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INFLUENCE OF GIBBERELLIC ACID ON SPINACH MORPHOLOGICAL TRAITS, GAS EXCHANGE ATTRIBUTES AND COPPER UPTAKE IN RESPONSE TO COPPER STRESS

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Abstract

Gibberellic acid (GA_3) is a pivotal plant hormone known for its extensive role in promoting growth and development. Its efficacy in ameliorating metal stress in plants has garnered considerable attention, positioning it as a potential mitigator of heavy metal toxicity. Therefore, we have conducted a pot experiment to explore the effects of GA_3 on spinach (*Spinacia oleracea* L.) under varying levels of copper (Cu) stress. The experiment was structured around three different Cu concentrations in the soil—0, 200, and 400 mg kg^{-1} coupled with the application of GA_3 at three concentrations—0, 50, and 100 mg L−1. Our results revealed that Cu stress markedly reduced plant growth, biomass, and photosynthetic pigment concentrations while elevating Cu accumulation in various parts of the spinach plants. Conversely, the application of GA_3 significantly counteracted these effects by enhancing plant growth and photosynthesis rates and reducing the Cu concentration within the plants. These findings underscore the protective and promotive effects of GA_3 in mitigating copper stress. In conclusion, GA_3 emerges as a promising agent for improving plant growth and composition under heavy metal stress conditions. Its application could potentially ameliorate the adverse effects of Cu toxicity. However, for a comprehensive understanding of its benefits and mechanisms, further detailed molecular studies are warranted in field environments. This study highlights the potential of GA_3 as a beneficial growth regulator in agricultural practices, especially in soils afflicted with heavy metal contamination.

Key words: Heavy metal contamination, Cereal crop, plant hormone, Gas exchange characteristics, Biomass.

Introduction

Metal contamination in agricultural soils poses a significant threat to crop productivity and ecosystem health (Wahab *et al.*, 2023). Among various metals, copper (Cu) toxicity stands out due to its widespread use and accumulation in agricultural lands, leading to detrimental impacts on plant growth and development (Rehman *et al.*, 2019). Excessive Cu levels in the soil can disrupt vital physiological and biochemical processes in plants, including photosynthesis, nutrient uptake, and water balance, ultimately leading to reduced biomass and yield (Saleem *et al.*, 2020). The mechanisms underlying Cu toxicity involve the generation of reactive oxygen species (ROS), leading to oxidative stress, damage to cellular structures, and impaired metabolic functions (Saleem *et al.*, 2020). Given the adverse effects of Cu toxicity, there is a pressing need for effective strategies to mitigate its impact on agricultural productivity and plant growth (Saleem *et al.*, 2020). Traditional methods such as soil amendments and phytoremediation have been explored, yet the search for more efficient and practical solutions continues (Parveen *et al.*, 2020). In this context, the application of plant growth regulators, such as gibberellic acid (GA_3), emerges as a promising approach to combat Cu toxicity in plants (Saleem *et al.*, 2021). By enhancing growth and stress tolerance mechanisms, these substances offer a potential means to safeguard crops against the negative consequences of metal stress and improve resilience in contaminated environments (Ghani *et al.*, 2021).

Spinach (*Spinacia oleracea* L.) is a leafy green vegetable that is widely cultivated for its nutritional value, rich in vitamins, minerals, and antioxidants (Ma *et al.*, 2022). This plant species has developed various tolerance mechanisms to cope with heavy metal stress, including the sequestration and compartmentalization of metals, activation of antioxidant defense systems, and regulation of metal uptake transporters (Zaheer *et al.*, 2020). Despite these adaptive strategies, metal toxicity, particularly from Cu, has been known to severely affect spinach growth globally, underscoring the need for effective approaches to combat this challenge (Ali *et al.*, 2023). Around the world, researchers and agriculturists have experimented with numerous methods to mitigate metal toxicity in crops. The use of GA_3 represents a novel, easy-to-adopt, and scientifically grounded method aimed at enhancing plant resilience to metal stress (Kaya *et al.*, 2020). For this purpose, our study focused on examining the impact of different levels of GA_3 under Cu stress in spinach, specifically looking at growth parameters, photosynthetic efficiency, and Cu accumulation in plant parts. The findings from the present study will significantly expand our understanding of the role of GA_3 in modulating plant responses to heavy metal stress.

Material and Methods

Plant material and growth conditions: In this study, a controlled greenhouse experiment was initiated to investigate the effects of GA_3 on spinach growth under Cu stress. We began by preparing mature spinach seeds, which were cleansed with a 0.1% HgCl solution to eliminate potential surface microbial contaminants. The soil, sourced from an experimental farm, was aerated, sieved through a 5-mm mesh, and then filled into pots for the experiment. These pots were then treated with $CuSO_4 \bullet 5H_2O$ at concentrations of 0 (no Cu), 200, and 400 mg kg^{-1} of soil to introduce Cu stress. Prior to planting, the treated soils were allowed to stabilize over a period of two months, during which they underwent four cycles of moistening with distilled water followed by air drying to ensure even addition of Cu. Throughout the experiment, spinach plants were irrigated with distilled water devoid of Cu to maintain soil moisture at approximately 70% of its water-holding capacity. Regular intercultural operations, including weeding, was performed when needed. To explore the effect of GA_3, we applied GA_3 treatments at concentrations of 0, 50, and 100 mg L^{-1} by spraying the solution directly onto the spinach seedlings two weeks post-sowing. This application was carried out in the morning hours, between 9:00 and 10:00 AM, ensuring thorough coverage of the plants (Sun *et al.*, 2013). The GA_3 treatment was applied only once during the entire study experiment. Each pot, measuring (20-cm-tall × 15-cm-wide), was filled with five kg of the prepared soil. Four seeds were sown per pot. The experimental design was a completely randomized design (CRD), with each treatment replicated four times to ensure statistical reliability of the results.

Plant harvesting: Spinach plants were meticulously uprooted 30 days post-treatment, equating to 60 days after germination, and were carefully washed with distilled water to remove any surface dust and deposits. Upon harvesting, the plants were segregated into roots and shoots to facilitate the analysis of various biological traits. Immediately following harvest, the lengths of the shoots and roots were measured using a scale. Fresh biomass for each plant part was determined using a precision weighing scale. Subsequently, the plant samples were thoroughly rinsed with de-ionized water, oven-dried at 70 °C for three days, finely ground in a stainless-steel mill, and sifted through a 0.1 mm nylon sieve, preparing them for further analytical processes.

Determination of chlorophyll pigments and gas exchange characteristics: The total contents of chlorophyll and carotenoids in seedlings were determined following the procedure outlined by Arnon (1949). This involved grinding the frozen leaves in 80% acetone and allowing the mixture to stand overnight. The clear supernatant was then obtained by centrifuging the mixture at 10,000 ×g for 15 minutes at a temperature of 4°C. The absorbance levels were measured using a spectrophotometer (Hitachi U-2910, Tokyo, Japan) at wavelengths of 645 and 663 nm for chlorophyll, and 480 nm for carotenoids. Net photosynthesis, leaf stomatal conductance, transpiration rate, and intercellular carbon dioxide concentration were recorded from four distinct plants within each experimental group. These measurements were carried out under clear sky conditions, specifically between the hours of 8 and 10:30 AM. These parameters were determined using a LI-COR gas-exchange system (model LI-6400; LI-COR Biosciences, Lincoln, NE, USA), which was equipped with a red-blue LED light source to illuminate the leaf chamber. Within the LI-COR system's cuvette, the CO_2 concentration was maintained at 380 mmol mol^{-1}, and the LED light intensity was set to 1000 mmol m^{-2} s^{-1}. This light intensity is recognized as the average level required to saturate photosynthesis in wheat, according to Austin (1990).

Determination of Cu uptake from different parts of plant: To determine the total Cu concentration from all parts of the plant, the samples were first oven-dried at 65°C for a duration of 24 hours and subsequently dried in a muffle furnace at 550°C for 20 hours. Following this process, the dried mixture was treated with a mixture of 31% (m/v) nitric acid (HNO_3) and 17.5% (v/v) hydrogen peroxide (H_2O_2) and incubated at 70°C for approximately 2 hours, after which distilled water was added. The concentration of Cu in the resulting solution was measured using an atomic absorption spectrophotometer (AAS).

Statistical analysis: The collected experimental data were analysed using analysis of variance (ANOVA) through the statistical software CoStat version 6.2 (Cohorts Software, 2003, Monterey, CA, USA). Differences between treatment means were assessed using the least significant difference method (Fisher's LSD) at a significance level of $p<0.05$. Graphical representations (including PCA and Pearson's correlation) of the data were organized using RStudio.

Results

Growth and biomass: In the presence of Cu stress, we observed a significant decrease in spinach growth and biomass parameters (Table 1). Specifically, Cu stress led to a reduction in shoot length by approximately 18.6% to 32.6%, root length by about 25% to 45.2%, shoot fresh weight by 17.9% to 35.7%, root fresh weight by 24.3% to 46.4%, shoot dry weight by 23.1% to 38.5%, and root dry weight by 24.4% to 38.2%, across the increasing Cu concentrations from 200 to 400 mg kg^{-1} as compared to the control group with no added Cu. Conversely, the application of GA_3 under Cu stress conditions led to an improvement in these parameters. The enhancements were quantifiable, with shoot length increasing by approximately 11.6% to 24.4%, root length by about 13.5% to 29.8%, shoot fresh weight by 14.3% to 28.6%, root fresh weight by 12.5% to 25%, shoot dry weight by 15.4% to 23.1%, and root dry weight by 14.7% to 25%, depending on the GA_3 concentrations (50 and 100 mg L^{-1}) compared to the Cu-stressed plants without GA_3 application.

Chlorophyll content and gas exchange parameters: For chlorophyll content and gas exchange parameters under Cu stress and GA_3 application, the results from Table 2 highlight significant variations across treatments. In the absence of Cu stress (Cu_0) with no GA_3 application (GA_0), baseline levels for total chlorophyll and carotenoid content, as well as net photosynthesis, stomatal conductance, transpiration rate, and intercellular CO_2 concentration, were established. The introduction of GA_3 led to observable improvements in these

parameters. Specifically, Cu stress reduced the total chlorophyll content by approximately 30.8% to 46.2% and carotenoid content by 17.3% to 21% at the highest level of Cu stress (Cu_2+GA_0) compared to the control group without Cu (Cu_0+GA_0). The application of GA_3 under Cu stress conditions counteracted these declines, with increases in total chlorophyll content by up to 15.4% to 28.1% and carotenoid content by 11.1% to 16.7%, depending on the GA_3 concentration used. Net photosynthesis, stomatal conductance, and transpiration rate also showed marked decreases under Cu stress, with reductions of approximately 15.4% to 30.8% in net photosynthesis, 20% to 42.9% in stomatal conductance, and 14% to 26.7% in transpiration rate from the lowest to the highest Cu stress levels compared to the control. However, GA_3 application improved these parameters, with enhancements in net photosynthesis by 15.4% to 30.8%, stomatal conductance by 14.3% to 37.1%, and transpiration rate by 11.6% to 21.7% compared to plants under the same level of Cu stress without GA_3. Intercellular CO_2 concentration remained relatively stable across all treatments, indicating that the observed changes in photosynthetic efficiency and gas exchange parameters were not significantly influenced by internal CO_2 levels.

Cu uptake in different parts of spinach: The investigation into Cu uptake in different parts of spinach under Cu stress and GA_3 application revealed distinct patterns of accumulation (Table 3). Consistently, Cu was found in higher concentrations in the roots compared to the stems, leaves, and flowers. The impact of Cu stress significantly increased Cu concentration in all plant parts, while the application of GA_3 was effective in reducing its accumulation. In the control group without Cu stress (Cu_0+GA_0), baseline Cu concentrations were observed across all plant parts. With the introduction of GA_3 (Cu_0+GA_1 and Cu_0+GA_2), there was a noticeable decrease in Cu accumulation, with reductions of approximately 8.9% in roots, 21.9% in stems, 16% in leaves, and 28.6% in flowers at the highest GA_3 concentration (Cu_0+GA_2) compared to the control group without GA_3. Under moderate Cu stress (Cu_1+GA_0), Cu concentrations in plant parts surged, showing an increase of over 2564% in roots, 2525% in stems, 3060% in leaves, and 3476% in flowers compared to the control without Cu stress. The application of GA_3 under moderate Cu stress conditions (Cu_1+GA_1 and Cu_1+GA_2) significantly reduced Cu accumulation by approximately 18.8% in roots, 19% in stems, 17.7% in leaves, and 26.7% in flowers at the highest GA_3 concentration (Cu_1+GA_2). The trend of GA_3 reducing Cu accumulation continued under high Cu stress (Cu_2). Without GA_3 (Cu_2+GA_0), Cu concentrations reached their peak across all plant parts. The application of GA_3 led to reductions in Cu accumulation by up to 27.3% in roots, 35.9% in stems, 40.7% in leaves, and 42.8% in flowers at the highest GA_3 concentration (Cu_2+GA_2) compared to high Cu stress without GA_3.

Table 1. Effect of different concentrations of Cu on plant growth and biomass under the foliar application of GA_3 in spinach.

Treatments	Root length (cm)	Shoot length (cm)	Root fresh weigh (g)	Shoot fresh weight (g)	Root dry weight (g)	Shoot dry weight (g)
$Cu_0 + GA_0$	8.4 ± 0.8^{cd}	43 ± 5^{c}	2.8 ± 0.2^{c}	4.2 ± 0.3^{c}	0.68 ± 0.05^{c}	1.3 ± 0.08^{c}
$Cu_0 + GA_1$	11.3 ± 1.3^{b}	48 ± 6^{b}	3.2 ± 0.3^{b}	5.3 ± 0.6^{b}	0.76 ± 0.07^{b}	1.6 ± 0.2^{b}
$Cu_0 + GA_2$	13.4 ± 1.5^{a}	53 ± 7^{a}	4.6 ± 0.2^{a}	6.8 ± 0.8^{a}	0.83 ± 0.1^{a}	1.9 ± 0.3^{a}
$Cu_1 + GA_0$	6.3 ± 0.3^{e}	35 ± 6^{f}	2.1 ± 0.3^{d}	3.5 ± 0.4^{d}	0.58 ± 0.08^{d}	1.1 ± 0.1^{cd}
$Cu_1 + GA_1$	8.2 ± 0.8^{d}	39 ± 5^{d}	2.8 ± 0.4^{c}	4.3 ± 0.6^{c}	0.68 ± 0.05^{c}	$1.4 \pm 0.08b^{c}$
$Cu_1 + GA_2$	10.4 ± 1.3^{bc}	42 ± 4^{c}	3.3 ± 0.3^{b}	5.2 ± 0.3^{b}	0.73 ± 0.04^{b}	1.6 ± 0.1^{b}
$Cu_2 + GA_0$	4.6 ± 0.2^{f}	31 ± 6^{g}	1.8 ± 0.2^{f}	2.7 ± 0.4^{f}	0.51 ± 0.05^{e}	0.8 ± 0.04^{e}
$Cu_2 + GA_1$	6.1 ± 0.4^{e}	33 ± 5^{fg}	2.3 ± 0.3^{ef}	3.5 ± 0.5^{e}	0.64 ± 0.08^{cd}	1.03 ± 0.06^{cd}
$Cu_2 + GA_2$	7.5 ± 0.9^{de}	37 ± 4^{ef}	2.6 ± 0.2^{cd}	3.7 ± 0.4^{de}	0.67 ± 0.06^{c}	1.4 ± 0.08^{bc}

Values in the table is just one harvest. Mean ± SD (n = 4). Different letters within a column indicate significant difference between the treatments ($p<0.05$). Different treatments used in the table are as follow: Cu_0+GA_0 (0 Cu + 0 GA_3), Cu_0+GA_1 (0 Cu + 50 GA_3), Cu_0+GA_2 (0 Cu + 100 GA_3), Cu_1+GA_0 (200 Cu + 0 GA_3), Cu_1+GA_1 (200 Cu + 50 GA_3), Cu_1+GA_2 (200 Cu + 100 GA_3), Cu_2+GA_0 (400 Cu + 0 GA_3), Cu_2+GA_1 (400 Cu + 50 GA_3) and Cu_2+GA_2 (400 Cu + 100 GA_3)

Table 2. Effect of different concentrations of Cu on chlorophyll content and gas exchange attributes under the foliar application of GA_3 in spinach.

Treatments	Total chlorophyll (mg g^{-1} FW)	Carotenoid (mg g^{-1} FW)	Net photosynthesis (µmol $m^{-2}s^{-1}$)	Stomatal conductance (µmol $m^{-2}s^{-1}$)	Transpiration rate (µmol $m^{-2}s^{-1}$)	Intercellular CO_2 (µmol $m^{-2}s^{-1}$)
$Cu_0 + GA_0$	2.6 ± 0.3^{c}	0.81 ± 0.09bc	13 ± 1.3^{cd}	0.35 ± 0.02^{c}	8.6 ± 0.9^{c}	234 ± 29^{a}
$Cu_0 + GA_1$	2.9 ± 0.4^{b}	0.86 ± 0.1^{b}	16 ± 1.8^{b}	0.46 ± 0.04^{b}	9.3 ± 1.2^{b}	238 ± 31^{a}
$Cu_0 + GA_2$	3.2 ± 0.3^{a}	0.95 ± 0.1^{a}	18 ± 2.3^{a}	0.53 ± 0.06^{a}	10.3 ± 1.1^{a}	243 ± 35^{a}
$Cu_1 + GA_0$	2.4 ± 0.2^{d}	0.75 ± 0.08^{d}	11 ± 1.6^{d}	0.31 ± 0.04^{e}	8.1 ± 0.6^{d}	231 ± 34^{a}
$Cu_1 + GA_1$	2.7 ± 0.3^{c}	0.83 ± 0.09^{bc}	14 ± 2.3^{c}	0.43 ± 0.05^{cd}	8.9 ± 0.8^{c}	235 ± 36^{a}
$Cu_1 + GA_2$	2.9 ± 0.4^{b}	$0.89 \pm 0.1a^{b}$	16 ± 1.5^{b}	0.48 ± 0.06^{b}	9.2 ± 0.7^{b}	238 ± 39^{a}
$Cu_2 + GA_0$	1.8 ± 0.3^{f}	0.67 ± 0.08^{e}	9 ± 1.3e	0.28 ± 0.04^{f}	7.4 ± 0.6^{f}	225 ± 34^{a}
$Cu_2 + GA_1$	2.1 ± 0.2^{e}	0.73 ± 0.07^{d}	12 ± 1.3^{d}	0.33 ± 0.03^{de}	7.9 ± 0.5^{e}	229 ± 43^{a}
$Cu_2 + GA_2$	2.3 ± 0.3^{de}	0.79 ± 0.09^{cd}	14 ± 1.5^{c}	0.35 ± 0.04^{d}	8.2 ± 0.7^{de}	233 ± 34^{a}

Values in the table is just one harvest. Mean ± SD (n = 4). Different letters within a column indicate significant difference between the treatments ($p<0.05$). Different treatments used in the table are as follow: Cu_0+GA_0 (0 Cu + 0 GA_3), Cu_0+GA_1 (0 Cu + 50 GA_3), Cu_0+GA_2 (0 Cu + 100 GA_3), Cu_1+GA_0 (200 Cu + 0 GA_3), Cu_1+GA_1 (200 Cu + 50 GA_3), Cu_1+GA_2 (200 Cu + 100 GA_3), Cu_2+GA_0 (400 Cu + 0 GA_3), Cu_2+GA_1 (400 Cu + 50 GA_3) and Cu_2+GA_2 (400 Cu + 100 GA_3)

Table 3. Effect of different concentrations of Cu-on-Cu accumulation id different parts of the plant under the foliar application of GA_3 in spinach.

Treatments	Roots (mg kg^{-1} DW)	Stems (mg kg^{-1} DW)	Leaves (mg kg^{-1} DW)	Flowers (mg kg^{-1} DW)
$Cu_0 + GA_0$	5.6 ± 0.8^{g}	3.2 ± 0.8^{g}	2.5 ± 0.6^{g}	2.1 ± 0.4^{f}
$Cu_0 + GA_1$	5.1 ± 0.6^{g}	2.8 ± 0.7^{g}	2.3 ± 1.3^{g}	1.8 ± 0.6^{f}
$Cu_0 + GA_2$	4.8 ± 0.5^{g}	2.5 ± 1.2^{g}	2.1 ± 1.1^{g}	1.5 ± 0.2^{f}
$Cu_1 + GA_0$	149 ± 18^{d}	84 ± 9^{d}	79 ± 8^{d}	75 ± 8^{c}
$Cu_1 + GA_1$	$121 \pm 16e^{f}$	68 ± 7^{e}	65 ± 6^{e}	61 ± 6^{d}
$Cu_1 + GA_2$	106 ± 11^{f}	51 ± 6^{f}	46 ± 5^{f}	48 ± 5^{e}
$Cu_2 + GA_0$	326 ± 36^{a}	164 ± 18^{a}	145 ± 12^{a}	138 ± 16^{a}
$Cu_2 + GA_1$	279 ± 28^{b}	124 ± 16^{b}	109 ± 14^{b}	101 ± 10^{b}
$Cu_2 + GA_2$	237 ± 34^{c}	105 ± 12^{c}	86 ± 9^{cd}	79 ± 8^{c}

Values in the table is just one harvest. Mean ± SD (n = 4). Different letters within a column indicate significant difference between the treatments (p<0.05). Different treatments used in the table are as follow: Cu_0+GA_0 (0 Cu + 0 GA_3), Cu_0+GA_1 (0 Cu + 50 GA_3), Cu_0+GA_2 (0 Cu + 100 GA_3), Cu_1+GA_0 (200 Cu + 0 GA_3), Cu_1+GA_1 (200 Cu + 50 GA_3), Cu_1+GA_2 (200 Cu + 100 GA_3), Cu_2+GA_0 (400 Cu + 0 GA_3), Cu_2+GA_1 (400 Cu + 50 GA_3) and Cu_2+GA_2 (400 Cu + 100 GA_3)

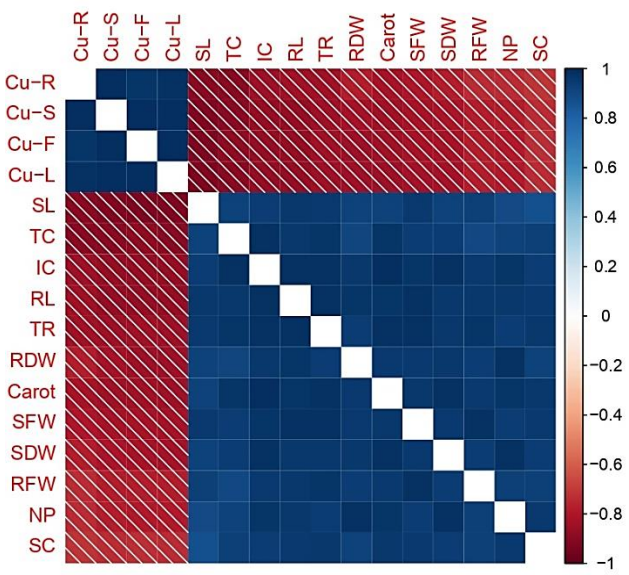

Fig. 1. Correlation between Cu uptake with chlorophyll contents, gases exchange and growth parameters in spinach. Different abbreviations used in the figure are as follow: Cu-R: Copper content in roots, Cu-S: Copper content in stems, Cu-F: Copper content in flowers, Cu-L: Copper content in leaves, SL: Shoot length, TC: Total chlorophyll, IC: Intercellular CO_2 concentration, RL: Root length, TR: Transpiration rate, RDW: Root dry weight, Carot: Carotenoids, SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, NP: Net photosynthesis and SC: Stomatal conductance.

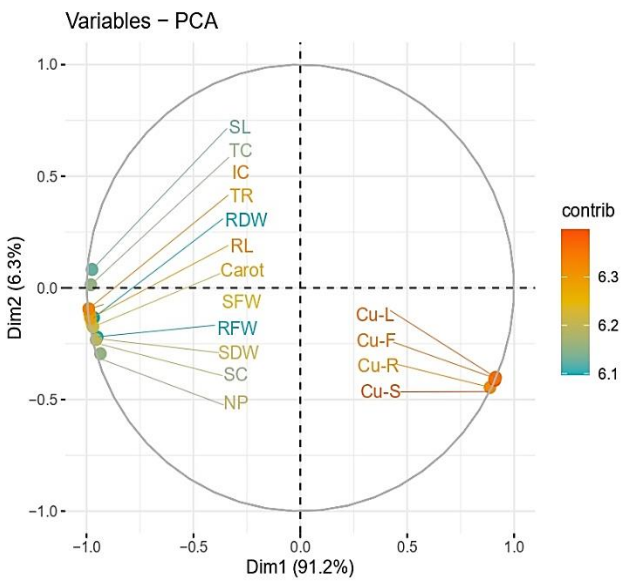

Fig. 2. Loading plots of principal component analysis (PCA) on different studied attributes of spinach grown under various stress levels of Cu in the soil. Different abbreviations used in the figure are as follow: Cu-R: Copper content in roots, Cu-S: Copper content in stems, Cu-F: Copper content in flowers, Cu-L: Copper content in leaves, SL: Shoot length, TC: Total chlorophyll, IC: Intercellular CO_2 concentration, RL: Root length, TR: Transpiration rate, RDW: Root dry weight, Carot: Carotenoids, SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, NP: Net photosynthesis and SC: Stomatal conductance.

Pearson's correlation: The Pearson's correlation heatmap indicates the relationships between Cu accumulation in various parts of the spinach plant and several growth and photosynthetic efficiency parameters (Fig. 1). The correlation shows positive correlations within the Cu uptake across different plant parts—meaning Cu in the roots (Cu-R) is positively correlated with Cu in the stems (Cu-S), leaves (Cu-L), and flowers (Cu-F). On the other hand, Cu accumulation in both the roots and stems is negatively correlated with parameters such as root length, shoot length, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight, total chlorophyll, carotenoids, net photosynthesis, stomatal conductance, transpiration rate, and intercellular CO_2 concentration. This correlation demonstrates a close connection between Cu stress and the physiological and biochemical responses of spinach plants.

Principal component analysis: The principal component analysis (PCA) visualized in the figure 2 provides insight into the relationships and contributions of various parameters under Cu stress in the study. The PCA is structured into two principal components, Dim1 and Dim2, which cumulatively explain 97.5% of the total variance within the dataset. Dim1 accounts for a substantial portion, at 91.2%, while Dim2 accounts for 6.3%. In this analysis, certain growth and photosynthetic parameters such as shoot length, total chlorophyll content, intercellular CO_2 concentration, transpiration rate, root dry weight, root length, and carotenoid content are predominantly associated with Dim1 and are oriented negatively on the PCA biplot. On the other hand, the parameters associated with Cu accumulation in different plant parts, including Cu in the roots (Cu-R), stems (Cu-S), leaves (Cu-L), and

flowers (Cu-F), are positively correlated and align with Dim2. Overall, the PCA results illustrate a clear distinction between the parameters associated with plant growth and photosynthesis, which are adversely affected by Cu stress, and those parameters directly related to Cu accumulation within the plant tissues.

Discussion

Metal toxicity in agricultural soils is a pervasive problem that can lead to significant reductions in crop yield and quality (Li *et al.*, 2020). Copper toxicity, in particular, poses a severe threat to plant growth, as it can negatively impact various physiological and biochemical processes (Rehman *et al.*, 2019). When plants are exposed to excess Cu, it can bind with important protein molecules, thereby disrupting enzymatic activities and causing deficiencies in vital nutrients (Rehman *et al.*, 2019). The accumulation of Cu within plant tissues can lead to the production of reactive oxygen species, which damage cellular structures, including membranes, lipids, proteins, and nucleic acids (Saleem *et al.*, 2020; Alshegaihi *et al.*, 2023). This oxidative stress impairs photosynthetic machinery and reduces chlorophyll synthesis, which is critical for plant energy production (Rehman *et al.*, 2020). Additionally, high levels of Cu can displace other essential metal ions at binding sites, further inhibiting metabolic functions and leading to stunted root and shoot growth, ultimately resulting in decreased biomass (Rehman *et al.*, 2021). The culmination of these effects underscores the complex challenge that Cu toxicity presents to maintaining plant growth and productivity (Saleem *et al.*, 2019).

Gibberellic acid is a plant growth hormone that has been increasingly recognized for its potential in alleviating metal stress in plants (Alatawi *et al.*, 2022). Under metal stress conditions, GA_3 is known to enhance plant growth and biomass by modulating gene expression related to cell division and elongation, thereby promoting stem and root growth even in the presence of toxic metals (Kaya *et al.*, 2020). It also improves photosynthetic efficiency by upregulating the synthesis of chlorophyll and enhancing the activity of photosynthetic enzymes, thus supporting better energy capture and utilization under stress conditions (Saleem *et al.*, 2021). Moreover, GA_3 application has been observed to reduce the uptake of Cu in different parts of the plant. This is possibly through the upregulation of metal chelators and transporters that sequester excess Cu, preventing its translocation to aerial parts, and facilitating its compartmentalization in less sensitive parts of the cell. By doing so, GA_3 helps in maintaining the ion homeostasis within the plant tissues, thereby mitigating the toxic effects of excessive Cu accumulation (Saleem *et al.*, 2020).

Conclusion

In conclusion, the detrimental effects of Cu stress on plant growth are multifaceted, leading to compromised growth, biomass, and photosynthetic capacity. However, the application of GA_3 has emerged as a promising strategy to counteract these negative impacts. GA_3 not only enhances growth and photosynthetic efficiency but also appears to restrict Cu uptake and distribution within the plant, contributing to improved metal stress tolerance. Future research should focus on elucidating the molecular mechanisms by which GA_3 mediates these protective effects and explore the potential of GA_3 as a part of integrated management strategies to ameliorate metal stress in crops. Additionally, field studies are recommended to validate the efficacy of GA_3 under varied environmental conditions and soil types, ensuring the practical applicability of these findings for sustainable agricultural practices.

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