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Effect of zinc stress on antioxidant defense system of finger millet

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Abstract
Zinc is an essential micronutrient in plants which is involved in protein synthesis, RNA metabolism and DNA formation, but high concentration of Zn has adverse effect on the growth and metabolism of plants. In the present work finger millet (VL-147 variety) were treated with different concentration 100mM, 200mM, and 300mM of $ZnSO_4$. The germinated seeds of VL-147 variety were grown in the pots containing sand and supplied with Hoagland nutrient solution. Untreated plants were kept as control. Under zinc stress condition with increase in concentration and age of plants the activities of antioxidant enzymes catalase and peroxidase increased significantly as compared to control plant. Decrease proline accumulation under Zn stress condition was observed as compared to control plant. Membrane Stability Index and Yield parameter decreased with increasing $ZnSO_4$ concentration but 100mM $ZnSO_4$ concentration increased yield parameter.

Keywords: Zn stress, finger millet, proline content**,** anti oxidative enzymes, MSI, Yield

1. Introduction

Zinc is an important micronutrient for plant growth, development and productivity. High concentration of zinc account for large decrease in the yield of a variety of crop plants (Wang *et a*l., 2003) [24]. This constraint effects are through osmotic inhibition, ionic toxicity and disturbance in the uptake and translocation of nutritional ions leading to alterations in physiological and biochemical functions of the plant cell (Misra and Gupta, 2005) [13]. De Singh and Kanagarj (2007) [5] reported that the two cotton varieties accumulated more proline and glycine betaine with increasing salinity stress. Kotapati *et al.,* (2014) [10] reported proline content increases in leaves of finger millet more than 3fold upon exposer to drought condition in test sample on comparison with control sample. Qi-Lin *et al.,* (2009) [18] reported activity of catalase, peroxidase and superoxide dismutase during 0-24 h under 200 m mol -1 sodium chloride in leaves of *Brassica napus.* After 24 h, the activites of these antioxidase were maxium and subsequently decreased. Raza *et al*., (2015) [19] reported that the antioxidant enzymes such as catalase (CAT), peroxidase (POX), ascorbate oxidase increased up to the optimum level in saline condition when compared to control plant in Millet. Finger millet (*Eleusine coracana* (L.) Gaertn) is one of the ancient millets in India (2300 BC), of all the cereals and has highest amount of calcium (344 mg %) and potassium (408 mg %). It has higher dietary fiber, minerals, and sulfur containing amino acids compared to white rice. Finger millet and kodo millet are a rich source of Polyphenols and exhibit significant antioxidant activity (Singh, and Raghuvanshi 2012) [15]. The coexistence of heat and drought stresses affects plant biochemical and physiological processes including cell membrane function. The increased permeability and leakage of ions out of the cell has been used as a measure of cell membrane stability (CMS) and as a screen test for stress tolerance. (Rehman *et a*l., 2016) [20]. The peroxidation unsaturated lipids in biological membranes is the most prominent symptom of oxidative stress in plants by which the functionally and integrity of the membrane is affected and can produce irreversible damage to cell function, hence also considered to be a biomarker of metal induced oxidative stress (Ferrat *et a*l 2003) [7]. Arif *et al*., (2013) [1] reported that yield characteristics (number of pods per plant, number of seeds per pod, 1000 seed mass and seed yield per plant) were significantly affected and exhibited a linear decrease in their values in response to the NaCl present in the soil.

2. Materials and Methods

Seeds of finger millet were obtained from Narendra Dev Agricultural University Faizabad Uttar Pradesh, India. Seeds were surface sterilized and allowed to germinate in growth chamber at tempareture 27± 2^0c. 7 day old seedling with uniform length were transferred to earth in ware pots containing sterilized sand. Plants were supplemented with Hogland nutrients solution at every 7 day interval. Plants were treated with varying concentration of $ZnSO_4$ (viz 100 mM, 200 mM, 300 mM). All the sets were watered daily to keep the sands moist untreated plants were kept as control. Plants were analysed for 20, 40, 60, 80 days. The periodic collection of samples was done at each 20 day interval. Membrane stability index was estimated by method of Deshmukh *et al.* (1991) [4]. Antioxidant enzyme activity was studied in the terms of Peroxidase activity (POD) and Catalase activity (CAT) POD was determined by the method of Shannon *et al*., (1996) [21] and CAT was estimated by method of Chance and Maehly (1955) [3]. For the estimation of Proline content, plants sample were oven dried at 65± 2^0c and Proline was determined by the method of Bates *et al*., (1973) [2]. Yield was studied in terms No. of ear/plant, Finger length, grain yield and weight of 1000 grains at 90 days of plant growth treatment. The data was analyzed statistically.

3. Results and Discussion

In the present work membrane stability index (MSI) was measured at different days of plant growth under different concentration (control, 100 mM, 200 mM, 300 mM) of $ZnSO_4$. A significant decrease was observed in MSI of (100 mM, 200 mM, 300 mM) of $ZnSO_4$ concentration. (Fig-1) A gradual increase in MSI was found from day 20 up to day 80. Minimum MSI was observed in plants treated with 300 mM $ZnSO_4$ concentration as compared to control. Nader *et al.*, (2014) observed that membranre stability index in *Allium cepa* decreased by metal stress. The enzyme activity was measured at different days of plant growth under different concentration (control, 100 mM, 200 mM, 300 mM) of $ZnSO_4$. A significant increase was observed in enzyme activity (100 mM, 200 mM, 300 mM) of $ZnSO_4$ concentration. (Fig-2 and 3). A gradual increase CAT and POD was found from day 20 up to day 80. Maximum CAT and POD enzyme activity was observed in plants treated with 300 mM $ZnSO_4$ concentration as compared to control. The result support the hypothesis that antioxidative enzyme play a central protective role in the detoxification of O_2 and H_2O_2 (Lian *et, al.*, 2003) [11]. An increased capacity of the antioxidant system is one of the possible mechanisms responsible for zinc stress tolerance as demonstrated by the existence of stress-resistant lines with naturally enhanced antioxidant systems (Fan *et al.*, 2015) [6]. CAT in finger millet plants. Such an idea is supported by recent evidence that H_2O_2 as a signal molecule plays a key role in antioxidant enzymes mediated plant defense under many abiotic stress factors (Maruta *et al.*, 2016) [12]. Solanki *et al.*, (2011) [23] reported increased peroxidase activity in response to elevated zinc concentration in *Vigna mungo*. Antioxidant enzymes play a vital role in the plant antioxidative defense system. Proline content was measured at different days of plant growth under different concentration (control, 100 mM, 200 mM, 300 mM) of $ZnSO_4$. A significant variation was observed in proline with (100 mM, 200 mM, 300 mM) of $ZnSO_4$ concentration. (Fig-4) A gradual increase in proline content from day 20 up to day 80 with maximum proline content observed in 100 mM $ZnSO_4$ concentration compared to control followed by decline at higher concentration. Proline is storage of nitrogen and carbon and it is implicated in facilitating scavenging of free radicals plants under zinc stress condition (Parida *et. al.*2002) [16]. Proline is believed to function as compatible solute in balancing cytoplasmic and vascular water potentials (Hassine *et al.*, 2008) [8]. Number of ears, Length of finger and seed yield observed at 90 day under different concentration of $ZnSO_4$. (Table-1) Number of ears, Length of finger, weight of 1000 seeds and seed yield increased to 100 mM $ZnSO_4$ followed by decrease at 200 mM, 300 mM $ZnSO_4$ concentration compared to control. The result support that) effect of Zn stress reduced growth and yield compared to control plant (Prasad *et al.*, 2012) [17]. Importance of Zn nutrition on water relations of crop plant is likely to be related to the severity of Zn deficiency and water deficit conditions. In chickpea the decreased seed yield, root growth during Zn deficiency has reduced the plants ability to exploit soil (Khan *et al* 1998) [9].

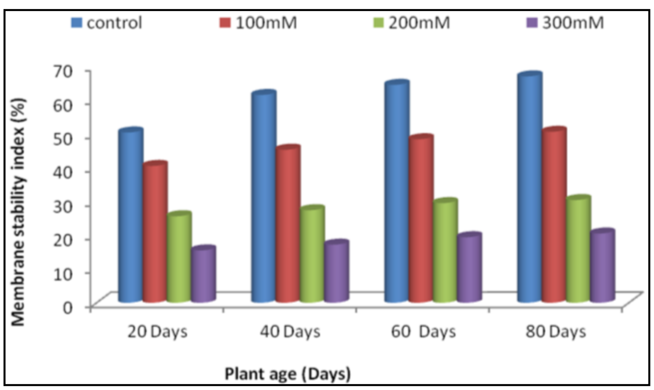

Fig 1: *Eleusine coracana*: Membrane stability at different age of plant growth under different $ZnSO_4$ concentration.

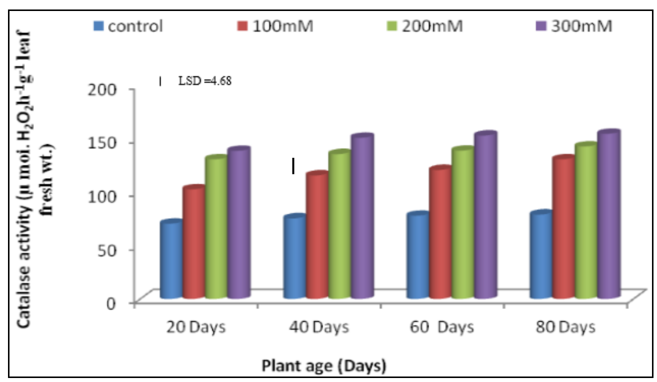

Fig 2: *Eleusine coracana*: Catalase activity at different age of plant growth under different $ZnSO_4$ concentration.

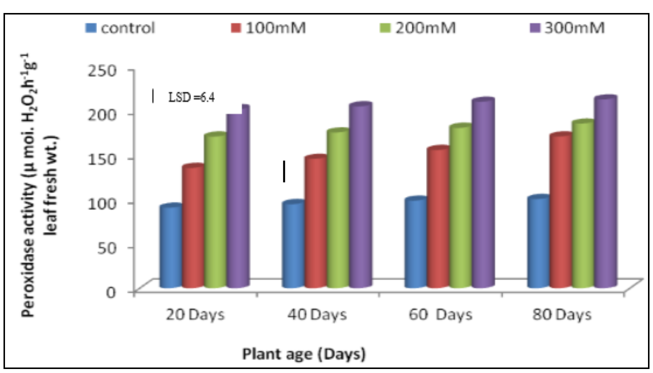

Fig 3: *Eleusine coracana*: Peroxidase activity at different age of plant growth under different $ZnSO_4$ concentration.

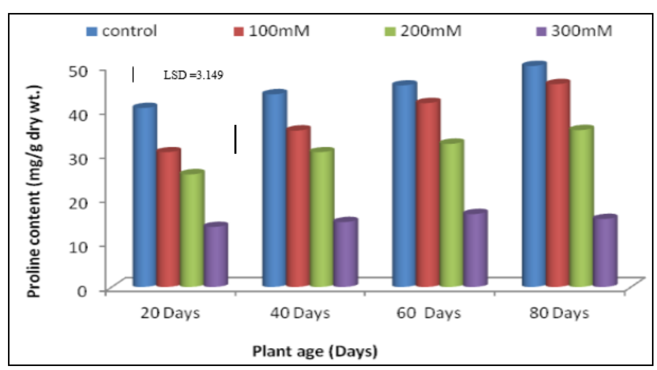

Fig 4: *Eleusine coracana*: Proline content at different age of plant growth under different $ZnSO_4$ concentration.

Table 1: *Eleusine coracana*: Number of ears per plant, Grain weight g/1000 seed, Length of finger (cm), Seed yield per plant (gm) under different concentration of $ZnSO_4$

Treatment	Number of Ears/plant	Seed weight /1000 grain(g)	Length of finger (cm)	Seed yield per plant (g)
Control	3.8±0.75	2.17±1.01	4.4±0.36	11.59±0.29
100 mM $ZnSo_4$	4.2±1.05	3.7±1.01	5.6±0.35	13.01±0.58
200 mM $ZnSo_4$	2.7±0.70	2.3±0.57	4.09±0.08	10.43±0.37
300 mM $ZnSo_4$	2.5±0.15	2.14±0.51	3.86±0.30	9.96±0.47

*Mean ± (n=3)

4. Conclusion

Conclusively, our results show that $ZnSO_4$ at higher concentration decreased MSI, Proline, and yield parameters. Our results suggest that presence of $ZnSO_4$ in low concentration increased MSI, Proline, and yield parameters of the test plant. But at high $ZnSO_4$ concentration, were observed growth inhibition, decline of physiological and biochemical activity of plants. But as a response of $ZnSO_4$ generated stress, plants increase their antioxidant enzyme activity but higher concentration cause oxidative stress.

5. Acknowledgments

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