Coşkun *et al. BMC Plant Biology* (2025) 25:1348
https://doi.org/10.1186/s12870-025-07287-y

BMC Plant Biology

RESEARCH

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Enhancing cucumber tolerance to zinc stress through grafting: a comprehensive study on biochemical, hormonal, and metabolic responses

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Abstract

Background Zinc (Zn) is an essential micronutrient for plant growth and development, yet excessive or deficient Zn levels can disrupt physiological processes and reduce yield by impairing photosynthesis, nutrient uptake, and antioxidant balance. Grafting has been proposed as a promising strategy to enhance plant resilience against abiotic stresses, including heavy metal toxicity, by improving root system efficiency, regulating hormone signaling, and enhancing antioxidant defense. This study aimed to investigate the effects of grafting and different rootstocks on mineral uptake, biochemical responses, and metabolic adjustments in cucumber (*Cucumis sativus* L.) plants exposed to zinc stress under controlled greenhouse conditions.

Results Under zinc-deficient conditions, manganese (Mn) content increased to 26.3 mg/kg in plants grafted onto the TZ148 rootstock, which was approximately 135% higher than in non-grafted plants (11.2 mg/kg). At 10 ppm Zn, catalase (CAT) activity reached 300 units in TZ148-grafted plants (22% higher), while superoxide dismutase (SOD) activity peaked at 760 units in Maximus-grafted plants (53% higher), relative to non-grafted controls. Proline content peaked under 50 ppm Zn, especially in TZ148-Çağla and non-grafted plants, indicating a strong osmotic adjustment response. At 50 ppm Zn, vitamin C content reached 0.12 units in TZ148-grafted plants (50% higher than non-grafted plants). Under zinc deficiency, gallic acid content in the Maximus-Çağla combination increased from 1.8 to 2.4 units, corresponding to a 33% increase compared to the control. Correlation analysis revealed strong positive associations, such as between gallic acid and quercetin ($r = 0.91$) and between delphinidin-3-glucoside and petunidin-3-glucoside ($r = 0.98$), indicating coordinated regulation of related metabolic pathways.

Conclusions The results demonstrate that grafting, especially using the TZ148 rootstock, enhances cucumber tolerance to zinc stress by improving mineral uptake, boosting antioxidant enzyme activities, and promoting the accumulation of stress-related metabolites such as proline, phenolics, and anthocyanins. Rootstock selection, especially TZ148 and Maximus, represents an effective strategy for improving cucumber productivity and quality in Zn-stressed environments, contributing to sustainable agricultural practices.

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Keywords Cucumber, *Cucumis sativus* L., Zinc stress, Grafting, Rootstock, Antioxidant enzymes, Mineral uptake, Proline, Phenolic compounds, Oxidative stress

Introduction

Environmental stresses are among the primary limiting factors for plant growth and productivity. Among these, heavy metal contamination has become increasingly concerning because of its widespread presence in agricultural soils, primarily as a result of industrial activities, urbanization, and the excessive use of agrochemicals [1]. Both essential and non-essential mineral elements may accumulate in soils through fertilizers, pesticides, and industrial waste, thereby disrupting soil-plant interactions and crop performance [2].

Zinc (Zn) is an essential micronutrient involved in numerous physiological and biochemical processes, including carbohydrate metabolism and the activation of several key enzymes such as dehydrogenases, aldolases, and DNA and RNA polymerases [3]. It also contributes to maintaining membrane stability and regulating antioxidant defenses under stress conditions [4]. However, like many other heavy metals, Zn exhibits a narrow optimal concentration range; when present in excess, it becomes toxic to plants by inducing oxidative stress and a nutrient imbalance [5]. High Zn availability in the rhizosphere can reduce the uptake of other essential micronutrients such as iron (Fe), manganese (Mn), and copper (Cu) by affecting membrane transport processes and modifying the soil pH buffering capacity [6]. Zn toxicity can also impair photosynthetic capacity, inhibit Fe uptake, and alter chloroplast structure, ultimately resulting in growth inhibition and physiological dysfunction [6].

The recognition of the adverse effects of heavy metals on plant performance and human health has intensified efforts to understand their behavior in agroecosystems and to develop effective mitigation strategies. Plants naturally possess adaptive mechanisms such as the activation of antioxidant systems, reinforcement of cell walls, and associations with mycorrhizal fungi to limit the damage caused by heavy metal toxicity [1, 6]. Additional strategies include vacuolar sequestration of metals and synthesis of chelating molecules such as phytochelatins [7]. Strengthening such defense responses in agricultural crops is crucial for maintaining yield stability and ensure environmental sustainability. In recent years, grafting has emerged as a promising approach for enhancing plant tolerance to abiotic stresses, including heavy metal toxicity [8, 9]. Grafting involves the union of two different plant segments: the rootstock, responsible for water and nutrient uptake, and the scion, which contributes to photosynthesis and fruit production [10]. This technique not only improves plant vigor but also enhances resistance to environmental stresses through rootstock-mediated physiological and biochemical modifications [11]. Recent advances show that grafting enhances stress tolerance at both physiological and molecular levels, including modulation of antioxidant pathways, hormonal signaling, and gene expression associated with stress responses [8, 12, 13]. Additionally, grafting has been reported to mitigate heavy metal translocation in crops while maintaining nutrient balance, thereby preserving photosynthetic efficiency under stress [14]. In cucumber (*Cucumis sativus* L.), a major horticultural crop with a global production exceeding 97 million tons annually [15], grafting is commonly performed using Cucurbita species as rootstocks due to their robust root systems and tolerance to various soil-borne stresses, including heavy metal contamination [16]. It has been reported that certain rootstocks can restrict the translocation of toxic elements such as Zn from roots to shoots, thereby reducing oxidative damage and improving plant resilience [10].

Despite the economic importance of cucumber and the increasing prevalence of soil Zn contamination, there remains a significant knowledge gap regarding the physiological responses of grafted cucumber plants to Zn stress. Moreover, limited data are available on the comparative performance of different rootstocks under Zn toxicity. Therefore, this study aims to investigate the effects of zinc stress on nutrient uptake, oxidative stress responses, and metabolic processes in cucumber plants grafted onto different rootstocks. The primary objective is to evaluate the ability of these rootstocks to alleviate zinc-induced nutrient imbalances and oxidative damage. The findings are expected to provide valuable insights into the use of grafting as a sustainable strategy for improving crop performance in Zn-stressed environments, particularly in regions facing micronutrient toxicity or deficiency in soils.

Materials and methods

This research was conducted during the summer and fall of 2023 in a controlled greenhouse at the Faculty of Agriculture, Hatay Mustafa Kemal University. The greenhouse conditions, including average temperature (25 ± 2 °C) and average relative humidity (70 ± 5%), were monitored daily and maintained as consistently as possible throughout the experiment. The experiment was arranged in a factorial design (4 zinc levels × 5 grafting combinations) using a Completely Randomized Design (CRD) with three replicates per treatment. Non-grafted cucumber plants served as the control group, while grafting was applied to both self-grafted and heterologous rootstock combinations. All plant materials used in the experiment were cultivated varieties sourced from certified commercial suppliers. The scion cultivar used in this study was Çağla (mini cucumber type), selected for its commercial importance and known graft compatibility, and the rootstocks employed were Cremna, Maximus, and TZ148. The scion cultivar (*Cucumis sativus* L. cv. Çağla, mini cucumber type) was obtained from Smyrna Seeds (İzmir, Türkiye). The rootstocks used in this study were Cremna F1 (provided by Seminis, a Bayer Crop Science company), TZ148 (obtained from HM.Clause), and Maximus F1 (provided by Syngenta), selected for their strong root systems, resistance to Fusarium spp. and other soil-borne pathogens, and adaptability to various abiotic stresses. All three are commercially available interspecific hybrids derived from *Cucurbita maxima* × *C. moschata*.

To ensure optimal graft compatibility, the cucumber scion seeds were sown four days earlier than the rootstock seeds. Grafting was carried out using the single cotyledon inclined-cut method, and the grafted seedlings underwent an acclimatization phase under high humidity and low light conditions. Following successful healing, the seedlings were transferred to 8-liter hydroponic containers. The plants were subjected to four different zinc treatments: control (0.05 ppm Zn, ZnC), zinc deficiency (Zn−), and zinc excess (10 ppm and 50 ppm Zn, denoted as Zn10 and Zn50, respectively). The nutrient solution, prepared according to a modified Hoagland and Arnon [17] formulation, contained all essential macro- and micronutrients and was adjusted for zinc levels according to the treatments. The experimental period lasted for four weeks after transplanting.

Mineral Element Profiling

Plant samples were collected at the end of the 4-week zinc treatment period, corresponding to 28 days after transplanting, with no intermediate sampling points. Following harvesting, samples were either snap-frozen in liquid nitrogen for biochemical analyses or oven-dried at 65 °C for mineral determination. For the analysis of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), manganese (Mn), and copper (Cu), 100 mg of dried plant tissue was boiled in 5 mL of Milli-Q water, filtered, and analyzed by high-performance liquid chromatography (HPLC) as described previously [18].

Analysis of Antioxidant Enzymes and Lipid Peroxidation

The level of lipid peroxidation was quantified by measuring malondialdehyde (MDA) content using the thiobarbituric acid (TBA) method. Enzyme activities of peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) were measured in protein extracts prepared with phosphate buffer (pH 7.0). CAT activity was determined based on the rate of hydrogen peroxide decomposition, while SOD activity was measured via inhibition of nitro-blue tetrazolium (NBT) reduction at 560 nm [19]. All spectrophotometric measurements were carried out using a Shimadzu 1208 UV spectrophotometer.

Hormone Analysis

Plant hormone levels were analyzed following the protocol of Kuraishi et al., 1991 [20]. Leaf and root tissues were homogenized and purified using Sep-Pak C-18 cartridges. Hormonal compounds were eluted with 80% methanol and analyzed by HPLC using a Zorbax Eclipse-AAA C-18 column. The detection wavelength was set at 265 nm for quantification of gibberellic acid (GA_3), salicylic acid (SA), indole acetic acid (IAA), and abscisic acid (ABA) [21].

Phenolic Compounds Analysis

Homogenized fruit samples were prepared for the determination of phenolic compounds including gallic acid, vanillic acid, trans-p-coumaric acid, ferulic acid, quercetin, and rutin. Quantification was performed using an Agilent 1100 HPLC system following established protocols [22].

Organic Acid Profiling

Plant samples collected at 28 days after transplanting were analyzed for organic acids such as oxalic, propionic, tartaric, butyric, malic, and lactic acids. The analysis was conducted using an Agilent 1200 HPLC system, with detection carried out at a wavelength of 220 nm [23].

Amino Acid Profiling

One gram of lyophilized plant tissue was dissolved in 0.1 M hydrochloric acid and sonicated to extract amino acids. The resulting supernatant was derivatized with o-phthaldialdehyde (OPA) and analyzed by HPLC using a C18 reverse-phase column as described in the literature [24].

Anthocyanin Profiling

Anthocyanin content in plant tissues was determined using a modified version of the HPLC method described by Durst and Wrolstad, 2001 [25]. The analysis was conducted with Agilent ChemStation software, and anthocyanin concentrations were calculated based on comparisons of retention times with known standards.

Statistical Analysis

Statistical analyses were performed using SAS software (version 9.1) and SPSS software (version 17.0). A significance level of $p < 0.01$ and $p < 0.001$ was applied for all tests. Each treatment group consisted of three biological replicates ($n = 3$), with 10 plants per replicate. Data were subjected to analysis of variance (ANOVA), and mean comparisons were conducted using Duncan's multiple range test. Correlation analyses were also performed to examine relationships among variables.

Results

Effect of zinc levels and grafting status on element accumulation

The application of varying zinc (Zn) levels in combination with different grafting strategies significantly influenced the accumulation of macro- and microelements in cucumber plants. Zn deficiency (Zn−) markedly reduced the uptake of essential elements, particularly manganese (Mn) and copper (Cu), in non-grafted plants. Under Zn – conditions, Mn content in non-grafted plants was 11.2 mg/kg, while Cu decreased to 2.02 mg/kg. In contrast, TZ148-grafted plants maintained Mn levels at 26.3 mg/kg and Ca content at 1.05 g/kg, representing a substantial increase compared to non-grafted controls. Cremna-grafted plants also showed moderate improvements, especially in Cu (2.83 mg/kg) and N (0.96 g/kg) accumulation. Under ZnC (control) conditions, TZ148-grafted plants exhibited the highest Ca (1.23 g/kg) and N (1.18 g/kg) contents among all treatments. When exposed to excess Zn (Zn10 and Zn50), the differences between grafted and non-grafted plants became more pronounced. TZ148-grafted plants reached 59.8 mg/kg Mn and 8.65 mg/kg Cu under Zn10, while non-grafted plants recorded 52.1 mg/kg Mn and 8.02 mg/kg Cu. P content in TZ148-grafted plants peaked at 54.2 mg/kg under Zn50, compared to 48.1 mg/kg in non-grafted plants. K content was also highest in TZ148 (8.31 mg/kg) under Zn50. Maximus rootstock performed particularly well in Mn and Cu accumulation under Zn toxicity, with Mn reaching 57.6 mg/kg and Cu 9.02 mg/kg under Zn10. Cremna's improvements were less pronounced overall but still contributed to higher Cu and N levels compared to non-grafted plants under Zn stress. Overall, grafted plants—especially TZ148—maintained more balanced mineral profiles under both Zn deficiency and excess. TZ148 exhibited superior uptake of N, Mn, P, and K, indicating strong nutrient acquisition and redistribution capacity under Zn stress. Maximus followed closely for Mn and Cu, while Cremna offered moderate benefits. These results confirm that rootstock genotype plays a critical role in modulating nutrient uptake efficiency and homeostasis in cucumber under zinc stress (Fig. 1).

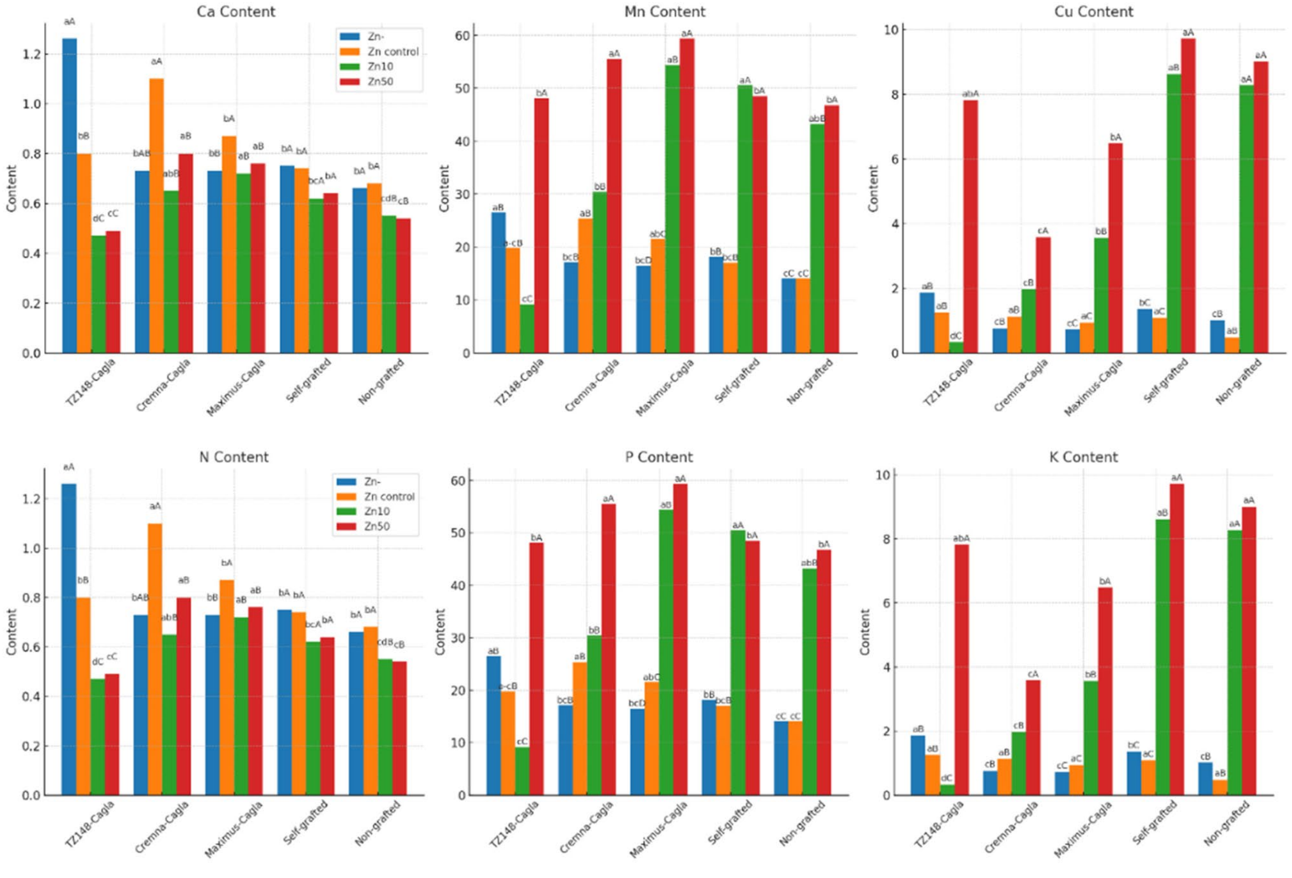

Fig. 1 Effect of grafting on element content in cucumber under zinc stress

Effect of zinc levels and grafting status on antioxidant enzyme activity

Antioxidant enzyme activities varied markedly with Zn treatments and grafting status. Under Zn deficiency (Zn–), CAT and SOD activities were highest in TZ148-grafted plants, reaching 320 units and 1250 units, respectively, compared to 260 units and 1020 units in non-grafted controls. POX activity under Zn – was also substantially greater in TZ148-grafted plants (15,800 units) than in non-grafted plants (2,300 units), indicating stronger antioxidant capacity. Under Zn10, enzyme activities remained relatively high in grafted combinations, particularly TZ148 (300 CAT units, 1200 SOD units, 16,200 POX units) and Maximus (290 CAT units, 1180 SOD units, 14,900 POX units). At Zn50, non-grafted plants exhibited slightly higher CAT activity (285 units) than most grafted plants, whereas grafted plants—especially TZ148—maintained high POX levels (17,500 units) and elevated SOD activity (1150 units), suggesting a shift towards alternative antioxidant pathways. Oxidative stress markers confirmed these trends. H_2O_2 content varied little between Zn– (18–21 units) and ZnC treatments (20–22 units), but under Zn50 it was highest in non-grafted plants (48 units), while TZ148-grafted plants limited accumulation to 30 units. MDA content peaked under Zn50 in non-grafted plants (24.5 units) and was markedly lower in TZ148-grafted plants (16.2 units), indicating reduced lipid peroxidation. Proline accumulation was highest under Zn50 in TZ148-grafted plants (0.85 units) and non-grafted plants (0.75 units), corresponding to an 13% increase over control, supporting enhanced osmoprotective responses in grafted combinations (Fig. 2).

Effect of zinc stress on hormonal changes and the role of grafting

Zinc availability markedly influenced the endogenous hormone profiles of cucumber plants, with grafting status playing a critical role in modulating these responses. Under zinc deficiency (Zn−), reductions were observed in key phytohormones such as indole-3-acetic acid (IAA), abscisic acid (ABA), cytokinins, jasmonic acid (JA), vitamin C, and vitamin E across all genotypes. In non-grafted plants, IAA levels decreased to 0.10 units, whereas TZ148-grafted plants maintained significantly higher levels at 0.18 units. ABA concentrations in non-grafted plants were 5,000 units, while TZ148-grafted plants retained slightly higher values (5,500 units).

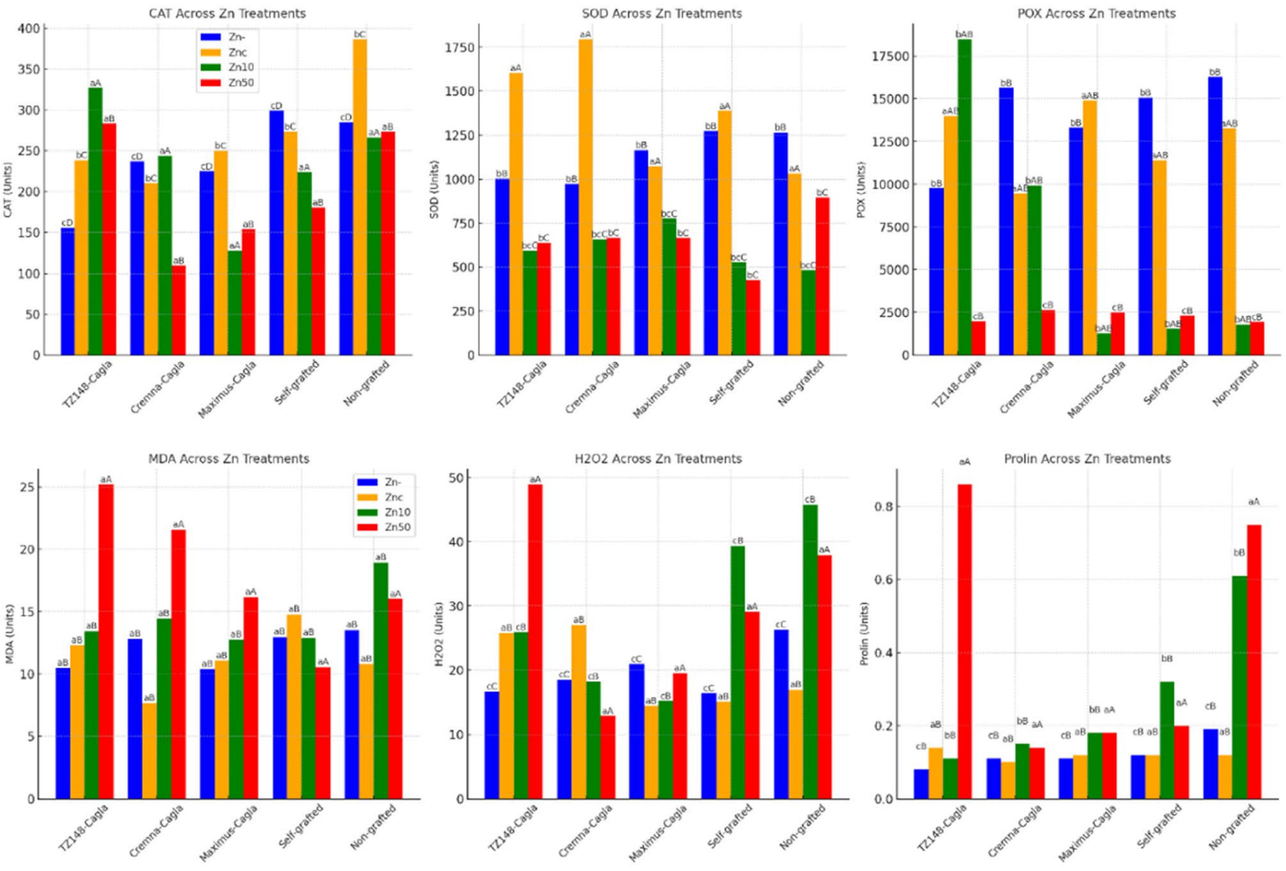

Fig. 2 Effect of grafting on antioxidant enzyme content in cucumber under zinc stress

Cytokinin levels were 0.28 units in non-grafted plants compared to 0.35 units in TZ148-grafted plants, indicating a rootstock-mediated stabilization of hormonal status under micronutrient stress. As Zn availability increased (ZnC, Zn10, Zn50), hormone concentrations rose, particularly in grafted plants. Under ZnC, TZ148-grafted plants reached 0.24 units IAA, 5,200 units ABA, and 0.35 units cytokinins. In Zn10 treatments, IAA increased to 0.32 units in TZ148-grafted plants, with ABA levels of 5,500 units and cytokinins at 0.45 units. Under Zn50 conditions, IAA levels peaked at 0.36 units in TZ148-grafted plants, representing a 3.6-fold increase compared to Zn− in non-grafted controls. Cytokinin content reached 1.10 units in TZ148-grafted plants, while Maximus-grafted plants exhibited elevated cytokinin concentrations between 0.80 and 1.00 units. Jasmonic acid (JA), a hormone linked to stress signaling, rose with increasing Zn levels. In non-grafted plants, JA increased from 1.15 units under Zn− to 1.50 units under Zn50. In contrast, Maximus-grafted plants reached 2.00 units JA under Zn50, while TZ148-grafted plants had 1.85 units. Vitamin C content was generally stable across treatments but showed slight increases under Zn50, with TZ148-grafted plants reaching 0.135 units compared to 0.125 units in non-grafted plants. Vitamin E content followed a similar trend, with non-grafted plants increasing from 0.15 units under Zn− to 0.175 units under Zn50, and TZ148-grafted plants increasing from 0.15 units to 0.18 units over the same range. In terms of ABA regulation, non-grafted plants maintained values close to 5,000 units across Zn treatments, whereas grafted plants—particularly TZ148—showed a moderate increase under high Zn, reaching 6,800 units at Zn50. Cremna-grafted plants displayed intermediate hormonal profiles, with cytokinin values ranging between 0.50 and 0.70 units across Zn treatments (Fig 3).

Effect of zinc levels and grafting status on phenolic compound accumulation

Zinc stress markedly affected the accumulation of phenolic compounds, with grafted plants generally maintaining higher concentrations than non-grafted ones under both deficiency and toxicity conditions. Under Zn deficiency (Zn−), gallic acid content was 1.8 µg/g in TZ148-grafted, 2.0 µg/g in Cremna-grafted, 1.9 µg/g in Maximus-grafted, 1.7 µg/g in self-grafted, and 1.8 µg/g in non-grafted plants. Vanillic acid ranged from 4.0 µg/g in Maximus-grafted to 4.8 µg/g in TZ148-grafted plants. Trans-p-coumaric acid content varied between 1.8 µg/g (Maximus-grafted) and 2.1 µg/g (TZ148-grafted), while

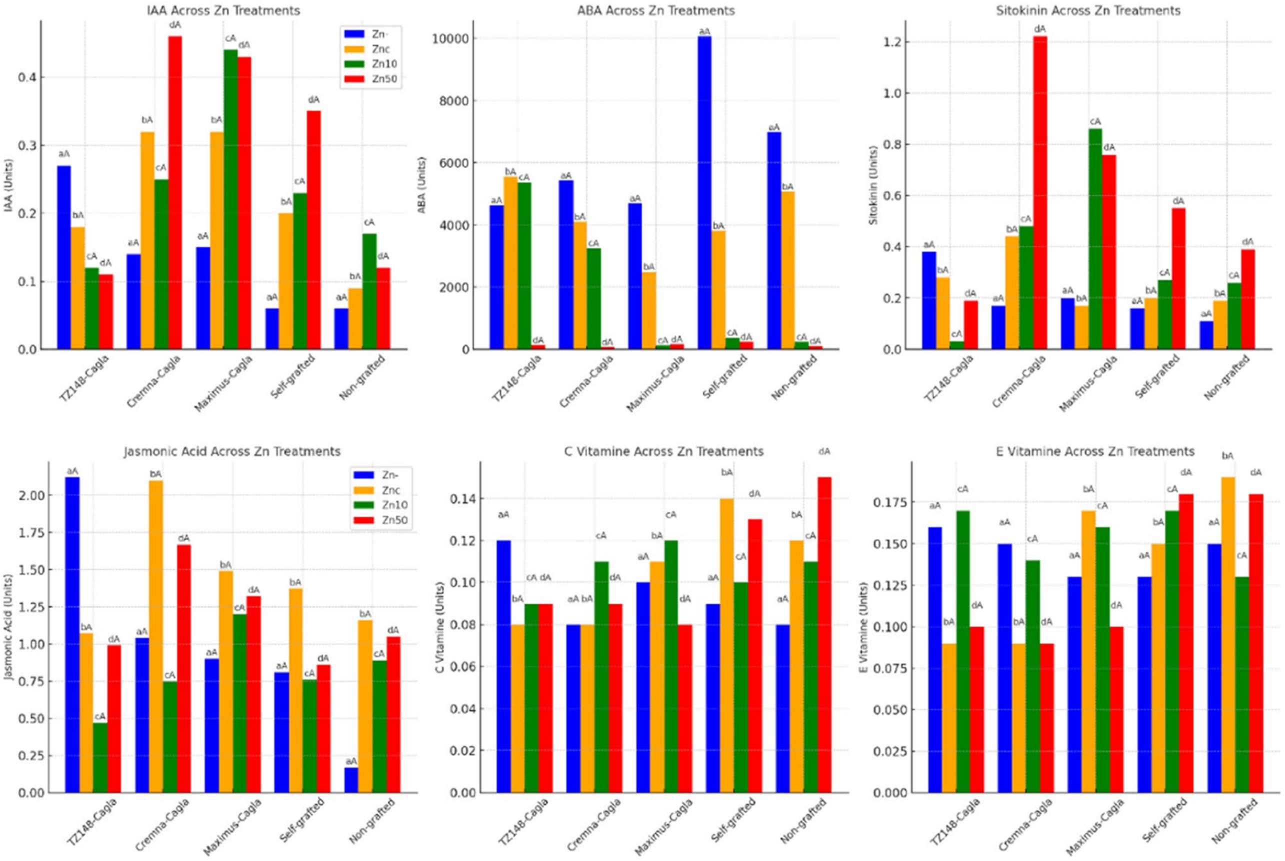

Fig. 3 Effect of grafting on hormone content in cucumber under zinc stress

ferulic acid levels were lowest in Maximus-grafted plants (1.8 µg/g) and highest in TZ148-grafted plants (2.2 µg/g). Under Zn50 treatment, phenolic compound levels increased sharply. Gallic acid reached 4.7 µg/g in TZ148-grafted plants, 4.4 µg/g in Cremna-grafted, 4.5 µg/g in Maximus-grafted, 4.3 µg/g in self-grafted, and 4.1 µg/g in non-grafted plants. Vanillic acid peaked at 8.6 µg/g in Maximus-grafted plants, followed by TZ148-grafted at 8.0 µg/g. Trans-p-coumaric acid reached 6.7 µg/g in self-grafted and 6.6 µg/g in TZ148-grafted plants. Ferulic acid content was highest in non-grafted plants (9.1 µg/g) and TZ148-grafted plants (8.3 µg/g). Flavonoid accumulation was also stimulated under Zn50. Quercetin levels peaked at 8.2 µg/g in self-grafted plants, followed by 7.9 µg/g in Cremna-grafted and 7.8 µg/g in Maximus-grafted plants. Rutin content was highest in self-grafted plants (5.9 µg/g) and Cremna-grafted plants (5.8 µg/g) (Fig 4).

Effect of zinc levels and grafting status on organic acid contents

Zinc availability significantly affected organic acid metabolism in cucumber plants, with distinct responses observed among grafted and non-grafted genotypes. Under Zn deficiency (Zn−), a general reduction in organic acid accumulation was evident across all treatments. In TZ148-grafted plants, oxalic acid levels decreased to 18.0 mg/g under Zn – but rose to 30.0 mg/g under Zn50, representing a 40% increase with Zn toxicity. Similarly, lactic acid content in Maximus-grafted plants increased from 15.0 mg/g under Zn – to 24.0 mg/g under Zn50, suggesting that elevated Zn levels stimulate the biosynthesis or accumulation of key organic acids. Butyric acid content showed substantial variation among genotypes. Under Zn50, Maximus-grafted plants recorded the highest butyric acid levels at 34.0 mg/g, indicating a marked increase compared to both Zn – and ZnC conditions. Propionic acid concentrations also followed an upward trend, rising from 18.0 mg/g under Zn – to 31.0 mg/g under Zn50 in Cremna-grafted plants. The accumulation of malic acid was notably higher in Maximus-grafted plants under Zn10 (19.0 mg/g) compared to non-grafted plants (10.0 mg/g), suggesting an enhanced carbon flow toward the tricarboxylic acid cycle and related pathways. Additionally, lactic acid content in TZ148-grafted plants was 5% higher under Zn50 compared to non-grafted plants, reflecting improved metabolic activity under stress (Fig. 5). Overall, the data indicate that grafted plants, particularly those grafted onto Maximus and TZ148, exhibited superior organic acid responses under Zn stress. These organic acids are

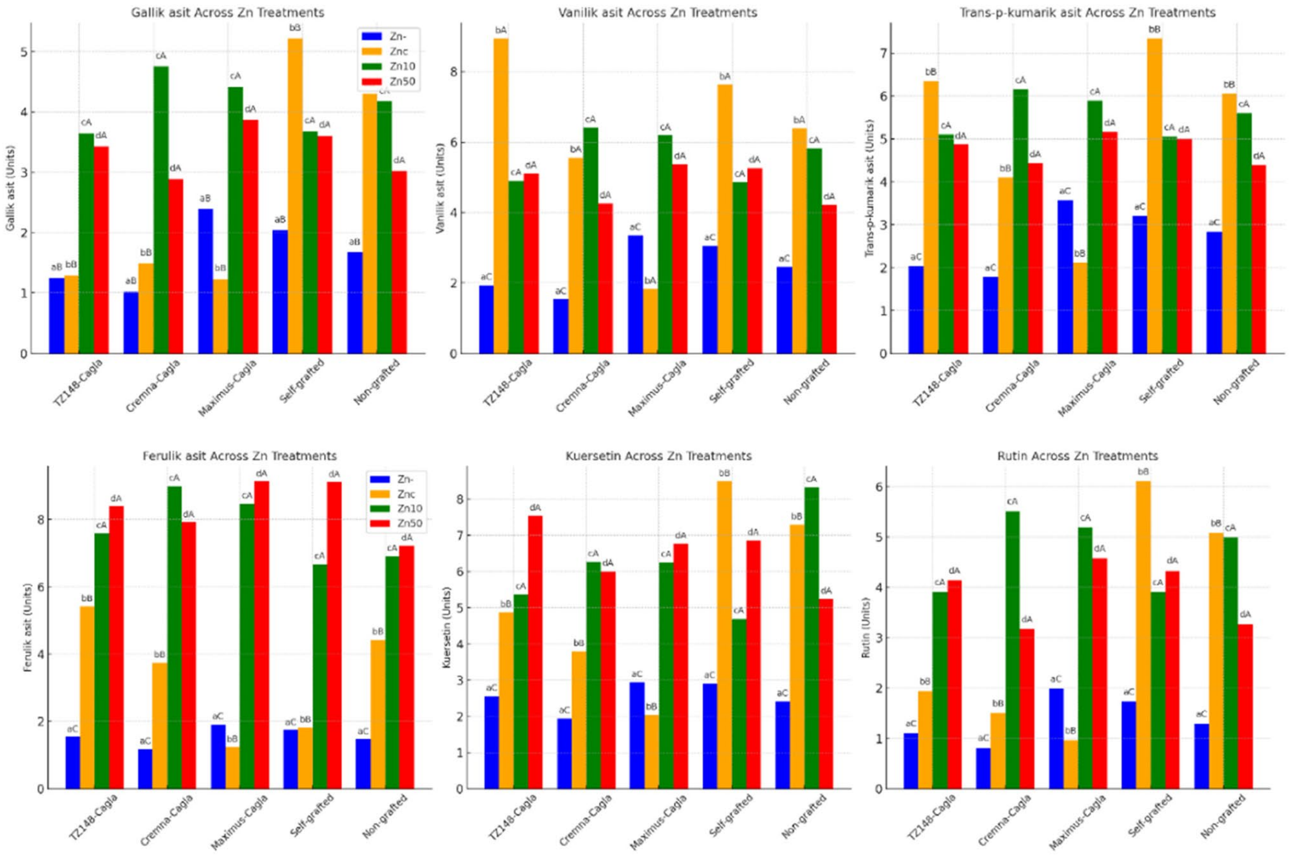

Fig. 4 Effect of grafting on phenol content in cucumber under zinc stress

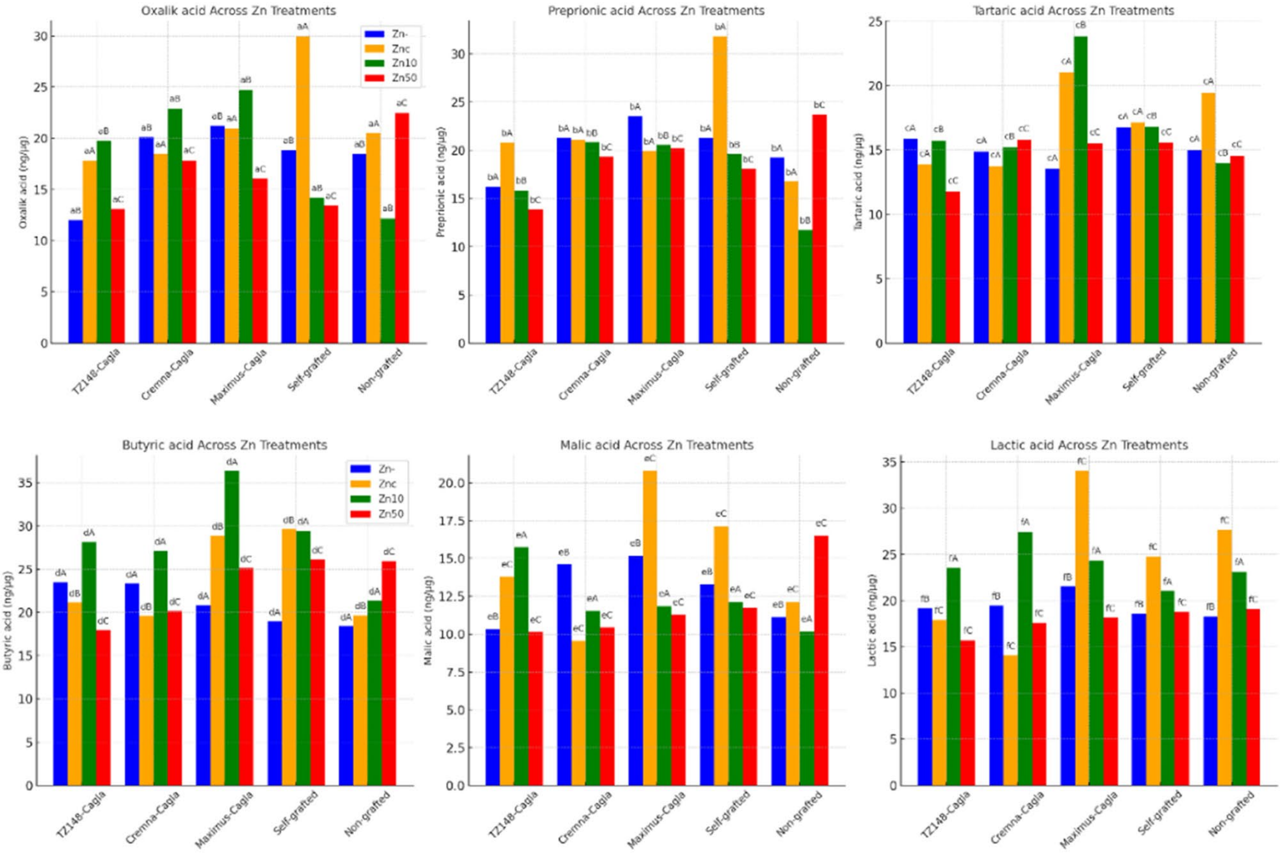

Fig. 5 Effect of grafting on organic acid content in cucumber under zinc stress

known to play crucial roles in metal chelation, pH regulation, and cellular redox buffering.

Effect of zinc levels and grafting status on amino acid content

Zinc stress markedly altered the amino acid profile of cucumber plants, with grafted combinations generally maintaining higher concentrations than non-grafted controls. Under Zn deficiency (Zn−), aspartate content in non-grafted plants was 455 µmol L^{-1}, whereas TZ148- and Maximus-grafted plants accumulated 560 µmol L^{-1} and 540 µmol L^{-1}, respectively. Glutamate levels in non-grafted plants were 190 µmol L^{-1}, while TZ148- and Maximus-grafted plants reached 255 µmol L^{-1} and 250 µmol L^{-1}, respectively. Tryptophan content under Zn – was 280 µmol L^{-1} in non-grafted plants, increasing to 500 µmol L^{-1} in Cremna-grafted plants and 480 µmol L^{-1} in Maximus-grafted plants under Zn50. Phenylalanine content under Zn – was 780 µmol L^{-1} in Maximus-grafted plants and 800 µmol L^{-1} in Cremna-grafted plants, compared to 620 µmol L^{-1} in non-grafted controls. Under Zn toxicity (Zn50), aspartate levels reached 680 µmol L^{-1} in TZ148-grafted plants and 660 µmol L^{-1} in Maximus-grafted plants but remained at 510 µmol L^{-1} in non-grafted plants. Arginine content peaked at 430 µmol L^{-1} in Maximus-grafted plants, while non-grafted plants contained 370 µmol L^{-1}. Methionine concentrations under Zn50 were 200 µmol L^{-1} in TZ148-grafted plants compared to 170 µmol L^{-1} in non-grafted plants. These results indicate that grafted plants, particularly those grafted onto TZ148 and Maximus, are more effective in sustaining or enhancing amino acid synthesis under both Zn deficiency and toxicity, supporting their improved metabolic resilience under stress (Fig 6).

Effect of zinc levels and grafting status on anthocyanin profile

Zinc stress and grafting significantly affected anthocyanin accumulation, with clear genotype-dependent differences. Under Zn deficiency (Zn−), anthocyanin biosynthesis was reduced in all plants; however, TZ148-grafted plants maintained comparatively higher delphinidin-3-glucoside (1.8%) and cyanidin-3-glucoside (1.2%) than non-grafted controls (1.5% and 0.8%, respectively). High zinc (Zn50) markedly stimulated anthocyanin accumulation, with delphinidin-3-glucoside reaching 5.2% in TZ148-grafted and 5.0% in Maximus-grafted plants versus 4.8% in non-grafted plants. Petunidin-3-glucoside peaked at 12.0% in TZ148-grafted plants, while peonidin-3-glucoside reached 110% relative absorbance

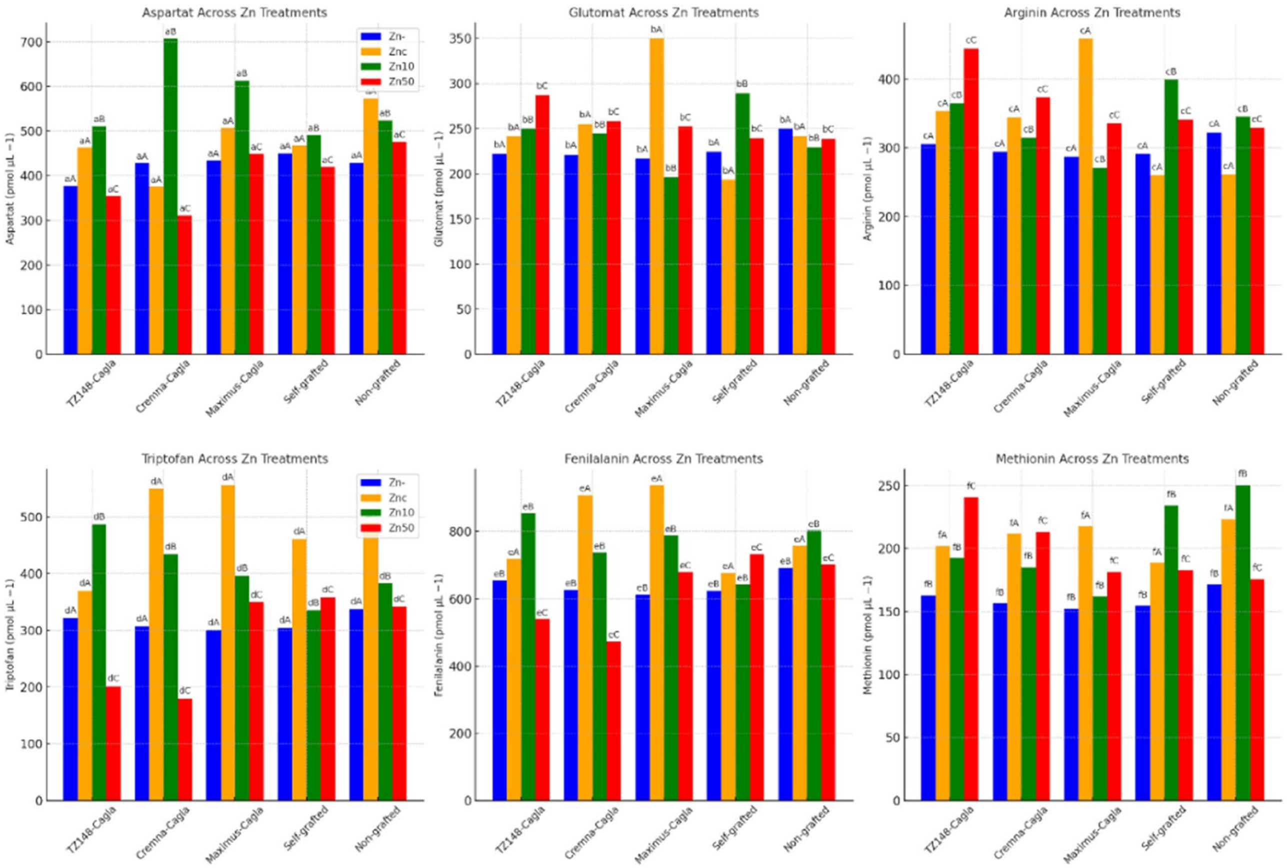

Fig. 6 Effect of grafting on amino acid content in cucumber under zinc stress

in self-grafted plants and 105% in non-grafted plants. Malvidin-3-glucoside content was highest in non-grafted plants (88%) under Zn50, followed closely by TZ148-grafted (82%) and Maximus-grafted (80%) plants. Peonidin-3-glucoside acetyl content peaked at 100% in non-grafted plants under Zn50, while TZ148-grafted plants reached 92%. Grafting consistently enhanced anthocyanin stability and content, with TZ148 showing the strongest response, followed by Maximus and Cremna. Self-grafted plants displayed intermediate values, suggesting a general grafting-induced stimulation of flavonoid metabolism (Fig 7).

Correlations among biochemical markers

Correlation analysis was conducted to identify potential interactions and co-regulation among key biochemical traits influenced by zinc stress and grafting. The resulting heatmap revealed strong associations between several oxidative stress-related parameters, osmoprotectants, and secondary metabolites, highlighting the integrated nature of the plant's stress response system (Fig. 8). The most pronounced positive correlation was observed between malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) ($r = 0.78$), reflecting their common role as markers of oxidative damage. Proline content also showed a positive correlation with H_2O_2 ($r = 0.38$), suggesting that osmoprotectant accumulation is linked to oxidative stress signaling. In contrast, catalase (CAT) and superoxide dismutase (SOD) exhibited a weak negative correlation ($r = -0.22$), indicating that their activities may not be directly co-regulated under zinc stress. Negative correlations were also evident. Abscisic acid (ABA) and cytokinins displayed an inverse relationship ($r = -0.55$), consistent with their antagonistic roles in stress and growth regulation. A moderate negative correlation was detected between potassium (K^+) and MDA ($r = -0.35$), implying that K^+ homeostasis may mitigate oxidative lipid damage. Additionally, calcium (Ca^{2+}) and proline showed a negative correlation ($r = -0.42$), suggesting possible crosstalk between calcium signaling and osmotic adjustment mechanisms. Phenolic compounds demonstrated highly coordinated behavior. For example, gallic acid and quercetin exhibited a correlation coefficient of $r = 0.92$, while ferulic acid and vanillic acid were strongly correlated ($r = 0.85$), indicating shared regulatory pathways in secondary metabolism. Similarly, rutin and quercetin ($r = 0.88$) also displayed strong co-accumulation trends. Organic acids also showed significant

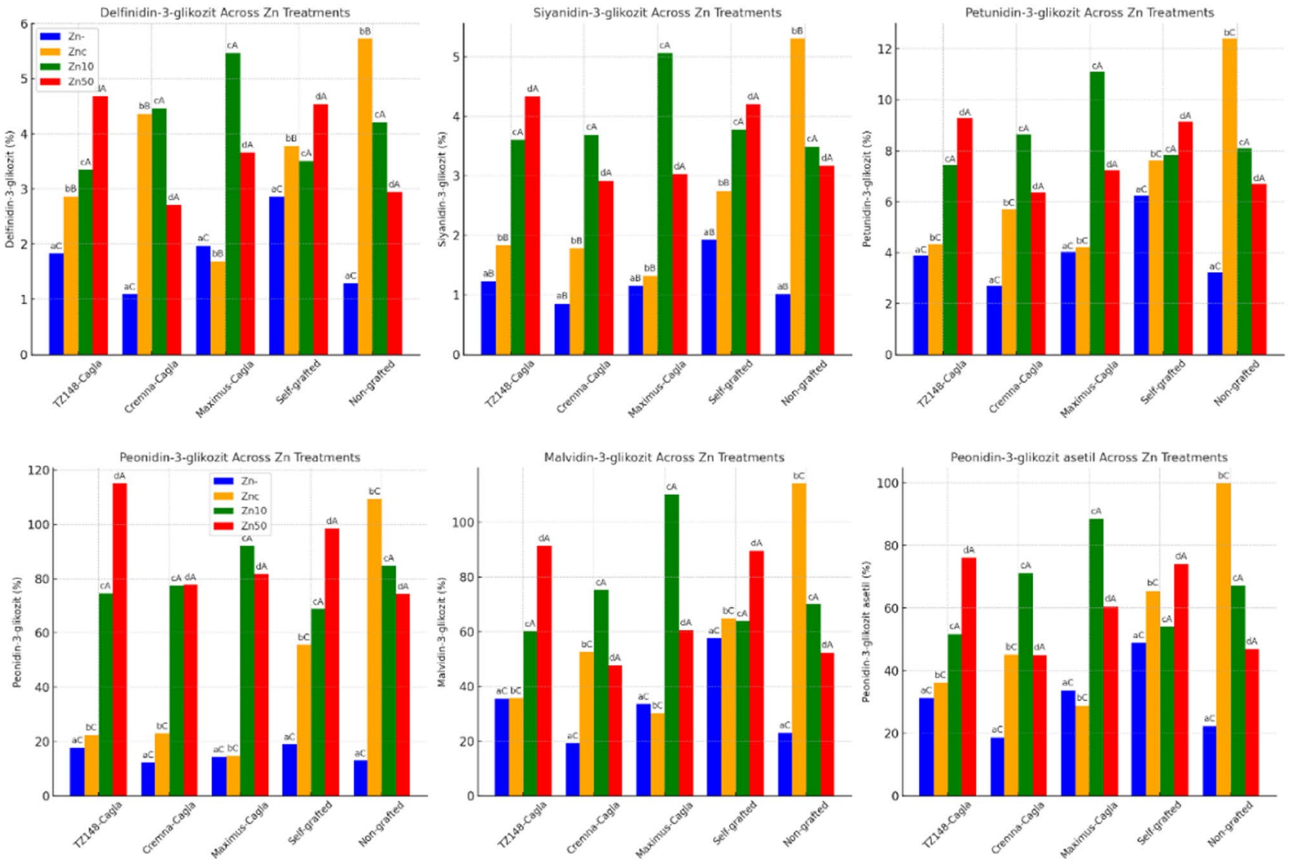

Fig. 7 Effect of grafting on anthocyanin content in cucumber under zinc stress

associations; tartaric acid and malic acid were positively correlated ($r = 0.85$), reflecting linked roles in energy metabolism and pH buffering. Oxalic acid and malic acid ($r = 0.80$) as well as lactic acid and butyric acid ($r = 0.77$) further supported this metabolic connectivity. In contrast, several weak correlations pointed to independently regulated traits. Tryptophan and H_2O_2 ($r = 0.05$) and arginine and vitamin C ($r = 0.11$) had negligible associations, suggesting distinct regulation patterns. Similarly, methionine and CAT ($r = -0.08$) showed almost no relationship. Overall, these findings highlight the complexity of physiological responses under zinc stress and demonstrate the value of correlation analysis in revealing potential interaction networks among biochemical parameters.

Discussion

Heavy metal pollution, particularly involving elements such as zinc, is a major environmental challenge that adversely affects plant growth, metabolism, and productivity by causing oxidative damage, disrupting nutrient balance, and inhibiting physiological processes [26]. In response, plants have evolved a variety of defense mechanisms to mitigate the toxic effects of heavy metals. One such strategy, grafting, has emerged as a promising agronomic technique to improve plant resilience, productivity, and overall physiological performance under stressful conditions [27]. The present study provides compelling evidence supporting the efficacy of *C. maxima* × *C. moschata* hybrid rootstocks in mitigating Zn-induced stress in cucumber plants by enhancing nutrient uptake, maintaining oxidative balance, and regulating stress-related metabolic pathways. These rootstocks not only contributed to stress tolerance but also positively influenced phytochemical accumulation, nutrient uptake, and oxidative balance [28]. The observed benefits of grafting in this study are consistent with previous reports involving other crops, such as watermelon [29] and tomato [30], where grafted plants demonstrated enhanced resistance to heavy metal-induced stress. For instance, the reduction of cadmium accumulation in grafted tomato plants highlights the potential of specific rootstocks in minimizing heavy metal translocation and enhancing plant defense mechanisms [31]. The variability in response among different rootstocks emphasizes the critical role of genotype selection in tailoring rootstock-scion combinations for optimal stress adaptation [32].

Nutrient uptake and transport processes in grafted plants are modulated by complex interactions between rootstock and scion, encompassing long-distance signaling pathways and rootstock-specific ion regulation

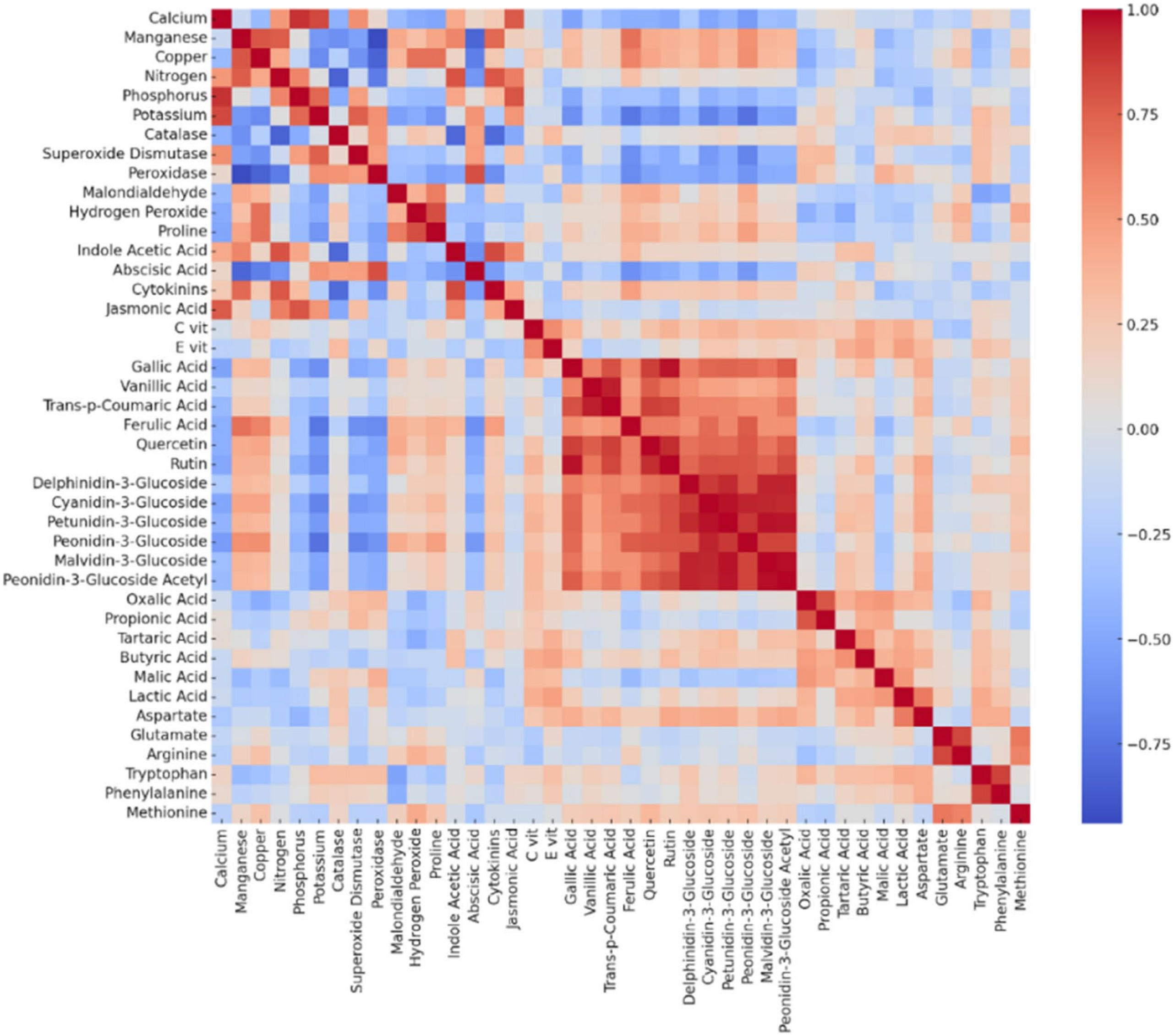

Fig. 8 Correlation Heatmap of Biochemical Parameters in Stress Response

mechanisms [33]. Certain rootstocks exhibit the capacity to sequester heavy metals in root tissues, effectively reducing their translocation to aerial parts and limiting toxicity [34]. Since Zn is primarily translocated from roots to leaves, rootstock selection becomes pivotal in managing Zn uptake and distribution [35]. In this context, our findings indicate that as Zn stress levels increased, the concentrations of Mn, Cu, P, and K generally increased, while Ca and N showed variable responses depending on rootstock and Zn level, with notable declines observed in non-grafted plants, suggesting an ion imbalance associated with metal stress. This pattern aligns with earlier studies reporting grafting-mediated improvements in potassium uptake in cucumbers [36] and enhanced macronutrient acquisition through specific rootstocks [16]. The superior performance of TZ148 and Maximus in maintaining mineral homeostasis under high Zn stress conditions confirms their potential as effective tools for nutrient management. An important component of rootstock-induced stress tolerance is the enhancement of antioxidant defense systems [37]. Grafted plants activate both enzymatic (e.g., APX, CAT, SOD) and non-enzymatic (e.g., ascorbate, glutathione, carotenoids, tocopherols) antioxidant systems to scavenge reactive oxygen species (ROS) and minimize oxidative damage. In our study, the rise in oxidative stress markers such as H_2O_2 and MDA under Zn deficiency was accompanied by a compensatory increase in proline levels which functions as an osmoprotectant, stabilizes proteins and membranes, and scavenges ROS, particularly in grafted combinations like TZ148-Cağla. TZ148-grafted plants exhibited higher POX activity under Zn−, reinforcing the notion that rootstocks bolster ROS detoxification pathways. The increased SOD activity observed in this study aligns well with the findings of earlier studies [38], supporting the role of grafting in strengthening antioxidant defense. In a similar vein, Liu et al. [39] reported elevated SOD and CAT activity as well as proline accumulation in grafted tobacco plants under abiotic stress. Under Zn deficiency, impaired Zn-dependent enzyme function (e.g., Cu/Zn-SOD) can intensify ROS generation, triggering compensatory upregulation of antioxidant enzymes like POX and SOD. This aligns with findings in grafted cucumber where drought stress enhanced both gene expression and enzyme activity of antioxidants [40]. In contrast, under Zn excess, displacement of essential cofactors by Zn and disrupted photosynthetic electron transport can overwhelm antioxidant systems and reduce enzyme efficiency—consistent with observations in other abiotic stress contexts where grafting modulates stress-responsive gene expression and antioxidant pathways [8, 41].

The present results also demonstrated that MDA levels, a key indicator of lipid peroxidation, rose under Zn stress but were significantly lower in grafted plants under both Zn10 and Zn50 treatments, with the strongest reduction observed in TZ148-grafted plants at Zn50. This observation is consistent with earlier findings showing a decrease in MDA concentrations in grafted plants under moderate metal stress [32]. Enhanced CAT activity under Zn10, particularly in TZ148-grafted plants, underscores the importance of catalase in ROS detoxification and stress tolerance—a function well-documented in prior research [42]. In contrast, under excessive Zn (Zn50), displacement of essential cations such as Mg^{2+}, Fe^{2+}, and Mn^{2+} from active sites of antioxidant enzymes can lead to partial enzyme inactivation. High Zn levels may also inhibit chlorophyll biosynthesis and photosynthetic electron transport, promoting photo-oxidative stress and overwhelming the plant's antioxidant capacity, which explains the reduced POX and SOD activities and elevated oxidative stress markers observed in this study. Hormonal regulation plays a crucial role in plant adaptation to stress. Rootstocks can influence hormonal crosstalk and metabolic pathways, including stomatal conductance (via ABA) and leaf senescence (via cytokinins) [43]. The role of IAA in sustaining meristematic activity and ABA in promoting antioxidant enzyme synthesis and stress-responsive gene expression has been widely established. Our findings confirm that under Zn stress, grafted plants, particularly those with TZ148 rootstock, maintained elevated ABA levels which is known to trigger antioxidant enzyme synthesis, regulate stomatal closure, and activate stress-responsive genes. Elevated ABA levels in these plants likely contributed to improved osmotic adjustment and stress tolerance. Hormone synthesis and precursor allocation, which are regulated at the rootstock level, influence the plant's capacity to withstand metal stress. Grafting has been shown to modulate hormonal crosstalk, including interactions among auxin, cytokinin, and ethylene, at early stages of graft union formation [44]. Such complex hormonal signaling suggests that ABA regulation under Zn stress may similarly be influenced by rootstock-mediated modulation of hormone activity. Declining ABA under high Zn could result from inhibition of biosynthetic pathways or disruption of signaling networks, which warrants further targeted research.

In non-grafted plants, increased Zn stress was associated with inconsistent trends in hormone levels, including elevated IAA and cytokinins but reduced ABA under certain conditions [45]. The decline in ABA under high Zn stress may be due to Zn-induced inhibition of key enzymes in the ABA biosynthetic pathway, such as 9-cis-epoxycarotenoid dioxygenase (NCED), or altered carotenoid metabolism. Additionally, membrane damage and disrupted water relations under Zn toxicity can impair root-to-shoot ABA transport, leading to suboptimal stomatal regulation and greater oxidative stress. In contrast, TZ148-grafted plants displayed more stable hormonal responses, particularly with respect to ABA, whose levels remained high across Zn stress gradients. ABA is known to regulate stress-responsive genes that mitigate the osmotic and oxidative damage caused by abiotic stressors [46], and previous studies have shown that ABA concentrations increase in cucumber leaves exposed to environmental stress [47]. Our results corroborate this, as grafted plants exhibited significantly higher ABA levels under Zn50, supporting the role of ABA biosynthesis in graft-mediated stress amelioration. Hormonal stability and flexibility in grafted plants under Zn stress were markedly higher compared to non-grafted controls. TZ148 consistently maintained or increased levels of stress-responsive hormones such as ABA, IAA, and cytokinins, reflecting superior hormonal homeostasis. Maximus rootstock also demonstrated effective modulation of stress-associated hormones, especially jasmonic acid (JA), which peaked under high Zn conditions. This suggests that JA-mediated pathways contribute to the enhanced defense capacity conferred by Maximus.

The synthesis and accumulation of phenolic compounds are also crucial for plant defense under metal stress. The type and abundance of phenolics are determined by genotype, grafting strategy, and environmental stress intensity. In our study, grafted plants maintained higher levels of total phenolic compounds than non-grafted ones under Zn stress. Notably, flavonoids and related polyphenols, including gallic acid, quercetin, ferulic acid, and rutin, were elevated in TZ148- and Maximus-grafted plants, even at Zn50. These molecules play a non-enzymatic antioxidant role by neutralizing ROS and inhibiting lipid peroxidation. Flavonoids contribute to stress mitigation by chelating heavy metals, regulating stomatal function, and enhancing water retention [48]. Their antioxidant activity involves suppression of ROS generation and stabilization of cell membranes. While heavy metal toxicity may inhibit flavonoid biosynthesis at extreme concentrations [49], our findings show that grafted plants were capable of sustaining high flavonoid levels even under elevated Zn stress. In contrast, non-grafted plants showed increased phenolic accumulation under Zn10, but this increase was less pronounced than in grafted plants and did not extend to Zn50, except for ferulic acid, which remained elevated. In support of these findings, a previous study on squash reported a 29.54% reduction in flavonoid content following exposure to 50 mg/kg cadmium [50]. The contrasting trend observed in this study, wherein grafted cucumber plants maintained or even increased flavonoid and anthocyanin content under high Zn stress, underscores the protective effect of robust rootstocks. The activation of the phenylpropanoid pathway under metal stress enhances biosynthesis of key phenolic acids, including caffeic, gallic, ferulic, cinnamic, malic, and vanillic acids [51]. Anthocyanins—such as cyanidin, petunidin, and delphinidin—are known to chelate metals via hydroxyl groups, contributing further to the antioxidant system [52]. Their synthesis is often regulated at the transcriptional level by stress-induced expression of biosynthetic genes, providing an additional layer of defense under metal toxicity [53]. For example, anthocyanin accumulation has been reported to alleviate cadmium stress in wheat [54] and peaked in tomato leaves treated with 100 μM Cr [55]. In our study, although anthocyanin levels declined in non-grafted plants under Zn50, grafted plants—especially those grafted onto TZ148—maintained elevated concentrations of key anthocyanins, including delphinidin-3-glucoside, petunidin-3-glucoside, and cyanidin-3-glucoside, even under Zn50 stress. These findings clearly indicate that grafting improves anthocyanin accumulation by stimulating the phenylpropanoid pathway and increasing the activity of key biosynthetic enzymes and enhances zinc stress tolerance in cucumbers [56]. Thus, the selection of appropriate rootstocks such as TZ148 and Maximus plays a critical role in optimizing physiological and biochemical traits to improve cucumber productivity under heavy metal stress. The integrative effects on mineral uptake, antioxidative capacity, hormonal balance, and secondary metabolite biosynthesis confirm the utility of grafting as a sustainable agronomic strategy for managing heavy metal contamination in agricultural systems. These integrative effects align with recent findings demonstrating that grafting enhances stress tolerance in cucumbers and other crops via coordinated improvements in nutrient uptake, antioxidant defense, hormonal homeostasis, and secondary metabolite biosynthesis [40, 57, 58].

Conclusion

This study demonstrates that grafting is an effective strategy to mitigate zinc stress in cucumber by enhancing nutrient acquisition, maintaining metabolic and hormonal balance, and strengthening antioxidative defense mechanisms, thereby supporting its potential as a sustainable approach to manage heavy metal contamination. Among the tested rootstocks, *Cucurbita maxima* × *C. moschata* hybrids TZ148 and Maximus significantly improved physiological and biochemical resilience under both zinc-deficient and zinc-toxic conditions, confirming their potential for commercial use in stress-prone production systems. In particular, TZ148 consistently exhibited superior performance by maintaining mineral homeostasis, reducing oxidative damage, and promoting the accumulation of protective compounds such as proline, phenolics, and anthocyanins. These findings highlight the pivotal role of rootstock selection in conferring stress resilience, offering a practical approach for optimizing cucumber production in areas affected by heavy metal exposure. Grafting onto stress-tolerant rootstocks not only contributes to crop productivity but also offers a sustainable agronomic practice for reducing the negative impacts of soil contamination in intensive agricultural systems. Future research should focus on evaluating the long-term efficacy of such grafting strategies under field conditions, and on identifying specific molecular and genetic markers linked to rootstock-mediated zinc stress tolerance, which could guide breeding programs for stress-resilient cucurbits. Such insights will be instrumental in breeding and deploying elite rootstocks tailored to specific environmental constraints. Overall, the take-home message is that selecting stress-tolerant rootstocks such as TZ148 and Maximus can substantially improve cucumber productivity and quality in zinc-stressed environments, contributing to sustainable agriculture.

Abbreviations

CAT	catalase
POX	peroxidase
SOD	superoxide dismutase
HPLC	high-performance liquid chromatography
MDA	malondialdehyde
NBT	nitro-blue tetrazolium
GA_3	gibberellic acid
SA	salicylic acid
IAA	indole acetic acid
ABA	abscisic acid
OPA	o-phthaldialdehyde
H_2O_2	hydrogen peroxide
ROS	reactive oxygen species

Author contributions

Ö.F.C and A.A. wrote the manuscript and conducted the experiments. Ö. F. C. conducted bioinformatics analysis and analyzed the data. S.T and H.B provided experimental materials. M.T. and Ö.F.C. contributed to the study of concepts and designs. All the authors read and approved the final manuscript.

Funding
Authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Data availability

Data available on request from the corresponding author.

Declarations

Ethics approval and consent to participate

This article does not require any ethical approval.

Consent for publication

Author read the manuscript and showed his willingness to publish this study.

Competing interests

The authors declare no competing interests.

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Received: 24 June 2025 / Accepted: 22 August 2025

References

1. Kumar M, Seth A, Singh AK, Rajput MS, Sikandar M. Remediation strategies for heavy metals contaminated ecosystem: a review. Environ Sustain Indic. 2021;12:100155.
2. Guo XX, Zhao D, Zhuang MH, Wang C, Zhang F-S. Fertilizer and pesticide reduction in Cherry tomato production to achieve multiple environmental benefits in Guangxi, China. Sci Total Environ. 2021;793:148527.
3. Yadav B, Dubey R, Gnanasekaran P, Narayan OP. OMICS approaches towards understanding plant's responses to counterattack heavy metal stress: an insight into molecular mechanisms of plant defense. Plant Gene. 2021;28:100333.
4. Umair Hassan M, Aamer M, Umer Chattha M, Haiying T, Shahzad B, Barbanti L, Nawaz M, Rasheed A, Afzal A, Liu Y, Guoqin H. The critical role of zinc in plants facing the drought stress. Agriculture. 2020;10(9):396.
5. Wang C, Zhang SH, Wang PF, Hou J, Zhang WJ, Li W, et al. The effect of excess Zn on mineral nutrition and antioxidative response in rapeseed seedlings. Chemosphere. 2009;75(11):1468–76.
6. Gupta R. Manganese repairs the oxygen-evolving complex (OEC) in maize (*Zea mays* L.) damage during seawater vulnerability. J Soil Sci Plant Nutr. 2020;20:1387–96.
7. Seregin IV, Kozhevnikova AD. Phytochelatins: sulfur-containing metal(loid)-chelating ligands in plants. Int J Mol Sci. 2023;26(3):2430.
8. Razi K, Suresh P, Mahapatra PP, Al Murad M, Venkat A, Notaguchi M, et al. Exploring the role of grafting in abiotic stress management: contemporary insights and automation trends. Plant Direct. 2024;15(12):e70021.
9. Yue P, Wang H, Jia Y, Xiong R, Zhang S, Li T, et al. Effect of grafting on cd tolerance and accumulation characteristics of cucumber. Sci Hortic. 2025;339:113859.
10. Yuan H, Sun L, Tai P, Liu W, Li X, Hao L. Effects of grafting on root-to-shoot cadmium translocation in plants of eggplant (*Solanum melongena*) and tomato (*Solanum lycopersicum*). Sci Total Environ. 2019;652:989–95.
11. Tomaz de Oliveira MM, Lu S, Zurgil U, Raveh E, Tel-Zur N. Grafting in Hylocereus (Cactaceae) as a tool for strengthening tolerance to high temperature stress. Plant Physiol Biochem. 2021;160:94–105.
12. Yang L, Xia L, Zeng Y, Han Q, Zhang S. Grafting enhances plants drought resistance: current understanding, mechanisms, and future perspectives. Front Plant Sci. 2022;6:13:1015317.
13. He J, Zhou J, Wan H, Zhuang X, Li H, Qin S, et al. Rootstock–scion interaction affects cadmium accumulation and tolerance of *Malus*. Front Plant Sci. 2020;11:1264.
14. Fullana-Pericàs M, Conesa MÀ, Pérez-Alfocea F, Galmés J. The influence of grafting on crops' photosynthetic performance. Plant Sci. 2020;295:110250.
15. FAO. Statistical yearbook of the food and agriculture organization. FAO; 2023.
16. Savvas D, Colla G, Rouphael Y, Schwarz D. Amelioration of heavy metal and nutrient stress in fruit vegetables by grafting. Sci Hort. 2010;127:156–61.
17. Hoagland DR, Arnon DI. The water-culture method for growing plants without soil. Circ Calif Agric Exp Stn. 1950;347:1–32.
18. Bremner JM. (2018) Nitrogen-total. In Methods of Soil Analysis: Part 3, Chemical Methods (pp. 1085–1121).
19. Abedi T, Pakniyat H. Antioxidant enzymes changes in response to drought stress in ten cultivars of oilseed rape (*Brassica napus* L). Czech J Genet Plant Breed. 2010;46:27–34.
20. Kuraishi S, Tasaki K, Sakurai N, Sadatoku K. Changes in levels of cytokinins in etiolated squash seedlings after illumination. Plant Cell Physiol. 1991;32:585–91.
21. Turan M, Ekinci M, Yildirim E, Gunes E, Karagoz K, Kotan R, Dursun A. Plant growth-promoting rhizobacteria improved growth, nutrient, and hormone content of cabbage (*Brassica oleracea*) seedlings. Turkish J Agric Forestry. 2014;38:327–33.
22. Sagdic O, Ozturk I, Ozkan G, Yetim H, Ekici L, Yilmaz MT. RP-HPLC–DAD analysis of phenolic compounds in pomace extracts from five grape cultivars: evaluation of their antioxidant, antiradical, and antifungal activities in orange and Apple juices. Food Chem. 2011;126:1749–58.
23. Olgun M, Turan M, Budak Başçiftçi Z, Ayter NG. Determination of organic acids on the development periods in bread wheat genotypes. Biol Divers Conserv. 2017;10:142–9.
24. Barrado E, Rodriguez JA, Castrillejo Y. Determination of primary amino acids in wines by high-performance liquid magnetochromatography. Talanta. 2009;78:672–5.
25. Durst RW, Wrolstad RE. Separation and characterization of anthocyanins by HPLC. Curr Protoc Food Anal Chem. 2001. https://doi.org/10.1002/0471142913.faf0103s00.
26. Abu-Muriefah SS. Growth parameters and elemental status of cucumber (*Cucumis sativus*) seedlings in response to cadmium accumulation. Int J Agric Biol. 2008;10:261–6.
27. Fernández-Paz J, Cortés AJ, Hernández-Varela CA, Mejía-de-Tafur MS, Rodriguez-Medina C, Baligar VC. Rootstock-mediated genetic variance in cadmium uptake by juvenile Cacao (*Theobroma cacao* L.) genotypes, and its effect on growth and physiology. Front Plant Sci. 2021;12:777842.
28. Bayoumi Y, Abd-Alkarim E, El-Ramady H, El-Aidy F, Hamed ES, Taha N, et al. Grafting improves fruit yield of cucumber plants grown under combined heat and soil salinity stresses. Horticulturae. 2021;7:61.
29. Jordana CN, Stapleton SC, Colee JC, Lee S, Gao Z, Ray ZT, Anrecio LR, Freed DJ, Zhao X. How does watermelon grafting impact fruit yield and quality? A systematic review. HortScience. 2023;58:836–45.
30. Xia H, Wang YN, Liao MA, Lin L, Zhang F, Tang Y, et al. Effects of different rootstocks on cadmium accumulation characteristics of the post-grafting generations of *Galinsoga parviflora*. Int J Phytoremediation. 2020;22:62–8.
31. He L, Wang HT, Zhao Q, Cheng Z, Tai P, Liu W. Tomato grafting onto Torubamu (*Solanum melongena*): miR166a and miR395b reduce scion cd accumulation by regulating sulfur transport. Plant Soil. 2020;452:267–79.
32. Coşkun ÖF. The effect of grafting on morphological, physiological, and molecular changes induced by drought stress in cucumber. Sustainability. 2023;15:875.
33. Lu X, Liu W, Wang T, Zhang J, Li X, Zhang W. Systemic long-distance signaling and communication between rootstock and scion in grafted vegetables. Front Plant Sci. 2020;11:460.
34. Zou Y, Yuan Y, Tian Y, Han D, Liao MA. (2020) Different rootstocks decrease the cadmium accumulation of the post-grafting generation of *Brassica juncea* var. *megarrhiza*. International Journal of Environmental Analytical Chemistry 1–19.
35. Bernardi LGP, Ferreira IEP, Silva JR, Mattos-Jr D, Baron D. How do cr and Zn modify cucumber plant re-establishment after grafting? Sci Hort. 2022;304:111278.

36. Zhu J, Bie ZL, Huang Y, Han XY. Effect of grafting on the growth and ion concentrations of cucumber seedlings under NaCl stress. Soil Sci Plant Nutr. 2008;54:895–902.
37. Louws FJ, Rivard CL, Kubota C. Grafting fruiting vegetables to manage soilborne pathogens, foliar pathogens, arthropods, and weeds. Sci Hort. 2010;127:127–46.
38. Shehata SA, Omar HS, Elfaidy AG. Grafting enhances drought tolerance by regulating stress-responsive gene expression and antioxidant enzyme activities in cucumbers. BMC Plant Biol. 2022;22:408.
39. Liu J, et al. Grafting improves drought tolerance by regulating antioxidant enzyme activities and stress-responsive gene expression in tobacco. Environ Exp Bot. 2014;107:173–9.
40. Shehata SA, Omar HS, Elfaidy AG et al. (2022) Grafting enhances drought tolerance by regulating stress-responsive gene expression and antioxidant enzyme activities in cucumbers. BMC Plant Biol 22, 408 (2022).
41. Feng M, Augstein F, Kareem A, Melnyk CW. Plant grafting: molecular mechanisms and applications. Mol Plant. 2024;17(1):75–91.
42. Hameed A, Goher M, Iqbal N. Heat stress-induced cell death, changes in antioxidants, lipid peroxidation, and protease activity in wheat leaves. J Plant Growth Regul. 2012;31:283–91.
43. Albacete A, Martinez-Andujar C, Martinez-Pérez A, et al. Unravelling rootstock × scion interactions to improve food security. J Exp Bot. 2015;66:2211–26.
44. Huang Q, Liu H, Shen Q, Yuan H, Cui F, Yan D, et al. Hormone fluctuation and gene expression during early stages of the hickory grafting process. Plants. 2025;14(14):2229.
45. Basak H, Aydın A, Yetişir H. Salt stress effects on hybrid bottle gourd (*Lagenaria siceraria*) rootstock candidates: plant growth, hormones, and nutrient content. J Crop Health. 2025;77:28.
46. Botella MA, Rosado A, Bressan RA, Hasegawa PM. (2007) Plant adaptive responses to salinity stress. In Plant Abiotic Stress (pp. 37–70).
47. Talanova VV, Titov AF. Endogenous abscisic acid content in cucumber leaves under the influence of unfavorable temperatures and salinity. J Exp Bot. 1994;45:1031–3.
48. Khlestkina EK. The adaptive role of flavonoids: emphasis on cereals. Cereal Res Commun. 2013;41:185–98.
49. González-Mendoza D, Troncoso-Rojas R, Gonzalez-Soto T, et al. Changes in the phenylalanine ammonia lyase activity, total phenolic compounds, and flavonoids in *Prosopis glandulosa* treated with cadmium and copper. An Acad Bras Cienc. 2018;90:1465–72.
50. Wang Y, Lei Z, Ye R, et al. Effects of cadmium on physiochemistry and bioactive substances of muskmelon (*Cucumis Melo* L). Molecules. 2022;27:2913.
51. Ben-Abdallah S, Zorrig W, Amyot L, et al. Potential production of polyphenols, carotenoids, and glycoalkaloids in *Solanum villosum* mill. under salt stress. Biologia. 2019;74:309–24.
52. Tang P, Giusti MM. Metal chelates of Petunidin derivatives exhibit enhanced color and stability. Foods. 2020;9:1426.
53. Wu Q, Su N, Zhang X, et al. Hydrogen peroxide, nitric oxide, and UV resistance locus8 interact to mediate UV-B-induced anthocyanin biosynthesis in radish sprouts. Sci Rep. 2016;6:1–12.
54. Shoeva OY, Khlestkina EK. Anthocyanins participate in the protection of wheat seedlings against cadmium stress. Cereal Res Commun. 2018;46:242–52.
55. Sun S, Liu A, Li Z, et al. Anthocyanin synthesis is critical for melatonin-induced chromium stress tolerance in tomato. J Hazard Mater. 2023;453:131456.
56. Janeeshma E, Rajan VK, Puthur JT. Spectral variations associated with anthocyanin accumulation: an Apt tool to evaluate zinc stress in *Zea Mays* L. Chem Ecol. 2020;36:32–49.
57. Amerian M, Palangi A, Gohari G, et al. Enhancing salinity tolerance in cucumber through selenium biofortification and grafting. BMC Plant Biol. 2024;24:24.
58. Razi K, Muneer S. Grafting enhances drought tolerance by regulating and mobilizing proteome, transcriptome and molecular physiology in Okra genotypes. Front Plant Sci. 2023;14:1178935.

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