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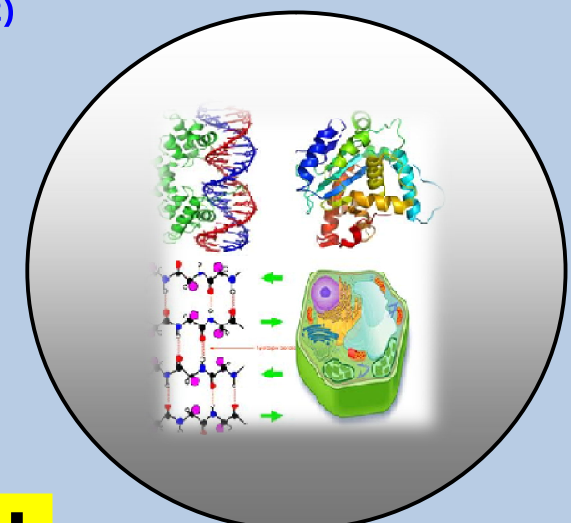
ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

Index Copernicus International Value
IC Value of Journal 4.21 (Poland, Europe) (2012)
Global Impact factor of Journal: 0.587 (2012)
Scientific Journals Impact Factor: 2.597

J. Biol. Chem. Research
Volume 31 (2) 2014 Pages No. 713-722



Journal of Biological and Chemical Research

(An International Journal of Life Sciences and Chemistry)

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 31, No. 2: 713-722 (2014)
(An International Journal of Life Sciences and Chemistry)

ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

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RESEARCH PAPER

Received: 12/04/2014 Revised: 12/05/2014 Accepted: 16/05/2014

Amelioration of Salt Stress in Onion (*Allium cepa* L.) based on Zn Application

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ABSTRACT

A soil pot culture experiment was carried out in wire house condition to investigate the response of Onion (Allium cepa cv. N-53) grown in saline water and amended with supplementary Zinc (Zn). Plants were subjected to different electrical conductivity (EC) solutions viz. 0, 2, 4, 6 and 8 dS/m prepared by mixing NaCl, Na_2SO_4, $CaCl_2$ and $MgCl_2$. While for remediation approach two salinity levels (EC 6 and 8 dS/m) were subjected with combination of 5ppm Zinc. The results demonstrated that salt stress hampered the plant growth such as fresh and dry weight of plant. Increased accumulation of malondialdehyde content in the leaf of plant exposed to salty solution suggested that salinity promoted the oxidative stress. The other stress indicator parameters such as enzymatic and non enzymatic antioxidant were also analyzed. The content of cysteine and non-protien thiol groups were found to gradually increase with increased in salinity levels. Zinc application comparatively (salt treated plants) decreased the content of cysteine and NP-SH in the leaf of plant treated with salt+Zn. Salt stress decreased the activity of catalase (CAT) and peroxidase (POX) but Zn amendment increased their activity. Activity of ascorbate peroxidase (APX) and glutathione reductase (GR) were found to increase at lower levels of salinity while decreased at higher levels. In this study activity of superoxide dismutase (SOD) was increased at all tested salinity levels while Zn supply decreased their activity. The results indicated that supplementary Zinc can mitigate the negative influence of water salinity on plant growth and physiological development.
Keywords: Salinity, Enzymes, Cysteine, MDA, NP-SH, and Zinc

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INTRODUCTION

Salinity is one of the most prominent abiotic factors affecting crop yield in arid and semiarid regions (Subramanyam *et al*., 2010). It is estimated that of the 230 million ha of arable land, 45 million are affected by salinity (FAO, 2008). Salt contaminated soils (ECe >4 dSm^{-1} or 40 mM NaCl or osmotic potential<0.117MPa) are defined as saline lands which directly affect plant growth and development particularly the vegetative growth (Ashraf, 2010). Most of crop species i.e. beans, eggplant, onion, pepper, corn, sugarcane, potato and cabbage are sensitive to salinity (ECe 1.0-1.8 dSm^{-1}), which reduce crop productivity about 6-19 % (Chaum and Kirdmanee, 2009). Salinity stress caused generation of excessive reactive oxygen species (ROS), which leads to cell toxicity, membrane damage and cell death (Chookhampaeng, 2011). To control the level of ROS and to protect the cells, plants possess low molecular weight antioxidants compounds and antioxidant enzymes such as CAT, POX and SOD which scavenge the ROS (Mishra *et al*., 2009). A high NaCl concentration causes reduction in growth parameters (Pessarakli and Touchane, 2006; Turhan *et al*., 2008) such as fresh and dry weight of leaves, shoots and roots along with a decrease in moisture content (Parvaiz and Riffat, 2005). The effectiveness of oxidative defense system in plants can be measured by the activities of antioxidant enzymes and levels of non-enzymatic antioxidants (Geebelen *et al*., 2002). Changes in activities of various antioxidant enzymes under salinity stress have been reported earlier (Dolatabadian & Saleh 2009). Zinc is an essential micronutrient required for optimum plant growth. Zn is considered to play a critical physiological role in the structure and function of membrane lipids especially under salt stress (Aktas *et al*. 2006). Zinc also plays an important role in the production of biomass (Cakmak, 2008) and in controlling the generation and detoxicification of free oxygen radicals which can damage membrane lipids and sulphydryl groups (Alloway, 2004). Alpaslan *et al*. (1999) concluded that, in salt affected areas, zinc application could alleviate Na and Cl injuries in plants. In rice (Aslam *et al*. 2000) it was reported that zinc application repressed Na^+ transport in plants grown in saline solutions, with concomitant improvement in plant growth. The aim of current experiment was to determine whether Zn is involved in regulation of antioxidant enzymes and non-enzymatic antioxidants like NP-SH and Cysteine under salt stress conditions, and to elucidate the physiological mechanisms by which salt stress is alleviated by Zn in Onion plant. In previous work on salinity stress done by other people has been focused on salinity impact on plant growth and yield, and management practices for better performance. But in this work the micronutrient zinc has been used to minimize the effect of salinity on growth and yield in onion plants.

MATERIAL AND METHODS

A soil culture experiment was carried out in wirehouse condition at the Department of Botany, University of Lucknow, Lucknow. Surface-sterilized (with 0.1% $HgCl_2$ solution for 5 min and washed thoroughly with distilled water) seeds were sown in earthen pots (320 cm^2) lined with polyethene bags and filled with a mixture of garden soil and farmyard manure (3:1).

Sixty day's old plants were supplied with various salinity levels (0, 2, 4, 6 and 8 dSm-1). The two higher salinity levels i.e. 6 and 8 dSm^{-1} were subjected to combination of 5 ppm of Zn. Treatment was given weakly for four months. The saline solution was made using a mixture of NaCl, Na_2SO_4, $MgCl_2$ and $CaCl_2$ in the eqimolar basis of various electrical conductivity levels. After four months of treatment, plants were harvested and used for analyzing various morphological and physiological parameters. The level of lipid peroxidation in plant tissue was measured as thiobarbituric acid reaction by the method of Heath and Packer (1971) in terms of malondialdehyde content. CAT activity was assayed by the modified method of Euler and Josephson (1927). The POX activity was determined by the method of Luck (1963). The activity of SOD was assayed by the method of Beauchamp and Fridovich, 1971 by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazoliun (NBT). The Glutathione reductase activity was assayed by the method of smith *et al*. (1988) and the activity of enzyme was expressed as µmoles of GSSG reduced min^{-1} g^{-1} fresh weight. The activity of APX was measured according to the Nakano and Asada (1981) by estimating the rate of ascorbate oxidation (extinction coefficient 2.8 mM^{-1} cm^{-1}). Non protein thiol group was estimated by the method of Ellman (1959). Cysteine content was estimated following the method of (Gaitonde, 1967).

Statistical analysis

The experimental data were tested for significance by using least significant difference (LSD) to compare means of different treatments that have an equal number of replications. All statistical test were performed with analysis tool from Microsoft office excel 2007.

RESULTS AND DISCUSSION

A decrease in whole plant FW and DW was noted due to salinity stress (Table-1). The shoot FW was significantly retarded by 43.8, 46.9 and 67.9 % under salinity treatments of 4, 6 and 8EC levels, respectively. Whereas, Bulb FW was significantly ($P<0.05$ and $P<0.01$) declined by 25.6, 45.9, 58.9, 64.3% at 2, 4, 6 and 8EC levels respectively. Aghaleh and Niknam (2009) also had reported decrease in the growth parameters of soyabean plants under high salinity levels (100, 150 and 200 mM Nacl). Decrease in growth parameter can be due to disturbance in mineral uptake, photosynthesis, water potential, defense system and specific ion toxicity arising from higher concentration of Na^+ and Cl^- (Khan and Ashraf, 1988 and Marschner, 1995). Zinc combination increased the FW of shoot by 3.1 and 17.7% and by 22.1 and 17.4% of bulb at 6 and 8EC levels. Salinity levels of 2, 4, 6 and 8EC caused 38.9, 50, 68.8 and 82.7 and 25.5, 47.1, 69.4 and 81.3% reduction in shoot and bulb DW respectively while application of Zn on increased the shoot DW at 6 and 8EC levels by 111 and 100% and bulb DW by 98 and 82.9 % respectively. This protective role of zinc was ascribed to its role in maintenance of the structural integrity of the plasma membrane and thus controlling the uptake of Na^+ and other toxic ions (Cakmak and Marschner, 1988b).

The role of Zn in maintenance and in various physiological processes was also reported by many workers (Marschner, 1995; Ali *et al*., 1999, 2000; Cakmak, 2000; Doncheva *et al*., 2001; Stoyanova and Doncheva, 2002; Di Baccio *et al*., 2005; Broadley *et al*., 2007). Malondialdehyde content (MDA) which can be used as an indicator to assess the tolerance of plants against oxidative damage and sensitivity of plant to salinity stress. MDA content in shoot and bulb was significantly ($P<0.05$ and $P<0.01$) higher under all salinity levels compared with control plant (Fig-1). Increase in MDA contents under salt stress was also found in rice (Tijen and Ìsmail, 2005), alfalfa (Wang and Han, 2007), cotton (Diego *et al*., 2003) and wheat (Sairam and Srivastava, 2002). The higher level of MDA under salinity stress suggests that onion plant is sensitive to salt stress. The cysteine content was significantly enhanced in shoot and bulb of salt stressed onion plants. A more pronounced increment was observed under 8EC level in shoot and bulb (Fig-2). The present observation revealed that the increased level of cysteine mitigates toxicity imposed by salt stress. However, Zn application decreased cysteine content by 35.1% at 8EC in shoot and 17.7 and 29.6% at 6 and 8 EC respectively, in bulb. Since Zn application minimizes the synthesis of cysteine that suggests Zn prevents the excessive uptake of ions. The level of non protein thiol was significantly ($P<0.05$ and $P<0.01$) stimulated in shoot up to 6EC by 50% and then decreased 4.6% at 8EC level, similar trend was observed in bulb. (Fig.2). It could be due to its role against salt-induced oxidative stress. The results showed conformity to the finding of Mishra *et al*. (2006) in Bacopa monnieri. The antioxidant property of NP-SH depends on the oxidation of -SH group of the tripeptide to disulfide form (Noctor and Foyer, 1998; Sanita di Toppi and Gabbrielli, 1999). The increased level of NP-SH may also be due to stimulation of sulphate reduction pathway such as APS reductase and serine acetyltransferase (Noctor and Foyer, 1998). Zn addition significantly ($P<0.01$) minimized the NP-SH level in shoot at 8EC by 28.6% while it was significantly declined 18.2% at 6EC level in bulb. Thus, this result suggested that salinity reduced oxidative stress which was controlled efficiently by Zn supplementation. Activity of CAT and POX were decreased in both shoot and bulb as salinity level increased (Fig-1). However, CAT activity was significantly increased at 6 and 8EC levels by 5.4 and 61.8% in shoot and 22.6 and 20% in bulb with the use of Zn. CAT activity in maize (Azevedo *et al*., 2006) and *Sesamum indicum* (Koca *et al*., 2007) was found to be differing under the influence of salt. Decrease in CAT activity under salt stress has also been observed by Saha and Gupta (1997) in sunflower and Bishnoi and Singh (1997) in cluster bean. The decrease in CAT activity suggests a greater accumulation of H_2O_2 which often generated in stressed plants (Elstner, 1982). This finding suggested that Onion is very sensitive to salt stress and Zn plays mitigatory role in maintaining the defense system of Onion plant. POX activity in shoot and bulb was significantly ($P<0.05$ and $P<0.01$) reduced (Fig-1). This decrease in POX activity suggested that Onion was sensitive plant in response to salinity. A gradual decline in POX activity was observed by Saha and Gupta (1997) in salt stressed sunflower seedlings. The reduced POX activity indicated that it had a lower capacity for the decomposition of H_2O_2 generated by SOD. But with the application of Zn, it was raised at 8EC by 116.6% in shoot and 105% at 6EC % in bulb.

Table 1. Effect of salinity and its interaction with Zn on FW and DW of shoot and bulb of onion (*Allium cepa* cv. N-53) observed at 120 days.

Salinity level(dSm^{-1})	Control	2EC	4EC	6EC	8EC	6EC+Zn	8EC+Zn
Shoot FW (g $plant^{-1}$)	16.25±1.58	14.71±1.10	9.13±0.63**	8.62±0.98**	5.21±0.21**	8.89±0.39**	6.55±0.29*
Bulb FW (g $plant^{-1}$)	18.50±0.69	13.77±0.68*	10.01±1.43**	7.6±0.36**	6.61±0.16**	9.28±0.59**	7.66±0.39*
Shoot DW (g $plant^{-1}$)	1.44±0.14	0.88±0.007**	0.72±0.009**	0.45±0.02**	0.25±0.02**	0.95±0.02**	0.35±0.39
Bulb DW (g $plant^{-1}$)	13.15±0.13	9.80±0.32*	6.96±1.44**	4.02±0.75**	2.46±0.76**	7.96±0.58**	3.33±0.39*

± represent. S.E. Statistically significant * value at (P<0.05) and **value at (P<0.01).

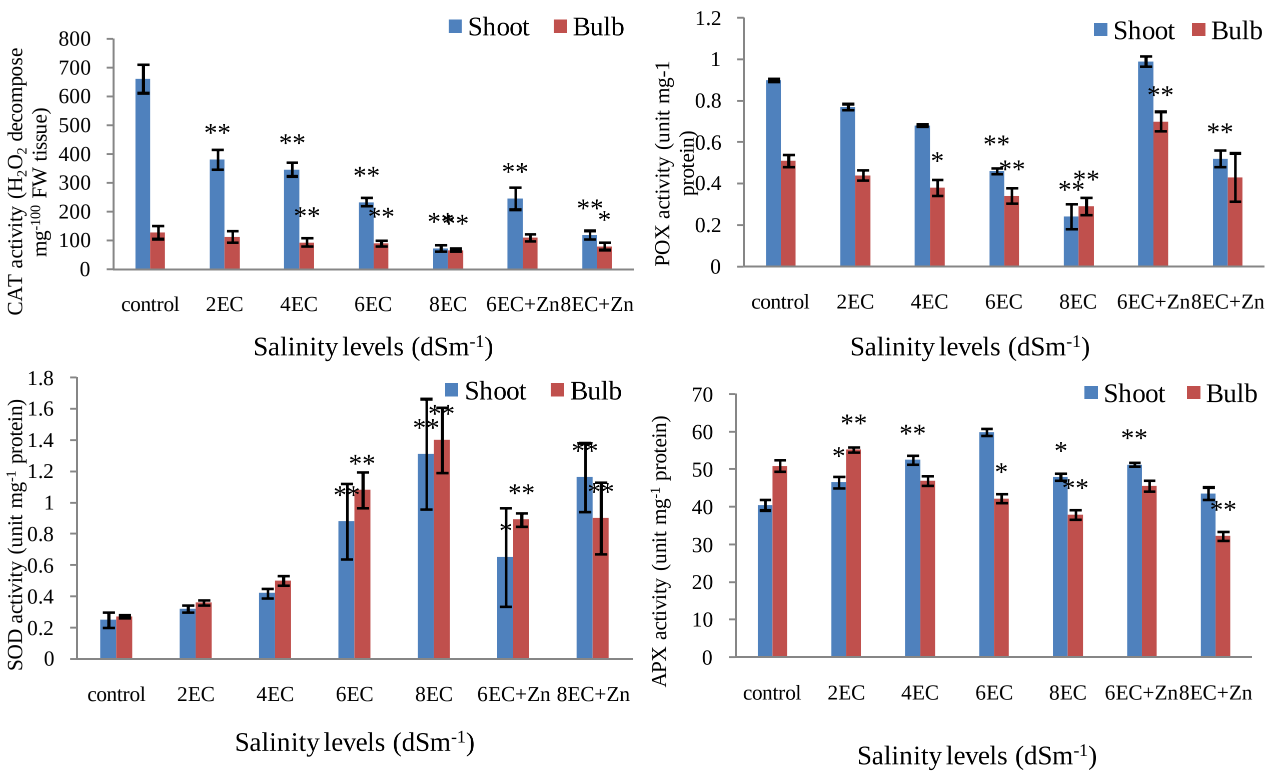

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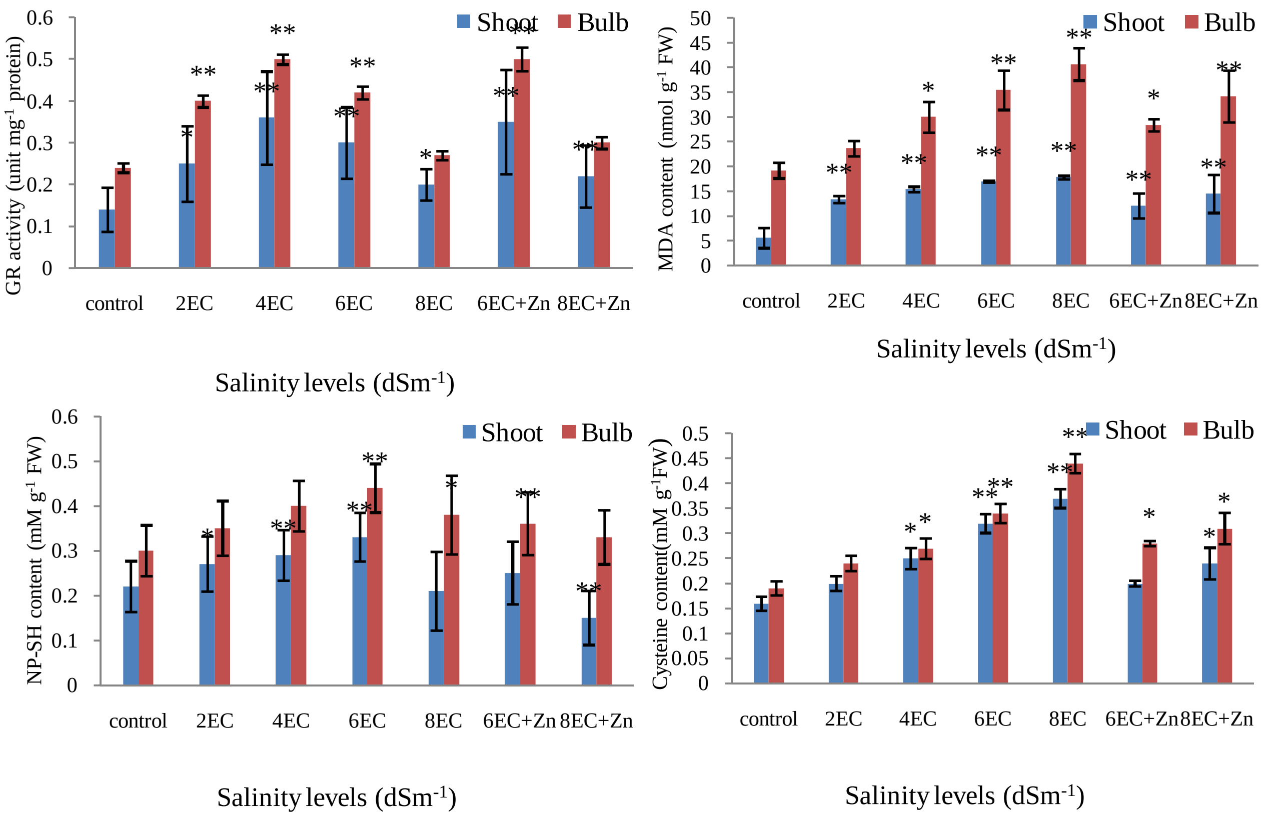
Figure 1. Effect of salinity and its interaction with Zn on CAT, POX, SOD and APX activity in shoot and bulb of onion (*Allium cepa* cv. N-53) observed at 120 days.



± represent. S.E. Statistically significant * value at (P<0.05) and **value at (P<0.01).

Figure 2. Effect of salinity and its interaction with Zn on GR activity and content of MDA, NP-SH and cysteine in shoot and bulb of onion (*Allium cepa* cv. N-53) observed at 120 days.

But enhancement in POX activity has also been observed in rice (Lee *et al*., 2001), pea (Shahid *et al*., 2011) and mulberry (Harinasut *et al*., 2003). Under stress condition enhanced activity of POX are related to tolerance while in current study it was observed that salt stress reduces the activity of the POX in Onion that suggested Onion is very sensitive to salt stress. When Zn was supplied to salt stressed Onion plants, the activity of POX found to be increased that suggested Zn helps in the mitigation of oxidative damage. SOD is one of that enzyme catalyze the disproportionation of two O_2^{-} radicals to H_2O_2 and O_2. The SOD activity was significantly (P<0.01) elevated in shoot at higher salinity levels (6 and 8EC) by 252 and 424% while in case of bulb, it was 300 and 418%. Whereas Zn application on plants exposed to salinity stress, significantly minimized SOD activity was noticed in shoot at 6 and 8EC by 6.1 and 11.5% and it was 17.6 and 35.7% in bulb. Zn application reduced the SOD activity in salt stressed plants. Reduction in SOD synthesis suggested reduction in the O_2^{-} means Zn helps in minimizing the production of O_2^{-} in plants exposed to salt stress.

APX activity was significantly (P<0.05 and P<0.01) increased in shoot up to 6 EC level while at 8EC level it was decreased by 18.2%. In case of bulb, it was enhanced at 2EC by 8.4% then progressively declined at all salinity levels. Since. Results showed that decrease in the activity of the POX was found to be compensated by the APX activity. Zn addition significantly minimized the activity of APX at 6EC by 14.5% in shoot and in bulb it was improved by 7.8 and 6.1% at 6 and 8EC levels respectively. This result revealed that decreased activity of APX with Zn plays an important role in ameliorating oxidative damage by quenching hydrogen peroxide provoked by salinity stress. The activity of GR was significantly increased in shoot upto 4EC by 157.1% then reduced upto 8EC by 42.9%. Similar trend was obtained in bulb. Increased activity of GR has been reported to play a role in tolerance to salt stress in pea (Hernandez *et al*., 1999), egg plant (Yasar, 2003), maize (Zacchini *et al*., 2003) and rice (Maribel and Tobita, 2003). Zn supply significantly (P<0.01) improved the activity of GR in shoot at 6 and 8EC levels by 66.7 and 50% whereas; it was significantly increased in bulb only at 6EC by19.5%. Similarly, in this study GR activity was moderately increased. Elevated activity resulted in accumulation of GSH levels and ultimately confers tolerance of plant (Sheela and Robina, 2007).

CONCLUSION

In this pragmatic investigation, plants irrigated with saline solution adversely affect growth and biochemical attributes in onion plants. Results of this experiment lead to the conclusion that possible salt toxicity, especially in Zn deficient areas, could be alleviated by Zn treatment and thus it increases tolerance against salinity stress in sensitive plant like onion.

ACKNOWLEDGEMENTS

The author is expressing her gratitude to Department of Botany, University of Lucknow, Lucknow (India) for providing laboratory facilities to this research work.

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