



Research Consortium Archive

P(ISSN) : 3007-0031

E(ISSN) : 3007-004X

<https://rc-archive.com/index.php/Journal/about>



Effect of Flood Stress on Antioxidant Enzyme Activities and Growth Parameters *Zea mays* L. and *Triticum aestivum* L. Seedlings

Areesha Ahmad*

Department of Microbiology, University of Karachi, Pakistan

Email: areesha_ahmad799@yahoo.com

Hiralal Hatesh

Jinnah Postgraduate Medical Karachi

Tehmina Bashir

Lahore Medical Research Center Lahore, Pakistan

Adnan Mehmood*

Department of Microbiology, Gulab Devi Educational Complex, Lahore

Noor Muhammad

Department of Zoology, Government College University Lahore, Pakistan

Publisher: EDUCATION GENIUS SOLUTIONS

Review Type: Double Blind Peer Review

ABSTRACT

Background: Due to the significant impact of climate change, flooding also endangers the production of cereals by means of oxygen deficiency in roots and the build-up of reactive oxygen species (ROS), which damages cellular components. **Objectives:** The study objective was to assess the impacts of flood stress on the important antioxidant enzyme activities and the growth parameters in maize and wheat seedlings and to compare their time responses to varying periods of flooding. **Methods:** The seedlings of maize and wheat were planted under controlled conditions and flooded after 24, 48, and 72 hours, with the well-watered seedlings as controls. SOD, CAT, POD, and APX activities were determined spectrophotometrically in the cases of the leaf samples, where the units were established as units/mg protein. Among the growth parameters measured were fresh and dry weight, the shoot length and root length, and total chlorophyll content. The data was analyzed using one-way ANOVA, and the post-hoc test of Tukey was done at $p < 0.05$. **Results:** Flood stress significantly increased SOD, CAT, POD, and APX activities in both crops, with peak levels observed at 48 hours, followed by a slight decline at 72 hours. Prolonged flooding reduced shoot and root length, fresh and dry weight, and chlorophyll content in both crops. Wheat maintained relatively higher enzyme activities and better growth than maize, indicating stronger physiological tolerance. **Conclusions:** Flood stress induces antioxidant enzyme activity as a protective mechanism against oxidative damage in maize and wheat seedlings. Peak enzyme activity at 48 hours indicates temporal regulation, while interspecies differences suggest crop-specific tolerance strategies.

Keywords: Flood Stress, Antioxidant Enzymes, Maize, Wheat, SOD, CAT, POD, APX, Seedling Growth

INTRODUCTION

One of the extreme weather events being intensified by climate change is flooding, which is becoming a significant threat to the global cereal production due to its negative impact on the growth, development, and production of plants (Wang et al., 2018; Farooq et al., 2023; Rhaman et al., 2022, Elhabashy et al., 2025). In large cereals like wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.), a long-term exposure to surplus water not only reduces the oxygen supply to the roots but also inhibits the usual exchange of gases through the air in the aerial tissues. In such low-oxygen environments, plants produce an increased amount of reactive oxygen species (ROS) that disrupts photosynthesis and nutrient uptake. When the hypoxia or anoxia becomes more severe, ROS such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) accumulate in the cell and cause oxidative damage to critical biomolecules such as lipids, proteins, and nucleic acids. A response of plants to oxidative stress involves the activation of a set of antioxidant enzymes, such as superoxide dismutase, catalase, peroxidases, and ascorbate peroxidase. Superoxide dismutase is the first line of defense, and it transforms the harmful superoxide radicals to hydrogen peroxide, which is, in turn, destroyed by catalase and ascorbate peroxidase to save the cells against oxidative damage (Caverzan et al., 2016; Khalid et al., 2024). Although the behavior of such enzymes during drought and heat stress in cereals has been more extensively studied (Tang et al., 2025), their behavior during flooding is less studied. The available research indicates that catalase, peroxidase, and ascorbate peroxidase activities can be highly enhanced by flooding, but the activity of superoxide dismutase appears to be determined by the genotype of the plant and the duration of exposure to the stress (Wang et al., 2023; Elhabashy et al., 2025). Moreover, wheat genotypes that are more resistant to salt and waterlogging

stresses together have better antioxidant activity and morpho-physiological characteristics of their seedlings (Elhabashy et al., 2025). Enzyme-based mechanisms of the flood tolerance are important to understand so that crop resilience can be enhanced. Crop-specific differences in antioxidant defenses in maize and wheat seedlings under controlled flooding conditions can be compared to provide breeding and management strategies (Toulotte et al., 2022; Youssef et al., 2016). By tracking enzyme activities at different times (e.g., 24, 48 and 72 h) this can also be used to evaluate temporal control of the antioxidant system. Flooding will decrease the oxygen in roots and cause oxidative stress in the cereals. The response of antioxidant enzymes (SOD, CAT, POD, APX) in maize and wheat could be investigated to identify how the flooding tolerance was achieved and how the crop can be strengthened. In our research, we hypothesized that the effect of flood stress would cause a temporary effect on the activity of SOD, CAT, POD, and APX in maize and wheat seedlings, and the difference in the magnitude and time in their responses would show their mechanisms of differing tolerance to the flood stress.

Methodology

Plant Material and Growth Conditions

Maize (*Z. mays* L.) and wheat (*T. aestivum* L.) seeds were sourced from the Punjab Seed Corporation, Lahore. The seeds were surface-sterilized in 1% sodium hypochlorite for five minutes and then rinsed thoroughly with distilled water. Ten seeds were sown per pot, and seedlings were grown under controlled conditions, maintaining a 16-hour light/8-hour dark cycle, a temperature of $25 \pm 2^{\circ}\text{C}$, and 60–70% relative humidity. To ensure adequate nutrition, they were supplied with half-strength Hoagland solution every two days.

Experimental Design and Flood Stress Treatment

Uniform seedlings were placed in pots with sand and Hoagland solution after ten days of germination, with three replicates per treatment in a fully randomized design. Flood stress was applied as follows: **Control:** Seedlings maintained under normal watering (field capacity).

Flood Treatment: Pots were submerged in water to 2–3 cm above the soil surface for 24 h, 48 h, and 72 h.

After the respective stress periods, leaf samples were collected immediately for enzyme assays.

Enzyme Extraction

Fresh leaf tissue (0.5 g) from each seedling was ground in 5 mL of chilled 50 mM phosphate buffer (pH 7.0) supplemented with 0.1 mM EDTA and 1% PVP. The mixture was then centrifuged at $12,000 \times g$ for 20 minutes at 4°C , and the clear supernatant was collected and used as the enzyme extract.

Enzyme Assays

Antioxidant enzyme activities were assessed using standard spectrophotometric procedures. Superoxide dismutase activity was determined by monitoring how effectively the enzyme prevented the photochemical reduction of nitroblue tetrazolium at 560 nm; the amount of enzyme required to inhibit this reaction by 50% was defined as one unit of activity. Catalase activity was evaluated separately by recording the decline in absorbance at 240 nm as hydrogen peroxide decomposed in a reaction

mixture containing 50 mM phosphate buffer (pH 7.0) and 10 mM H₂O₂. Peroxidase activity was measured by tracking the formation of tetraguaiacol at 470 nm, using guaiacol as the substrate. For ascorbate peroxidase, the assay focused on the decline in absorbance at 290 nm as ascorbate was oxidized in the presence of hydrogen peroxide. All enzyme activities were expressed relative to total protein levels, which were quantified using the Bradford method.

Statistical Analysis

The data were analyzed using SPSS version 27.0. Differences in enzyme activities under the various stress durations were assessed using one-way ANOVA, followed by Tukey's post-hoc test to compare the groups. All results are presented as the average of three biological replicates with their standard deviations, and differences were considered statistically significant when the p-value was below 0.05.

Results

All of the enzyme activities measured under flood stress showed an increase in both crops over the control, indicating an induced antioxidant defense response. As an illustration, in maize, SOD activity rose to 66.2 ± 3.0 units/mg protein in 48 hours of flooding compared to 45.3 ± 2.1 units/mg protein in the control, and in wheat, the SOD activity rose to 70.4 ± 3.2 units/mg protein in 48 hours of flooding compared to 48.1 ± 1.9 units/mg protein in the control. The same was noted about the CAT, POD, and APX activities, where the highest activity was usually recorded at a 48 h flooding period. The enzyme activities had slight decreases of the 72h flood stress, but still were higher than the control levels. This implies that long-term flooding could partially remove the antioxidant system of the seedlings, lowering the efficiency of the enzymes. The root length, fresh weight, and dry weight of maize and wheat in flooded seedlings also decreased progressively in both crops with a long-term flooding condition, which implied inhibited growth by the lack of oxygen and metabolic constraints caused by the stress. The total chlorophyll content also declined during flood stress, and the results of maize seedlings declined with increased stress duration (the results of 24, 48, and 72 h follow the same downward pattern). The growth of wheat and the chlorophyll content were usually higher than that of maize at all durations of stress, implying that the physiological responses of wheat to flooding were better than those of maize.

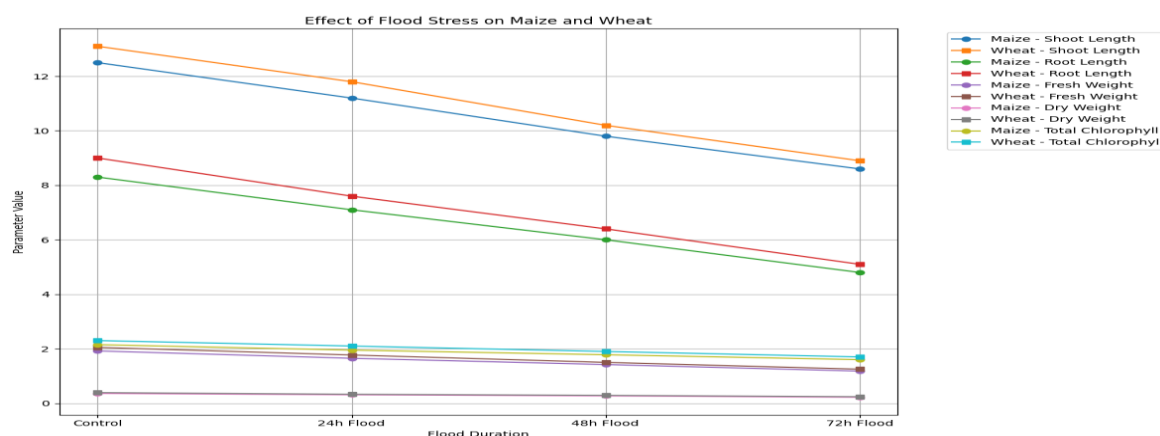


Figure 1: Effect of Flood Stress on Growth and Physiological Parameters in Maize
The enzyme activities are represented in units per milligram of protein (Unit/mg protein) and are expressed as mean and standard deviation (SD). The findings show that flood stress tended to enhance the activities of all the enzymes in comparison to the conditions

under control and the activities of all the enzymes were maximum at the 48 h of flooding, and a minor decline was observed at the 72 h period. Enzyme activities were observed to be slightly higher in wheat seedlings compared to maize at all stress periods indicating that there is a mechanism of variation in tolerance of the two crops to flood stress.

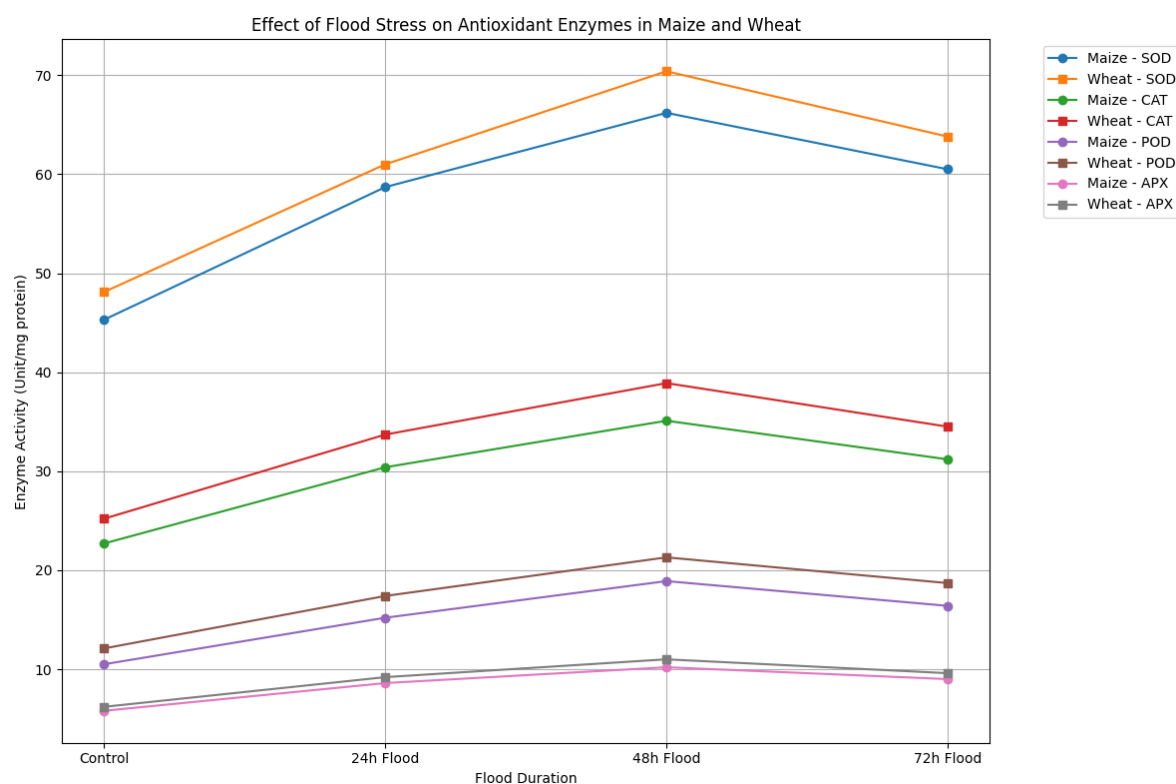


Figure 2: Effect of Flood Stress on Enzyme Activities in Maize and Wheat Seedlings

Discussion

Our results indicate that the antioxidant enzyme system, in particular, the SOD, CAT, POD, and APX, is strongly triggered by flood stress in maize and wheat seedlings, which is consistent with the results of waterlogging and flooding stress in plants. The presence of reactive oxygen species (ROS) in flood stress under conditions of low oxygen supply in the root zone (hypoxia) results in the development of superoxide (O_2^-), hydrogen peroxide (H_2O_2). Too much ROS may cause damage to membranes, proteins, and other cell elements. Plant's response to this oxidative stress is the up-regulation of antioxidant defences (Liaqat et al., 2023). In the experiment, SOD, CAT, POD and APX activities were greatly enhanced after 48h of flooding, that was followed by a slight decrease after 72h. The trend aligns with the fact that antioxidant enzymes are originally able to neutralize ROS, but can be overwhelmed by the stressor in the long term (Hasanuzzaman et al., 2020; Chen et al., 2024; Bashir et al., 2025). SOD takes the first defense whereby the superoxide radicals are changed into H_2O_2 that is less detrimental. We found that the peak of SOD activity was at 48 h of flooding, which is in line with results of other species, including *Styrax tonkinensis*, which under flood-drought conditions upregulated both SOD and CAT (Chen et al., 2024, Yue et al, 2024; Hu et al, 2020). CAT has a major role in the degradation of H_2O_2 into water and oxygen and it peaked similarly at 48 h in our experiment, which helps in oxidative detoxification (Hasanuzzaman et al., 2020; Anjum et al., 2016; Ighodaro et al., 2018). POD and APX that also detoxify H_2O_2 were also greatly increased, indicating a multi-

enzyme antioxidant response. Waterlogging stress in *Sesamum indicum* stimulated the activity of APX and other antioxidant enzyme activities, but not all of the times CAT activity increased with stress (Anee et al., 2019). Enzyme activities dropped but were not under the control ranges after 72 h of flooding. The depreciation can be either a sign of the partial depletion of the antioxidant system or regulatory feedback. The same has been found in other plant species under stress over an extended period (Anee et al., 2019). Regarding growth, shoot and root length, the fresh and dry weight reduced with extended flooding. These pathways indicate the physiological price of stress: despite the active antioxidant defence, ROS potentially can interfere with photosynthesis and reduce biomass growth. It has been previously demonstrated that waterlogging decreases growth parameters in maize, and the stress tolerance corresponds to increased antioxidant enzyme activities. The total chlorophyll content also decreased during flooding, especially in maize, and was a sign of oxidative damage or inhibition of chlorophyll production, as it was done in other studies (Wang et al., 2023). Wheat seedlings kept relatively high enzyme activities and enhanced growth and chlorophyll retention compared to maize, which implies an efficient antioxidant defense mechanism or increased stress tolerance which is what interspecific and genotypic variation in other studies has found. Flood tolerance could be improved by enhancing antioxidant capacity by breeding or exogenous antioxidant treatments (e.g., melatonin) (Anee et al., 2019). Waterlogging damage can also be alleviated via agronomic techniques (i.e., ridge tilling in maize) that support the maintenance of high SOD, CAT, and POD (Muhammad et al., 2025; Kumar et al., 2021; Sheoran et al., 2024). The knowledge of timing of antioxidant responses and especially the highest point of 48 hour might be used to advance the use of protective strategies. In this study, enzyme activity and not the ROS levels and gene expression were measured. Transcriptional regulation, ROS quantification and downstream damage assessment (lipid peroxidation, membrane integrity) must be included in future work (Khan et al., 2025). Moreover, the stress length or investigating the recovery may give an idea of the resilience in the long run. Further increase in flood resistance by exploring genotypic variation in maize and wheat and evaluating exogenous regulators to increase enzyme activity may be considered (Chen et al., 2024; Anee et al., 2019).

Conclusions

Maize and wheat are prone to antioxidant defense induced by flood stress with a peak of SOD, CAT, POD, and APX activities at 48h. Stress overwhelm is manifested by prolonged flooding that suppresses growth and chlorophyll. Wheat was more tolerant as enzyme activity and growth was higher, compared to that of maize. Increasing antioxidant response would increase crop resilience to waterlogging.

REFERENCES

- Anee TI, Nahar K, Rahman A, Mahmud JA, Bhuiyan TF, Alam MU, Fujita M, Hasanuzzaman M. Oxidative damage and antioxidant defense in *Sesamum indicum* after different waterlogging durations. *Plants*. 2019 Jun 29;8(7):196.
- Anjum NA, Sharma P, Gill SS, Hasanuzzaman M, Khan EA, Kachhap K, Mohamed AA, Thangavel P, Devi GD, Vasudhevan P, Sofo A. Catalase and ascorbate peroxidase—representative H₂O₂-detoxifying heme enzymes in plants. *Environmental science and pollution research*. 2016 Oct;23(19):19002-29.
- Bashir T, Mehmood A, Muhammad N. Antibiotic Resistance Profiles of Lactic Acid Bacteria Isolated from Dairy Products in Lahore, Pakistan: Antibiotic

- Resistance in LAB from Dairy Products in Lahore. *MARKHOR (The Journal of Zoology)*. 2025 Sep 30;14:8.
- Caverzan A, Casassola A, Brammer SP. Antioxidant responses of wheat plants under stress. *Genetics and molecular biology*. 2016;39(1):1-6.
- Chen H, Han C, Cui L, Liu Z, Yu F. Transcriptome analysis of antioxidant system response in *Styrax tonkinensis* seedlings under flood-drought abrupt alternation. *BMC Plant Biology*. 2024 May 17;24(1):413.
- Elhabashy S, Zhang S, Qiu CW, Shi SH, Holford P, Wu F. Identification of Wheat Genotypes with High Tolerance to Combined Salt and Waterlogging Stresses Using Biochemical and Morpho-Physiological Insights at the Seedling Stage. *Plants*. 2025 Apr 22;14(9):1268.
- Farooq A, Farooq N, Akbar H, Hassan ZU, Gheewala SH. A critical review of climate change impact at a global scale on cereal crop production. *Agronomy*. 2023 Jan 4;13(1):162.
- Hasanuzzaman M, Bhuyan MB, Zulfiqar F, Raza A, Mohsin SM, Mahmud JA, Fujita M, Fotopoulos V. Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. *Antioxidants*. 2020 Jul 29;9(8):681.
- Hu H, Zhang P, Wang L, Liang H, Liang J, Lyu R. Physiological, Biochemical and Gene Expression Analyses of *Halimodendron halodendron* Responding to Drought Stress. *Genes*. 2025 Nov;16(11):1274.
- Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria journal of medicine*. 2018;54(4):287-93.
- Khalid H, Tariq A, Jurrat H, Musaddaq R, Liaqat I, Muhammad N. Actinomycetes: ultimate potential source of bioactive compounds production: actinomycetes for bioactive compounds production. *Futuristic Biotechnology*. 2024 Dec 31:02-11.
- Khan HR, Muhammad N, Tariq A, Bashir T, Jurrat H, Javeed M, Javaid A. Microbial Contamination of Healthcare Personnel and Devices: A Critical Route for Pathogen Transmission: Microbial Contamination of Healthcare Personnel and Devices for Pathogen Transmission. *Pakistan BioMedical Journal*. 2025 Jul 31:49-55.
- Kumar S, Mahapatra S, Chakraborty S, Mukharjee S. Effect of abiotic stresses and mitigation strategy associated with their tolerance in wheat. *Journal of Cereal Research* 13 (1): 16-37.
- Liaqat I, Muhammad N, Ara C, Hanif U, Andleeb S, Arshad M, Aftab MN, Raza C, Mubin M. Bioremediation of heavy metals polluted environment and decolourization of black liquor using microbial biofilms. *Molecular biology reports*. 2023 May;50(5):3985-97.
- Muhammad N, Liaqat I, Andleeb S, Naseem S, Arshad M, Khalid A. Indigenous ascel chicken-derived probiotics as biofactories of antifungal metabolites to control mycotoxin contamination in poultry feed. *Molecular Biology Reports*. 2025 Dec;52(1):724.
- Rhaman MS, Kibria MG, Hoque A. Climate Change and Its Adverse Impacts on Plant Growth in South Asia: Current Status and Upcoming Challenges. *Phyton (0031-9457)*. 2022 Mar 1;91(4).
- Sheoran AR, Lakra N, Saharan BS, Luhach A, Mandal NK, Seth CS, Sharma D, Santal AR, Sadh PK, Rajput VD, Duhan JS. Enduring drought: effects and strategies for Brassica crop resilience. *Academia Biology*. 2024 Jul 19;2(3).

- Tang H, Zhang L, Xie X, Wang Y, Wang T, Liu C. Resilience of Maize to Environmental Stress: Insights into Drought and Heat Tolerance. *International Journal of Molecular Sciences*. 2025 May 30;26(11):5274.
- Toulotte JM, Pantazopoulou CK, Sanclemente MA, Voesenek LA, Sasidharan R. Water stress resilient cereal crops: Lessons from wild relatives. *Journal of Integrative Plant Biology*. 2022 Feb;64(2):412-30.
- Wang GY, Ahmad S, Wang Y, Wang BW, Huang JH, Jahan MS, Zhou XB, Shi CQ. Multivariate analysis compares and evaluates drought and flooding tolerances of maize germplasm. *Plant Physiology*. 2023 Sep;193(1):339-55.
- Wang J, Vanga SK, Saxena R, Orsat V, Raghavan V. Effect of climate change on the yield of cereal crops: A review. *Climate*. 2018 May 15;6(2):41.
- Wang Y, Xu Y, Xu J, Sun W, Lv Z, Manzoor MA, Liu X, Shen Z, Wang J, Liu R, Whiting MD. Oxygenation alleviates waterlogging-caused damages to cherry rootstocks. *Molecular Horticulture*. 2023 Dec;3(1):1-23.
- Youssef MS, Mira MM, Renault S, Hill RD, Stasolla C. Phytoglobin expression influences soil flooding response of corn plants. *Annals of Botany*. 2016 Oct 1;118(5):919-31.
- Yue L, Yu C, Abdoussalami A, Li X, Lv K, Huang G, Hu M, Yang Z. Growth, Morphological Alterations, and Enhanced Photosynthetic Performance Promote Tolerance of *Distylium chinense* to Alternate Drought–Flooding Stresses. *Forests*. 2024 Jan 8;15(1):125.