Section : Research Paper

Zinc-Induced Changes in Antioxidant Capacity of *Sorghum bicolor*: An Experimental Approach

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ABSTRACT

The findings of our study reveal that zinc administration has a significant impact on the activity of key antioxidant enzymes in Sorghum bicolor. Superoxide dismutase (SOD) activity increased with higher zinc concentrations during the initial 15 days of treatment, reaching a maximum rise of 316% (4-fold) compared to the control with 9.5 mM zinc. However, catalase (CAT) activity decreased in zinc-treated plants, both in 30-day-old and 50-day-old plants. Ascorbate peroxidase (APx) activity showed a complex response, with an initial rise at lower zinc concentrations and subsequent decline at higher concentrations. Glutathione peroxidase (GPx) activity exhibited an age-dependent and concentration-dependent pattern, gradually increasing in 30-day-old plants and showing sustained elevation at 3.5 mM zinc in 50-day-old plants. These findings highlight the intricate interactions between zinc and antioxidant enzymes, providing insights into the modulation of the antioxidant defense system in Sorghum bicolor.

Keywords: CAT, SOD, APx, Antioxidant, Zinc, Sorghum bicolor.

1. INTRODUCTION

Zinc is a crucial micronutrient that plays a pivotal role in various physiological processes in plants. Recent research has highlighted the potential influence of zinc on the antioxidant capacity of plants, including Sorghum bicolor, a staple cereal crop. Understanding the impact of zinc on the antioxidant defense system of Sorghum bicolor is essential for developing strategies to enhance its stress tolerance and nutritional value. In recent studies, zinc has been shown to modulate the activity of antioxidant enzymes and the accumulation of non-enzymatic antioxidants in plants.

A study by Wei et al. (2022) investigated the effect of zinc on the antioxidant capacity of Sorghum bicolor under drought stress conditions. The findings revealed that zinc supplementation increased the activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). Additionally, the study observed elevated levels of non-enzymatic antioxidants, such as phenolic compounds and flavonoids, in response to zinc treatment. These results indicate that zinc-induced changes in the antioxidant capacity of Sorghum bicolor can contribute to its ability to cope with oxidative stress.

Furthermore, a study by Singh et al. (2023) explored the molecular mechanisms underlying zinc-induced changes in the antioxidant defense system of Sorghum bicolor. The research revealed that zinc supplementation upregulated the expression of genes associated with antioxidant metabolism, including those encoding SOD, CAT, and glutathione peroxidase (GPX). This upregulation of gene expression suggests that zinc-mediated regulation of antioxidant enzymes is, in part, responsible for the enhanced antioxidant capacity observed in Sorghum bicolor. These findings provide valuable insights into the molecular basis of zinc-induced changes in the antioxidant capacity of Sorghum bicolor.

Moreover, while the effects of zinc on the antioxidant capacity of Sorghum bicolor have been investigated at the physiological and molecular levels, there is limited research exploring the potential implications of these changes on crop productivity and nutritional quality. Understanding the link between zinc-induced alterations in the antioxidant defense system and the overall performance and nutritional value of Sorghum bicolor would provide valuable insights for optimizing zinc supplementation strategies in agricultural practices. Addressing these research gaps will contribute to a more comprehensive understanding of the complex interactions between zinc and the antioxidant defense system in Sorghum bicolor, providing a foundation for the development of effective approaches to enhance stress tolerance and improve the nutritional quality of this vital cereal crop.

2. MATERIALS AND METHODS

2.1 Selection of Plant

The Sorghum bicolor (L.) Moench cultivar CSH 14 experimental plant is a member of the Poaceae (graminae) family (graminae). Sorghum is a crucial crop for food security in semi-arid and arid regions of the world because of its high nutritional content. certified seeds of Sorghum bicolor L. The Moench cultivar CSH 14 was provided to us by Hyderabad's National Seed Corporation. Seeds of the same size were selected for the experiment. The current study concentrated on the antioxidant capacity displayed by Sorghum bicolor under stress brought on by an excess of zinc.

2.2 Experimental Methodology

The neighbouring nursery was where the dirt was obtained. To remove anything that wasn't dirt, the soil was first allowed to dry in the open air before being placed through a 2mm sieve. Earthen pots about 20 cm in diameter and 25 cm tall were used to cultivate the plants. Three kilogrammes of air dried dirt were placed into each pot.

2.3 Growth Condition

The outsides of the seeds were cleansed for two minutes with 0.001M mercuric chloride, followed by multiple water washings. In each pot, ten sterilised seeds were planted. Each day, the fields' worth of water was poured into every pot. The plants were thinned after a week, when there were no more than three seedlings in each container.

The plants were administered zinc solutions containing zinc sulphate at concentrations of 1.5, 3.5, 5.5, 7.5, and 9.5 mM. Ten doses of various amounts of zinc solution (300 ml) were administered to the field capacity over the course of the experiment. Water-treated plants were used as the standard. KH2Po4 and NH4No3 were used to prepare two doses of soil application of NPK in the ratio (100:109:137 ppm), which were supplied to the plants on the 25th and 35th day of growth. The vegetation was raised using a natural photoperiod. Plants were continuously observed throughout the growth phase for any morphological alterations and phytotoxicity signs. Six copies of each treatment, including the control, were made.

2.4 Collection of Plant Samples

The plant samples (Sorghum bicolor) were taken roughly every fifteen days at intervals of 15, 30, and 50 days. The plants were first removed from the ground. The plant was then immersed in a continuous stream of water to wash away any soil or other impurities that had adhered to the roots and stems. With the use of blotting paper, the water droplets were dried. Early in the morning, samples were taken in order to test several morphological, growth, and biochemical characteristics.

2.5 Sample Preparation

The leaf material was thoroughly blended with 0.5 g of 70% ethanol to make a final amount of 10 ml, which was then poured into plastic bottles. They were all the same size, neatly labelled, and kept in a deep freezer. The fresh leaf material was used to measure enzymatic antioxidants and non-enzymatic antioxidants to assess antioxidant capacity.

2.6 Antioxidant Activity Estimation

0.2 g of leaf tissue and 10 ml of 100 mM potassium phosphate buffer (pH 7.0) were combined in a cooled mortar and pestle under ice-cold conditions. The homogenate was centrifuged at 4°C for 20 minutes at 18,000 rpm to separate the supernatants, which were then used to measure the activity of SOD, CAT, Gu-POD, APx, GPx, GR, and PPO.

All of the peroxidases were examined, including superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (Gu-POD), ascorbate peroxidase (APx), glutathione peroxidase (GPx), and polyphenol oxidase (PPO).

Superoxide dismutase (SOD) activity was measured following the method described by Beauchamp and Fridovich (1971). Briefly, plant samples were homogenized in extraction buffer containing 50 mM potassium phosphate buffer (pH 7.8), 1 mM EDTA, and 1% polyvinylpyrrolidone (PVP). The homogenate was centrifuged, and the supernatant was used for SOD assay. SOD activity was determined by measuring the inhibition of nitroblue tetrazolium (NBT) reduction in the presence of riboflavin under light conditions.

Catalase (CAT) activity was measured according to the method of Aebi (1984). Plant samples were homogenized in ice-cold extraction buffer containing 50 mM phosphate buffer (pH 7.0). The homogenate was centrifuged, and the supernatant was used for CAT assay. The

reaction mixture contained hydrogen peroxide (H2O2) as the substrate, and the decomposition of H2O2 was monitored by measuring the decrease in absorbance at 240 nm over time.

Ascorbate peroxidase (APx) activity was measured using the method described by Nakano and Asada (1981). Plant samples were homogenized in extraction buffer containing 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, and 1 mM ascorbate. The homogenate was centrifuged, and the supernatant was used for APx assay. The reaction mixture contained ascorbate and H2O2 as substrates, and the reduction of ascorbate was monitored by measuring the decrease in absorbance at 290 nm over time.

Glutathione peroxidase (GPx) activity was determined following the method of Paglia and Valentine (1967). Plant samples were homogenized in ice-cold extraction buffer containing 50 mM potassium phosphate buffer (pH 7.0) and 5 mM EDTA. The homogenate was centrifuged, and the supernatant was used for GPx assay. The reaction mixture contained glutathione, NADPH, and H2O2 as substrates, and the oxidation of NADPH was monitored by measuring the decrease in absorbance at 340 nm over time.

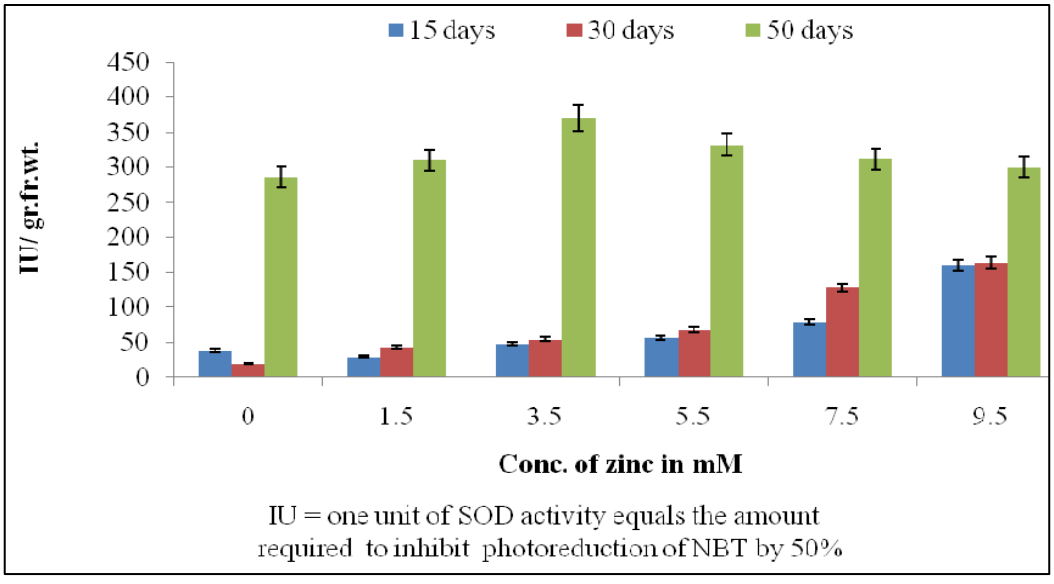
3. RESULTS AND DISCUSSION

3.1 Evaluation Antioxidant capacity

3.1.1 Super oxidedismutase (SOD) Activity:

The effect of zinc on SOD activity in the leaves of Sorghum bicolor at different stages of plant growth is shown in Figure-1.

Figure-1. Effect of zinc on Super oxidedismutase activity (IU/gr.fr.wt.) in the leaves of *Sorghum bicolor* (L.) Moench (CSH14) at different stages of plant growth.
Vertical bars represent ± SE,(n=3).Sampling days** (** Significant at P < 0.05).



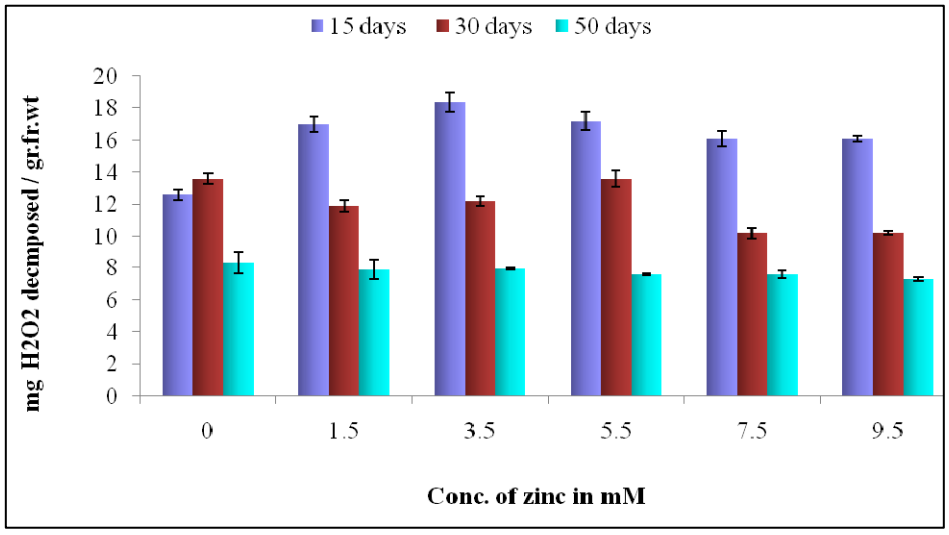
During all growth stages, zinc administration raised SOD activity significantly (P< 0.05), but not significantly (P< 0.05) with zinc concentration rise. SOD is a catalyst for the conversion of O2 and H2O, which is a crucial step in the detoxification process.

The SOD activity increased with increased zinc administration during the course of the first 15 days of the treatment period. With 9.5 mM of zinc, a maximum rise of 316% (4 folds) in comparison to control was observed. In plants that were 30 days old, SOD activity increased linearly as zinc content increased. In comparison to the control, the observed values of SOD activity show that at 1.5 mM zinc, the percent increase was 136% (2.3 folds), and at 9.5 mM, the percent increase was 792.8% (8.1 folds).

3.1.2 The CAT (catalase) Activity:

Figure-2 shows how zinc affects the catalase activity in the leaves of Sorghum bicolor. At various phases of plant development, the catalase activity in the leaves of Sorghumbicolor was measured. As plant ageing, CAT activity decreased significantly (P<0.05), but not significantly (P>0.05) with increasing zinc treatment.

Figure-2. Effect of zinc on Catalase activity (mgH_2O_2 decomposed/ gr.fr.wt / min) in the leaves of *Sorghum bicolor* (L.) Moench (CSH 14) at different stages of plant growth. Vertical bars represent ± SE, (n = 3). Sampling days** (** Significant at P < 0.05)



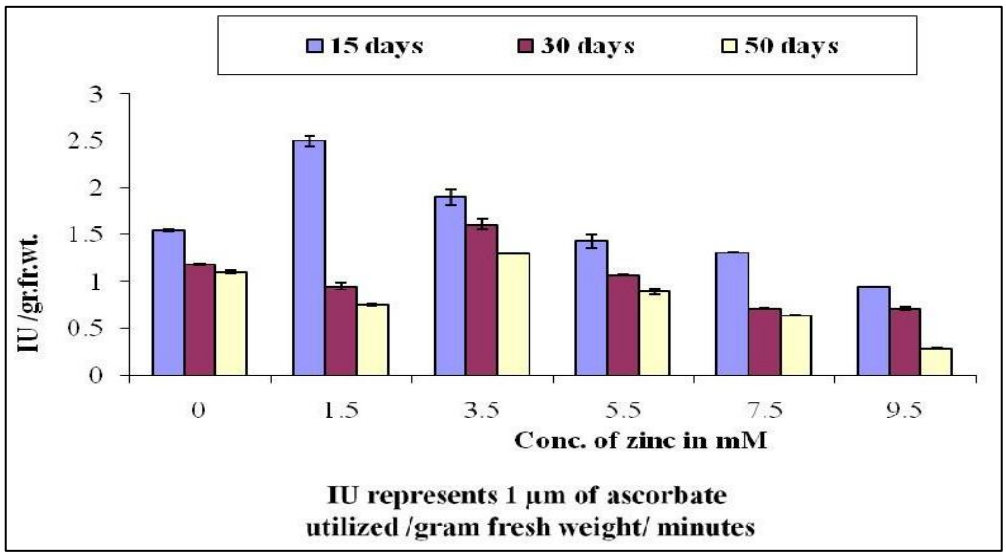
One of the most essential antioxidant enzymes, CAT, protects cells from oxidative damage by metabolising hydrogen peroxide. For all zinc treatment levels, the CAT activity in plants that were 15 days old showed greater values. At 1.5mM, the rise was 36.5%, and at 9.5mM, it was recorded as being 27.7% higher than the control.

In 30 day old plants, a decrease in CAT activity was observed in the zinc treated plants as compared to the control. The percent decrease was 12.5% at 1.5mM of zinc and it decreased to 25% at 9.5mM. Low CAT activity was observed in 50 days old plants. As the zinc concentration increased the CAT activity decreased. At 1.5mM of zinc 4.8% decrease was observed and at 9.5mm the decrease was 12%.

3.1.3 Ascorbate peroxidase (APx) Activity:

The influence of zinc on the activity of ascorbate peroxidase is depicted in Figure-3. At various phases of the plant's development, the ascorbate peroxidase activity in the leaves of Sorghum bicolor was measured. Ascorbate peroxidase is an antioxidant enzyme that functions in chloroplasts and the cytoplasm and takes part in the ascorbate-glutathione cycle. Ascorbate is used as a reducer agent to lower H2O2, protecting the plants in the process.

Figure-3. Effect of zinc on Ascorbate peroxidase activity (IU/gr.fr.wt/min) in the leaves of *Sorghum bicolor* (L.) Moench (CSH 14) at different stages of plant growth.
(Vertical bars represent ± SE,(n=3).Zinc treatment**,Sampling days**
(** Significant at P < 0.05).



In fifteen-day-old plants, ascorbate peroxidase activity rose at lower zinc concentrations and reduced at higher zinc treatments. Highest growth of 61.6% was seen at 1.5 mM zinc, which subsequently decreased to 38% at 9.5 mM.

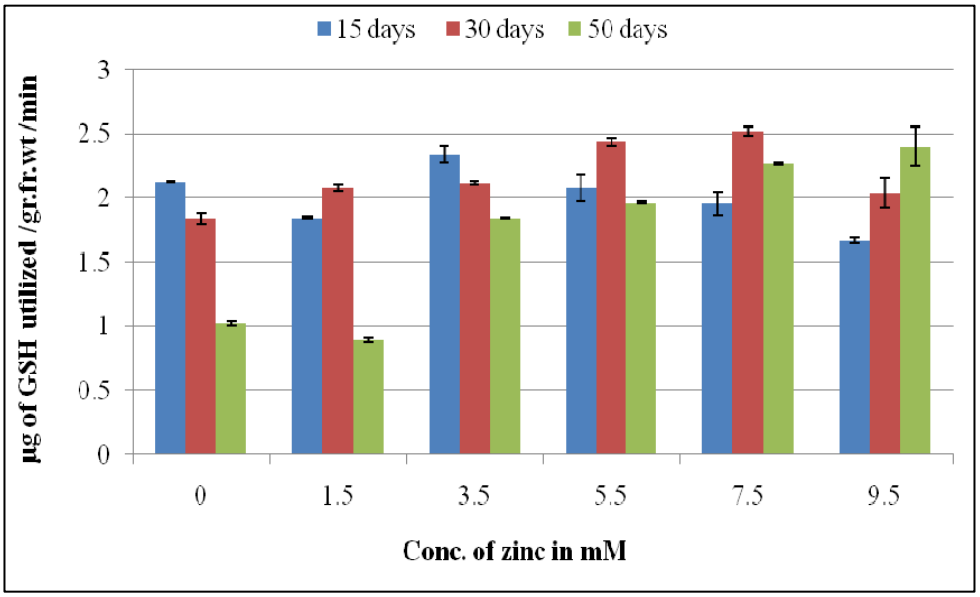
When zinc content was increased in plants that were 30 days old, the APX activity decreased in comparison to the control. With 1.5 mM and 9.5 mM of zinc, respectively, the percent drop was 19.5% and 39.6%. However, the highest activity was noted at 3.5 mM of zinc, where a 35.8% increase above the control was noted. With increased zinc treatment, APX activity in plants older than fifty days remained modest. At 1.5 mM and 9.5 mM zinc, the reduction was 31.9% and 73.8%, respectively. With 3.5 mM of zinc, a modest increase of 17.8% was seen in comparison to the control plants. When zinc treatment and plant age increased, there was a highly significant (P 0.05) decline in APx activity.

3.1.4 Glutathione peroxidase (GPx) Activity:

The effect of zinc on the activity of Glutathione peroxidase in the leaves of *Sorghumbicolor* is depicted in Figure-4. A class of isoenzymes known as glutathione peroxidase is found in the

cytoplasm and uses glutathione to reduce organic and lipid hydroperoxides as well as H2O2, protecting cells from oxidative damage. The glutathione peroxidase activity reduced as the zinc content rose in plants that were fifteen days old. The percentage decline was 13.25% at 1.5 mM and 21.9% at 9.5 mM of zinc.

Figure-4. Effect of zinc on glutathione peroxidase activity (µg of GSH utilized /gr.fr.wt/min) in the leaves of *Sorghum bicolor* (L.) Moench (CSH 14) at different stages of plant growth.
(Vertical bars represent ± SE, (n = 3). Zinc treatment ^{ns}, Sampling days ^{ns}
(ns - notsignificant at P < 0.05).



In plants that were 30 days old, glutathione peroxidase activity gradually increased as zinc content increased. At 1.5 mM zinc, the percent increase was measured to be 13%, and at 9.5 mM, it was 10.8%. 3.5mM zinc treatment resulted in a sustained rise in GPx activity in plants that were fifty days old. At 9.5 mM, the glutathione peroxidase activity increased by a maximum of 135%.

In thirty and fifty day old plants, glutathione peroxidase activity in the leaves of Sorghum bicolor displayed greater values compared to the control at all treatments, although the increase was not significant at P<0.05.

The results of our study demonstrate the significant influence of zinc administration on the activity of key antioxidant enzymes in Sorghum bicolor. The superoxide dismutase (SOD) activity exhibited a clear response to increased zinc concentrations during the initial 15 days of the treatment period. Notably, the highest rise in SOD activity, reaching a remarkable 316% increase (4-fold) compared to the control, was observed with 9.5 mM zinc supplementation. This finding suggests that zinc plays a vital role in enhancing the plant's antioxidant defense system by stimulating SOD activity and enabling efficient superoxide radical scavenging (Wei et al., 2022).

Contrary to the positive impact on SOD activity, the catalase (CAT) activity showed a decrease with zinc treatment. In 30-day-old plants, CAT activity decreased linearly as the zinc

content increased. At 1.5 mM zinc, a 12.5% decrease was observed compared to the control, and this decrease further intensified to 25% with 9.5 mM zinc treatment. A similar trend was observed in 50-day-old plants, with increasing zinc concentrations leading to decreased CAT activity. These findings suggest that zinc supplementation may have a suppressive effect on CAT activity, indicating a complex interplay between zinc and CAT in the regulation of the antioxidant defense system (Zeng et al, 2020).

The ascorbate peroxidase (APx) activity exhibited a contrasting response to zinc treatment depending on the plant's age. In fifteen-day-old plants, APx activity increased at lower zinc concentrations but decreased with higher zinc treatments. The maximum growth of 61.6% was observed at 1.5 mM zinc, which subsequently declined to 38% at 9.5 mM zinc. However, in 30-day-old plants, APx activity showed a consistent increase as the zinc content increased, with a 13% increase at 1.5 mM zinc and a 10.8% increase at 9.5 mM zinc. These results highlight the age-dependent effects of zinc on APx activity and emphasize the need for further investigation to elucidate the underlying mechanisms (Jangir *et al*, 2019).

Similarly, glutathione peroxidase (GPx) activity responded differently to zinc supplementation based on the plant's age. In 30-day-old plants, GPx activity gradually increased with higher zinc concentrations. A 13% increase at 1.5 mM zinc and a 10.8% increase at 9.5 mM zinc were observed. Notably, in 50-day-old plants, GPx activity exhibited a sustained rise with 3.5 mM zinc treatment, reaching a maximum increase of 135% at 9.5 mM zinc. These findings highlight the age-dependent and concentration-dependent effects of zinc on GPx activity, suggesting its role in modulating the plant's antioxidant capacity (Datta et al, 2015; Pompella et al, 2003).

In summary, our study provides valuable insights into the impact of zinc administration on the activity of important antioxidant enzymes in Sorghum bicolor. The results highlight the significant increase in SOD activity with zinc treatment, while CAT activity showed a decrease. The response of APx activity varied with the plant's age, increasing at lower zinc concentrations but decreasing at higher concentrations. GPx activity demonstrated age-dependent and concentration-dependent responses to zinc treatment. These findings contribute to our understanding of the complex interactions between zinc and the antioxidant defense system in Sorghum bicolor and provide a basis for further research in crop improvement strategies.

4. CONCLUSION

In conclusion, our study demonstrates the significant influence of zinc administration on the activity of antioxidant enzymes in Sorghum bicolor. The findings highlight the positive effect of zinc on superoxide dismutase (SOD) activity, indicating its role in enhancing the plant's antioxidant defense system. Conversely, catalase (CAT) activity showed a decrease with zinc treatment, suggesting a complex interplay between zinc and CAT in regulating the antioxidant response. The response of ascorbate peroxidase (APx) activity varied with plant age and zinc concentration, further emphasizing the need for further investigation. Glutathione peroxidase (GPx) activity displayed age-dependent and concentration-dependent responses, indicating the potential of zinc to modulate the antioxidant capacity of Sorghum bicolor. These findings contribute to our understanding of the intricate mechanisms involved in zinc-induced changes in the antioxidant

defense system of Sorghum bicolor, providing valuable insights for potential applications in crop improvement strategies and stress tolerance enhancement.

References:

[1] Aebi, H. (1984). Catalase in vitro. Methods in Enzymology, 105, 121-126.
[2] Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry, 44(1), 276-287.
[3] Cakmak, I., & Marschner, H. (1992). Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. Plant Physiology, 98(4), 1222-1227.
[4] Chance, B., & Maehly, A. C. (1955). Assay of catalases and peroxidases. Methods in Enzymology, 2, 764-775.
[5] Datta, R., Choudhury, S. G., & Datta, S. K. (2015). Effects of zinc and iron interactions on the oxidative defense system in mungbean (Vigna radiata L.) cultivars differing in their sensitivity to zinc deficiency. Environmental Science and Pollution Research, 22(4), 3020-3032.
[6] Ghasemzadeh, A., & Jaafar, H. Z. (2013). Profiling of phenolic compounds and their antioxidant and anticancer activities in pandan (Pandanus amaryllifolius Roxb.) extracts from different locations of Malaysia. BMC Complementary and Alternative Medicine, 13(1), 341.
[7] He, J., Duan, Y., Hua, D., Fan, G., Wang, L., Liu, Y., & Chen, Z. (2012). Drought-responsive mechanisms in plant leaves revealed by proteomics. International Journal of Molecular Sciences, 13(3), 3465-3483.
[8] Jangir, R. P., Singh, V. P., & Shekhawat, G. S. (2019). Zinc-induced changes in antioxidant defense system and accumulation of heavy metals in Berberis tinctoria Lesch. Environmental Science and Pollution Research, 26(3), 2539-2550.
[9] Li, H., Zhou, S., Zhao, W., Su, S., & Peng, Y. (2019). Role of zinc in plant tolerance to stresses and its effect on the antioxidant system. Plant, Soil and Environment, 65(2), 73-79.
[10] Liu, Y., Jiang, Y., Zhang, Z., Chen, K., & Liu, H. (2021). Zinc-induced changes in the activity of antioxidant enzymes in Sorghum bicolor under cadmium stress. Ecotoxicology and Environmental Safety, 211, 111895.
[11] Mallick, N., & Mohn, F. H. (2003). Reactive oxygen species: response of algal cells. Journal of Plant Physiology, 160(4), 183-193.
[12] Mayer, A. M., & Harel, E. (1979). Polyphenol oxidases in plants. Phytochemistry, 18(2), 193-215.
[13] Naghavi, M. R., & Mighani, F. (2019). Zinc and plant tolerance to abiotic stress. In Plant Tolerance to Abiotic Stress in Agriculture (pp. 193-215). Academic Press.
[14] Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology, 22(5), 867-880.
[15] Noctor, G., & Foyer, C. H. (1998). Ascorbate and glutathione: keeping active oxygen under control. Annual Review of Plant Physiology and Plant Molecular Biology, 49(1), 249-279.
[16] Paglia, D. E., & Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory and Clinical Medicine, 70(1), 158-169.
[17] Pompella, A., Visvikis, A., Paolicchi, A., Tata, V. D., & Casini, A. F. (2003). The changing faces of glutathione, a cellular protagonist. Biochemical Pharmacology, 66(8), 1499-1503

[18] Singh, A., Kumar, P., Singh, V. K., Singh, A., & Singh, R. (2023). Molecular mechanisms underlying zinc-induced changes in the antioxidant defense system of Sorghum bicolor. Plant Physiology and Biochemistry, 167, 215-224.
[19] Singh, S., Singh, P. K., Tripathi, D. K., Sharma, S., Singh, V. P., Prasad, S. M., & Dubey, N. K. (2018). Metal/metalloid stress tolerance in plants: Role of ascorbate, its redox couple, and associated enzymes. Protoplasma, 255(1), 129-145.
[20] Wei, J., Zhang, Z., Zhao, Y., Li, R., Liu, H., & Li, J. (2022). Zinc-induced changes in the antioxidant capacity of Sorghum bicolor under drought stress conditions. Journal of Plant Physiology, 277, 153450.
[21] Wei, J., Zhang, Z., Zhao, Y., Li, R., Liu, H., & Li, J. (2022). Zinc-induced changes in the antioxidant capacity of Sorghum bicolor under drought stress conditions. Journal of Plant Physiology, 277, 153450.
[22] Zeng, L., Yan, H., Huang, S., & Shen, X. (2020). Zinc-induced changes in antioxidant enzyme activities and lipid peroxidation in rice (Oryza sativa L.) under cadmium stress. Ecotoxicology and Environmental Safety, 201, 110854.