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Impact of Nanoparticles on Morpho-Biochemical Changes of Wheat (*Triticum aestivum* L.) Under Moisture Stress

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Abstract

Moisture stress negatively influences various morphological and biochemical processes resulting in poor growth, oxidative stress, and hampered photosynthetic activity ultimately resulting in decreased crop yield. Nanotechnology is a new strategy that can be used to address these problems. Nanoparticles when applied to crops plants, they improve the growth of plants under unfavorable conditions by activating several physiological and biochemical mechanisms. A study was carried out during the Rabi season of 2022-23 and 2023-24, using a randomized block design, with three replications and ten treatments. Wheat variety HD 2967 was used. Different combinations of nano zinc, nano titanium and calcium phosphate were used. Foliar spray was done at 45 days after sowing (DAS). ZnO (50ppm) was found most effective in increasing plant height and shoot dry biomass of the crop under conditions of moisture stress followed by ZnO (40ppm). Contrary to this, when considering antioxidant enzyme activity for SOD and catalase and also various yield attributing characters and yield ZnO (50ppm) showed a significantly higher value followed by TiO2 (40ppm)+Ca_3PO_4 (40ppm). The current study concludes that nanoparticles effectively reduce moisture stress in wheat particularly treatment T10 i.e., ZnO (50ppm) followed by treatment T4 i.e. TiO_2 (40ppm) + Ca_3PO_4 (40ppm) are recommended to alleviate the negative impacts of moisture stress on wheat crops.

Keywords: wheat, antioxidants, zinc oxide nanoparticles, titanium oxide nanoparticles, grain yield, moisture stress

1. Introduction

Agriculture is among the most vital sectors of the national economy in developing nations, most notably India. The food production rate increases considerably, contributing to the expansion of the national gross domestic product. Furthermore, over sixty per cent of the populace depends on it for nutrition, fodder, fuel, and fiber (Bisht *et al*., 2022) [2]. Reducing the productivity of food grains may result in a food shortage and a compromise on nutrition security. The decline in food productivity trends is primarily ascribed to water and agricultural land availability constraints. However, climate change, water deterioration, and soil nutrient depletion can further exacerbate this challenge (Van Nguyen *et al*., 2022) [23].

Climate change exacerbates the potential for drought to lead to severe food scarcity (Kah *et al*., 2019) [7]. Drought is the most severe abiotic stress that inhibits plant growth and causes increased crop yield losses, according to (Lambers *et al*., 2008) [10]. The adverse impact on the plant's morphological, physiological, biochemical, and molecular attributes reduces the quantity and quality of the harvested crops (Farooq *et al*., 2009) [4]. Therefore, it is essential to construct and improve the drought resistance of plants using practical, risk-free techniques. It has been demonstrated that nanomaterials (NMs) are among numerous effective methods for combating plant mortality caused by drought (Khan *et al*., 2017) [8]. Reportedly, many NMs have increased crop yields by practicing sustainable agriculture to meet the increasing global demand for fuel, food, and sustenance (Kah *et al*., 2019) [7]. It has been documented that nanoparticles (NPs) enhance the activities of enzymatic and non-enzymatic antioxidants, including glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD), in order to modulate defense mechanisms (Taran *et al*., 2017; Djanaguiraman *et al*., 2018) [22, 3]. Recent research has demonstrated that nanoparticles (NPs) mitigate the detrimental effects of drought stress by enhancing the concentrations of osmolytes and osmoprotectants-namely prolines, glycine betaine, and soluble sugars and augmenting the levels of osmolytes and ROS (H_2O_2 and O_2^-) that aid in osmotic adjustment under conditions of drought stress (Mustafa *et al*., 2021; Van Nguyen *et al*., 2022) [15, 23].

Applying titanium dioxide (TiO_2) nanoparticles (NPs) to specific plant species profoundly affects their morphological, physiological, and biochemical properties. In 2008, Lei *et al*. documented that utilising TiO_2 nanoparticles enhanced chlorophyll formation, photosynthetic rate, and activities of antioxidant and rubisco enzymes, all contributing to an overall increase in crop productivity. In addition to reducing H_2O_2 and MDA levels, Latef *et al*. (2018) [11] found that TiO_2 NPs improved broad bean plant growth, antioxidant enzyme activities, soluble carbohydrates, amino acids, and proline content when grown in saline conditions. Khan (2016) [9] documented how applying TiO_2 NPs to tomato plants alleviated salt stress by increasing yield, agronomic characteristics, leaf chlorophyll content, phenolics, antioxidant capacity, and antioxidant enzyme activities. (Lei *et al*., 2008) [12] TiO_2 may be regarded as a plant stimulant due to its ability to activate diverse defence mechanisms implicated in plant tolerance to various abiotic stressors. Zinc (Zn), a vital micronutrient, is indispensable for the growth and development of plants. It comprises more than 300 proteins and enzymes synthesised by plants without excessive water stress or under normal water conditions (Noreen *et al*., 2021; Yao & Tang, 2022) [16, 25]. Zinc is essential for numerous physiological processes, including protein synthesis, growth regulation, pollination, and antioxidant activity. Furthermore, in conditions of water scarcity, it is essential for photosynthesis, cell membrane maintenance, free radical detoxification, and gene expression (Suganya *et al*., 2020) [21].

2. Material and methods

Study design

The present field study was conducted during the Rabi season of 2022-23 and 2023-24 at Student's Instructional Farm of Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh. Geographically the experimental site is situated 42km away from Ayodhya on Ayodhya-Raibarielly Road between latitude of 26.47° North and longitude of 81.12° East on an elevation of 113 meters in the gangetic alluvium of eastern Uttar Pradesh. The experiment was conducted in randomized block design (RBD) with three replications and ten treatments. Wheat variety HD 2967 was used and the sowing was done in November. Moisture stress was applied to the crop by abstaining irrigation after the CRI (Crown Root Initiation) stage. Full dose of fertilizer was applied as basal @ 120:60:40 N:P_2O_5:K_2O respectively. Zinc oxide nanoparticles and titanium dioxide nanoparticles used in the study were purchased from SRL (Sisco Research Laboratory), Maharashtra, India. Zinc and Titanium nanoparticle solutions were prepared by sonicating the solution at 40 KHz for 20 min and 20 KHz for 15 min respectively. The spray of nanoparticles was done 45 days after sowing (DAS).

Treatments

T1: Control (foliar spray of distilled water 45 DAS).

T2: Calcium phosphate Ca_3PO_4 (40ppm).

T3: Titanium oxide TiO_2 (40ppm).

T4: Titanium oxide TiO_2 (40ppm) + Calcium phosphate Ca_3PO_4 (40ppm).

T5: Titanium oxide TiO_2 (50ppm)

T6: Titanium oxide TiO_2 (50ppm) + Calcium phosphate Ca_3PO_4 (40ppm)

T7: Zinc oxide ZnO (40ppm).

T8: Zinc oxide ZnO (40ppm) + Calcium phosphate Ca_3PO_4 (40ppm).

T9: Zinc oxide ZnO (50ppm)

T10: Zinc oxide ZnO (50ppm) + Calcium phosphate Ca_3PO_4 (40ppm)

Biochemical analysis

The antioxidant enzyme activities were recorded at three different stages i.e. 60, 75 and 90 DAS.

Catalase activity: Catalase activity can be assayed calorimetrically according to method given in analytical biochemistry (Sinha 1972). Catalase facilitates the dismutation of H_2O_2 to water and O_2 according to the reaction.

$$H_2O_2 \longrightarrow H_2O + \frac{1}{2} O_2$$

200 mg of fresh leaves material was homogenized with 10 ml of phosphate buffer 0.1 M (pH 7.0) and centrifuged at 10000 rpm for 30 minutes at 4^0C. The reaction mixture was taken in Erlenmeyer flask and mixed rapidly at 37 ^oC. At 3 minutes' interval, 2.0 ml of reaction mixture was withdrawn in test tube and to it, 2.0 ml potassium dichromate acetic acid solution was added and kept on water bath for 10 minutes and color intensity was measured after cooling at 570 nm.

SOD activity: Superoxide dismutase (SOD) activity in plant tissue was measured, method described by (Asada 1974) and modified by (Giannopolitis and Ries 1977) [5]. Superoxide dismutase was assayed on the basis of its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) into blue formazan which has a wavelength maximum at 560 nm. Superoxide dismutase (SOD) essentially inhibited the formation of the blue formazan and could be quantitated on this basis.

Statistical analysis: The statistical analysis was carried out for each parameter studied based on the randomized block design following the method of Gomez & Gomez (1984) [6]. Associations between parameters were studied using correlation analysis. Means were compared by least significant difference (LSD) at 5% level of significance.

Result & Discussion

Effect of nanoparticles on antioxidants activity of wheat under moisture stress condition

Plants exposed to drought stress accumulate excessive ROS, and maintaining the balance between the formation and degradation of ROS is critical to avoiding oxidative damage (Meneguzzo *et al*., 1999) [13]. ROS is detoxified in plants by ROS-scavenging antioxidative enzymes like SOD, and CAT (Schutzendubel *et al*., 2002) [18]. Foliar spray with ZnO and TiO_2 NPs along with Ca_3PO_4 significantly increases the antioxidant enzyme SOD and catalase activity compared with untreated plot (Table 1). Among the various combinations of TiO_2 applied it was observed that treatment T4 TiO_2 (40ppm) along with Ca_3PO_4 (40ppm) was most effective also for ZnO among the various combinations applied treatment T9 ZnO (50ppm) was most effective against moisture stress. Overall it was observed that among the various treatment combinations applied maximum SOD as well as catalase activity were measured for T_9 when the ZnO NPs (50 ppm) is applied under moisture stress conditions. Similarly at 75 DAS and 90 DAS maximum SOD and catalase activity were recorded in treatment T_9 (455.5) and (470.0) followed by T_4 TiO_2 (40ppm) along with Ca_3PO_4 (40ppm) (434.9) and (464.44) respectively. ZnO NPs were found to be more effective as compared with TiO_2 NPs under water-stressed condition. Wheat plants from untreated plot (control) record the minimum SOD and catalase activity at all the crop stages (Table 3). Our findings are in agreement with Raeisi Sadati *et al*., 2022 [17] who reported that at all stress levels, the nanoparticle treatments significantly increased the CAT antioxidant activities in cultivars; however, this increment was more pronounced in severe stress than the others. The highest amounts of the CAT (6.72 $\mu M\ g^{-1}\ min^{-1}$) activities were observed in Heidari and Gascogne by ZnO NPs 1 gL^{-1}, whereas the lowest level of CAT activities were observed by 0.5 gL^{-1} of ZnO NPs under mild and severe stress conditions. The results presented here also corroborate the findings of Waqas Mazhar *et al*. (2022) [24], which indicated that applying ZnO NPs during seed priming enhanced the activities of antioxidant enzymes, showcasing their efficacy in mitigating drought. The SOD, which serves as the initial line of defence in the presence of stress, increased substantially by 23.69-31% in leaves of Kargi and 35.51-52.06% in CSR 30 when exposed to saline conditions, in comparison to the levels observed with non-stressed treatment. The application of ZnO-NPs resulted in an additional elevation in SOD. Singh *et al*. (2022) [19] observed that the leaf SOD activity increased by 37.58-60.99% in Kargi and 47.42-70.23% in CSR 30, in comparison to the leaf SOD activity observed in non-stressed and stressed treatments, respectively.

TABLE 1: EFFECT OF NANOPARTICLES ON CATALASE ACTIVITY (G^{-1}FRESH WEIGHT MIN^{-1}) OF WHEAT UNDER MOISTURE STRESS CONDITION

Treatments	Catalase activity (g^{-1}fresh weight min^{-1})			SOD activity (unit g^{-1}fresh weight min^{-1})		
	60DAS	75DAS	90DAS	60DAS	75DAS	90DAS
T1	99.17	354.6	432.50	198.20	223.6	243.43
T_2	101.94	360.0	441.11	213.69	238.6	259.93
T_3	99.72	407.2	447.50	224.39	252.0	277.45
T_4	106.11	434.9	464.44	230.46	296.0	322.40
T_5	96.94	354.6	438.61	208.50	255.1	278.20
T_6	98.06	367.8	442.50	216.31	263.0	281.03
T_7	95.00	407.4	451.11	229.97	277.4	304.56
T_8	97.50	397.3	444.72	217.09	256.3	287.09
T_9	107.78	455.5	470.00	230.87	307.5	333.96
T_{10}	98.89	412.7	455.83	224.41	260.2	289.35
S.Em±	1.39	16.52	6.87	2.1	2.41	2.72
C.D. 5%	4.14	49.07	20.4	6.09	7.16	8.07

Effects of nanoparticles on yield attributes and yield of wheat under moisture stress condition

Moisture stress significantly affected the yield attributes and yield of the crop causing a reduction in the yield contributing attributes as well as the grain yield of the wheat variety however, foliar spray with ZnO and TiO_2 NPs along with Ca_3PO_4 significantly enhances the yield contributing attributes as well as the grain yield compared with untreated plot (Table 2). It was observed that among the various yield contributing attributing characters application of nanoparticles had no impact on the effective tillers of the wheat crop. However other characters i.e. ear length and test weight showed a significant increase. It was observed that maximum ear length was observed for treatment T9: ZnO (50ppm) i.e. (12.67 cm) followed by treatment T4: TiO_2 (40ppm) along with Ca_3PO_4 (40ppm) i.e. (12.20 cm). Similarly maximum test weight was observed for treatment T9 (37.13g) followed by treatment T4 (36.57 g). Overall it was observed that among the various combinations applied in the study maximum grain yield are obtained for T_9 when the ZnO NPs (50 ppm) was applied under moisture stress conditions i.e. (6.58 kg/plot) followed by T_4 [TiO_2 (40ppm) along with Ca_3PO_4 (40ppm)] having grain yield (5.72 kg/plot). ZnO NPs is more responsive as compared with TiO_2 NPs under water-stressed condition. Wheat plants from untreated plot (control) record the minimum grain yield as well as yield attributing characters at all the crop stages (Table 2). The findings acquired are consistent with the observations documented by Muhammad *et al*. (2022) [14]. They found that the combination of ZnO NPs and PGPR (T4) increased tillering yield under drought conditions (D2) by 7% and 10%, spike length increased by 14% and 18%, grain weight increased by 1.2% and 4%, and grain yield increased by 3% and 6%, respectively, when compared to the use of PGPR (T2) and ZnO NPs (T3) alone. T4 achieved an 8.5% and 9.3% increase in Productive Tiller, a 23.5% and 24.3% increase in Spike Length, a 7% and 14% increase in 1000 grain weight, and a 24% and 28% increase in GY, respectively, at the water-stressed grain-filling stage (D3). Consequently, the findings above validated the adverse impacts of drought on tillering growth and grain-filling stage yield characteristics, which T4 subsequently remedied in comparison to T2 and T3.

TABLE 2: EFFECT OF NANOPARTICLES ON YIELD ATTRIBUTES AND YIELD OF WHEAT UNDER MOISTURE STRESS CONDITION

Treatments	Effective Tillers/Plant	Length of Ear (cm)	Grain Yield (kg/plot)	Test Weight (g)
T_1	6.67	11.70	3.62	34.65
T_2	7.33	12.10	5.47	33.37
T_3	7.00	11.57	5.26	34.55
T_4	6.33	12.20	5.72	36.57

T_5	6.67	11.47	5.16	31.96
T_6	8.00	11.43	5.56	35.96
T_7	7.67	12.17	5.17	33.32
T_8	8.00	12.10	4.72	36.53
T_9	8.33	12.67	6.58	37.13
T_{10}	8.00	11.53	5.46	33.02
S.Em±	0.79	0.25	0.12	0.92

Conclusion

Insufficient water results in a decline in stomatal conductance, gaseous exchange, and plant metabolism, all contributing to unfavourable agronomic characteristics. Water scarcity typically results in the generation of reactive oxygen species (ROS), which have the potential to damage plant metabolism and function as mutagens by causing enzymatic denaturation. Biological membrane lipid peroxidation caused by reactive oxygen species disrupts membrane transport processes. It is noteworthy that the utilization of nanoparticles managed to mitigate the decline in growth and yield by enhancing resistance to arid conditions and optimizing antioxidant concentrations (specifically, catalase and SOD). Based on the present study's findings, it is possible to conclude that interventions T10 and T8 significantly improved the wheat crop's morphological development. Furthermore, implementing zinc and titanium nanoparticles resulted in enhanced yield-attributing properties, ultimately lifting the cereal yield. In contrast to morphological characteristics, it was noted that the implementation of treatments T9 and T4 significantly increased the wheat yield when subjected to moisture stress. Furthermore, it was determined that zinc nanoparticles exhibited a greater capacity to alleviate the consequences of moisture stress than titanium nanoparticles. After this, additional field research is necessary to validate the diverse nanoparticle combinations utilized in investigating methods for enhancing crop resilience.

Conflicts of Interest

The authors declare no conflicts of interest.

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Credit taxonomy

Deeksha Tiwari: Data Curation, Investigation, Visualization, Writing- original draft.

A. K. Singh: Principal Investigator, Planning, Methodology, Conceptualization, Supervision.

A.K. Pandey: Investigation, Data Curation, Data Analysis, Validation, Writing-original manuscript, review and editing.

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