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Response of Yield and Yield-Related Traits to Exogenous Foliar Application of 6-Benzyladenine (6-BA) in Wheat

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ABSTRACT

Cytokinins are a class of plant hormones that play a pivotal role in regulating growth and development by promoting cell division, delaying senescence, and enhancing stress tolerance, thereby influencing key physiological processes that determine plant productivity. Wheat is a crucial crop that significantly contributes to global food security. This study investigates the effects of foliar application of the synthetic cytokinin 6-benzyladenine (6-BA) at three distinct concentrations 15, 30, and 45 ppm over two consecutive growing seasons (2016-2018) at a research farm located in Barsian village, Ziar on wheat cultivar M7318, aiming to evaluate its impact on yield and yield-related traits, including grain yield, 1000-grain weight, biological yield, harvest index, and grain weight per plant, with the objective of identifying optimal application strategies to enhance wheat. The results reveal a clear trend of increasing grain yield and 1000-grain weight with rising 6-BA concentrations, peaking at 45 ppm, though the improvement plateaus with no significant difference between 30 and 45 ppm, while biological yield significantly increases only at 45 ppm, harvest index declines at this higher dose, and grain weight per plant reaches its maximum at 30 ppm, highlighting the concentration-specific effects of 6-BA on wheat performance. These findings underscore the potential of cytokinin hormones, particularly 6-BA, in wheat cultivation as a sustainable approach to boost yield, offering a promising avenue for precision agriculture by tailoring application rates to optimize grain production and biomass allocation.

Keywords: Yield, yield component traits, Wheat, 6-benzyladenine

INTRODUCTION

Wheat (*Triticum aestivum* L.), a cornerstone cereal crop, serves as a primary source of carbohydrates and calories (Shewry and Hey, 2015). This grain, enriched with proteins, minerals (e.g., iron and zinc), and vitamins (e.g., B vitamins), is indispensable for food security (Gupta *et al*., 2024). Despite advancements by researchers and plant breeders in enhancing wheat yield through genetic improvement and agronomic practices, current production struggles to meet the demands of a burgeoning global population (Tilman *et al*., 2011). To address these challenges and reduce reliance on chemical fertilizers and pesticides, plant growth regulators, particularly cytokinins such as 6-benzyladenine (6-BA), have emerged as a promising strategy (Mok, 2019). Cytokinins increase plant resistance by regulating vital processes, including bud proliferation, branching, flowering, fruiting, and delaying senescence, thereby improving functional traits and ultimately plant yield (Hussain *et al*., 2025).

6-Benzylaminopurine (6-BA), a synthetic cytokinin, modulates biochemical and physiological pathways when applied as a foliar spray and offers significant potential for yield in wheat (Zheng *et al*., 2016). The compound also modulates signaling pathways, such as strigolactones, which can optimize resource allocation and increase yield, regulate water use efficiency, and increase root biomass (Gulzar *et al*., 2024). Beyond promoting growth, 6-BA facilitates physiological improvement, making it a key tool in sustainable agriculture. In cereals such as rice, exogenous application of 6-BA has been shown to promote spikelet development and endosperm filling, and improve grain quality and yield (Wang *et al*., 2020a). Furthermore, 6-BA maintains chloroplast structural stability, delays chlorophyll degradation, and reduces leaf senescence, preserving photosynthetic capacity under adverse conditions (Zhang *et al*., 2023).

The application of 6-BA enhances the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) in wheat, boosting photosynthetic capacity (Li *et al*., 2025). Wang *et al*. (2020b) found that pre-waterlogging 6-BA spraying sustains high flag leaf photosynthetic rates, mitigating grain yield losses. Moreover, 6-BA increases the number of fertile florets in basal and middle spikelets, elevates grain number per spikelet, and accelerates average and maximum grain-filling rates, resulting in fuller grains, increased thousand-grain weight, and ultimately higher yields (Jing *et al*., 2024). Yang *et al*. (2016) reported that 6-BA application during wheat flowering significantly enhanced grain-filling speed and grain weight, effectively counteracting yield reductions due to high-temperature stress. In rice, Wang *et al*. (2022) observed that 6-BA increased yield by boosting spikelet number and grain weight, with a more pronounced effect in low-temperature-sensitive cultivars, suggesting genotype-specific responses to environmental stresses.

Incorporating 6-BA into wheat cultivation practices can promise to increase yield stability and improve performance traits. Despite the extensive effects of cytokinin on plants, there is insufficient information on the effect of different concentrations of 6-BA foliar spray on important performance traits and yield value of wheat. This study seeks to fill the scientific gap in this regard.

MATERIALS AND METHODS

The current study was carried out over the 2016-2018 cropping seasons at an experimental field situated in Barsian Village, Ziar County, Isfahan Province. The field's precise location, determined using the World Geodetic System (WGS84), is marked by a longitude of 51°83'E'

E and a latitude of 32°43’ N, positioned at an altitude of 1,570 meters above sea level. The area experiences a semi-arid climate, characterized by an average annual precipitation of about 120 mm and a mean annual temperature of 16°C.

The study was implemented using a randomized complete block design with three replicates. Experimental treatments consisted of foliar spraying with the cytokinin hormone at three distinct concentrations (15, 30, and 45 ppm), administered at key developmental phases as defined by the Zadoks-Tattman growth and phenology classification system (Weaver, 1972): the end of the tillering phase, the completion of flag leaf emergence, and the full flowering/pollination stage. The wheat cultivar M7318, renowned for its high yield potential and adaptability to diverse environmental conditions, served as the experimental material. This cultivar, specifically bred for winter wheat production, exhibits unique traits that make it suitable for cultivation across various Iranian regions. Developed through an intricate hybridization program involving the established Azadi cultivar and several advanced international wheat lines, M7318 underwent a rigorous three-year evaluation period before receiving official approval and registration.

The sowing date for wheat in this study was established as November 20th, tailored to the climatic conditions of Isfahan County, the developmental cycle of the wheat cultivar, and its specific thermal requirements. This timing was chosen to allow the plants to leverage early soil moisture and autumn precipitation for effective initial establishment and tillering, while minimizing exposure to extreme winter cold. Before initiating the experiment and planting, the land underwent preparatory measures, including deep plowing to a depth of 25-30 cm, followed by disking to create a fine seedbed and disintegrate soil clumps. Each experimental plot comprised 320 sowing rows, with each treatment replicated across five distinct rows, spaced 15 cm apart and extending 4 meters in length. Soil analyses, conducted prior to planting for both experimental years, provided detailed soil data, which are summarized in Table 1.

Table 1- Mean of chemical and physical properties of the soil at the field (depth 0 to 60 cm)

Nitrogen (%)	Potassium ($mg.kg^{-1}$)	Soil texture	pH	Phosphorus ($mg.kg^{-1}$)	EC ($dS.m^{-1}$)
0.05	585	Loam-clay	6.99	7.58	0.63

Based on soil analysis, a basal application of 150 kg/ha of potassium fertilizer, in the form of potassium sulfate (K_2SO_2) with a 50% K_2O content, and 100 kg/ha of phosphorus fertilizer, as triple superphosphate ($Ca(H_2PO_2)_2$) containing 46% P_2O_5 , was incorporated into the field prior to sowing. These nutrients were evenly distributed across the soil surface and mixed into the topsoil layer (10-15 cm) using light tillage. To enhance soil structure and boost organic matter, fully decomposed farmyard manure was integrated into the soil during pre-planting plowing. Nitrogen fertilizer was supplied as a split top-dressing application at key developmental phases: tillering, stem elongation, and flowering. Post-planting irrigation was conducted using a flood irrigation technique to ensure initial moisture availability. Weed management involved the application of Pumasuper for controlling broadleaf weeds and

Granstar for grassy weeds throughout the growing season. The evaluated parameters encompassed yield (measured in grams per square meter) and associated yield components, such as biological yield, harvest index, 1000-grain weight, grain weight per plant, and spike length. Biological yield is determined by harvesting plants from a defined area (e.g., 1 m^2), drying them to a constant weight, and recording the total above-ground biomass in grams. Harvest index is calculated as the ratio of grain yield to biological yield, expressed as a percentage, by weighing the harvested grain and dividing it by the total biomass after drying. The 1000-grain weight is assessed by randomly selecting 1000 grains from a representative sample, weighing them on a precision balance, and recording the result in grams to evaluate grain size and quality. Grain weight per plant is measured by individually harvesting mature plants and weighing the total grain output per plant in grams to reflect individual productivity. Spike length is determined by measuring the length of the spike (from the base to the tip) using a ruler on a sample of mature spikes, recorded in centimeters.

In the physiological stage, after the collection of experimental data and before conducting statistical analysis, the assumptions underlying the analysis of variance (ANOVA) were rigorously evaluated. These assumptions included the normality of data distribution, the normality of experimental residuals, and the additive nature of block and treatment effects. The ANOVA was carried out using a compound analysis in time and with a basic block design with three replicates per treatment. Pairwise mean comparisons were performed employing the Least Significant Difference (LSD) test at a 5% significance level, utilizing SAS 9.4 software to identify significant differences among the traits.

RESULTS

Analysis and estimation of variation in yield and yield-related traits

Table 2 presents the results of the analysis of variance (ANOVA) for a set of yield and yield-related traits, encompassing biological yield, harvest index, 1000-grain weight, grain weight per plant, and spike length, in response to varying concentrations of cytokinin 6-benzylaminopurine (6-BA) applied as a foliar spray. The results indicate that the application of 6-BA exerted significant effects ($P < 0.01$) on all evaluated traits, with the notable exception of spike length, which did not show a significant response to the different 6-BA concentrations. This differential response suggests that while 6-BA effectively modulates key yield components such as biological yield reflecting total above-ground biomass and harvest index, which indicates the efficiency of partitioning assimilates to grains, as well as 1000-grain weight and grain weight per plant, its influence on spike morphology may be limited or influenced by other genetic or environmental factors.

Table 2. Analysis of variance regarding the effects of foliar spraying of cytokinin (6-BA) at concentrations of 15, 30, and 45 ppm on grain yield and yield-related traits in the wheat cultivar M7318.

Source of variation	df	MS					
		Grain yield	Biological yield	Harvest index	1000-grain weight	Grain weight per plant	Spike length
Year	1	3^{ns}	101^{ns}	0.102^{ns}	0.04^{ns}	11.79^{ns}	4.73^{**}
Rep(Year)	4	353	130	0.001	1.06	1.11	0.07
Cytokinin (6-BA)	2	2326^{**}	7208^{**}	0.137^{**}	29.15^{**}	36.32^{**}	0.21^{ns}
Year × Cytokinin (6-BA)	2	1287^{**}	10568^{**}	0.071^{ns}	41.57^{**}	37.26^{**}	2.33^{**}
Error	8	94	299	0.006	2.88	1.52	0.07
CV (%)		12.02	9.56	7.22	7.63	9.54	6.57

**, * 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

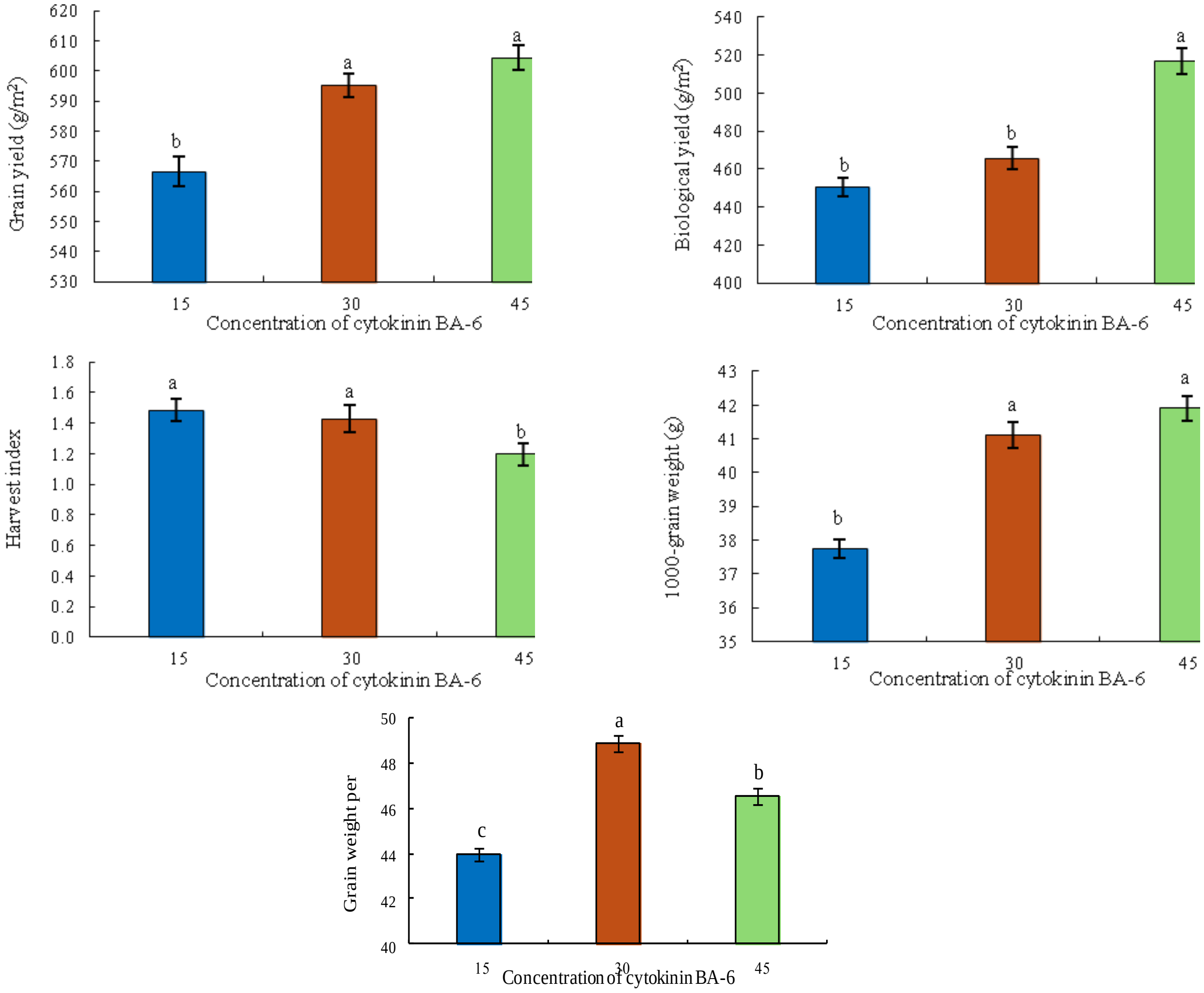

Figure 1. Analysis of variance regarding the effects of foliar spraying of cytokinin (6-BA) at concentrations of 15, 30, and 45 ppm on grain yield and yield-related traits in the wheat cultivar M7318.
In each common means followed by the same letter are not significantly different according to the LSD test at an alpha level of *0.05*.

The impact of 6-benzyladenine (6-BA) on yield and yield-related traits wheat

The results of the mean values for study traits, including grain yield, biological yield, harvest index, 1000-grain weight, and grain weight per plant, are presented in Figure 1.

The evaluation of three distinct concentrations of cytokinin 6-benzyladenine (6-BA), 15 ppm, 30 ppm, and 45 ppm, revealed notable effects on grain yield and 1000-grain weight. The results indicate a clear trend of significant grain yield and 1000-grain weight enhancement with increasing 6-BA concentration, with the highest yields and 1000-grain weight observed at 45 ppm. Statistical analysis confirmed that the increment from 15 ppm to 30 ppm and subsequently to 45 ppm was associated with progressively higher grain yields and 1000-grain weight, reflecting the compound's role in mitigating stress-induced yield losses. However, a detailed examination of the data, as depicted in Figure 1, highlights that while the grain yield and 1000-grain weight increased significantly from 15 ppm to 30 ppm and from 30 ppm to 45 ppm, there was no statistically significant difference between the yields and 1000-grain weight at 30 ppm and 45 ppm ($P > 0.05$). The result of the impact of cytokinin 6-benzyladenine (6-BA) on the biological yield of wheat revealed nuanced responses across varying application concentrations. Statistical analysis indicated that foliar applications of 6-BA at concentrations of 15 ppm and 30 ppm did not exert a significant effect on biological yield, suggesting that these lower doses may fall below the threshold required to stimulate substantial biomass accumulation under the experimental conditions. In contrast, the application of 45 ppm 6-BA resulted in the highest biological yield, demonstrating a marked improvement in total above-ground biomass, which encompasses both grain and straw components.

The findings pertaining to the harvest index (HI), revealed nuanced responses to varying concentrations of 6-benzylaminopurine (6-BA) foliar spray in wheat. The result indicated that there was no significant difference in HI between the 15 ppm and 30 ppm treatments, suggesting that these lower concentrations do not substantially alter the allocation of photosynthates to grain relative to vegetative biomass under the experimental conditions. However, the application of 6-BA at a higher concentration of 45 ppm resulted in a significant reduction in the harvest index value ($P < 0.05$). This decline may be attributed to an over-stimulation of vegetative growth or altered source-sink relationships, potentially leading to a greater proportion of biomass being directed toward non-reproductive tissues, such as stems or leaves, rather than grains.

The findings pertaining to grain weight per plant revealed significant variations influenced by the foliar application of cytokinin 6-benzyladenine (6-BA) at different concentrations. The lowest grain weight per plant was recorded with the application of 15 ppm 6-BA, suggesting that this concentration may be suboptimal for promoting grain development, potentially due to insufficient hormonal stimulation or an inadequate threshold for eliciting a robust physiological response. In contrast, the highest grain weight per plant was achieved with a foliar spray concentration of 30 ppm, indicating an optimal dosage that likely enhances cellular division, grain filling, and overall biomass accumulation. This observed peak at 30 ppm aligns with the role of cytokinins in regulating sink strength and delaying senescence, which are critical for maximizing grain yield. These results underscore the importance of concentration-specific effects of 6-BA, highlighting the need for precise application rates to optimize wheat productivity under varying environmental conditions.

DISCUSSION AND CONCLUSION

The findings of this study indicated that cytokinin foliar application significantly affects the yield and related traits in wheat plant. The evaluation of three distinct concentrations of cytokinin 6-benzyladenine (6-BA), including 15, 30, and 45 ppm, demonstrates its significant influence on key agronomic traits in wheat, including grain yield, 1000-grain weight, biological yield, harvest index, and grain weight per plant. The results reveal a pronounced trend of increasing grain yield and 1000-grain weight with escalating 6-BA concentrations, with the peak performance observed at 45 ppm. The results indicate a progressive increment in these traits from 15 ppm to 30 ppm and further to 45 ppm, aligning with findings by Zhu *et al*. (2022), who noted that exogenous cytokinin applications enhance grain-filling rates and yield components in cereals. However, a detailed scrutiny reveals that while the transition from 15 ppm to 30 ppm and from 30 ppm to 45 ppm yielded significant improvements, the differences between 30 ppm and 45 ppm were not statistically significant, suggesting a potential plateau effect where higher concentrations may not proportionally augment yield benefits. This observation is consistent with dose-response studies indicating that excessive cytokinin levels might saturate physiological responses (Qi *et al*., 2024). The biological yield, encompassing total above-ground biomass (grain and straw), exhibited a nuanced response, with 15 and 30 ppm showing no significant effect, likely due to insufficient hormonal stimulation to drive substantial biomass accumulation (Alvi *et al*., 2022). In contrast, the 45 ppm treatment markedly elevated biological yield, underscoring 6-BA's role in enhancing vegetative and reproductive biomass, a finding supported by Ghaleh *et al*. (2020), who reported improved biomass with optimal cytokinin doses.

The harvest index is a critical indicator of photosynthate allocation to grain versus vegetative tissues, and displayed varied responses to 6-BA concentrations. No significant differences were observed between 15 and 30 ppm treatments, suggesting that these lower doses do not substantially alter the partitioning of assimilates under the experimental conditions, possibly due to a threshold below which cytokinin effects on source-sink dynamics remain minimal (Khosravi-Nejad *et al*., 2022). However, the application of 45 ppm resulted in a significant reduction in harvest index, which may reflect an over-stimulation of vegetative growth, redirecting resources toward non-reproductive tissues such as stems and leaves rather than grains. This shift could indicate an imbalance in source-sink relationships, where excessive cytokinin promotes excessive vegetative biomass at the expense of grain filling, a phenomenon noted in high-dose cytokinin studies by Raza *et al*. (2020). Such findings highlight the need to balance cytokinin application to optimize grain yield without compromising harvest index, an important consideration for breeding programs aiming to enhance harvest efficiency.

Grain weight per plant further elucidates the concentration-dependent effects of 6-BA, with the lowest values recorded at 15 ppm, indicating that this concentration may be suboptimal for grain development, potentially due to inadequate hormonal stimulation or failure to surpass a critical physiological threshold (Zhou *et al*., 2024). Conversely, the highest grain weight per plant was achieved at 30 ppm, suggesting an optimal dosage that enhances cellular division, grain filling, and biomass accumulation, likely through the regulation of sink strength and delayed senescence, as supported by Vedenicheva and Kosakivska (2024). This peak at 30 ppm aligns with the role of cytokinins in improving grain quality and yield under adverse conditions, a mechanism elucidated by Mughal *et al*. (2024), who emphasized cytokinins' protective effects on photosynthetic efficiency. The observed concentration-specific responses underscore the importance of tailoring 6-BA application rates to environmental and genotypic contexts, as excessive doses (e.g., 45 ppm) may not yield proportional benefits and could disrupt resource allocation, necessitating further investigation into dose optimization.

These findings collectively affirm 6-BA's potential as a strategic tool for enhancing wheat productivity and resilience, consistent with global efforts to promote sustainable agriculture amid climate change (Mangena, 2022). The gradual increase in grain yield and thousand-grain weight up to 45 ppm, despite the decrease in harvest index, indicates changes between biomass accumulation and grain division that require further investigation. The lack of significant biological yield enhancement at lower concentrations (15 and 30 ppm) indicates a threshold effect, while the optimal grain weight at 30 ppm highlights a critical concentration for grain-specific benefits. Integrating these insights with molecular studies on cytokinin signaling pathways (e.g., strigolactones) and genotypic variability could guide the development of precision agriculture practices.

CONCLUSION

This study underscores the significant impact of foliar application of cytokinin 6-benzyladenine (6-BA) at concentrations of 15, 30, and 45 ppm on agronomic traits of wheat, including grain yield, 1000-grain weight, biological yield, harvest index, and grain weight per plant, affirming its potential as a vital tool for enhancing productivity and resilience under drought stress. The progressive increase in grain yield and 1000-grain weight, peaking at 45 ppm, alongside the optimal grain weight per plant at 30 ppm, highlights the dose-dependent efficacy of 6-BA in promoting grain-filling rates and biomass accumulation, though the lack of significant differences between 30 and 45 ppm suggests a saturation threshold. The marked improvement in biological yield at 45 ppm, contrasted with negligible effects at lower concentrations, underscores a critical hormonal threshold for biomass enhancement, while the significant reduction in harvest index at 45 ppm indicates a potential over-stimulation of vegetative growth, redirecting resources from grain to non-reproductive tissues. These concentration-specific responses emphasize the need for precise application strategies tailored to environmental and genotypic contexts, as excessive doses may disrupt source-sink dynamics, necessitating further optimization.

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