Physiol Biochem 2006; 44: 25-37.
24. Balestrasse KB, Gardey L, Gallego SM, Tomaro ML. Response of antioxidant defence system in soybean nodules and roots subjected to cadmium stress. Aust J Plant Physio 2001; 28: 497–504.
25. Zhang X, Li C, Nan Z. Effects of cadmium stress on growth and antioxidative systems in *Achnatherum inebrians* symbiotic with *Neotyphodium gansuense*. J Hazard Mat 2010; 175: 703-09.
26. Romero-Puertas MC, Zabalza ANA, Corpas FJ, Gómez M, Del Rio LA, Rodríguez-Serrano M. Cadmium effect on oxidative metabolism of pea (*Pisum sativum* L.) roots. Imaging of reactive oxygen species and nitric oxide accumulation in vivo. Plant Cell Environ 2006; 29: 1532–44.
27. Bates LS, Waldren RP, Teari D. Rapid determination of free proline for water stress studies. Plant Soil 1973; 39: 205-07.
28. Salama FM, Awadalla AA. The effect of different kinetin application methods on some chlorophyll parameters of two crop plants grown under salinity stress. Phyton 1987; 27: 181- 93.
29. Gadallah MAA. "Effects of cadmium and kinetin on chlorophyll content, saccharides and dry matter accumulation in sunflower plants." Biol Planta 1995; 37: 233-40
30. Mansour MMF. Nitrogen containing compounds and adaptation of plants to salinity stress. Biol Plant 2000; 43: 491-500.
31. Dogan M. Investigation of the effect of salt stress on the antioxidant enzyme activities on the young and old leaves of salsola (Stenoptera) and tomato (Lycopersicon esculentum L.). Afric J Plant Sci 2012; 6: 62-72.
32. Kachout SS, Hamza KJ, Bouraoui NK, Leclerc JC, Ouergh Z. Salt-induced changes in antioxidative enzyme activities in shoot tissues of two Atriplex varieties. Not Bot Horti Agrobo 2013; 41: 115-21.
33. Carrasco-Rios L, Pinto M (2014). Effect of salt stress on antioxidant enzymes and lipid peroxidation in leaves in two contrasting corn, 'Lluteno' and 'Jubilee'. Chilean J Agric Res 2014; 74: 89-95.
34. Desingh R, Kanagaraj G. Influence of salinity stress on photosynthesis and antioxidative systems in two cotton varieties. Gen Appl Plant Physiol 2007; 33: 221-34.
35. Nagesh Babu R, Devaraj VR (2008). High temperature and salt stress response in French bean (Phaseolus vulga-ris). Aus J Crop Sci 2008; 2: 40-48.
36. Ramachandran H, Vincent SGT (2013). Biochemical response of Capsicum annum under cadmium stress. Inter J Environ Monit Anal 2013; 3: 99-104.
37. Khosravinejad F, Heydari R, Farboodnia T. Antioxidant responses of two barley varieties to saline stress. Pak J Biol Sci 2008; 11: 905-9.
38. De Souza MO, Pelacani CR, Willems, LAJ, De Castro RD, Hilhorst HWM, Ligterink W. Effect of osmopriming on germination and initial growth of Physalis angulata L. under stress and on expression of associated genes. Annals Brazil Acad Sci 2016; 88: 503-16.
39. Ben-Hayyim G, Gueta-Dahan Y, Avsian-Kretchmer O, Weichert H, Feussner I. Preferential induction of a 9-lipoxygenase by salt in salt-tolerant cells of Citrus sinensis L. Osbeck. Planta 2001; 212: 367-75.
40. Silva-Ortega CO, Ochoa-Alfaro AE, Reyes-Agüero JA, Aguado-Santacruz GA, Jiménez-Bremont JF. Salt stress increases the expression of p5cs gene and induces proline accumulation in cactus pear. Plant Physiol Biochem 2008; 46: 82-92.
41. Nazarbeygi E, Yazdi HL, Naseri R, Soleimani R. The effects of different levels of salinity on proline and A-, B-chlorophylls in canola. Amr-Euras J Agric Environ Sci 2011; 10: 70-74.
42. Celik O and Atak C (2012). The effect of salt stress on antioxidative enzymes and proline content of two Turkish tobacco varieties. Turk J Biol 2012; 36: 339-56.
43. Huang Z, Zhao L, Chen D, Liang M, Liu Z, Shao H, Long X. Salt stress encourages proline accumulation by regulation proline biosynthesis and degradation in Jerusalem artichoke plantlets. PlosOne 2013; 8: 80-85.
44. Lopez-Gómez M, Hidalgo-Castellanos, Inbarna C, Lluch C. Proline accumulation has prevalence over polyamines in nodules of Medicago sativa in symbiosis with

Sinorhizobium meliloti during the initial response to salinity. Plant Soil 2014; 374: 149-59.
45. Sozharajan R, Natarajan S. Biochemical constituents of Excoecaria agallocha L. Under different levels of NaCl stress. Curr Bot 2015; 6: 9-14.
46. Differential growth and yield responses of salt-tolerant and susceptible rice cultivars to individual (Na^+ and Cl_2) and additive stress effects of NaCl. Acta Physiol Plant 2016; 38: 170.
47. Sivasankaramoorthy S. Effect of salinity on sodium, potassium and proline content of chickpea seedlings. Int Res J Pharm 2013; 4: 147-50.

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