

Exploring the Potential of Foliar-Applied Organic Acids in Enhancing Iron Contents in Maize Grain Through Translocation

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Abstract

Iron deficiency affects over one-third of the world's population and is one of the most common micronutrient deficiencies globally. Although agronomic biofortification has made progress, there is still a lack of research on the synergistic effects of combining organic acids with iron to evaluate iron translocation when applied in combination with organic acids. A pot experiment was conducted at the University of Agriculture, Faisalabad, under a completely randomized design with three replications. An iron spray (0.1%) was applied alone and in combination with different organic acids (citric, tartaric, oxalic, and malic acids) at a concentration of 0.1 ppm on maize hybrid (P-3875). Seeds were sown in 10-kg soil-filled plastic pots measuring 12 inches in height and 8 inches in diameter. The primary nutrient requirements were fulfilled using a standard nitrogen:phosphorus:potassium fertilizer ratio mixed into the soil. The recorded data were analyzed using analysis of variance, and treatment means were compared at the 5% probability level using the least significant difference test. Iron dissolved in tartaric acid showed significant improvements in leaf turgor pressure (0.83 MPa), leaf water potential (1.3 MPa), 100-grain weight (35.9 g), grain yield per plant (160 g), and grain iron content (44.5 mg/kg). These results suggest that mixing iron with a tartaric acid solution enhances its availability and translocation to maize grains. Further research under different environmental conditions is recommended to validate these findings.

Categories: Organic agriculture, Field & Horticultural crops sustainable production, Food nutrition

Keywords: food security, malnutrition, hidden hunger, grain iron concentration, tartaric acid, citric acid, oxalic acid, malic acid

Introduction

More than two billion people worldwide suffer from micronutrient deficiency. Hidden hunger, largely caused by declining soil nutritional quality and the preference for macronutrients over micronutrients, is a major concern [1]. Micronutrients are essential for plant physiological processes, including photosynthesis, respiration, enzyme activation, and stress tolerance [2]. Low micronutrient availability, particularly iron (Fe) and zinc (Zn), in calcareous soils is considered a major abiotic stress and a challenge to cereal productivity across the globe. Alarming, nearly 50% of the world's soils are deficient in Fe and Zn [3]. Fe plays a central role in chlorophyll synthesis, electron transport, and protein metabolism [4]. In calcareous and alkaline soils, Fe becomes insoluble and unavailable, leading to chlorosis, reduced photosynthetic efficiency, impaired growth, and lower yields [5]. Fe deficiency anemia, which particularly affects women and children and impairs cognitive development, immune function, and overall productivity, is a global problem that coincides with soil limitations [6]. These challenges underscore the urgent need for strategies that enhance both human health and

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of the most common micronutrient limitations worldwide. An imbalance between the solubility of Fe in soil and the Fe requirements of plants is the primary cause of Fe chlorosis [7]. Foliar application of micronutrients has been documented as a more efficient alternative compared to soil or broadcast application [8].

In many developing regions, maize (*Zea mays* L.) serves as a major staple food, providing a significant proportion of daily caloric intake. However, its inherent low levels of essential micronutrients such as Fe, Zn, and folate frequently contribute to micronutrient deficiency [9]. Biofortification of maize, therefore, has emerged as a promising, sustainable intervention to enhance the micronutrient density of staple crops such as maize. Agronomic biofortification, genetic engineering, and conventional breeding have all been extensively studied to increase mineral concentration and bioavailability [10]. Applying organic acids to crops grown in Fe-limiting soils has recently drawn interest as an economical and environmentally beneficial method to increase Fe solubility, mobility, and uptake. Exogenous organic acids may facilitate Fe-biofortified crops and increase the uptake of Fe by monocot and dicot plants [11].

Soils contain organic acids inherently because of microbial activity, organic matter turnover, and root exudation. Predominantly in nutrient-deficient environments, these organic acids improve nutrient solubilization, chelation, and translocation in soil and plant parts [12,13]. Additionally, they play crucial roles in stress tolerance and metabolic regulation. For example, oxalic acid increases stress tolerance and activates defense-related enzymes [14], whereas tartaric acid decreases oxidative damage and enhances metal absorption. The tricarboxylic acid cycle depends on malic and citric acids to maintain energy metabolism, photosynthesis, and food uptake [15]. Emerging evidence suggests that foliar application of organic acids improves plant nutritional quality by enhancing micronutrient uptake, maintaining chlorophyll and carotenoid content, boosting physiological performance, and reducing oxidative stress [16]. These findings highlight the potential of organic-acid-based agronomic strategies to enhance Fe accumulation in maize grains.

Although agronomic biofortification has made progress through the use of organic acids and Fe in many crops, data on grain Fe concentration and Fe application via foliar methods remain limited [17]. Some previous studies have indicated that organic acids may be an effective tool to enhance Fe availability in maize during growth and development; however, there has been little work evaluating the effect of different organic acids on Fe translocation from shoot to grain. While synthetic Fe chelates such as Fe-EDTA and Fe-DTPA are commonly used to correct Fe deficiency, comparative evaluations of foliar-applied organic acids as alternative Fe sources remain scarce. Organic acids may differ from conventional chelates in terms of complex stability, mobility, and interactions with plant tissues, yet these mechanistic aspects are not fully understood. Therefore, this study was designed to evaluate the effects of different organic acids applied with Fe solutions on maize grain Fe concentration and yield. We hypothesized that (i) foliar Fe supplied with different organic acids would result in differential Fe concentrations in the grain, and (ii) certain organic acid treatments would enhance grain Fe accumulation without negatively affecting maize grain yield.

Materials And Methods

Experimental site description

The experiment was conducted in the Wire House, Department of Agronomy, University of Agriculture, Faisalabad, during the spring season of 2023. Maize hybrid P-3875, recommended for the spring season and characterized by vigorous growth and good nutrient-use efficiency, was used. Primary nutrient requirements were fulfilled using the recommended dose of nitrogen:phosphorus:potassium (N:P:K; 200-120-125 kg ha⁻¹), and this requirement was fulfilled by adding 0.62:0.31:0.31 g N:P:K per 10 kg soil in a pot. All management practices were kept consistent to ensure the establishment of a healthy crop. Three seeds were sown per pot, and thinning was performed 10 days after emergence to retain only one plant per pot. Treatments were arranged in a completely randomized design with three replications and consisted of control (no application), 0.1% Fe sulphate spray (Fe), 0.1% Fe sulphate dissolved in 0.1 ppm citric acid solution (Fe+CA), 0.1% Fe sulphate dissolved in 0.1 ppm tartaric acid solution (Fe+TA), 0.1% Fe sulphate dissolved in 0.1 ppm oxalic acid solution (Fe+OA), and 0.1% Fe sulphate dissolved in 0.1 ppm malic acid solution (Fe+MA). Equal volumes of each acid and solution were used for treatment preparation and application. Foliar sprays were applied at 150 mL per pot, equivalent to 120 L/acre, using a hand sprayer to ensure uniform leaf coverage. The 0.1% FeSO₄ concentration was selected based on previous findings indicating that foliar application at this level effectively improves maize growth without causing phytotoxicity [18].

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Experimental design and treatments

Treatments were applied at the reproductive stage, starting at tasseling. A measuring tape was used to record the length and width of leaves and stems. Relative water content (RWC) was determined using the method of Barrs and Weatherley [19].

Sampling and analytical methods

Leaf samples were randomly collected from three plants in each replication early in the morning to avoid water loss due to transpiration. The samples were transported in sealed polythene bags and kept in an ice box until they reached the laboratory for analysis. Fresh leaf weight was recorded using a digital balance. After recording the fresh weight, the leaves were soaked in distilled water for 24 h. Subsequently, the leaves were removed from the water, surface water was gently blotted with tissue paper, and turgid weight was measured using the digital electrical balance. The leaves were then dried in an oven at 65°C for 72 h, and dry weight was recorded. RWC for each replication was calculated using the following formula:

$$\text{RWC} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100$$

where, FW is the leaf's fresh weight, DW is its dry weight, and TW is its turgid weight.

Grain Fe contents were analyzed using an atomic absorption spectrophotometer. For the determination of Chl *a*, Chl *b*, and carotenoids, a technique proposed by Lichtenthaler and Buschmann [20] was followed. A 0.5 g of fresh leaf sample was used as an extract, which was treated with 5 mL of an 80% acetone solution. The extract was centrifuged at 12,000 rpm for 5 min. Spectrophotometer measurements of the supernatant's absorbance were made at 645, 652, and 663 nm using the following formula:

$$\text{Chl } a = (12.7 (\text{A}_{663}) - 2.69 (\text{A}_{645}) \times V / 1000 \times W$$

$$\text{Chl } b = 22.9 (\text{A}_{645} - 4.68 (\text{A}_{663}) \times V / 1000 \times W$$

$$\text{Carotenoids} = (\text{OD}_{470} + 0.114 (\text{OD}_{663}) - 0.638 (\text{OD}_{645})) / 2500 \times 1000$$

where "A" stands for absorbance (nm), "V" for extracted volume (mL), "W" for fresh leaf tissue weight (g), and "OD" for optical density. A pressure chamber was used to measure leaf water potential. Leaves were cut early in the morning to minimize water loss through evapotranspiration, following the method of Ahmad et al. [21]. Fully grown plant leaves were placed in the chamber so that the cut surface protruded from the slot. Compressed nitrogen was applied to increase chamber pressure until xylem fluid appeared at the cut surface. The values of the chamber were recorded by converting the original value, which appeared in pounds per square inch, into megapascals (MPa). Maize grain Fe concentration was determined using an atomic absorption spectrophotometer according to the Association of Official Analytical Collaboration International method [22]. In order to determine grain Fe content, grain samples were collected and rinsed with deionized water to remove surface contaminants. Samples were dried in an EYELA windy oven (WFO 600ND) at 70°C until a constant weight was achieved, then ground into a fine powder. One gram of each powdered sample was weighed, transferred to digestion flasks, and treated with a mixture of nitric acid and hydrogen peroxide (2:1). Briefly, 1 g sample was digested in an acid mixture of HNO₃ and HClO₄ (2:1). After digestion, the sample was diluted to 50 mL in a volumetric flask with double-distilled water. The measurements of Fe were made using an atomic absorption spectrophotometer (Agilent Technologies 200 Series AA), and readings were recorded for each sample one by one.

Data analysis

The recorded data were managed using Microsoft Excel (365) and analyzed using Statistix (v8.1) through analysis of variance (ANOVA). When ANOVA results were significant, treatment means were compared at the 5% probability level using the least significant difference test.

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Results And Discussion

The results of our study highlight that the combined application of organic acids and Fe had significant effects on chlorophyll a, chlorophyll b, and carotenoid contents ($p \leq 0.05$). The highest chlorophyll a content (2.53 mg/g) was observed with Fe dissolved in tartaric acid (TA+Fe), representing a 69.7% increase compared to the control, which had the lowest chlorophyll a content (1.49 mg/g). This was followed by OA+Fe, showing a 46.9% increase over the control (Figure 1a). Similarly, the highest chlorophyll b content (0.69 mg/g) was recorded with TA+Fe, followed by OA+Fe and MA+Fe, which produced 136% and 104% higher chlorophyll b, respectively, compared to the control (0.25 mg/g) (Figure 1b). Carotenoid content was also highest (0.45 mg/g) under TA+Fe, representing a 45.1% increase over the control, which had the lowest value (0.31 mg/g) (Figure 1c). Grain iron content was significantly affected by treatment application ($p \leq 0.05$), with TA+Fe producing the highest value (44.5 mg/kg), corresponding to a 125.8% increase compared to the control (19.7 mg/kg) (Figure 1d). These results strongly support the hypothesis that Fe dissolved in organic acids performs better than traditional applications in increasing iron content in maize grains. Iron partitioning within the plants was improved with the application of organic acids, further supporting the hypothesis and highlighting the importance of organic acids for Fe biofortification. Increased grain Fe contents were mainly observed with the application of Fe dissolved in organic acids because foliar fertilization promoted adequate Fe uptake by the leaves and translocation through the plant, resulting in improved crop growth and development [23]. Fe molecules should have been taken up by the mesophyll cells, initiating a two-step mechanism involving a plasma membrane Fe(III) chelate-reductase and Fe(II) transporters [24]. Although plant studies on foliar iron nutrition primarily focus on traditional chelates, organic acids enhance iron solubility and uptake by maintaining Fe in a soluble, chelated form that penetrates the leaf cuticle more effectively. Tartaric acid, while less studied in plant systems, is recognized as a viable organic chelating agent capable of forming soluble iron complexes, thereby potentially facilitating foliar iron uptake. Some organic acids have been investigated as complexing agents for micronutrients in foliar sprays and nutrient solutions [25]. Due to their varying chelation strengths, citric acid and malic acid also enhance Fe absorption, but to a lesser extent. Malic acid forms weak complexes that are more likely to re-precipitate, whereas citric acid binds Fe too strongly, reducing its bioavailability. Higher concentrations of Fe in leaves and grains under TA indicate its greater chelating efficiency and biological compatibility, allowing effective Fe remobilization through phloem transport via nicotianamine and citrate [26]. The significant increases in carotenoid and chlorophyll content under TA+Fe result directly from improved chlorophyll synthesis, which is an Fe-dependent process [27,28]. Improved osmotic balance and membrane function are reflected by significant increases in leaf water potential and turgor pressure with TA+Fe. Iron also enhances cellular water transport by promoting the development of aquaporins [29,30]. As suitable solutes, organic acids support the production of osmoprotectants such as proline and soluble sugars, which protect proteins and membranes from temporary stress [31,32]. Fe also affects aquaporin development and activity, which helps move water across membranes [33]. These combined effects likely enhanced sustained photosynthetic activity and stomatal function, enabling treated plants to perform better physiologically.

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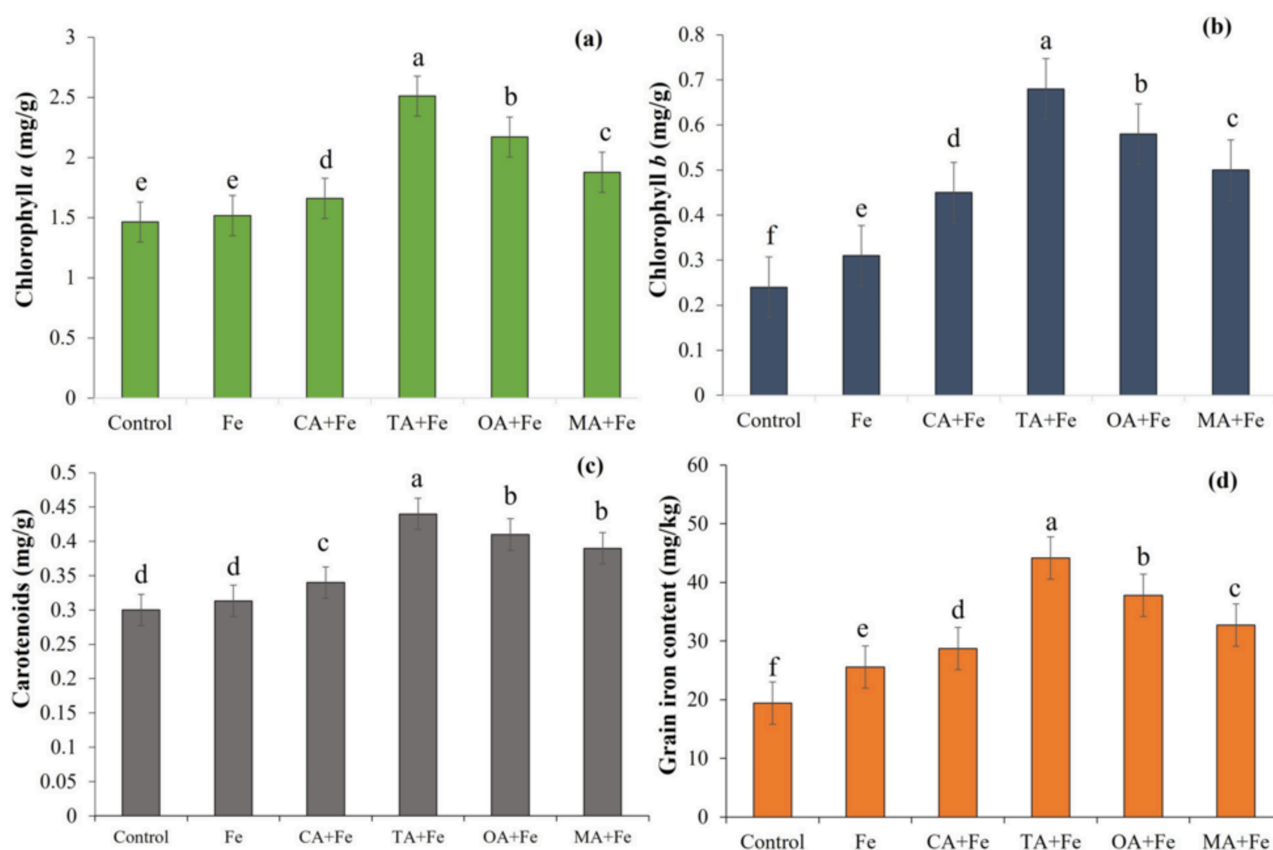


FIGURE 1: Chlorophyll a, chlorophyll b, carotenoid content, and grain iron content of maize in response to different organic acids and iron sulphate.

The lowest leaf water potential (0.69 MPa) was observed in the TA+Fe treatment, representing a 63.7% decrease relative to the control, while the smallest reduction was recorded in Fe alone (18.1%). Control plants showed the highest leaf water potential (1.13 MPa) (Figure 2a). However, the results from the sole Fe treatment were comparable to the control. Leaf turgor pressure (0.83 MPa), grain yield (160 g), and 100-grain weight (36 g) were highest under the TA+Fe treatment, representing increases of 76.5%, 36.7%, and 135.2% over the control (0.47 MPa, 117 g, and 15.3 g, respectively) (Figure 2b-d). Grain production and biomass accumulation were significantly associated with improved photosynthetic efficiency and Fe nutrition. Compared to the control, the TA+Fe treatment provided roughly 36.75% more grain and 135% heavier grains. This highlights the critical role of Fe-containing enzymes in enhancing pollen viability and starch deposition processes [34,35]. Although they were less successful than tartaric acid, malic acid and citric acid treatments also significantly improved yield attributes, highlighting the importance of adequate Fe chelation stability and metabolic integration for maximizing reproductive performance. Additionally, iron is necessary for the assembly of photosystems I and II, where electron transport is mediated by heme groups and Fe-S clusters [36]. These Fe containing elements promote increased carbon fixation and biomass growth by ensuring effective electron flow for the synthesis of NADPH and ATP [35]. TA+Fe performed better because of synergistic interactions compared to additive effects. Through chelation, tartaric acid increases the bioavailability of iron, facilitating important metabolic functions [37,38]. The importance of crop nutrition and micronutrient fortification has already been well documented in grasses [39-42]. Fe deficiency is immediately overcome by foliar tartaric acid and Fe. Consistent improvements across all measures are made clear by this integrated approach, which simultaneously addresses immediate physiological demands.

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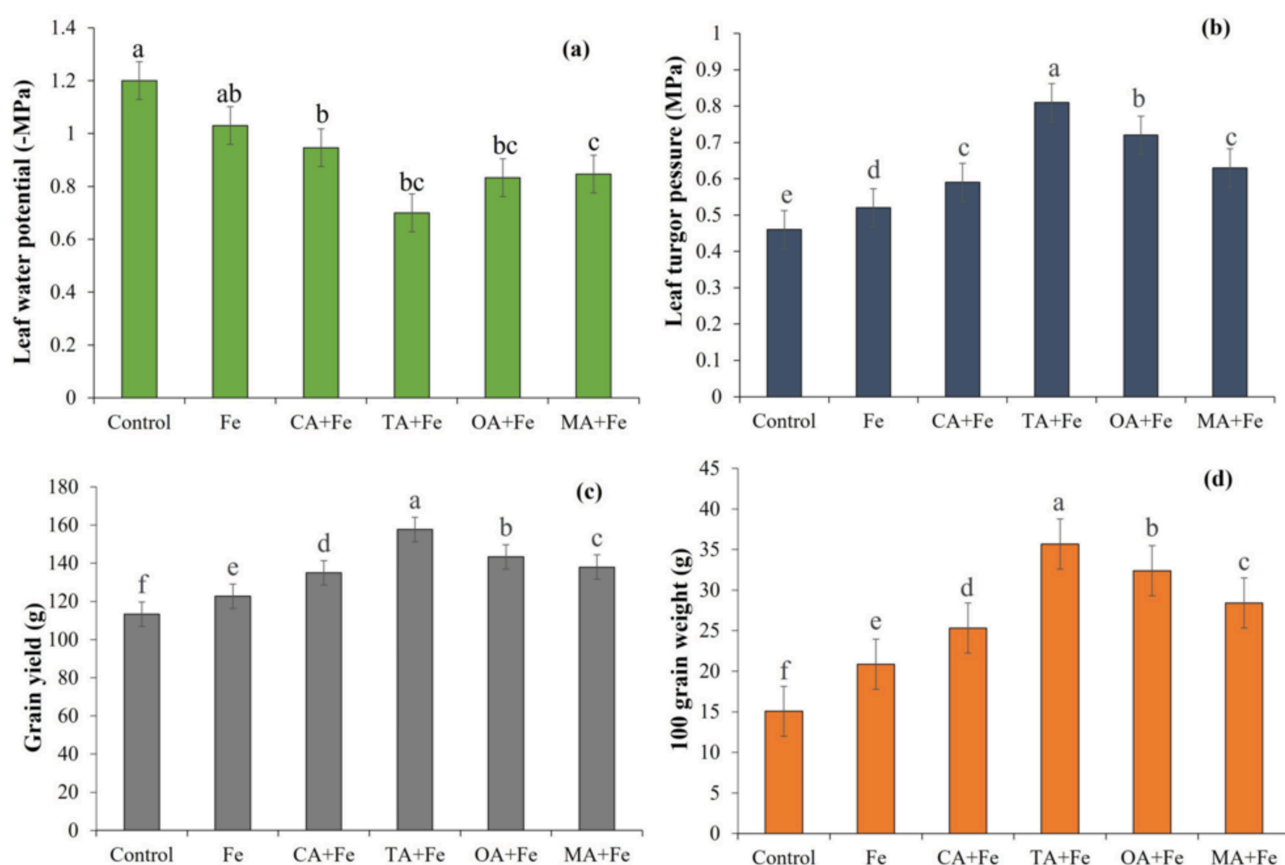


FIGURE 2: Leaf water potential, leaf turgor pressure, grain yield and 100 grain weight content of maize in response to different organic acids and iron sulphate.

Heatmap analysis

The heatmap clearly shows improvements in physiological and yield-related variables across treatments, with higher values represented by darker shades (Figure 3). From the control to the TA+Fe treatment, a variety of parameters, such as grain yield, chlorophylls, carotenoids, grain iron, and 100-grain weight, show significant increases, indicating that the combination of Fe and organic acids significantly enhances plant performance. Under these treatments, leaf water potential becomes less negative, indicating improved plant water status, while leaf turgor pressure also increases. Overall, the pattern indicates that organic acids effectively enhance the availability and uptake of Fe, which improves crop production, nutritional quality, and photosynthetic efficiency.

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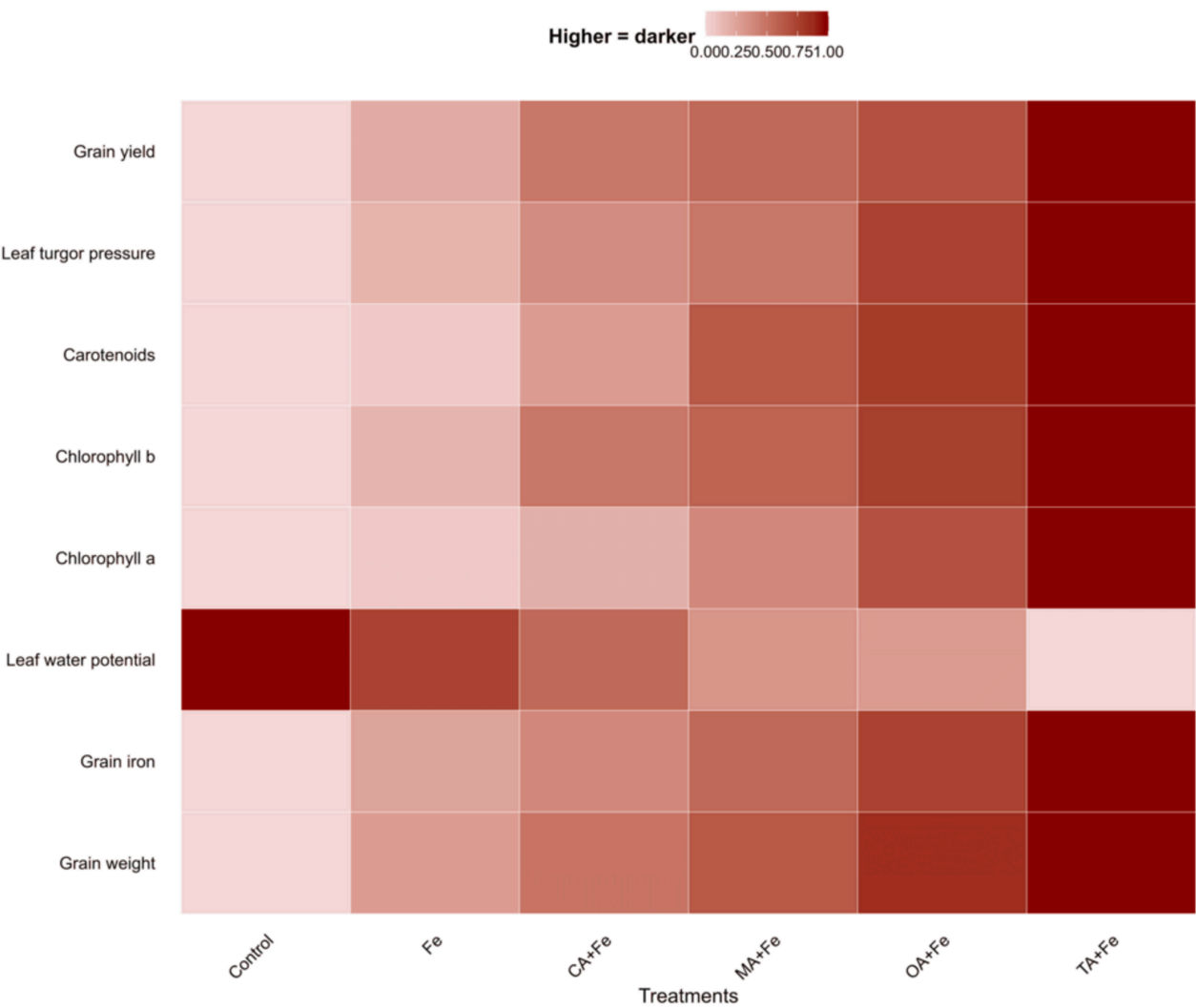


FIGURE 3: Heatmap showing the correlation among observed parameters with higher values at darker color while lower values at lighter color. The x-axis shows treatment combinations, while y-axis shows list of parameters observed.

Conclusions

The results of the experiment led to the acceptance of the hypothesis that all the organic acids performed differently in enhancing Fe bioavailability and translocation to the grain, with tartaric acid dissolved in FeSO₄ outperforming all other acids by increasing maize grain yield and Fe concentration. Further controlled and multi-location field experiments, including comparisons with commercial iron chelates and optimization of organic acid concentrations, are necessary to validate these findings and to more clearly define their broader applicability for nutrient enrichment strategies.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Muhammad Abdullah Saleem, Ayeza Zanib, Abid Shehzad, Muhammad Shahbaz Naeem

Acquisition, analysis, or interpretation of data: Muhammad Abdullah Saleem, Ayeza Zanib, Abid Shehzad

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Critical review of the manuscript for important intellectual content: Muhammad Abdullah Saleem, Ayeza Zanib, Abid Shehzad, Muhammad Shahbaz Naeem

Drafting of the manuscript: Ayeza Zanib, Abid Shehzad

Supervision: Muhammad Shahbaz Naeem

Disclosures

Plant subjects: All authors have confirmed that this study did not involve any herbarium specimen. **Human subjects:** All authors have confirmed that this study did not involve human participants or tissue. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Data Availability Statements

The datasets (and/or code) supporting this study are available from the corresponding author upon reasonable request.

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