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Article

Synergistic Effects of Bioactive Compounds and Nano-Enabled Fertilization on Growth and Physio-Biochemical Responses of Maize Under Drought Stress

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Abstract

Maize (*Zea mays* L.) is a major cereal crop of global significance, contributing substantially to food security and agricultural sustainability. Enhancing its physiological performance through innovative nutrient management and bioactive compounds is essential for improving productivity under changing environmental conditions. This study investigated the combined effects of γ-aminobutyric acid (GABA), kaempferol (KP), and nanoparticle (NP)–coated urea on maize growth and physiological responses. The experimental treatments were control (no KP or GABA), KP at 5 µM, KP at 10 µM, and GABA at 0.1 mM, applied individually or in combination with KP following a completely randomized design (CRD). Growth attributes, including shoot and root development and biomass accumulation, were assessed under varying concentrations of GABA and KP in combination with NP-coated urea. The results demonstrated a pronounced concentration-dependent response. Under non-GABA conditions, shoot length increased by 4.5% and 9% with 5 µM and 10 µM KP, respectively. In the presence of 0.1 mM GABA, root length exhibited substantial enhancement, increasing by 20.29% with 5 µM KP and by 39.72% with 10 µM KP. Leaf dry weight also increased markedly, showing gains of 21.35% and 53.26% at 5 µM and 10 µM KP, respectively. These findings reveal a synergistic interaction between GABA, kaempferol, and NP-coated urea, suggesting that their integrated application can effectively promote maize growth and improve physiological efficiency. The study provides a promising framework for developing advanced nutrient and stress-management strategies to enhance maize performance.

Keywords: Oxidative stress mitigation; gas exchange traits; nutrient uptake; abiotic stress.

1. Introduction

Ensuring sustainable agricultural production to meet the food demands of a rapidly expanding global population is one of the foremost challenges facing modern agriculture. Projections by the Food and Agriculture Organization indicate that global crop productivity must increase substantially by 2050 to satisfy escalating food and nutritional requirements. However, the yield potential of major crops is increasingly constrained by climatic variability and environmental stresses, including drought, salinity, and soil nutrient depletion (Hafsi *et al.*, 2014, Shafiq *et al.*, 2021). It has been estimated that crop yields in several regions may decline by up to 50% by the end of the century if current trends persist. In addition to climate-related factors, non-climatic pressures such as population growth and increased demand for animal-

derived products are intensifying stress on existing food systems (Liang *et al.,* 2017, Klimczyk *et al.,* 2021). These challenges require adopting innovative agronomic strategies to enhance crop resilience, nutrient-use efficiency, and physiological performance.

The application of indirect growth regulators and advanced fertilizer technologies has emerged as a promising approach to mitigate stress-induced limitations in crop productivity. Among these, γ-aminobutyric acid (GABA) plays a multifunctional role in plant metabolism and stress signaling. GABA is a non-protein amino acid that accumulates in plant tissues under abiotic stress conditions and participates in key physiological processes, including carbon–nitrogen balance regulation, osmotic adjustment, antioxidant defense, and signal transduction. Exogenous application of GABA has been reported to enhance antioxidant enzyme activity, reduce reactive oxygen species accumulation, and improve ionic homeostasis under stress conditions (Liang *et al.,* 2017, Khan *et al.,* 2021). In maize, GABA application under saline environments has been shown to improve seedling growth, increase enzymatic antioxidant activity, and promote osmolyte accumulation compared with untreated plants.

Another biologically active compound receiving increasing attention is kaempferol, a naturally occurring flavonoid that acts as an indirect plant growth regulator. Although kaempferol does not directly control plant growth, it plays an important role in modulating developmental and defense processes through its antioxidant properties and regulatory effects on signaling pathways (Gunathunga *et al.,* 2024). Kaempferol influences reactive oxygen species–mediated signaling associated with phytohormones such as auxin and abscisic acid, thereby contributing to improved growth regulation and stress adaptation (Rajendran *et al.,* 2014, Periferakis *et al.,* 2022). Furthermore, kaempferol exhibits metal-chelating properties in the rhizosphere, which can enhance micronutrient availability by converting less soluble forms, such as Fe^{3+}, into more plant-accessible forms like Fe^{2+}.

Recent advancements in nanotechnology have further improved fertilizer efficiency through the development of nanoparticle (NP)–coated fertilizers, particularly NP-coated urea. These fertilizers enhance nitrogen-use efficiency by minimizing volatilization and leaching losses while ensuring a sustained nutrient supply. In addition to improving nutrient availability, nanoparticles have been reported to alleviate abiotic stress by inducing physiological and biochemical adjustments, including enhanced photosynthetic activity, improved carbon assimilation, and stabilization of the photosynthetic apparatus (Dawar *et al.,* 2011, Wang *et al.,* 2020). Moreover, NP-based applications reduce oxidative damage by limiting the accumulation of malondialdehyde and hydrogen peroxide and by regulating stress-responsive metabolic pathways (Chandrashekar *et al.,* 2023, Dimkpa *et al.,* 2023).

Maize (*Zea mays* L.) is one of the most important cereal crops globally and serves as a major source of food, feed, and industrial raw materials. It provides essential nutrients, including vitamins, minerals, and bioactive phytochemicals, and contributes significantly to food security and economic stability (Li *et al.,* 2019, Qaderi *et al.,* 2023). Despite its high productivity potential, maize is highly sensitive to environmental stresses, which adversely affect growth, physiological efficiency, and yield stability.

Although the individual effects of GABA, kaempferol, and nanoparticle-based fertilizers on plant growth and stress tolerance have been previously documented, their combined and interactive effects on maize physiological performance remain insufficiently explored. Understanding how these components interact to influence growth, photosynthetic efficiency, nutrient uptake, and stress-response mechanisms is essential for developing sustainable crop management strategies. Therefore, the present study was designed to evaluate the integrated impact of GABA, kaempferol, and NP-coated and non-coated urea on maize physiological parameters. It was hypothesized that varying concentrations of these treatments would induce distinct and synergistic physiological responses, ultimately enhancing maize growth and stress resilience. The findings of this study are expected to contribute to the development of environmentally sustainable and efficient nutrient management practices for maize production.

2. Materials and Methods

2.1. Experimental site

A pot experiment was conducted to evaluate the effects of γ-aminobutyric acid (GABA) and kaempferol (KP) foliar application on the growth, nutrient status, and antioxidant activity of maize (*Zea mays* L.) under drought stress, with or without nanoparticle-coated urea. The site is located at 30°09'41.6"N latitude and 71°36'38.0"E longitude. Prior to the experiment, ten soil samples were randomly collected from the experimental area, combined to form a composite sample, and analyzed to determine baseline soil characteristics. Detailed information on soil properties and irrigation practices is provided in Table 1.

2.2. Nanoparticles

Zinc oxide nanoparticles (ZnO NPs) were synthesized using an aqueous extract of *Moringa oleifera* leaves through a green synthesis approach. A 0.25 M precursor solution was prepared by dissolving 4.5 g of zinc acetate

dihydrate in 100 mL of deionized water, followed by the gradual addition of the leaf extract under continuous magnetic stirring at 70°C for two hours. The resulting mixture was dried on a hot plate to obtain a semi-solid mass, then calcined at 200°C in a muffle furnace for 2 hours to stabilize the phase and remove organic impurities. For nano-coating, 25 g of urea granules were first mixed with 0.5 mL vegetable oil as a binder to enhance adhesion. Subsequently, 1.0 g of synthesized ZnO nanoparticles (equivalent to 4% w/w coating percentage) along with 1.5 g $Ca(OH)_2$ as a stabilizing agent were added and homogenized using a rotating shaker until uniform coating and free-flowing granules were obtained. The coated granules were air-dried at room temperature for 24 hours to ensure proper adhesion and then stored in airtight polyethylene containers under dry conditions until further use to prevent moisture absorption and agglomeration.

Table 1. Pre-experimental soil and irrigation characteristics

Soil	Values	Irrigation	Values
pH	8.19	pH	7.29
EC*e* (dS/m)	3.11	EC*e* (µS/cm)	249
Organic matter (%)	0.40	Carbonates (meq/L)	0.00
Total nitrogen (%)	0.02	Bicarbonates (meq/L)	4.11
Ext. P (mg/kg)	5.11	Chloride (meq/L)	0.05
Ava. K (mg/kg)	101	Ca + Mg (meq/L)	3.19
Silt (%)	45	Sodium (mg/L)	64
Sand (%)	26	EC*e* = Electrical conductivity	
Clay (%)	29		
Texture	Clay Loam		

2.3. Treatment Plan and Experimental Design

The experiment was arranged in a completely randomized design (CRD) with three replications. Two types of urea were applied: recommended-dose uncoated urea and NP-coated urea. The treatments included control (no KP or GABA), KP at 5 µM, KP at 10 µM, and GABA at 0.1 mM, applied individually or in combination with KP. Drought stress was maintained at 50% of field capacity of soil as per the irrigation requirement for maize using the gravimetric method.

2.4. Seed Selection, Sterilization, and Sowing

Maize seeds were obtained from a licensed supplier in Punjab, Pakistan. Only healthy and intact seeds were selected, and damaged seeds were discarded. Surface sterilization was performed by soaking seeds in 5% sodium hypochlorite solution, followed by three washes with 95% ethanol and three rinses with sterilized deionized water to remove residual sterilants (Ahmad *et al.*, 2014). Twenty seeds were sown in pots containing 5 kg of soil, and seedlings were thinned to ten per pot after germination to maintain uniform growth.

2.5. Data Collection and Harvesting

Data collection commenced 50 days after sowing, coinciding with the harvest. Fresh shoot and root weights were recorded, and samples were oven-dried at 65°C for 72 hours to determine dry biomass.

2.6. Analysis of Chlorophyll

Chlorophyll a, chlorophyll b, and total chlorophyll were determined using standard method (Arnon, 1949). Fresh leaves were homogenized in 80% acetone, and absorbance readings were taken at 663 nm and 645 nm for chlorophyll a and b, respectively.

2.7. Measurements of Gas Exchange

Leaf gas exchange parameters, including net photosynthesis, stomatal conductance, and transpiration rate, were measured using the CI-340 Photosynthesis System (CID, Inc., USA) between 10:30 and 11:30 a.m., when light conditions were optimal for photosynthetic activity (Nazar *et al.*, 2014). Measurements were taken from the fully expanded uppermost leaves under controlled chamber conditions. The environmental parameters inside the leaf chamber were maintained as follows: Photosynthetically Active Radiation (PAR) at 1000 µmol m^{-2} s^{-1}, leaf chamber temperature at 25 ± 2°C, relative humidity at 60–65%, and CO_2 concentration at 400 µmol mol^{-1}. Prior to recording, leaves were allowed to acclimate in the chamber for approximately 2–3 minutes to ensure steady-state readings.

2.8. Antioxidant Analysis

Antioxidant enzyme activities were determined using standard protocols. Superoxide dismutase (SOD) activity was assayed based on the inhibition of nitroblue tetrazolium reduction in the presence of riboflavin, with absorbance recorded at 560 nm (Dhindsa *et al.*, 1982). Peroxidase (POD) activity was measured by monitoring guaiacol oxidation, while catalase (CAT) activity was determined by monitoring hydrogen peroxide decomposition at 240 nm (Nakano & Asada, 1981). Ascorbate peroxidase (APX) activity was assayed by following ascorbate oxidation in the presence of hydrogen peroxide. Lipid peroxidation was estimated by measuring malondialdehyde (MDA) content using the thiobarbituric acid reaction.

2.9. Electrolyte Leakage

Leaf samples (~1 g) were cut into uniform 1 cm diameter pieces, rinsed with deionized water, and incubated in 20 mL deionized water at 25°C for 24 hours to allow electrolyte leakage. Initial conductivity (EC1) was recorded using a calibrated EC meter. Samples were then autoclaved at 120°C for 20 minutes, and final conductivity (EC2) was measured. Electrolyte leakage was calculated as EL (%) = (EC1/EC2) × 100 (Lutts *et al.*, 1996).

2.10. Ion analysis

Plant nutrient concentrations were determined following acid digestion of oven-dried and finely ground plant samples using a nitric–perchloric acid (HNO_3–$HClO_4$) wet digestion method. Briefly, 0.5 g of dried plant material was digested with a di-acid mixture (HNO_3:$HClO_4$, 2:1 v/v) on a hot plate until a clear solution was obtained. After cooling, the digest was filtered and diluted to a known final volume with deionized water. Nitrogen concentrations were quantified using Kjeldahl's distillation apparatus.

2.11. Statistical Analysis

All data were subjected to two-way analysis of variance (ANOVA) to assess the significance of treatment effects. Tukey's honest significant difference (HSD) test was used for pairwise comparisons at $p < 0.05$. Statistical analyses were conducted using R software (version 4.3.1).

3. Results

3.1. Shoot Growth and Biomass

Foliar application of kaempferol (KP) in combination with γ-aminobutyric acid (GABA) and nanoparticle-coated urea significantly enhanced maize shoot growth. Under 0 mM GABA, 5 μM and 10 μM KP with NP-coated urea increased shoot length by approximately 5% and 8%, respectively, compared to the untreated control. Shoot fresh and dry weights were similarly elevated, with increases ranging from 4% to 11% across treatment combinations. When GABA concentration was raised to 0.1 mM, the same KP treatments further increased shoot length by 7–14%, while fresh and dry biomass improved by 6–7%. Treatments without NP-coated urea also demonstrated substantial improvements, with shoot length rising by 5–13% and shoot fresh and dry weights by up to 62%, highlighting the additive effects of KP and GABA on shoot development (Table 2).

3.2. Root Growth and Biomass

Root growth was markedly enhanced by KP and GABA applications. With NP-coated urea at 0 mM GABA, 5 μM and 10 μM KP increased root length by 6% and 13%, respectively. At 0.1 mM GABA, root length increased by 20% and 40%. In treatments without NP-coated urea, root length improvements ranged from 9% to 27% depending on KP concentration and GABA level. Root fresh and dry weights followed similar trends, with NP-coated urea treatments increasing biomass by 4.8–11% and non-coated urea treatments achieving 6–15% improvements, demonstrating the positive, concentration-dependent effect of KP and GABA on root growth (Table 3).

3.3. Leaf Biomass

Leaf fresh and dry weights increased significantly with KP and GABA treatments. At 0 mM GABA with NP-coated urea, 5 μM and 10 μM KP increased leaf fresh weight by 8% and 14%, respectively, whereas at 0.1 mM GABA, increases reached 11% and 18%, respectively. Non-coated urea treatments produced even higher gains, with leaf fresh weight rising by 15–32% and dry weight by 40–62% at higher KP concentrations under 0.1 mM GABA (Table 4). These results indicate a synergistic effect of KP, GABA, and urea on leaf biomass accumulation.

3.4. Chlorophyll and Carotenoids

Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents were significantly increased by KP and GABA applications. NP-coated urea with 5–10 μM KP raised chlorophyll a by 11–22% under 0 mM GABA and 22–33% under 0.1 mM GABA. Non-coated urea treatments further amplified these effects, with increases of 49–65% at higher KP concentrations. Similar trends were observed for chlorophyll b and total chlorophyll. Carotenoid levels also increased, confirming that KP and GABA enhance photosynthetic pigment accumulation, particularly in combination with urea (Table 5).

3.5. Gas Exchange Parameters

Photosynthetic performance improved under KP and GABA treatments. Transpiration rates increased by 6–13% at 0 mM GABA, with greater gains (8–15%) at 0.1 mM GABA. Stomatal conductance showed modest enhancements (1–6%), while net photosynthetic rates improved by 6–16%, reflecting enhanced carbon assimilation and water-use efficiency under combined treatments (Table 6).

3.6. Nutrient Content

Leaf and root nutrient concentrations increased with KP, GABA, and NP-coated urea applications. Leaf phosphorus (P) and potassium (K) improved by 0.10–0.17%, while nitrogen content rose from 0.05% to 0.51% in leaves and 0.40% to 0.64% in roots across treatments. Non-coated urea treatments also enhanced nutrient accumulation, confirming that KP and GABA positively influence nutrient uptake and assimilation (Table 7; Figure 1).

Table 2. Impacts of kaempferol, GABA and NP coated Urea on the shoot length, shoot fresh weight, and shoot dry weight of maize cultivated in stress.

Kaempferol Levels (µM)	Shoot length (cm)		Shoot fresh weight (g)		Shoot dry weight (g)	
	GABA (mM)					
	0	0.1	0	0.1	0	0.1
	NP coated Urea					
0	45.92±0.6c	51.9±0.8c	190.4±3.6c	213.1±2.2c	13.9±0.2c	16.3±0.29c
5	48.08±0.6b	54.1±0.5b	197.8±1.4b	223.0±1.6b	14.8±0.2b	17.2±0.28
10	49.97±0.4a	55.69±0.4a	204.05±3.2a	232.0±4.6a	15.5±0.2a	18.1±0.24a
	No NP coated Urea					
0	34.5±0.6c	39.48±0.5a	130.1±3.2c	156.1±3.1a	9.1±0.4c	11.5±0.27a
5	36.3±0.5c	41.57±0.7a	136.4±3.1c	168.0±4.4a	9.9±0.2c	12.3±0.27a
10	37.4±0.5c	43.90±1.0a	145.1±2.9c	179.1±3.1a	10.8±0.3c	13.2±0.24a

Values are mean ± standard deviation (n=3) within NP coated or uncoated urea, kaempferol and GABA with the same letter (s) are statistically non-significant at $p<0.05$.

Table 3. Impacts of kaempferol, GABA, and NP coated Urea on the root length, root fresh weight, and root dry weight of maize cultivated under stress.

Kaempferol Levels (µM)	Root length (cm)		Root fresh weight (g)		Root dry weight (g)	
	GABA (mM)					
	0	0.1	0	0.1	0	0.1
	NP coated Urea					
0	23.14±0.5c	28.03±0.5c	29.2±0.3c	33.97±0.4c	7.5±0.07c	8.81±0.19c
5	24.67±0.50b	29.67±0.4b	30.6±0.4b	35.39±0.6b	7.9±0.07b	9.20±0.10
10	26.36±0.7a	26.0±0.4a	32.5±0.2a	36.89±0.3a	8.3±0.05a	9.53±0.18a
	No NP coated Urea					
0	14.1±0.4c	18.6±0.3a	20.8±0.2a	25.08±0.4a	5.0±0.2a	6.23±0.08a
5	15.4±0.6c	19.9±0.4a	21.9±0.4a	26.02±0.3a	5.3±0.06a	6.61±0.08a
10	16.1±0.4c	21.5±0.6a	23.3±0.3a	27.78±0.6a	5.7±0.1a	7.06±0.14a

Values are mean ± standard deviation (n=3) within NP coated or uncoated urea, kaempferol and GABA with the same letter (s) are statistically non-significant at $p<0.05$.

Table 4. Impacts of kaempferol, GABA and NP coated Urea on the leaf fresh weight and dry weight of maize cultivated in stress.

Kaempferol Levels (µM)	Leaf fresh weight (g)		Leaf dry weight (g)	
	GABA (mM)			
	0	0.1	0	0.1
	NP coated Urea			
0	50.9±1.4c	60.9±1.1c	9.9±0.2c	12.06±0.2c
5	54.6±1.0b	64.9±1.2b	10.6±0.1b	12.7±0.2b
10	57.7±1.3a	67.9±1.3a	11.2±0.2a	13.3±0.2a
	No NP coated Urea			
0	29.8±1.0a	40.14±1.3a	5.5±0.23a	7.84±0.23a
5	32.9±1.4a	43.58±1.2a	6.3±0.20a	8.68±0.24a
10	36.7±1.2a	47.08±1.2a	7.0±0.29a	9.26±0.26a

Values are mean ± standard deviation (n=3) within NP coated or uncoated urea, kaempferol and GABA with the same letter (s) are statistically non-significant at $p<0.05$.

Table 5. Impacts of kaempferol, GABA and NP coated Urea on the Chlorophyll content of maize cultivated in stress.

Kaempferol Levels (µM)	Chlorophyll a (mg/g)		Chlorophyll b (mg/g)		Total Chlorophyll (mg/g)		Carotenoids (mg/g)	
	GABA (mM)							
	0	0.1	0	0.1	0	0.1	0	0.1
	NP coated Urea							
0	0.9±0.01c	1.16±0.01	0.36±0.00c	0.46±0.005c	1.31±0.7c	1.63±0.02c	0.69±0.12c	0.84±0.01c
5	1.0±0.03b	1.21±0.02b	0.40±0.00b	0.49±0.01b	1.43±0.4b	1.71±0.03b	0.73±0.12b	0.88±0.01b
10	1.1±0.01a	1.26±0.01a	0.43±0.00a	0.52±0.008a	1.53±0.5a	1.76±0.02a	0.78±0.17a	0.94±0.01a
	No NP coated Urea							
0	0.37±0.02a	0.64±0.03a	0.19±0.00a	0.28±0.01a	0.56±0.02a	0.93±0.05a	0.41±0.01a	0.54±0.01a
5	0.45±0.02a	0.73±0.04a	0.21±0.00a	0.31±0.01a	0.67±0.03a	1.05±0.04a	0.45±0.01a	0.58±0.01a
10	0.55±0.02a	0.85±0.04a	0.24±0.01a	0.35±0.008a	0.80±0.04a	1.20±0.05a	0.5±0.008a	0.68±0.02a

Values are mean ± standard deviation (n=3) within NP coated or uncoated urea, kaempferol and GABA with the same letter (s) are statistically non-significant at $p<0.05$.

Table 6. Impacts of kaempferol, GABA, and NP coated Urea on transpiration rate, stomatal conductance, and photosynthetic rate of maize cultivated under stress

Kaempferol Levels (µM)	Transpiration rate (mmol H_2O m^{-2} s^{-1})		Stomatal conductance (mol H_2O m^{-2} s^{-1})		Photosynthetic rate (µmol CO_2 m^{-2} s^{-1})	
	GABA (mM)					
	0	0.1	0	0.1	0	0.1
	NP coated Urea					
0	1.6±0.009c	1.76±0.009c	2.51±0.01c	2.71±0.018c	16.5±0.004c	21.2±0.5c
5	1.7±0.01b	1.79±0.005b	2.57±0.02b	2.75±0.012b	18.3±0.006b	22.5±0.4b
10	1.7±0.009a	1.82±0.02a	2.64±0.02a	2.79±0.015a	19.6±0.004a	23.8±0.3a
	No NP coated Urea					
0	1.5±0.008a	1.56±0.01a	2.01±0.2a	2.32±0.02a	8.1±0.4a	11.83±0.4a
5	1.51±0.005a	1.59±0.009a	2.17±0.01a	2.38±0.02a	9.4±0.4a	13.56±0.6a
10	1.53±0.009a	1.63±0.009a	2.23±0.02a	2.44±0.02a	10.5±0.2a	15.02±0.3a

Values are mean ± standard deviation (n=3) within NP coated or uncoated urea, kaempferol and GABA with the same letter (s) are statistically non-significant at $p<0.05$.

Table 7. Impacts of kaempferol, GABA and NP coated urea on leaf phosphorous and potassium and root phosphorus and potassium of maize cultivated under stress

Kaempferol Levels (µM)	Leaf Phosphorus (%)		Leaf Potassium (%)		Root phosphorus (%)		Root potassium (%)	
	GABA (mM)							
	0	0.1	0	0.1	0	0.1	0	0.1
	NP coated Urea							
0	0.10±0.006c	0.15±0.008c	0.05±0.02c	1.25±0.012c	0.14±0.001c	0.18±0.002c	0.58±0.01c	0.68±0.008c
5	0.12±0.005b	0.16±0.002b	0.12±0.02b	1.30±0.017b	0.15±0.004b	0.19±0.004b	0.61±0.005b	0.70±0.01b
10	0.13±0.003a	0.17±0.001a	0.19±0.02a	1.37±0.017a	0.17±0.007a	0.20±0.002a	0.64±0.01a	0.73±0.005a
	No NP coated Urea							
0	0.06±0.003a	0.08±0.002a	0.28±0.02a	0.67±0.02a	0.06±0.001a	0.08±0.002a	0.27±0.095a	0.036±0.050a
5	0.07±0.001a	0.08±0.001a	0.4±0.06a	0.83±0.02a	0.07±0.002a	0.10±0.007a	0.29±0.015a	0.038±0.095a
10	0.07±0.0050a	0.09±0.000a	0.51±0.05a	0.96±0.03a	0.07±0.004a	0.12±0.006a	0.33±0.015a	0.040±0.057a

Values are mean ± standard deviation (n=3) within NP coated or uncoated urea, kaempferol and GABA with the same letter (s) are statistically non-significant at $p<0.05$.

Table 8. Impacts of kaempferol, GABA, and NP coated urea on electrolyte leakage, H_2O_2, and MDA Antioxidants of maize cultivated under stress.

Kaempferol Levels (µM)	Electrolyte leakage (%)		H_2O_2 (nmol/g FW)		MDA (nmol/mg Protein)	
	GABA (mM)					
	0	0.1	0	0.1	0	0.1
	NP coated Urea					
0	48.5±1.2c	35.75±1.7c	28.9±0.1c	16.18±1.4c	0.79±0.3c	0.44±0.03c
5	44.5±1.2b	31.5±1.2b	25.1±0.2b	12.65±0.7b	0.66±0.4b	0.35±0.02b
10	40.5±1.2a	29±0.8a	21.0±0.3a	9.60±0.01a	0.54±0.4a	0.28±0.02a
	No NP coated Urea					
0	70±0.81a	60.5±1.2a	55.6±1.3a	43.2±1.7a	1.30±0.02a	1.07±0.02a
5	66.75±0.95a	56.5±1.2a	51.8±1.1a	39.7±1.0a	1.23±0.02a	0.99±0.04a
10	64±0.81a	55.5±1.2a	48.2±1.1a	34.3±2.5a	1.16±0.02a	0.88±0.03a

Values are mean ± standard deviation (n=3) within NP coated or uncoated urea, kaempferol and GABA with the same letter (s) are statistically non-significant at $p<0.05$.

3.7. Antioxidant Enzyme Activity

Activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) were modulated by KP, GABA, and urea. NP-coated urea combined with 5–10 µM KP reduced POD and SOD activities from 38% to 23%, while CAT and APX activities decreased from 55% to 32%, indicating a reduction in oxidative stress. Non-coated urea treatments showed comparable reductions, confirming their protective role (Figures 2 and 3).

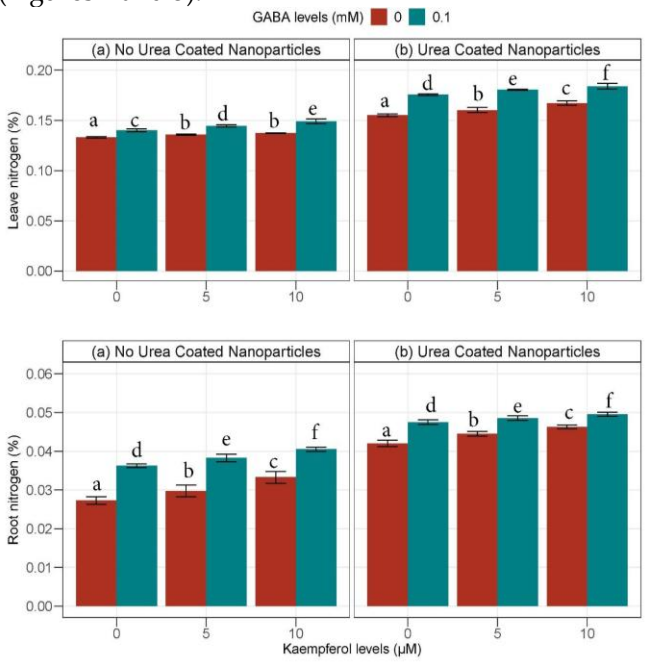

Figure 1. Impacts of Kaempferol, GABA, and NP coated urea on root and leave nitrogen. Error bars showed standard deviation (n=3). The bars with the same letters are statistically non-significant at $p<0.05$.

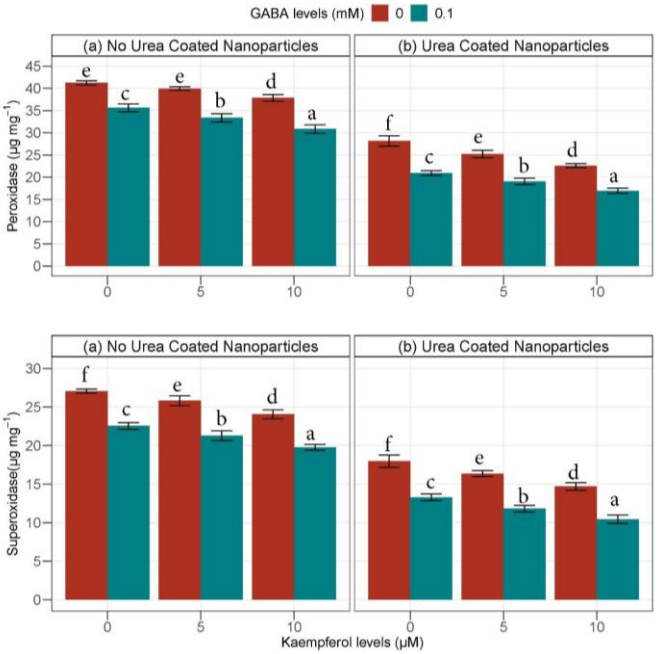

Figure 2. Impacts of Kaempferol, GABA, and NP coated urea on superoxidase and peroxidase. Error bars showed standard deviation (n=3). The bars with the same letters are statistically non-significant at $p<0.05$.

3.8. Oxidative Stress Markers

Electrolyte leakage, hydrogen peroxide (H_2O_2), and malondialdehyde (MDA) levels declined significantly with KP and GABA treatments. NP-coated urea combined with KP decreased H_2O_2 from 2.98% to 2.00%, MDA from 29% to 20%, and electrolyte leakage from 48% to 41%. Non-coated urea treatments showed slightly higher, yet consistent, reductions, demonstrating improved membrane stability and reduced oxidative damage under combined applications (Table 8).

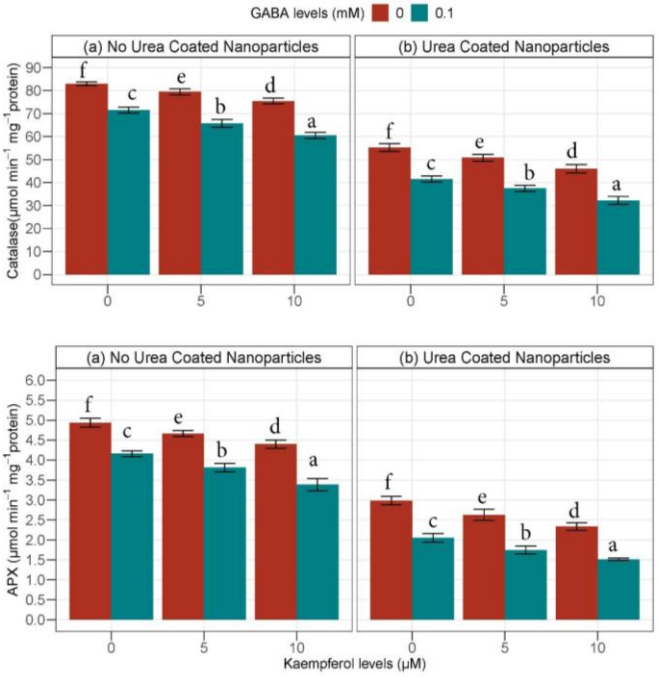

Figure 3. Impacts of Kaempferol, GABA, and NP coated urea on APX and catalase. Error bars showed standard deviation (n=3). The bars with the same letters are statistically non-significant at $p<0.05$.

4. Discussion

The results of this study show that kaempferol (KP), γ-aminobutyric acid (GABA), and nanoparticle (NP)-coated urea significantly influence maize growth, nutrient accumulation, and stress response. Kaempferol, a flavonoid with known antioxidant properties, appears to reduce oxidative damage by lowering hydrogen peroxide (H_2O_2), malondialdehyde (MDA) levels, catalase (CAT) and ascorbate peroxidase (APX). These findings suggest that KP enhances the plant's antioxidant system, consistent with reports that flavonoids protect plant tissues from oxidative stress and regulate stress-related gene expression (Jin *et al.*, 2023, Gunathunga *et al.*, 2024).

GABA, a non-protein amino acid, functions as a signaling molecule that modulates stress responses and nutrient transport. In this study, GABA treatment reduced stress indicators and improved nutrient uptake, likely by activating ion channels and regulating nutrient transporters. This supports previous evidence that GABA enhances plant tolerance to environmental stresses by maintaining membrane stability, regulating osmotic balance, and promoting antioxidant defense (Ramos-Ruiz *et al.*, 2018).

NP-coated urea further improved plant performance by providing controlled nutrient release, enhancing nitrogen use efficiency, and facilitating better nutrient absorption. The increases observed in leaf and root phosphorus and potassium indicate that NP coating allows a sustained supply of nutrients, which positively affects physiological traits and biomass accumulation (Mishra *et al.*, 2018).

The enhancement in net photosynthesis, stomatal conductance, and transpiration rate suggests improved stomatal regulation and chloroplast functionality under water-limited conditions (Hamzah Saleem *et al.*, 2022). These improvements can be attributed to efficient nano-mediated nutrient delivery, which enhances nutrient availability, uptake, and utilization efficiency compared to conventional fertilization (Khalid *et al.*, 2022). The increased Zn accumulation in plant tissues further confirms the effectiveness of nano-enabled fertilization in improving biofortification efficiency through better root absorption and internal translocation. Moreover, the moderated antioxidant enzyme activity along with reduced oxidative markers indicates improved reactive oxygen species homeostasis, reflecting lower oxidative damage under drought stress (Ma *et al.*, 2017).

The combination of KP, GABA, and NP-coated urea produced synergistic effects, enhancing chlorophyll and carotenoid content, biomass, and photosynthetic activity. Improved chlorophyll levels and carotenoid content likely contributed to higher photosynthesis rates and better stress tolerance, as supported by previous studies (Lv *et al.*, 2021). These combined effects may result from the slow release of KP and GABA via the nanoparticle coating, allowing extended biochemical activity and efficient modulation of stress-related pathways.

Responses were also concentration-dependent. Treatments with 5 µM and 10 µM KP, particularly when combined with NP-coated urea, significantly increased shoot length, leaf area, and biomass. Similar concentration-dependent improvements have been observed in other crops, indicating that optimal KP and GABA levels are crucial for maximizing growth and stress resilience (Haque *et al.*, 2001, Chandra *et al.*, 2023). Interestingly, root growth responded more strongly in some cases without NP-coated urea, suggesting that nutrient diffusion and root signaling dynamics may differ between treatments.

Conclusion

In conclusion, KP at 10 µM and GABA at 0.1 mM with NP-coated urea have potential to significantly improve growth, gas exchange attributes, nutrients uptake, and oxidative stress regulation in maize under drought condition (50%FC). The treatment KP (10 µM) and GABA (0.1 mM) with NP-coated urea caused such improvements via regulation of gas exchange attributes, chlorophyll contents and nutrient i.e., N, P and K uptake. However, more field-level investigations are required to

declare KP (10μM) + GABA (0.1 mM) with NP-coated urea as the best amendment for improving maize growth under drought stress.

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