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# EFFECTS OF IAA AND IAA–GRAPHENE OXIDE NANOENSEMBLE ON DROUGHT STRESS IN DURUM WHEAT (*TRITICUM DURUM*)

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## Abstract

*Wheat (Triticum durum) is one of the world's most important crops, supplying essential nutrients and serving as a key source of food and livestock feed. Drought stress is a major challenge that hampers wheat growth and yield by disrupting its physiological and biochemical processes. Recent developments in nanotechnology present new opportunities to boost plant resilience and productivity. In this study, we examined the effects of a newly developed indole-3-acetic acid–graphene oxide (IAA–GO) nanoensemble on wheat seedlings under both normal and drought conditions. Seeds of the Yasaman variety were treated with IAA and IAA–GO and grown under controlled phytotron settings. We evaluated seedling growth, photosynthetic pigment levels, and activities of antioxidant enzymes, including catalase and guaiacol peroxidase. The results revealed that IAA alone significantly improved germination, root and shoot growth, and catalase activity. In contrast, the IAA–GO nanoensemble showed mixed effects, slightly enhancing catalase activity but reducing peroxidase activity and chlorophyll b content. Under drought stress, both treatments positively affected photosynthetic pigments, indicating an adaptive response. These findings suggest that IAA–GO complexes could be a valuable tool for enhancing drought tolerance in wheat by supporting growth, photosynthesis, and antioxidant defense systems.*

***Key words:*** *wheat, graphene oxide, nanotechnology, antioxidant system, phytohormones, plant protection*

## INTRODUCTION

Plant-based products play a crucial role in ensuring human nutrition, accounting for approximately 90 % of the total food sources. A significant portion of these, around 60 % comes from cereal crops. On a global scale, about 75 % of the human dietary demand is met by wheat (Hajiyeva et al., 2019). Wheat demonstrates remarkable ecological adaptability compared to other cereal crops and stands as the most widely cultivated grain globally. It is also recognized as one of the primary sources of plant-derived protein essential for human nutrition (Hasanova et al., 2019; Vilayet & Boyukkhanim, 2024). Wheat undergoes extensive processing and serves as a key raw material in producing a variety of food products such as pasta, flour, pastries, starch, beer and alcoholic beverages. Moreover, cereal grains play a vital role in providing the main feed source for livestock and poultry (Mirzayeva et al., 2022). According to the data provided in the *Food Balances of Azerbaijan* compendium published by the State Statistical Committee of the Republic of Azerbaijan, the total wheat reserves in 2024 amounted to 3,343,240 tons. Of this amount, 111,447 tons were allocated for seed, 695,681 tons for livestock and poultry feed, 1,822,312 tons for flour and groats production, 34,779 tons for alcohol production, and 31 tons for beer production (Food balances of Azerbaijan, 2025).

Drought stress is among the most significant abiotic challenges that farmers across thr globe encounter due to the impacts of climate change (Mammadova et al., 2025; Mansour et al., 2020). Drought stress profoundly affects wheat by altering its physiology, morphology and biochemical processes, which in turn slows down its growth and lowers overall yield (Jafarzadeh et al., 2025; Nyaupane et al., 2024).

Recent studies highlight the great potential of nanotechnology to transform agriculture by boosting the effiency of inputs and offering innovative solutions to the environmental and farming challenges, ultimately supporting higher good production and improved food security (Hasanova et al., 2022; Usman et al., 2020). Nanotechnology holds diverse applications in agroecosystems and is an emerging technique contributing to sustainable agricultural development with various potential functions. In recent years, graphene oxide (GO) and similar nanomaterials have been increasingly utilizied to efficiently deliver agrochemicals and nutrients to plants. They possess distinctive physicochemical properties, such as a very high specific surface area (2620 $m^2/g$) excellent resistance to the chemical and thermal breakdown, and a strong ability to adsorb both agrochemicals and micronutrients (Zhang et al., 2020). The way nanomaterials interact with and influence plants is complex and constantly changing, depending on factors such as the type of nanoparticle, how it is applied (including concentration, duration, and method), and the balance of plant hormones (Cheng et al., 2016). Plants respond to GO mainly through the reactive oxygen species (ROS) pathway. While ROS are naturally produced during normal cellular metabolism, stress can trigger their excessive accumulation, leading to oxidative damage and cell death. Research indicates that nanomaterials can affect plant growth and development by modulating this ROS metabolism (Rashid et al., 2021; Siddiq & Husen, 2017). GO can be used to deliver a range of substances, including plant hormones, which helps improve nutrient availability for plants while reducing losses due to leaching, breakdown by light, or hydrolysis (Amrahov at el., 2025). One example of such a phytohormone is IAA, a natural auxin that is essential for controlling plant growth, boosting stress resistance, and activating antioxidant defenses. IAA helps plants produce more energy by promoting the accumulation of photosynthetic products and also strengthens their antioxidant defenses when they face stressful conditions. IAA lowers the buildup of reactive oxygen species (ROS), helping to prevent oxidative damage and boosting the plant's ability to withstand stress. (Wang et al., 2024). This study focused on exploring how a newly synthesized IAA–graphene oxide complex influences the growth and immune system components of one of the most important wheat species (*Triticum durum*).

## MATERIALS AND METHODS

The study was carried out using the Yasaman (Triticum durum) wheat variety, with seeds sourced from the Azerbaijan Research Institute of Crop Husbandry. Before sowing, the wheat seeds were sterilized in a 0.1% potassium permanganate solution for one minute. Then they were planted in plastic pots filled with cocopeat substrate.

Graphene oxide (GO) nanolayers were prepared using a modified Hummer method (Taghiyeva et al ., 2024). IAA (1 μM) and IAA-GO (1 μM) were applied continuously throughout the growth period, starting from the earliest seedling stage. To simulate drought stress, polyethylene glycol (PEG-6000) was used; a 15% solution was prepared by adding 150 g of PEG-6000 to 1 liter of Steiner hydroponic solution.

The experiments were carried out using wheat seedlings that had been grown for two weeks.

Enzymatic activity analysis. The activity of the catalase enzyme was determined using the Mosheva gasometric method (Mosheva, 1982).

Guaiacol peroxidase activity was determined based on the method developed by Chance and Maehly (Chance & Maehly, 1955).

Chlorophyll a, chlorophyll b, and carotenoid contents were measured according to the procedure established by Wellburn and Lichtenthaler (Wellburn & Lichtenthaler, 1984).

Statistical analyses. All adsorption experiments were carried out in triplicate, and the results are presented as the mean ± standard deviation (SD). Regression parametres ($R^2$) for the isotherm, kinetic models were generated using the statistical measures in Microsoft Excel (Office 11, Microsoft Corporation, USA).

## RESULTS

The impact of IAA and the IAA-GO nanoensemble on wheat seedling germination and growth and is illustrated in Figure 1 and summarized in Table 1.

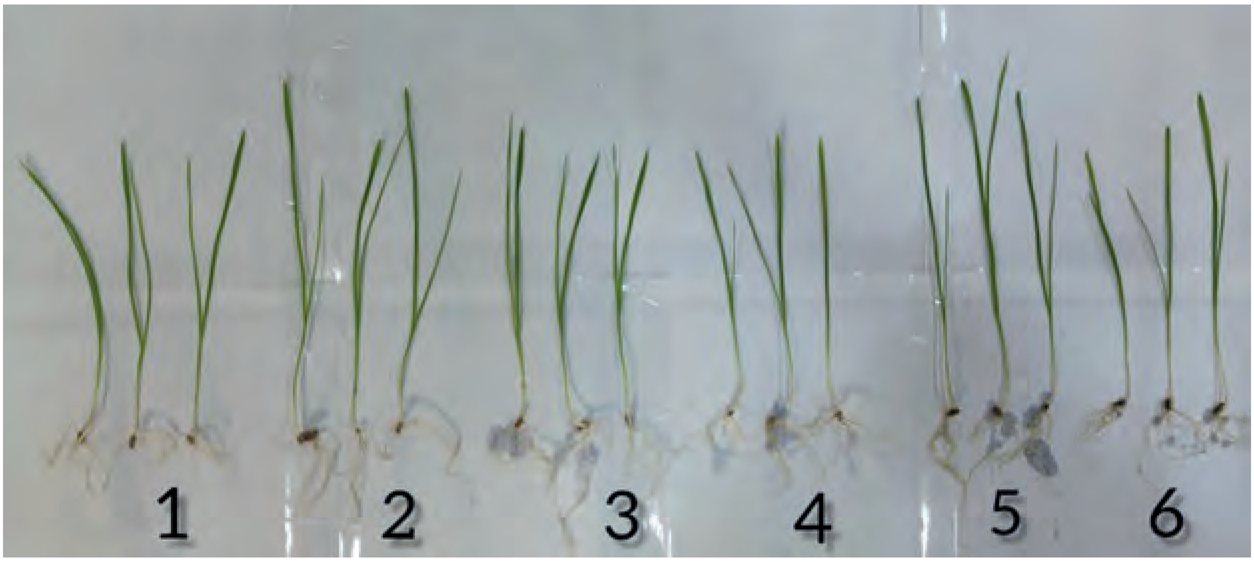

**Figure 1.** Effect of İAA and İAA-GO nanoensembles on the development of 14 day old Yasemen wheat genotype under drought stress. 1.Control. 2. 1 µM IAA. 3. 1 µM + GO. 4. 15 % PEG. 5. 15 % PEG + 1 µM IAA. 6. 15 % PEG + 1 µM IAA + GO

**Table 1.**
**Germination percentage, root, and stem lengths of the Trititucum durum Desf. Yasemen variety exposed to IAA and IAA + graphene oxide under drought stress**

| Samples | Number of seedlings ( total number of seeds – 72) | Germination percentage, % | Stem length (sm) | Root length (sm) |
|---|---|---|---|---|
| Control | 59 | 81.9 | 17.5±1,01 | 4.11±0.22 |
| 1 µM IAA | 65 | 90.2 | 19.26±1.22 | 7.68±1.21 |
| 1 µM IAA+ GO | 54 | 75 | 16.79±0,49 | 6.17±1.36 |
| 15 % PEG | 49 | 68.05 | 16.34±1.57 | 6.45±1.35 |
| 15% PEG+ 1 µM IAA | 58 | 80.5 | 19.28±0.21 | 10.06±2,01 |
| 15 % PEG +1 µM IAA + GO | 53 | 73.61 | 16.73±0.48 | 4.77±0.85 |

According to the results of the comparative germination analysis, the 1 µM IAA + GO treatment exhibited a minimal effect compared to the control variant. Although this treatment increased root length by 50%, it reduced leaf length by 4%. In contrast, the application of 1 µM IAA alone enhanced both leaf and root elongation by 10% and 86%, respectively. Under drought stress conditions, the 1 µM IAA + GO treatment slightly improved germination by 8.17% and leaf length by 2.38%. Regarding primary root development, compared with the 15% PEG variant, the nanoensemble caused a 26% reduction in root length, whereas 1 µM IAA exerted a positive influence on all three measured parameters, increasing germination by 18.29%, leaf length by 17.9%, and root length by 55.96%.

As a result of treating wheat seedlings with the IAA–GO phytohormone complex, a decrease in GPX enzyme activity was observed compared to the control variant (Figure 2). A similar inhibitory effect on the enzyme activity was also recorded during the application of the IAA–graphene oxide nanoensemble under drought stress conditions — with a 12.9% reduction before stress application and a 24.59% reduction afterward. IAA alone, however, did not affect peroxidase activity in either case. These data indicate that IAA exerted a stable (non-significant) effect, while the IAA–graphene oxide nanoensemble demonstrated an inhibitory effect on this enzymatic component of the antioxidant system in wheat plants.

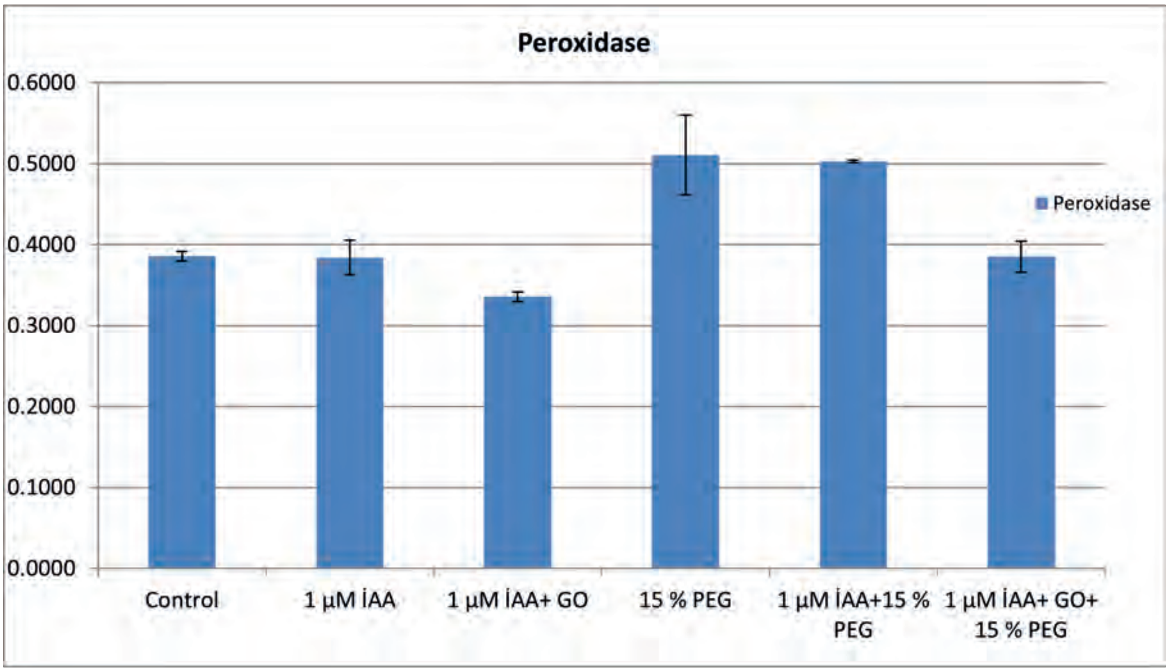

**Figure 2.** Guaiacol Peroxidase (GPX) activity in the leaves of Triticum durum Desf. Yasemen genotype treated by concentrations of İAA and İAA-GO nanoensemble**.**

The conducted analyses showed that, under normal conditions, treatment with the IAA+GO complex caused a 36.54% reduction in chlorophyll b content compared to the control group (Figure 3). This finding suggests that IAA–GO suppresses chlorophyll b synthesis, negatively affecting the plant's photosynthetic machinery by weakening the production of key photosynthetic pigments and diminishing its activity. When applied under drought stress, both IAA and the IAA–GO nanoensemble complex influenced the synthesis of all three components of the photosynthetic system. In particular, IAA treatment resulted in 0.3, 2.3, and 2.5 times increases, while IAA–GO treatment led to 3.25%, 31.28%, and 16.96% increases, respectively. However, these effects appear to be primarily associated with the influence of drought stress.

Overall, the results highlight the importance of further studies to better understand the interaction mechanisms between nanoensembles and drought stress in the regulation of photosynthetic activity in plants.

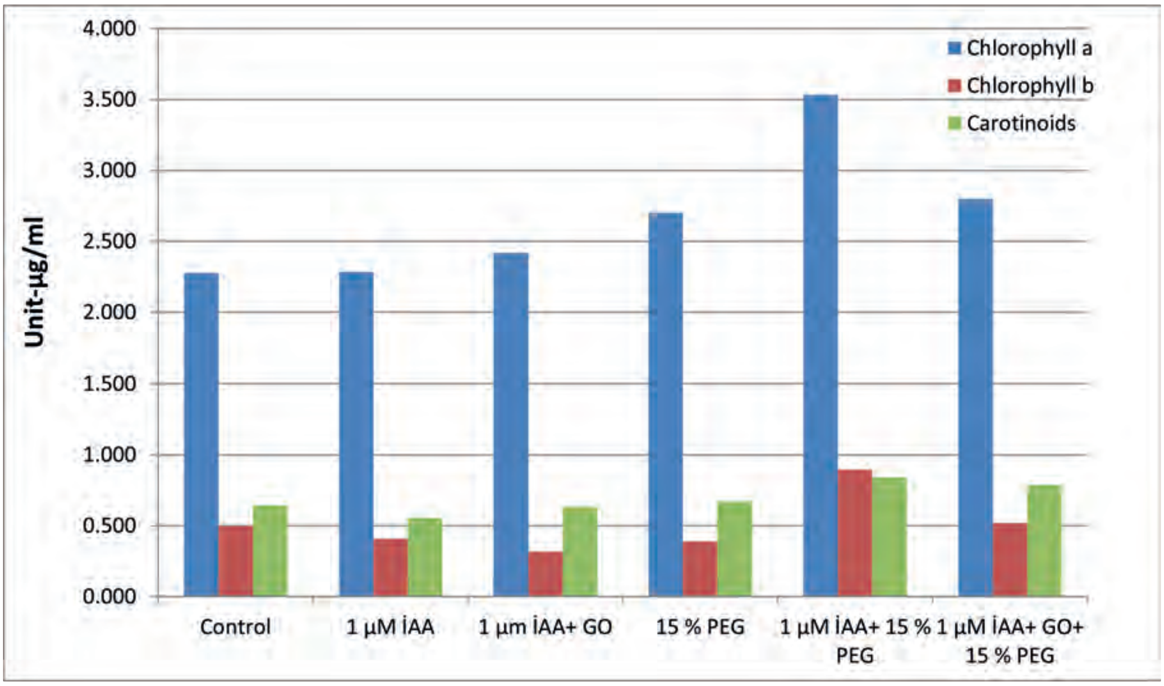

**Figure 3.** The impact of IAA and IAA-graphene oxide nanoensemble on the levels of chlorophyll a, b, and carotenoids in the leaves of Triticum durum Desf. Yasemen genotype.

Catalase is a vital antioxidant enzyme responsible for breaking down hydrogen peroxide into water and oxygen, thus helping to protect cells by preventing the accumulation of harmful reactive molecules. Under both normal and drought conditions, treatment with IAA enhanced catalase activity by 20% and 8.2%, respectively. Although the IAA–GO nanoensemble showed slightly lower catalase activity than IAA alone, it still caused a modest increase compared to the control group (Figure 4).

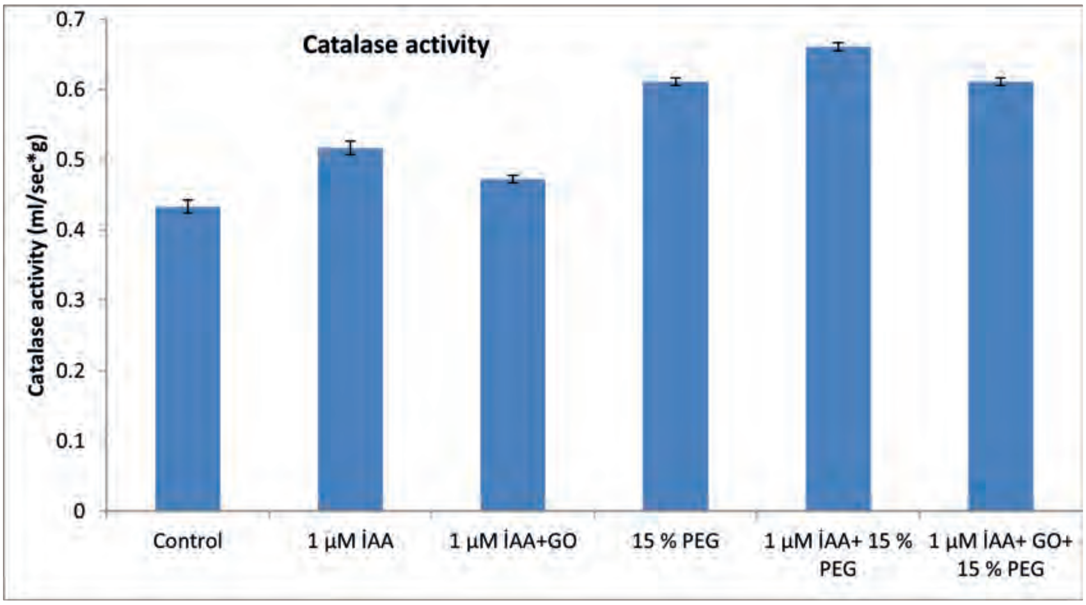

**Figure 4.** The effect of IAA and IAA-GO nanoensemble on catalase activity in the leaves of Triticum durum Desf., Yasemen genotype

## DISCUSSION

Auxins are key regulators of vegetative development and growth, controlling nearly all aspects of these processes, as well as mediating resistance to various stress factors (Amrahov et al., 2023). In addition to regulating fundamental aspects of cell division, cell elongation, and cell differentiation, they play a crucial role in diverse plant physiological processes (Zhang et al., 2022). Indole-3-acetic acid (IAA), as a member of the auxin group of phytohormones, possesses the characteristic properties described above. In previous studies, a single exogenous application of IAA resulted in a theoretical yield increase of 48.53% in *Pinus yunnanensis* (Zhu et al., 2025). In our research, exogenous IAA was also found to have an inductive effect on both leaf and root length, as well as seed germination, in wheat plants.

In recent years, numerous studies have explored the effects of nanoensemble structures on plant development. In particular, the use of nanomaterials such as carbon nanotubes, graphene, and graphene oxide has shown significant results. Recent research has demonstrated that graphene holds considerable potential in agricultural applications, including the delivery of plant nutrients and crop protection agents, as well as in post-harvest management processes (Bhattacharya et al., 2023). However, to ensure the safe development of nanotechnology, it is essential to investigate the potential negative effects of nanoparticles (Rzayev et al., 2022).

Recent studies have reported that a graphene oxide-based nanoensemble, non-covalently combined with kinetin, did not enhance stem growth in cotton plants, but accelerated seed germination by 13.3%, providing clear evidence of the positive effects of graphene oxide (Amrahov et al., 2025; Olatunbosun et al., 2023). In contrast, in our study, a graphene oxide-based nanoensemble combined with IAA negatively affected both seed germination and stem and root growth. This may be explained by the fact that graphene oxide can interfere with the distinct metabolic pathways of mono- and dicotyledonous plants through different mechanisms.

Plants in their natural environments are exposed to various abiotic stresses that trigger the production of reactive oxygen species (ROS), leading to oxidative stress and potential cellular damage. ROS perform a dual function in plant systems: while their excessive accumulation can cause oxidative damage, they also act as key signaling molecules, transmitting stress signals to the nucleus via redox-mediated activation of mitogen-activated protein kinase (MAPK) cascades (Agbektas et al., 2023). ROS can oxidize specific redox-sensitive proteins and initiate MAPK signaling. Through these mechanisms, ROS play a central role in plant acclimation to environmental stress and function as primary signal transducers interacting with multiple pathways (Rao et all, 2023). Studies on wheat and rapeseed seedlings have shown that modified graphene oxide can slightly enhance catalase activity (Wang et al., 2022). In our study, both IAA and the IAA–graphene oxide nanoensemble slightly increased catalase activity, suggesting that the nanoensemble may exert a synergistic effect on the antioxidant system.

Alongside catalase, peroxidases also play a crucial role in the antioxidant defense of plant cells, making it important to determine how the activity of these enzymes is affected by nanoensembles. In our study, a decrease in guaiacol peroxidase activity was observed, and this effect remained consistent under drought stress when plants were treated with the IAA–graphene oxide nanoensemble. Under 15% PEG-induced drought stress, PEG itself increased peroxidase activity due to the generation of reactive oxygen species, which lead to oxidative stress. The reduction in peroxidase activity under combined drought stress and IAA–graphene oxide treatment appears to be a direct effect of graphene oxide. Graphene oxide possesses a redox-active surface and can influence intracellular electron transfer processes. This interaction alters the redox environment and cofactor balance, indirectly affecting the active site of peroxidase and consequently weakening its overall activity.

Chlorophylls a and b, the main components of the photosynthetic apparatus, provide plants with energy and carbon for organic matter synthesis. In our study, the IAA–graphene oxide nanoensemble alone inhibited chlorophyll b formation. However, under drought stress, both IAA and the IAA–graphene oxide nanoensemble increased the levels of photosynthetic components. Previous studies have also reported that graphene oxide (GO) alone stimulated photosynthetic pigments in certain tolerant species, such as *Iris pseudacorus*, where chlorophyll a + b and carotenoid levels increased by 26–178% depending on GO concentration (Zhou et all, 2023). This effect has been associated with improved light capture, electron transport, and other photophysical processes, although it is strongly dependent on plant species and GO dosage (Aliyeva  et al., 2025; Nahida, 2020).

## CONCLUSION

The results of this study indicate that the IAA–graphene oxide (IAA–GO) phytohormone complex plays a significant role in enhancing drought tolerance in wheat plants. Under drought stress, it stimulates the components of the photosynthetic apparatus and regulates the activity of antioxidant enzymes such as catalase and peroxidase, thereby improving the plants' resistance to stress. Future research should further explore these mechanisms on a broader scale and compare the effects of the complex across different plant species.

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