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MITIGATING ARSENIC STRESS IN WHEAT WITH IRON-LYSINE FOLIAR APPLICATION: IMPACTS ON GROWTH, PHOTOSYNTHESIS, ORGANIC ACID EXUDATION, AND ARSENIC ACCUMULATION

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Abstract

Arsenic (As) toxicity poses a significant threat to plant growth, even at minute concentrations, compromising growth and physiological functions. However, the application of iron−lysine (Fe−lys) has shown potential in ameliorating these adverse effects, enhancing plant growth and composition. In this study, we conducted a pot experiment to investigate the effects of varying levels of Fe−lys (0, 5, and 10 mM) on wheat plants grown under As stress conditions (0, 50, and 100 µM). Our findings reveal that increasing As concentrations in the soil led to a decline in plant growth, biomass, chlorophyll content, and gas exchange characteristics, while simultaneously increasing the exudation patterns of organic acids and As accumulation in the plants. Conversely, the application of Fe−lys markedly improved plant growth and biomass, chlorophyll content, and gas exchange parameters, and reduced the exudation of organic acids and As accumulation. These results underscore the efficacy of Fe−lys in counteracting the detrimental effects of As stress, suggesting its utility as a viable strategy to boost crop yield and biomass in As-contaminated soils. The study highlights the potential of Fe-lys application as a practical agronomic intervention to mitigate arsenic toxicity in wheat, offering insights into improving plant resilience and productivity under environmental stress conditions.

Key words: Cash crop, Gas exchange characteristics, Heavy metal contamination, Iron−lysine.

Introduction

Metal contamination in agricultural soils is an escalating global issue, undermining food safety and ecosystem health (Tariq *et al.*, 2021). The toxicity of heavy metals, such as arsenic (As), to agricultural soils poses a critical challenge, affecting crop yield and quality, and thereby food security (Irshad *et al.*, 2021). Arsenic, a notorious metalloid, is particularly detrimental to plants, impairing growth, photosynthesis, and nutrient uptake even at low concentrations (Alsafran *et al.*, 2022). It induces oxidative stress, disrupts cellular functions, and can lead to the accumulation of toxic levels in edible plant parts, posing a significant risk to human health (Saleem *et al.*, 2022). Given the severe impacts of As toxicity on agriculture, there is a pressing need to explore advanced mitigation strategies that can effectively reduce its uptake and accumulation in crops, ensuring plant growth and productivity. There are various techniques employed in the agricultural sector to combat metal toxicity, ranging from soil remediation practices to genetic engineering of crops (Ali *et al.*, 2022). However, the chelation of micronutrients with amino acids represents an innovative approach to mitigate metal toxicity effectively (Zaheer *et al.*, 2020). Among these, iron-lysine (Fe-lys) chelation emerges as a novel, cost-effective, and scientifically endorsed method, offering multiple advantages (Zaheer *et al.*, 2020). By enhancing the bioavailability of iron, a crucial nutrient for plant growth, Fe-lys chelation can mitigate the adverse effects of metal toxicity, including As, by promoting healthier and more resilient plant development (Zaheer *et al.*, 2020). This method not only improves the nutritional status of plants but also reduces the bioavailability of toxic metals like As, thereby decreasing their uptake and accumulation in plant tissues.

Wheat (*Triticum aestivum* L.) is one of the world's most important food crops, serving as a staple for over a third of the global population and playing a crucial role in human nutrition and food security (Nawaz *et al.*, 2024). Characterized by its adaptability to a wide range of climatic conditions, wheat has shown remarkable resilience in agricultural practices (Ahmar *et al.*, 2020). This resilience extends to its ability to exhibit tolerance against metal toxicity, where innovative treatments, such as Fe-lys chelation, have demonstrated significant potential in enhancing wheat's ability to thrive in contaminated soils, further underscoring its vital role in global agriculture. The primary objective of our study is to investigate the impact of Fe-lys foliar application on the growth, photosynthesis, organic acid exudation, and As accumulation in wheat plants under varying levels of As contamination in the soil. By examining these parameters, this research aims to enhance our understanding of how Fe-lys can ameliorate As stress in wheat, potentially offering a novel approach to mitigate the adverse effects of heavy metal toxicity on this crucial crop. This study will contribute to expanding our knowledge about the mechanisms through which Fe-lys influences plant physiology and stress response, thereby providing insights into developing more effective strategies for improving plant resilience and productivity in As-contaminated soils.

Material and Methods

Experimental setup and plant material: Our methodology involved conducting a pot experiment within a greenhouse setting. Wheat seeds were initially treated with a solution of bleaching powder, followed by thorough rinsing with distilled water to ensure cleanliness prior to sowing. Soil for the experiment was collected from an experimental station, sieved through a 5 mm mesh to eliminate stones and debris, and then allocated into pots, each receiving 8 kg of soil. To introduce As stress, sodium arsenate (Na_2HAsO_4)•$7H_2O$ (sourced from Sigma) was applied to the soil in designated concentrations of 0 (control, with no As), 50, and 100 µM. The experiment utilized small pots, measuring 15 cm in height and 20 cm in width, each filled with 8 kg of sieved soil. These pots underwent two cycles of water saturation followed by air drying to prepare for seeding. Following seed germination, indicated by the emergence of seedlings beyond 2 cm in height, we commenced the exogenous application of Fe-lys at varying concentrations (0, 5, and 10 mM) using a hand sprayer (Zaheer *et al*., 2020). This application process distributed a total of 2 liters of Fe-lys solution across all treatments throughout the experiment, ensuring that each treatment was replicated four times and that each pot contained three plants. Additionally, to supplement micronutrient levels, tannery wastewater was applied at least once weekly, along with regular sprays of Fe-lys based on plant requirements, targeting all parts of the plant. To maintain optimal micronutrient levels within the plant tissues, predetermined quantities of phosphate and potassium sulphate fertilizers were also added as per established guidelines. To minimize any potential environmental biases within the greenhouse, the positioning of the pots was regularly altered.

Plant harvesting: Wheat 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, while the number of leaves per plant was recorded. The leaf area for each plant was also quantitatively assessed to provide insights into foliage growth. 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 organic acids and As uptake: The quantification of organic acids was performed using high-performance liquid chromatography (HPLC) with a Flexar FX-10 UHPLC isocratic pump (PerkinElmer, MA, USA). To prepare the samples for analysis, 80% ethanol was added to the frozen samples, and 20 µl of the resulting solution was injected into a C-18 column (Brownlee Analytical C-18, 3 µm; 150 mm × 4.6 mm^2, USA). The HPLC's mobile phase consisted of acetonitrile, acetic acid, and H_2SO_4 in a 15:1:4 ratio, respectively, with a fixed pH of 4.9. A flow rate of 1 ml per minute was maintained throughout the 10-minute analysis period for organic acids. The analysis was conducted at a column temperature of 45°C, using an absorption wavelength of 214 nm with a UV–VIS Series 200 detector (USA), as described by UdDin *et al*., (2015).

To determine the total As concentration in both shoots and roots, 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 As 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 of the data were organized using Origin 2017.

Results

Growth and biomass: The results of our investigation into the various growth and biomass parameters of wheat under As stress with the application of Fe-lys are presented in Fig. 1. As stress notably diminished the shoot length by approximately 44-57%, root length by about 48-65%, number of leaves by roughly 47-62%, leaf area by 47-57%, shoot fresh weight by 42-55%, root fresh weight by 44-59%, shoot dry weight by 46-58%, and root dry weight by 48-60% across the increasing As concentrations from 50 to 100 µM as compared to the control with no As. Conversely, the foliar application of Fe-lys demonstrated an ameliorative effect, with improvements in shoot length by up to 19-28%, root length by 16-26%, number of leaves by 15-24%, leaf area by 18-25%, shoot fresh weight by 17-26%, root fresh weight by 19-27%, shoot dry weight by 17-25%, and root dry weight by 18-27% in comparison to the plants that did not receive Fe-lys treatment under the same levels of As stress.

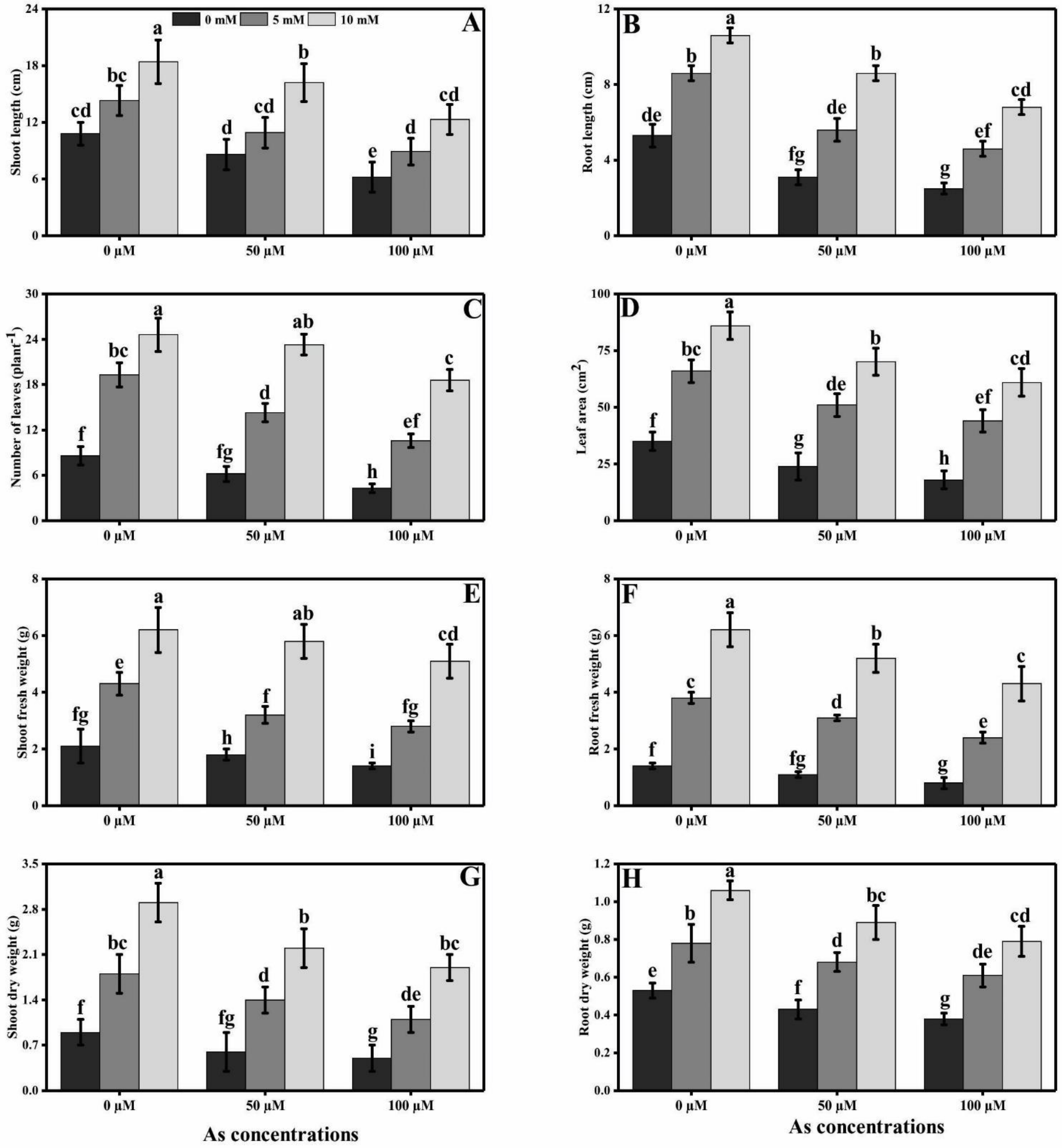

Fig. 1. Effect of various levels of iron−lysine (0, 5 and 10 mM) on shoot length (A), root length (B), number of leaves (C), leaf area (D), shoot fresh weight (E), root fresh weight (F), shoot dry weight (G), and root dry weight (H) of wheat grown under various stress levels of As (0, 50 and 100 µM). Values are demonstrated as means of four replicates along with standard deviation (SD; n=4). One-way ANOVA was performed and means differences were tested by HSD ($p<0.05$). Different lowercase letters on the error bars indicate significant difference between the treatments.

Chlorophyll content and gas exchange parameters: The assessment of chlorophyll content and gas exchange parameters in wheat plants under As stress conditions and their response to Fe-lys foliar application are summarized in Fig. 2. Chlorophyll content was significantly reduced under As stress, with chlorophyll a dropping by approximately 32-42% and chlorophyll b by 35-46%, across increasing As concentrations of 50 to 100 µM compared to the control. The application of Fe-lys was effective in mitigating this decline, with chlorophyll a increasing by up to 16-22% and chlorophyll b by 18-24% relative to plants not receiving Fe-lys. Gas exchange parameters were also adversely affected by As stress. Net photosynthesis was lowered by 28-38%, stomatal conductance by 29-39%, transpiration rate by 23-34%, and intercellular carbon dioxide concentration by 15-26% with the increase in As concentration. The application of Fe-lys improved these parameters with an increase in net photosynthesis by 14-19%, stomatal conductance by 15-21%, transpiration rate by 13-18%, and intercellular carbon dioxide concentration by 11-16% in comparison to plants not treated with Fe-lys under As stress.

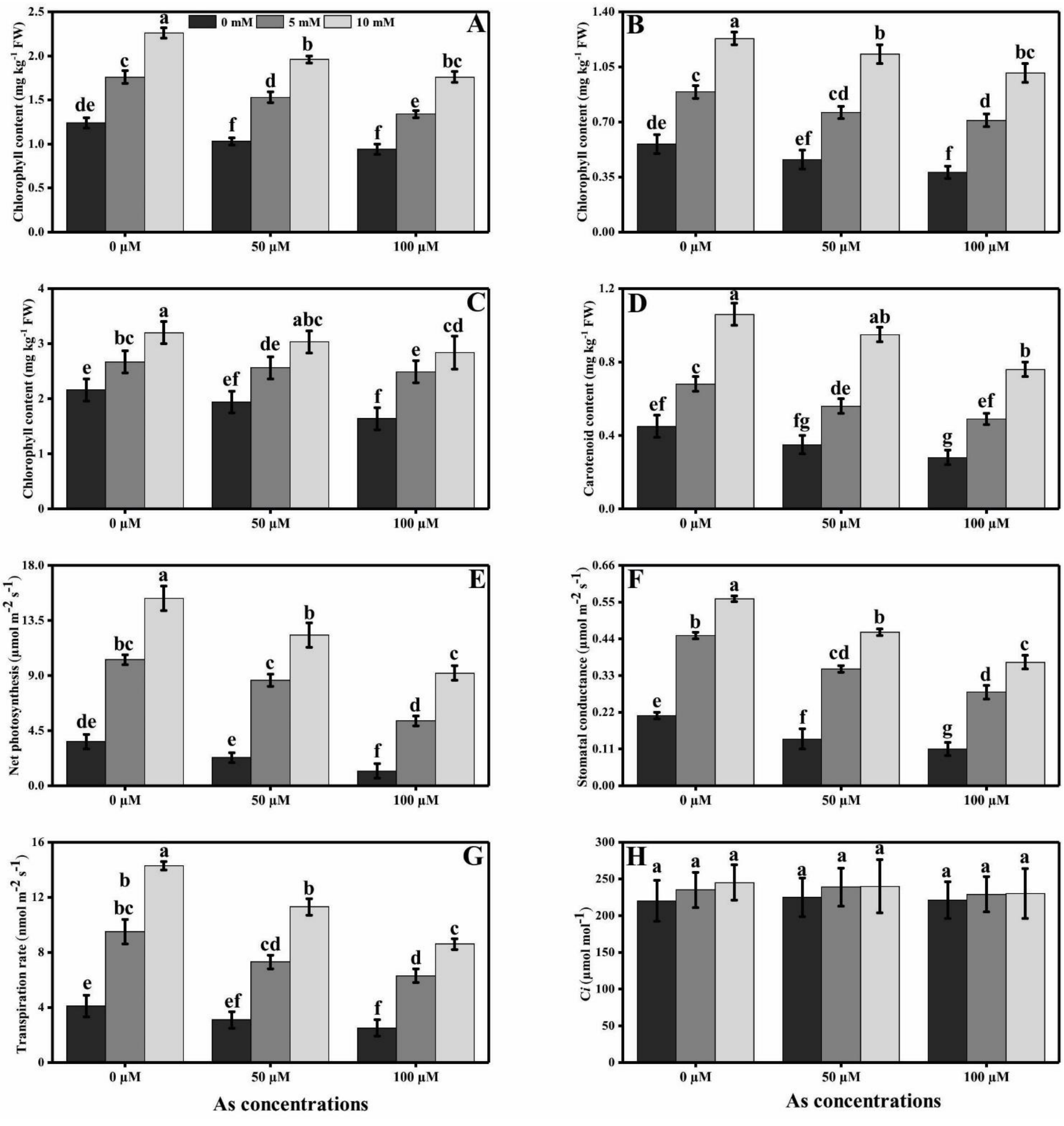

Fig. 2. Effect of various levels of iron−lysine (0, 5 and 10 mM) on chlorophyll-a content (A), chlorophyll-b content (B), total chlorophyll content (C), carotenoid content (D), net photosynthesis, € stomatal conductance (F), transpiration rate (G), and intercellular CO_2 (H) of wheat grown under various stress levels of As (0, 50 and 100 µM). Values are demonstrated as means of four replicates along with standard deviation (SD; n=4). One-way ANOVA was performed and means differences were tested by HSD (p<0.05). Different lowercase letters on the error bars indicate significant difference between the treatments.

Organic acids and As uptake: The influence of As stress and the application of Fe-lys on organic acid exudation and As uptake in wheat is detailed in Fig. 3. As stress prompted a notable increase in the exudation of organic acids, with citric acid levels rising by 58-170%, malic acid by 60-150%, and oxalic acid by 50-140% across the gradient of As concentrations from 50 to 100 µM when compared to the control. The application of Fe-lys effectively reduced the stress-induced exudation, with citric acid decreasing by up to 25-30%, malic acid by 20-28%, and oxalic acid by 22-27% relative to plants without Fe-lys treatment under the same As levels. Arsenic accumulation in the shoots and roots showed a marked increase under As stress, with shoot As concentration elevating by 150-300% and root As concentration by 200-400% as the As levels increased. However, Fe-lys treatment managed to attenuate the As uptake, resulting in a decrease of shoot As concentration by 20-25% and root As concentration by 18-22% compared to the untreated plants under equivalent As stress conditions.

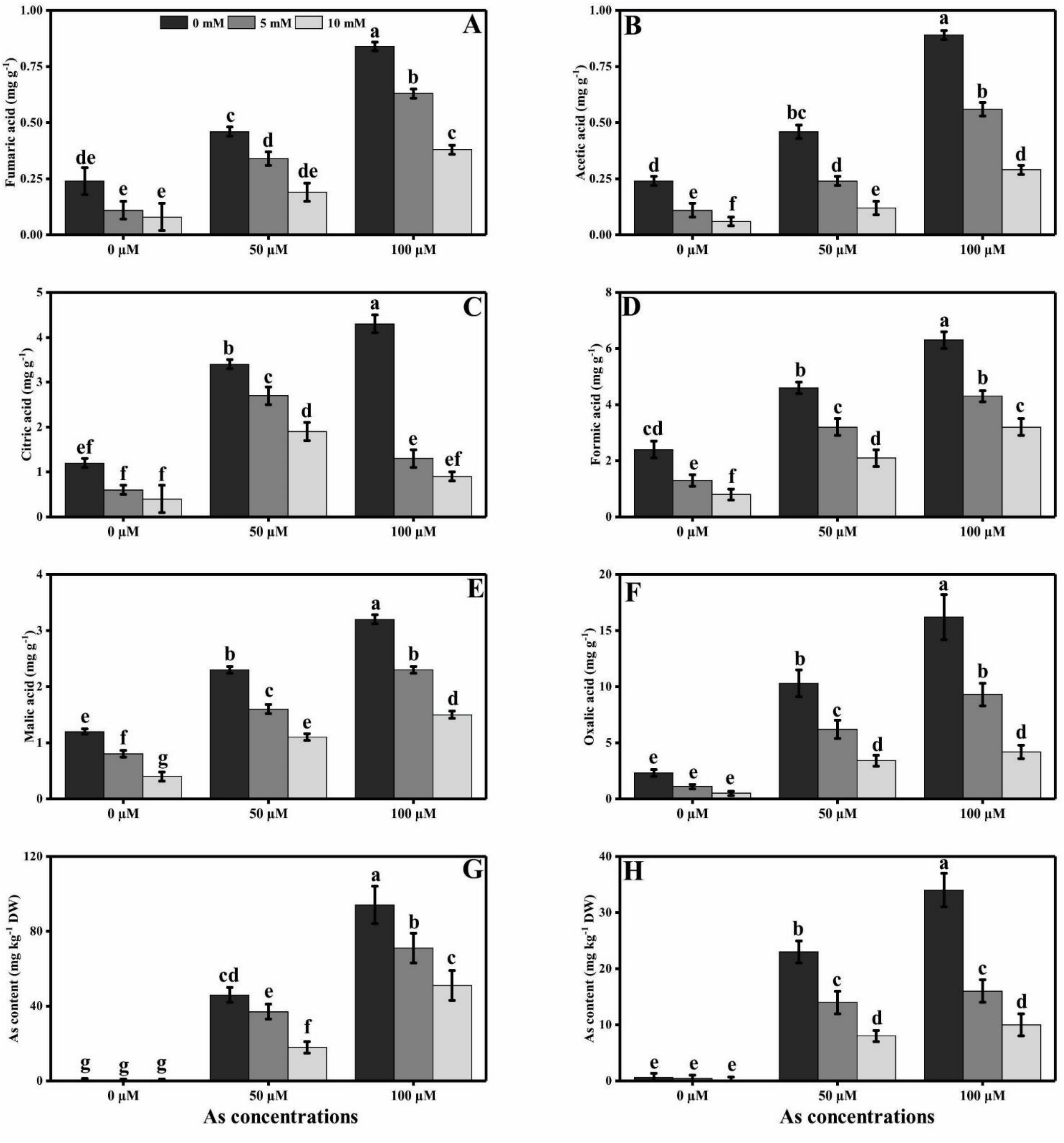

Fig. 3. Effect of various levels of iron−lysine (0, 5 and 10 mM) on fumaric acid contents (A), acetic acid contents (B), citric acid contents (C), formic acid contents (D), malic acid contents (E), oxalic acid contents (F), in the roots and As contents in the roots (G), and As contents in the shoots (H) of wheat grown under various stress levels of As (0, 50 and 100 µM). Values are demonstrated as means of four replicates along with standard deviation (SD; n=4). One-way ANOVA was performed and means differences were tested by HSD (p<0.05). Different lowercase letters on the error bars indicate significant difference between the treatments.

Principal component analysis: The principal component analysis under arsenic stress for all studied parameters is depicted in Fig. 4. The PCA is divided into two components, Dim1 and Dim2, which together account for 95.8% of the variance observed in the dataset, with Dim1 contributing 91% and Dim2 contributing 4.8%. Parameters such as root dry weight, root fresh weight, root length, intercellular CO_2 concentration, stomatal conductance, carotenoid content, chlorophyll b content, leaf area, total chlorophyll, chlorophyll a content, shoot dry weight, number of leaves, and shoot length were all found to be negatively correlated with the PCA. Conversely, the parameters related to organic acid content and arsenic accumulation—specifically, malic acid content, formic acid content, oxalic acid content, fumaric acid content, citric acid content, acetic acid content, arsenic concentration in the shoots, and arsenic concentration in the roots—were positively correlated within the PCA framework.

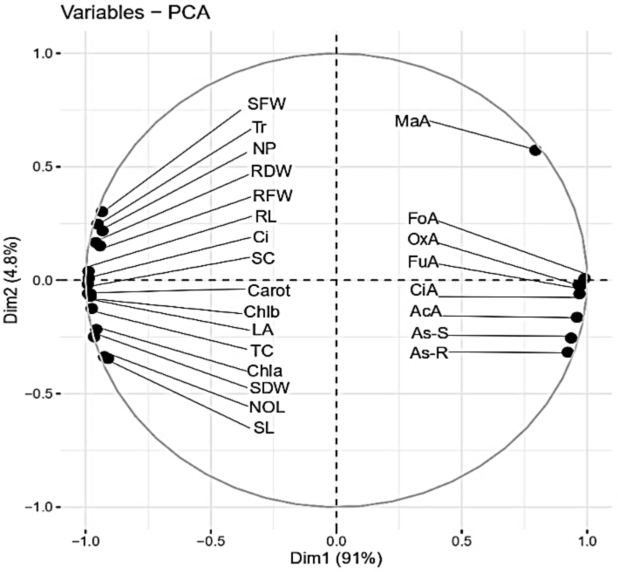

Fig. 4. Loading plots of principal component analysis (PCA) on different studied attributes of wheat grown under various stress levels of As in the soil. Different abbreviations used in the figure are as follows: SFW (shoot fresh weight), Tr (transpiration rate), NP (net photosynthesis), RDW (root dry weight), RFW (root fresh weight), RL (root length), Ci (intercellular CO_2), SC (stomatal conductance), Carot (carotenoid content), Chlb (chlrophyll b content), LA (leaf area), TC (total chlorophyll), Chla (chlorophyll a content), SDW (shoot dry weight), NOL (number of leaves), SL (shoot length), MaA (malic acid content), FoA (formic acid content), OxA (oxalic acid content), FuA (fumaric acid content), CiA (citric acid content), AcA (acetic acid content), As-S (arsenic concentration in the shoots) and As-R (arsenic concentration in the roots).

Discussion

Arsenic toxicity presents a formidable challenge for plant growth and development, with elevated concentrations proving to be alarming and detrimental to normal growth (Saleem *et al.*, 2024). As stress exerts toxic effects on plant physiology, notably impeding biomass accumulation and the photosynthetic capacity of plants (Alsafran *et al.*, 2022). The mechanism behind this involves As's interference with vital cellular functions; it disrupts enzyme activities within the photosynthetic pathway, leads to the generation of reactive oxygen species, and competes with essential nutrients for uptake, thereby stunting growth and reducing photosynthetic efficiency (Mondal *et al.*, 2022). The exudation of organic acids in plants is a recognized response to heavy metal stress, including As (Saleem *et al.*, 2022). These organic acids can bind with metal ions, which may help in sequestering and immobilizing arsenic within the rhizosphere, potentially reducing its uptake by the plants (Ma *et al.*, 2022). However, this process also reflects a stress response that can alter the plant's metabolic balance and energy status (Ma *et al.*, 2022). The observed pattern of increased organic acid exudation correlates with increased As accumulation, suggesting that while this response may be a defense mechanism, it is not sufficient to fully mitigate the uptake and toxicity of As within the plants.

In recent years, researchers have deployed a myriad of strategies to combat metal toxicity in plants, from traditional soil amendments to advanced genetic engineering (Al-Huqail *et al.*, 2023; Li *et al.*, 2023). These methods aim to enhance plant tolerance and reduce the uptake of toxic metals, thus safeguarding plant growth and crop yields (Chen *et al.*, 2024). Utilizing amino acid chelation with essential nutrients is emerging as a novel approach to address this issue. This technique involves coupling micronutrients with amino acids to form complexes that plants can more readily absorb and utilize, improving their resistance to metal-induced stress (Hussain *et al.*, 2018; Zaheer *et al.*, 2019). Iron-lysine is believed to significantly increase plant growth, biomass, and photosynthesis while concurrently decreasing organic acid excretion and As accumulation. The underlying mechanism of Fe-lys involves its ability to form stable chelates with iron, which are easily taken up by plants, thereby enhancing chlorophyll synthesis and overall photosynthetic activity (Zaheer *et al.*, 2020). Additionally, Fe-lys might facilitate the sequestration of As in less bioavailable forms, reducing its translocation to aerial parts of the plant (Okla *et al.*, 2023). This not only curtails the direct toxic effects of As on the plant but also potentially lowers the risk of As entering the food chain.

Conclusion

In conclusion, the present study underscores the significant threat posed by As toxicity to plant growth and highlights the vital need for innovative approaches to mitigate its effects. The use of Fe-lys chelation has emerged as a promising strategy, proving to not only enhance plant growth and photosynthesis but also to reduce the accumulation of As and the exudation of organic acids. This approach leverages the beneficial effects of stable micronutrient chelates, which facilitate improved nutrient uptake and stress resilience in plants, offering a practical solution to a pressing agricultural challenge. For future research, it is recommended to explore the long-term impacts of Fe-lys application on various crops and to assess its efficacy in different soil types and environmental conditions. Further investigation into the molecular mechanisms of Fe-lys's interaction with As could also provide deeper insights into its role in enhancing plant tolerance to heavy metal stress.

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