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Evaluation Of Photosynthetic Reactions and Antioxidant System Of Wheat Cultivar M7318 Using 6-Benzyladenine (6-BA) Under Water Deficit Stress Conditions

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ABSTRACT

Drought is one of the most significant constraints on agricultural productivity worldwide, and its severity is predicted to increase in the future. To combat drought stress in plants, employing effective strategies such as foliar application of plant hormones is essential. Wheat, as a strategic crop, plays a vital role in global food security; however, drought stress severely impacts both its quantitative and qualitative yield. This study was conducted over two consecutive growing seasons (2016-2017 and 2017-2018) at a research farm located in Barsian village, Ziar, with the objective of investigating the effects of different drought stress levels (including normal, mild drought stress, and drought stress) and foliar application of the cytokinin hormone 6-BA at three concentrations (15, 30, and 45 ppm) on yield value, photosynthetic traits (chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll), and enzymatic traits (catalase, ascorbate peroxidase, and peroxidase) of wheat cultivar M7318. ANOVA confirmed significant moisture regime effects on all traits, and cytokinin 6-BA impacting all except Chla, and significant interactions only for GY, POX, and CAR. Results reveal a 39% GY reduction under drought stress, alongside significant declines in traits such as Chla, Chlb, and CAR, indicating disrupted photosynthesis due to oxidative damage and reduced stomatal conductance. Drought-induced enzymatic increases (POX, CAT, APX) reflect an adaptive ROS-scavenging response, while 6-BA at 30-45 ppm enhanced CAT and APX but reduced POX, suggesting dose-dependent regulation. The 45 ppm 6-BA treatment significantly boosted GY under normal conditions (758 g/m²) and improved Chlb, Tchl, and CAR. Interaction effects showed the highest POX under drought with 45 ppm (15.5 min^{-1} mg^{-1} protein) and lowest under normal conditions (8.5 min^{-1} mg^{-1} protein), indicating context-specific responses. These findings indicate the usefulness of foliar application of cytokinin 6-BA as a useful method for reducing the effects of drought in wheat, and the results of this study can be used to improve yield, chlorophyll, and enzyme properties in agriculture and future studies.

Keywords: Drought stress, Enzymatic characteristics, Photosynthetic traits, Wheat, 6-benzyladenine

INTRODUCTION

Drought is one of the most destructive abiotic stresses that poses a fundamental challenge to global agricultural production. It particularly exerts severe impacts on the yield of strategic crops like wheat in arid and semi-arid regions (Kouchakkhani *et al*., 2024). This stress disrupts plants' physiological and enzymatic systems through complex mechanisms. At the cellular level, osmotic stress caused by drought disturbs ionic balance and osmotic homeostasis while altering the activity of key metabolic enzymes such as superoxide dismutase, catalase, and peroxidase (Pamungkas & Farid, 2022). Plants attempt to maintain metabolic homeostasis and adapt to stress conditions by regulating enzymatic activities and physiological parameters including leaf water potential, relative water content, and stomatal conductance (Haghpanah *et al*., 2024). Investigating these changes provides deeper insights into drought tolerance mechanisms and paves the way for developing effective stress management strategies to enhance agricultural productivity. Furthermore, it highlights the necessity of adopting innovative and sustainable approaches in agriculture.

Wheat (*Triticum aestivum* L.), the most important cereal crop and a primary source of carbohydrates and calories for humans, provides approximately 72% of the daily dietary energy intake. This grain, rich in carbohydrates, proteins, minerals, and vitamins, plays a vital role in global food security (Filip *et al*., 2023). However, current wheat production fails to meet the demands of a growing population. Although efforts by researchers and plant breeders have improved wheat yield, the challenge of increasing production under drought and climate change persists (Erenstein *et al*., 2022). To enhance cereal production and reduce reliance on pesticides and chemical fertilizers, the use of plant growth regulators such as cytokinins has emerged as a novel strategy (Prasad, 2022). Cytokinins, including 6-benzyladenine (6-BA), enhance plant resilience to environmental stresses by regulating processes like bud proliferation, branching, flowering, fruiting, and delaying tissue senescence (Kosakivska *et al*., 2022). 6-Benzylaminopurine (6-BA), a synthetic cytokinin, modulates biochemical pathways associated with drought tolerance when applied as a foliar spray (Qi *et al*., 2024). It improves water retention, enhances photosynthetic efficiency, reduces reactive oxygen species (ROS), and mitigates oxidative stress, thereby boosting plant resistance under water-deficient conditions. Additionally, 6-BA regulates water use efficiency and increases root biomass by influencing signaling pathways such as strigolactones, ultimately improving overall drought resilience (Liu *et al*., 2022). This approach not only promotes plant growth under stress but also facilitates physiological recovery post-stress, highlighting its role in sustainable agriculture. Studies demonstrate that exogenous cytokinin application in cereals, including rice, promotes spikelet growth and endosperm filling (Zhu *et al*., 2022). Sadat Hosseini *et al*. (2021) reported that 50 μM 6-BA treatment in six wheat cultivars enhanced photosynthesis rate, leaf area index durability, thousand-grain weight, biological yield, and grain yield. Another study showed that foliar cytokinin application improved mineral uptake and physiological traits in wheat under drought stress (Khosravi-Nejad *et al*., 2022).

Although significant information exists regarding the role of cytokinin in improving root and shoot growth as well as morphological traits, the photosynthetic and enzymatic responses of wheat to 6-BA under drought stress conditions have not been thoroughly investigated. This study focuses on evaluating the photosynthetic responses and antioxidant system of wheat under drought stress following 6-BA application. The findings of this research could serve as a foundation for promoting the use of plant hormones as an alternative mechanism to mitigate the effects of drought stress in wheat.

MATERIALS AND METHODS

The present experiment was conducted during the 2016-2017 and 2017-2018 growing seasons at a research farm located in Barsian Village, Ziar County, Isfahan Province. The exact geographical coordinates of the studied farm, based on the World Geodetic System (WGS84), were longitude 51°83' E and latitude 32°43' N, with an elevation of 1,570 meters above sea level. The region has a semi-arid climate, with an average annual rainfall of approximately 120 mm and a mean annual temperature of 16°C (Figure 1).

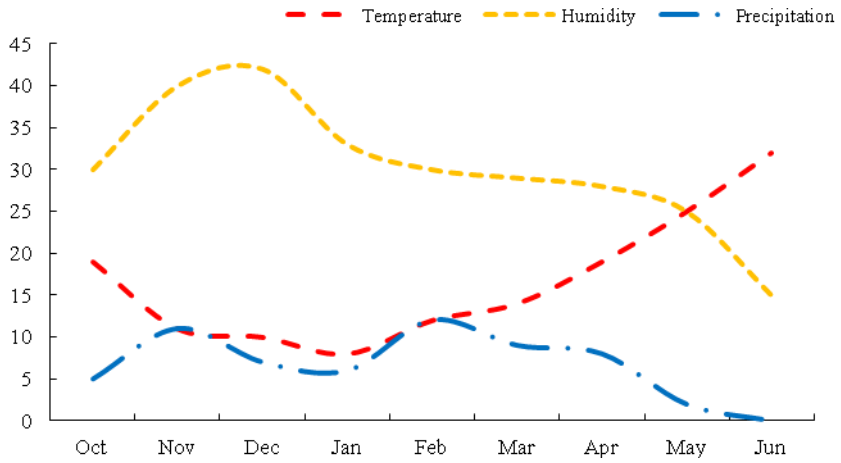

Figure 1. Average temperature, humidity and precipitation for the two crop years 2016-2017 and 2017-2018

The experiment was conducted as a split-plot arrangement within a randomized complete block design with three replications. The experimental treatments included foliar application of cytokinin hormone at three concentrations (15, 30, and 45 ppm) applied at three growth stages according to the Zadoks-Tattman growth and phenology classification system (Weaver, 1972): end of tillering stage, end of flag leaf emergence stage, and full flowering/pollination stage. The study utilized wheat cultivar M7318, which is considered a high-yielding and environmentally tolerant variety. Cultivar M7318 possesses distinctive characteristics that make it an ideal winter wheat for cultivation in various regions of Iran. This cultivar was developed through a complex hybridization process between the widely used Azadi cultivar

and several advanced foreign lines, and after three years of evaluation, it successfully obtained official approval and registration.

The wheat planting date in this study was set as November 20th, considering the climatic conditions of Isfahan County, the growth pattern of the plant, and the thermal requirements of the studied cultivar. This date was selected to enable the plants to utilize initial soil moisture and autumn rainfall for proper initial establishment and tillering while avoiding severe winter cold stresses. Prior to conducting the experiment and planting operations, preliminary land preparation was performed, including plowing (to a depth of 25-30 cm) and disking to soften the seedbed and break up clods. Each plot in this experiment consisted of 320 planting rows, with each treatment implemented in 5 separate planting rows spaced 15 cm apart and 4 meters in length. Based on the soil test results conducted before planting, the soil characteristics were determined for both years prior to cultivation (Table 1).

Table 1. Physical and chemical properties of the soil at the test site (depth 0 to 60 cm)

Year	Potassium ($mg.kg^{-1}$)	(%) Nitrogen	Soil texture	pH	EC ($dS.m^{-1}$)	Phosphorus ($mg.kg^{-1}$)
2016-2017	570	0.04	Loam-clay	6.97	0.65	7.14
2017-2018	601	0.06	Loam-clay	7.01	0.61	8.02

According to Table 1, 150 kg of potassium fertilizer (as potassium sulfate, $K2SO_4$, containing 50% potassium oxide) and 100 kg of phosphorus fertilizer (as triple superphosphate, $Ca(H_2PO4)_2$, containing 46% P_2O_5) were applied at the field level as basal fertilizers before planting. These fertilizers were broadcast-applied and then incorporated into the soil through light disking to a depth of 10-15 cm. Additionally, well-decomposed farmyard manure was added to the soil before plowing to improve soil physical structure and increase organic matter content. Nitrogen fertilizer was applied as top-dressing at different growth stages including tillering, stem elongation, and flowering. Initial irrigation was performed using the flood irrigation method after planting. For broadleaf weed control during the growing season, Pumasuper was used, while Granstar was applied for grassy weed control. Water stress was imposed during the flowering stage. Drought stress was applied at two levels (normal and drought stress). The moisture regimes consisted of a non-stressed condition with a MAD (mean fraction of total available water that can be depleted from the root zone without causing plant stress) coefficient of 50%, and a moisture-stressed condition with a MAD coefficient of 90% (Allen *et al.*, 1998). The measured traits included yield (expressed as grams per square meter), photosynthetic traits (protein content, chlorophyll a, chlorophyll b, carotenoids, total chlorophyll), and enzymatic traits (catalase, peroxidase, and ascorbate peroxidase activities).

The contents of chlorophyll a, chlorophyll b, and carotenoids in leaves were measured using the spectrophotometric method (Lichtenthaler and Buschmann). First, a leaf sample weighing 0.1 g was powdered and homogenized in 10 mL of 80% acetone in darkness. The samples were then centrifuged at 5000 × g for 15 minutes using an Eppendorf Refrigerated Centrifuge (model 5810R, Germany). The absorbance of the extracted solution was measured at 662 nm and 645 nm (for chlorophyll a and chlorophyll b, respectively) and at 470 nm (for

carotenoids) against a blank sample (80% acetone). Finally, the results were expressed as milligrams of pigment per gram of fresh leaf weight (Bhatta *et al.*, 2018):

Chl a = [12.21 (ABS_{663}) - 2.81 (ABS_{646})] × [(10 ml Acetone) / 1000]

Chl b = [20.13 (ABS_{646}) - 5.03 (ABS_{663})] × [(10 ml Acetone) / 1000]

CAR = [[[1000 (ABS_{470}) - 3.27 Chl a – 104Chl b] / 227] / Weight (g)] × [(10 ml Acetone) / 1000]

Tchl (mg/l) = Chl a + Chl b

Under freezing conditions, antioxidant enzyme activities were measured using 500 mg of powdered leaf tissue extracted with a buffer containing 1% polyvinylpyrrolidone (PVP) and 0.5% Triton X-100 in 100 mM potassium phosphate buffer (pH 7.0). The homogenate was centrifuged at 15,000 rpm for 20 min at 4°C, and the supernatant was collected for enzyme assays. Ascorbate Peroxidase (APX) Activity (μmol monodehydroascorbate formed min^{-1} mg^{-1} protein) was determined spectrophotometrically at 265 nm for 2 min following the method of Nakano and Asada (35). The 3 mL assay mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM H_2O_5, 5 mM ascorbate, and 50 μL enzyme extract. APX activity was calculated using an extinction coefficient of 2.8 mM^{-1} cm^{-1}. Peroxidase (POD) Activity (absorbance increase min^{-1} mg^{-1} protein) was measured by adding 50 μL enzyme extract to 2.95 mL of 50 mM potassium phosphate buffer (pH 7.5) containing 9 mM guaiacol and 10 mM $_{H2O2}$. Tetraguaiacol formation was monitored at 470 nm for 2 min, with activity calculated using an extinction coefficient of 26.61 mM^{-1} cm^{-1}. Catalase (CAT) Activity (μmol H_2 O_2 decomposed min^{-1} mg^{-1} protein) was determined by monitoring H_2O2 consumption at 240 nm for 2 min (2). The assay mixture consisted of 50 mM potassium phosphate buffer (pH 7.0), 15 mM H_2O_2, and 50 μL enzyme extract (Bradford, 1976).

After collecting the experimental data and prior to analysis, the assumptions of variance analysis, including normality of data distribution, normality of experimental errors, homogeneity of variances, and additivity of block and treatment effects, were examined for all yield, photosynthetic, and enzymatic traits. The analysis of variance was performed using a split-plot design based on randomized complete blocks with three replications. Mean comparisons were conducted using the LSD test at a 5% significance level for each trait with the assistance of SAS9.4 and MINITAB22.3.

RESULTS

Analysis of variation and estimation of variation for traits

Table 2 indicates the analysis of variance (ANOVA) for photosynthetic parameters and enzymatic activities in response to varying levels of cytokinin (6-BA) foliar application and drought stress treatments. Analysis of variance revealed that moisture regimes had significant effects on all studied traits at the 0.05 and 0.01 levels. Also, the effects of cytokinin BA-6 were significant for all traits except Chla. The results of interaction effects showed that only

three traits, GY, POX, and CAR, were affected by the interaction effect of drought stress on cytokinin, and other traits were not affected by the interaction effect of these two treatments (Table 2). Notably, the remaining traits did not show any significant response to the combined treatment effects, indicating independent rather than synergistic effects of these stress and hormonal factors (Table 2). These findings indicated the sensitivity of different physiological pathways to drought stress conditions and hormone application.

Table 2. Analysis of variance of the effect of foliar spraying of cytokinin (6-BA) (15, 30, and 45 ppm) on grain yield (GY), photosynthetic parameters and leaf enzyme content in wheat cultivar M7318 under two levels of irrigation (normal and drought stress)

S.V	df	MS							
		GY	CAT	APX	POX	Chla	Chlb	Tchl	CAR
Rep	2	214^{*}	0.01^{ns}	0.22^{ns}	0.03^{ns}	0.001^{ns}	0.001^{ns}	0.004^{ns}	0.0003^{ns}
Irrigation	1	385222^{**}	0.79^{**}	11.87^{**}	63.92^{**}	0.961^{**}	0.080^{**}	1.614^{**}	0.0600^{*}
Irrigation × Rep	2	2	0.01	0.02	0.01	0.001	0.001	0.001	0.0007
Cytokinin (6-BA)	2	1912^{**}	0.24^{**}	0.44^{**}	1.81^{**}	0.007^{ns}	0.010^{**}	0.015^{*}	0.0079^{**}
Irrigation × Cytokinin (6-BA)	2	4950^{**}	0.04^{ns}	0.09^{ns}	14.80^{**}	0.001^{ns}	0.004^{ns}	0.005^{ns}	0.0057^{**}
Error	8	37	0.01	0.05	0.02	0.002	0.001	0.003	0.0003
CV (%)		12.02	4.70	3.91	3.52	4.34	4.50	4.15	3.50

**, * and ns : Significant at the 0.01 and 0.05 probability level and non-significant
CV: coefficient of variation, df: degrees of freedom, MS: mean square, Rep: replication, S.V: source of variation CAT: (µmoles of H_2O_2 decomposed min^{-1} mg^{-1} protein) Catalase, APX (µmole of monodehydroascorbate formed min^{-1} mg^{-1} protein) Ascorbate peroxidase, POX: (increase in absorbance min^{-1} mg^{-1} protein) Peroxidase, Chl a: (mg/g) Chlorophyll a content, Chl b: (mg/g) Chlorophyll b content, Tchl: (mg/g) Total Chlorophyll content, CAR: (mg/g) Carotenoid content

The impact of water stress on wheat

The average comparison results revealed that grain yield (GY) was highest under normal conditions but experienced a substantial decline of 39% under drought stress conditions (Table 3). This reduction indicated the severe impact of water scarcity on wheat productivity. Under drought stress, wheat exhibited a significant rise in enzyme content, with all three measured enzymes showing a pronounced increase compared to normal conditions, suggesting an adaptive biochemical response to water deficit. Additionally, drought stress severely impaired the photosynthetic system, with notable reductions in photosynthetic pigments. Chlorophyll a decreased by 18%, chlorophyll b by 17%, and carotenoids by 18%, indicating a widespread disruption in photosynthetic efficiency.

Table 3. Mean for grain yield (GY) and photosynthetic parameters and leaf enzyme content in wheat cultivar M7318 under two levels of irrigation (normal and drought stress)

Irrigation	GY	CAT	APX	POX	Chla	Chlb	Tchl	CAR
Normal	744±15a	2.21±0.01b	5.31±0.41b	10.5±0.84b	2.55±0.84a	0.73±0.02a	3.29±0.19a	0.60±0.01a
Drought stress	452±10b	2.63±0.01a	6.93±0.48a	14.3±1.01a	2.09±0.67b	0.60±0.01b	2.69±0.18b	0.49±0.01b
LSD (0.05)	12.30	0.09	0.32	0.27	0.04	0.06	0.05	0.05

CAT: (μmoles of H_2O_2 decomposed min^{-1} mg^{-1} protein) Catalase, APX (μmole of monodehydroascorbate formed min^{-1} mg^{-1} protein) Ascorbate peroxidase, POX: (increase in absorbance min^{-1} mg^{-1} protein) Peroxidase, Chl a: (mg/g) Chlorophyll a content, Chl b: (mg/g) Chlorophyll b content, Tchl: (mg/g) Total Chlorophyll content, CAR: (mg/g) Carotenoid content

In each common means followed by a same letter are not significantly different according to the LSD test at an alpha level of *0.05*

The results of the effect of three levels of cytokinin BA-6 hormone foliar spray on the leaves of the wheat cultivar M7318 are shown in Table 3. The results indicated that the application of 45 ppm of cytokinin BA-6 significantly increased the GY value of wheat, whereas the applications of 15 and 30 ppm did not have a significant effect on GY. The application of cytokinin BA-6 at concentrations of 30 and 45 ppm resulted in increased levels of CAT and APX enzymes, while the same concentrations significantly decreased the levels of the POX enzyme. The results revealed that the highest and lowest levels of POX enzyme were measured at 15 and 45 ppm. The application of high levels of cytokinin BA-6 foliar spray resulted in an increase in the amount of the photosynthetic system. The results indicated that the application of 30 ppm foliar spray could lead to an increase in the amount of chlorophyll b and total chlorophyll, and the application of 45 ppm foliar spray could lead to an increase in the carotenoid content.

Table 4. Mean effects of three levels of cytokinin BA-6 foliar application on wheat cultivar M7318 on grain yield (GY), photosynthetic parameters, and leaf enzyme content

Cytokinin BA-6	GY	CAT	APX	POX	Chlb	Tchl	CAR
15 ppm	589±15b	2.19±0.01b	5.81±0.35b	12.9±0.99a	0.63±0.01b	2.93±0.19b	0.53±0.01b
30 ppm	586±11b	2.48±0.01a	6.24±0.31a	12.5±1.00b	0.71±0.01a	3.03±1.01a	0.52±0.01b
45 ppm	618±10a	2.57±0.01a	6.31±0.38a	11.8±0.92c	0.64±0.01b	3.01±0.18ab	0.59±0.02a
LSD (0.05)	8.19	0.15	0.31	0.27	0.04	0.07	0.02

CAT: (μmoles of H_2O_2 decomposed min^{-1} mg^{-1} protein) Catalase, APX (μmole of monodehydroascorbate formed min^{-1} mg^{-1} protein) Ascorbate peroxidase, POX: (increase in absorbance min^{-1} mg^{-1} protein) Peroxidase, Chl a: (mg/g) Chlorophyll a content, Chl b: (mg/g) Chlorophyll b content, Tchl: (mg/g) Total Chlorophyll content, CAR: (mg/g) Carotenoid content

In each common means followed by a same letter are not significantly different according to the LSD test at an alpha level of *0.05*

The results of the interaction effects of drought stress levels and foliar application of cytokinin BA-6 for the three traits including GY, POX and CAR are indicated in Table 5. The results indicated that the highest amount of GY was measured under normal condition with a foliar application of 45 ppm cytokinin BA-6 at a rate of 758 g/m², and the lowest GY was observed under drought stress condition with an application of 15 ppm cytokinin BA-6. The highest POX amount was observed under drought stress conditions and foliar application of 45 ppm cytokinin BA-6 (15.5 min−1 mg−1 protein), and the lowest POX amount was observed under normal conditions and foliar application of 45 ppm cytokinin BA-6 (8.5 min^{-1} mg^{-1} protein). The highest amount of CAR was observed under normal conditions with foliar application of cytokinin BA-6 at 15 and 45 ppm, and drought stress led to a decrease in the amount of CAR.

Table 5. Average interaction effects of three levels of cytokinin foliar application on wheat cultivar M7318 on grain yield (GY), peroxidase (POX), and carotenoid content (CAR) under two irrigation levels (normal and drought stress)

Irrigation	Cytokinin BA-6	GY	POX	CAR
Normal	15 ppm	732±14b	11.9±0.92d	0.62±0.03a
	30 ppm	741±16b	11.5±1.00e	0.58±0.02b
	45 ppm	758±17a	8.5±0.91f	0.62±0.02a
Drought stress	15 ppm	419±16e	13.9±1.04b	0.44±0.01b
	30 ppm	431±13d	13.4±0.94c	0.47±0.01c
	45 ppm	504±14c	15.5±1.11a	0.56±0.01b
LSD (0.05)		9.22	0.23	0.02

In each common means followed by a same letter are not significantly different according to the LSD test at an alpha level of *0.05*

DISCUSSION AND CONCLUSION

The results of this study indicated that drought stress and foliar application of cytokinin have significant effects on the performance of the photosynthetic apparatus and the activity of antioxidant enzymes in wheat plants. These results indicate that cytokinin can improve stress tolerance mechanisms in wheat plants by regulating the activity of antioxidant enzymes and maintaining the integrity of the photosynthetic apparatus, and has significant potential in increasing the drought resistance of this strategic crop. The severe decline in GY under drought stress conditions highlights the profound impact of water scarcity on wheat productivity, a finding consistent with studies on cereal crops under drought stress (Anwaar *et al.,* 2020; Ahmad *et al.,* 2022; Benito-Verdugo *et al.,* 2023). The reductions in photosynthetic pigments, including Chla (18%), Chlb (17%), and CAR (18%) under drought stress condition, reflect a widespread disruption in the photosynthetic apparatus, likely due to oxidative damage and reduced stomatal conductance (Zahra et al., 2023). This indicates that drought-induced osmotic stress impairs electron transport and chlorophyll synthesis. The lack of

significant interaction effects on most traits (except GY, POX, and CAR) indicates that drought drives these physiological changes independently, rather than synergistically with 6-BA application.

The pronounced increase in enzymatic activities (POX, CAT, APX) under drought stress underscores an adaptive response to mitigate oxidative stress, a common mechanism in plants facing water deficit (Sapakhova *et al*., 2022). This elevation likely reflects heightened reactive oxygen species (ROS) scavenging to protect cellular structures (Pour-Aboughadareh *et al.*, 2022). However, the differential response to 6-BA levels—where 30 and 45 ppm increased CAT and APX while decreasing POX suggests a complex regulation of the antioxidant system. The highest POX at 15 ppm and lowest at 45 ppm under drought stress (Table 5) indicate that 6-BA may modulate enzyme expression or activity thresholds, potentially optimizing ROS management at higher concentrations.

The application of 45 ppm 6-BA significantly enhanced GY under normal condition, demonstrating its potential to boost productivity, possibly by improving grain filling, as noted in prior studies on cytokinins in cereals (Vedenicheva and Kosakivska, 2024). The lack of significant GY improvement at 15 and 30 ppm may indicate a threshold effect, where higher concentrations are required to overcome metabolic limitations. Additionally, 6-BA positive influence on photosynthetic pigments Chlb and Tchl at 30 ppm, and CAR at 45 ppm indicates a protective role against drought-induced degradation, likely by enhancing chlorophyll stability or biosynthesis. This aligns with report revealing cytokinins delaying senescence and maintaining photosynthetic efficiency (Mughal *et al.,* 2024).

The significant interaction effects on GY, POX, and CAR reveal a context-specific response to combined drought and 6-BA treatments. The highest GY under normal conditions with 45 ppm 6-BA contrasts with the lowest under drought with 15 ppm, suggesting that optimal hormonal application can amplify yield potential but may be less effective under severe stress unless dosage is tailored. The highest POX under drought with 45 ppm (15.5 min^{-1} mg^{-1} protein) versus the lowest under normal conditions with the same dose (8.5 min^{-1} mg^{-1} protein) indicates that 6-BA may enhance POX activity as a stress-adaptive mechanism, while reducing it under non-stress conditions to avoid over-activation. Similarly, the higher CAR under normal conditions with 15 and 45 ppm, and its decline under drought, suggests that 6-BA supports pigment accumulation only when water is adequate, highlighting the need for integrated water management.

These findings emphasize the potential of cytokinin 6-BA foliar application as a useful tool in drought-prone areas, in line with global efforts to reduce chemical inputs and increase crop resilience in arid and semi-arid regions (Mangena, 2022; Wang *et al.*, 2022). The independent effects of drought and 6-BA on most traits suggest that irrigation management remains critical, but strategic 6-BA application (e.g., 45 ppm) can serve as a complementary strategy to mitigate yield losses. The lack of significant Chla response to 6-BA, despite its sensitivity to drought, indicates that chlorophyll a may be more genetically or environmentally regulated, a hypothesis requiring further genomic studies.

Conclusion

This study indicates the differential impacts of drought stress and 6-BA foliar application on wheat physiology and yield. While drought severely decreases GY and photosynthetic capacity, 6-BA at 45 ppm offers a promising avenue to enhance yield and antioxidant defenses, particularly under normal conditions. The interaction effects on GY, POX, and CAR emphasize the need for tailored agronomic practices, integrating water availability with hormonal treatments to maximize wheat productivity in changing climates.

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