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Synergistic effects of iron oxide nanoparticles and arbuscular mycorrhizal fungi through seed coating on maize growth and yield under drought stress

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ABSTRACT

Drought stress significantly hampers maize growth and productivity, especially in drylands. This study evaluated the potential of nano seed-coating enriched with arbuscular mycorrhizal fungi (AMF) to improve maize performance under drought stress. A split-plot randomized complete block design was used with two field capacities (100% and 60% FC) and four seed coating treatments: control, Fe_3O_4 nanoparticles (NPs), AMF, and Fe_3O_4 NPs+AMF. Results showed that Fe_3O_4 NPs enriched with AMF improved physiological traits such as photosynthetic rate and leaf relative water content while reducing proline accumulation under drought conditions. Although seed coating treatments did not significantly enhance yield parameters, a positive trend was observed, with higher cob weight and kernel weight per cob compared to the control. Pearson correlation analysis revealed strong positive correlations between kernel weight per cob and multiple growth and physiological parameters, as well as a strong negative correlation with proline content. These findings suggest that seed coating with Fe_3O_4 NPs and AMF can potentially enhance maize drought resilience through physiological improvements, warranting further field-scale validation.

Keywords: arbuscular mycorrhizal fungi, cob weight, iron oxide nanoparticles, photosynthetic rate, proline.

INTRODUCTION

Drought remains one of the most critical challenges in global agriculture, directly threatening food availability and crop productivity (Chukwuneme et al., 2020). Changes in the timing and intensity of rainy and dry seasons have exacerbated this issue by disrupting water availability for crops. Prolonged dry periods limit planting options, increase drought risk, and often lead to crop failure (Sukarman et al., 2018). In Indonesia, rainfall variability – largely influenced by the El Nino Southern Oscillation (ENSO) – poses a significant challenge. El Nino, typically associated with reduced precipitation, has been shown to severely affect maize production nationwide (Malau et al., 2000). Climate change exacerbates these stresses by degrading soil quality and reducing water availability, resulting in prolonged drought affecting crop performance (Rahmah et al., 2020).

In maize, drought stress during the early growth stages induces oxidative stress that can hinder seedling development, damage plant tissues, and ultimately result in plant death (Vardharajula et al., 2011; Zhang et al., 2018). Various strategies have been investigated to alleviate drought stress, including using micro- and macronutrient fertilizers (Matlok et al., 2022). However, conventional fertilizers often show low nutrient use efficiency and may contribute to environmental degradation. Nanotechnology offers a promising alternative. Nanoparticles – extremely small particles (1–100 nm) – are more reactive, efficient at delivering nutrients, and effective at lower doses than bulk materials (Widowati et al., 2012; Liu & Lal, 2015). In addition to enhanced nutrient uptake, nanoparticles reduce oxidative stress by decreasing reactive oxygen species (ROS) levels and increasing the activity of antioxidant enzymes, including superoxide dismutase, catalase, and guaiacol peroxidase (Guha et al., 2018).

This study focuses on iron oxide (Fe_3O_4) nanoparticles (NPs), selected for their magnetic properties, biocompatibility, and superior stability compared to other iron-based compounds such as $FeSO_4$ or Fe_2O_3 (Su, 2017; Raiesi-Ardali et al., 2022). Previous studies have reported that Fe_3O_4 NPs promote root elongation, stimulate dehydrogenase enzyme activity, reduce proline accumulation in maize, and support beneficial microbial activity in the soil (Yan et al., 2020). In wheat, they have also been shown to improve photosynthetic efficiency, enhance antioxidant defense mechanisms, and increase the availability of iron and manganese ions under saline conditions (Kreslavski et al., 2023).

Despite these advantages, limited research has explored the potential synergistic effects of combining Fe_3O_4 NPs with arbuscular mycorrhizal fungi (AMF), especially in maize under drought stress. AMFs are well-known to improve drought tolerance by increasing water and nutrient uptake and stimulating antioxidant production (Hameed et al., 2014). For instance, Bahraminia et al. (2020) reported that AMF increased chlorophyll content and biomass in maize under 70% and 50% field capacity (FC). Furthermore, the combination of AMF and titanium dioxide (TiO_2) NPs has been shown to improve the drought tolerance and productivity of *Salvia officinalis* L. (Ostadi et al., 2022a).

However, the application of AMF via seed coating is still underutilized, representing only about 4% of total uses among plant-beneficial microbes (PBMs) (Rocha et al., 2019a). Seed coating involves encapsulating seeds with specific materials that deliver active compounds such as symbiotic microbes, micronutrients, protective agents, growth regulators, or colorants (Palupi et al., 2016; Pedrini et al., 2017). This method can enhance seed quality, promote early seedling vigor, and increase plant resilience to biotic and abiotic stresses (Palupi et al., 2017; Rehman et al., 2020). Combining Fe_3O_4 NPs and AMF in a seed coating formulation may yield synergistic effects, yet to date, this strategy has not been reported in maize cultivated under drought conditions in Indonesia.

To date, no studies have explored the combined use of Fe_3O_4 NPs and AMF via seed coating in maize under drought stress, particularly in Indonesia. This study addresses that gap by evaluating their synergistic effects on maize growth and yield. We therefore hypothesized that the combination would enhance maize performance under drought stress. This seed-based integration of nanotechnology and microbial symbiosis offers a sustainable strategy to improve crop resilience in water-limited conditions.

MATERIALS AND METHODS

Research objects and experimental site

This research was conducted from September 2023 to January 2024 at the Seed Science and Technology Laboratory, Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University; the Laboratory of Mycorrhizal Technology and Seedling Quality, Department of Silviculture, Faculty of Forestry and Environment, Bogor Agricultural University; and the greenhouse of the Indonesian Institute of Soil and Fertilizer Standardization. The maize seeds used were of the Lamuru variety from the Indonesian Cereals Research Institute (ICERI), Maros, South Sulawesi, harvested in July 2022. The AMF inoculum was produced in July 2023, with a density of 30 spores g^{-1} of zeolite. The AMF inoculum contained four species: *Glomus etunicatum*, *Glomus manihotis*, *Gigaspora* sp., and *Acaulospora* sp. Analytical grade Fe_3O_4 NPs used for nano seed coating were purchased from Nano Research Company NRE Lab, ITNANO™ (Medan, Indonesia). According to the Certificate of Analysis (CoA) provided by NRE Lab, the Fe_3O_4 NPs had a particle size of 58 ± 14.01 nm (Figure 1a), crystallite size of 16 nm, and a molecular weight of 231.533 g mol^{-1}, with 99% purity. Scanning Electron Microscopy (SEM) was used to analyze the particle size distribution (Figure 1b), while X-ray diffraction (XRD) was employed to determine the crystallite size of the nanoparticles (Figure 1c).

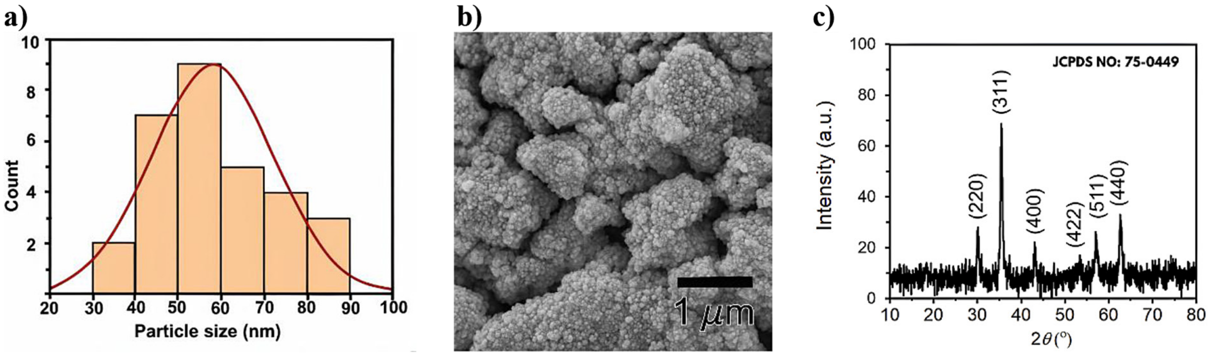

Figure 1. Characterization of Fe_3O_4 powder. (a) particle size distribution from SEM analysis, with an average size of 58 ± 14.01 nm, (b) SEM image showing the aggregated morphology of Fe_3O_4 NPs, and (c) XRD pattern confirming the cubic spinel structure (JCPDS No: 75-0449). All data in this figure were obtained from the NRE Lab, ITNANO™

Seed coating

The maize seeds were surface-sterilized using 0.5% sodium hypochlorite for 5 minutes, followed by thorough rinsing with distilled water three times and once with deionized water (Shah et al., 2021). The AMF inoculum in the zeolite and peat media was filtered using an 18-mesh sieve. Seeds were coated with AMF inoculum, zeolite flour, sterile peat, polymer, and Fe_3O_4 NPs (300 mg L^{-1}). The polymers used were 2.5% polyvinyl alcohol (PVA) and 5% tapioca starch. The maize seed, polymer + NPs, and inoculant carrier ratio were 10:1:1 (Marwanto et al., 2020). Seed coating was performed in a closed container for approximately 20 minutes. The seeds were air-dried for 7 days at 20 ± 1 °C with 50% relative humidity until the seed moisture content reached approximately 11%. Based on the study by Manggung et al. (2014), the number of AMF spores coated on each seed was approximately 50 spores.

Experimental design

The experiment was conducted using a split-plot randomized complete block design (RCBD), with field capacity (FC) levels (100% and 60% FC) as main plots and four seed coating treatments (control, Fe_3O_4 NPs, AMF, and Fe_3O_4 NPs+AMF) as subplots. Ultisol soil (pH 5.20, CEC 16.65 cmol kg^{-1}) was used and filled into 10 kg pots (29 × 28 cm). Soil moisture was maintained by daily weighing and adjusting the water supply based on the gravimetric method and soil moisture meter readings. Drought stress began 28 days after sowing (DAS), based on the research of Guler et al. (2016). Plants were fertilized with essential nutrients at a dose of urea 340 kg ha^{-1}, SP-36 100 kg ha^{-1}, and KCl 200 kg ha^{-1}. The first fertilization occurred at 10 DAS with a dose of 0.7 g urea, 0.5 g SP-36, and 1 g KCl per pot. The second fertilization occurred at 30 DAS with 1 g of urea per pot. Parameters measured included growth traits, photosynthetic rate, chlorophyll content, leaf relative water content, proline content, AMF root colonization, plant dry weight, and yield components (number of cobs, cob weight, kernel weight per cob, cob length, and cob diameter). Harvesting occurred when kernel moisture reached 22–25%, then reduced to 12% for yield measurements (García-Lara et al., 2019).

Statistical analysis

Data were analyzed using a Two-Way Analysis of Variance (ANOVA). Post-hoc analysis was performed using the Duncan Multiple Range Test (DMRT) at α = 5% significance level. Pearson correlation tests were employed to determine the relationships between variables. The statistical analyses were performed using Microsoft Excel 2019 and R Studio version 2023.06.1.

RESULTS AND DISCUSSION

Table 1 summarizes the F-test analysis on the effect of seed coating on maize growth under drought stress in a greenhouse. Field capacity (FC) as a single factor significantly affected all variables except plant height at 5 WAP, leaf number at 5 WAP, photosynthetic rate, leaf relative water content, and AMF colonization.

Table 1. Summary of Two-Way ANOVA results for the interaction effects of field capacity and seed coating on maize growth and yield components

Parameters	Treatments		Interaction	CV (%)
	Field capacity	Seed coating		
Plant height at 5 WAP	0.580^{ns}	0.311^{ns}	0.747^{ns}	7.5
Plant height at 6 WAP	0.001**	0.328^{ns}	0.990^{ns}	7.8
Plant height at 7 WAP	4.770***	0.202^{ns}	0.684^{ns}	7.5
Plant height at 8 WAP	8.781***	0.243^{ns}	0.850^{ns}	8.5
Plant height at 9 WAP	1.751***	0.127^{ns}	0.522^{ns}	8.6
Plant height at 10 WAP	1.391***	0.112^{ns}	0.555^{ns}	7.1
Stem diameter at 5 WAP	0.043*	0.489^{ns}	0.401^{ns}	8.5
Stem diameter at 6 WAP	0.000***	0.390^{ns}	0.581^{ns}	9.0
Stem diameter at 7 WAP	0.000***	0.740^{ns}	0.563^{ns}	9.1
Stem diameter at 8 WAP	0.000***	0.481^{ns}	0.910^{ns}	7.9
Stem diameter at 9 WAP	0.022*	0.936^{ns}	0.884^{ns}	8.9
Stem diameter at 10 WAP	0.009**	0.934^{ns}	0.950^{ns}	8.7
Number of leaves at 5 WAP	0.125^{ns}	0.187^{ns}	0.790^{ns}	7.3
Number of leaves at 6 WAP	2.751***	0.112^{ns}	0.112^{ns}	4.5
Number of leaves at 7 WAP	3.641***	0.214^{ns}	0.900^{ns}	6.0
Number of leaves at 8 WAP	7.321***	0.132^{ns}	0.249^{ns}	4.6
Number of leaves at 9 WAP	8.871***	0.586^{ns}	0.993^{ns}	4.8
Number of leaves at 10 WAP	9.871***	0.586^{ns}	0.993^{ns}	2.2
Photosynthetic rate	0.176^{ns}	0.000***	0.420^{ns}	16.5
Chlorophyll content	0.000***	0.451^{ns}	0.539^{ns}	7.5
Leaf area	5.407***	0.716^{ns}	0.761^{ns}	10.2
Leaf relative water content	0.688^{ns}	0.027*	0.037*	3.8
Proline content	9.011***	2.081***	4.761***	4.2
AMF colonization rate on roots	0.672^{ns}	0.000***	0.787^{ns}	15.3^{t}
Plant dry weight	9.672***	0.069^{ns}	0.116^{ns}	16.2
Number of cobs	0.021*	0.205^{ns}	0.205^{ns}	16.7
Cob weight	6.7211**	0.090^{ns}	0.381^{ns}	19.8
Kernel weight per cob	1.011***	0.549^{ns}	0.167^{ns}	28.7
Cob length	2.651***	0.178^{ns}	0.224^{ns}	17.1
Cob diameter	1.011***	0.333^{ns}	0.108^{ns}	16.8

Note: * – significant effect at the $\alpha = 5\%$ level, ** – significant effect at the $\alpha = 1\%$ level, *** – significant effect at the $\alpha = 0.1\%$ level, ns – no significant effect, CV – coefficient of variation, *t* – square root transformed data.

Conversely, seed coating alone significantly influenced photosynthetic rate, leaf relative water content, proline content, and AMF colonization. Moreover, the interaction between FC and seed coating significantly impacted leaf relative water content and proline content.

Drought stress at 60% FC significantly reduced plant height (Figure 2a), stem diameter (Figure 2c), and number of leaves (Figure 2e). Drought stress affects plant growth through decreased carbon accumulation, cell number, and tissue expansion (Tardieu et al., 2011). Moharramnejad et al. (2019) observed that drought stress reduced the height and diameter of maize stems. Jain et al. (2019) found that drought stress inhibits cell growth and decreases the number of leaves. The number and area of leaves are important factors in photosynthesis, transpiration, and plant interception. In this study, the diameter of maize stems tended to increase until 8 weeks after planting (WAP) and decreased in the following observations (Figure 2b). Sabiel et al. (2014) reported similar findings, with maize stem diameters of 14 mm, 20 mm, and 19 mm recorded at 30, 45, and 60 DAS, respectively. These measurements likely indicate the plants' transition into the generative phase. Center et al. (1970) indicated that the dry weight of maize stems, leaves, and cob decreased during seed ripening. Shodikin and Wardiyati (2018) explained that in the generative phase, maize assimilates translocation and focuses on cob formation.

In this study, coating treatment had no significant effect on height (Figure 2b), stem diameter (Figure 2d), or leaf number (Figure 2f) of maize under drought stress conditions. These findings differ from previous studies that reported the positive effects of nano-priming and AMF application on crop growth under abiotic stress. For instance, Haq et al. (2023) reported that nano seed priming Fe 150 mg L^{-1} increased the number of leaves of mung bean plants both under drought and without stress. Kreslavsi et al. (2023) showed that nano-priming Fe_3O_4 NPs 200 mg L^{-1} and 500 mg L^{-1} improved wheat growth by increasing photosynthetic ability, antioxidant balance, and availability of iron and manganese ions under salinity stress. Similarly, Naseer et al. (2022) reported that combining nano seed priming Fe (10 mg L^{-1}) with *Glomus intraradices* inoculation increased wheat growth and photosynthetic rate. Previous studies on maize have also reported that AMF inoculation enhanced plant height and stem diameter under drought conditions (Song et al., 2021a), supporting the potential role of AMF in improving plant resilience.

The absence of significant effects in the current study could be attributed to several factors. Maize as a species may respond differently to Fe_3O_4 NPs and AMF, influenced by differences in physiology, stress tolerance thresholds, and nutrient requirements. Additionally, the nanoparticle concentration, AMF inoculum viability, or colonization rate may not have reached an effective threshold to elicit measurable growth responses. Environmental variability, particularly in drought stress intensity and soil nutrient status, could also have influenced the effectiveness of the seed coating treatments.

Although significant improvements were not observed in the measured parameters, previous research has established the important roles of AMF

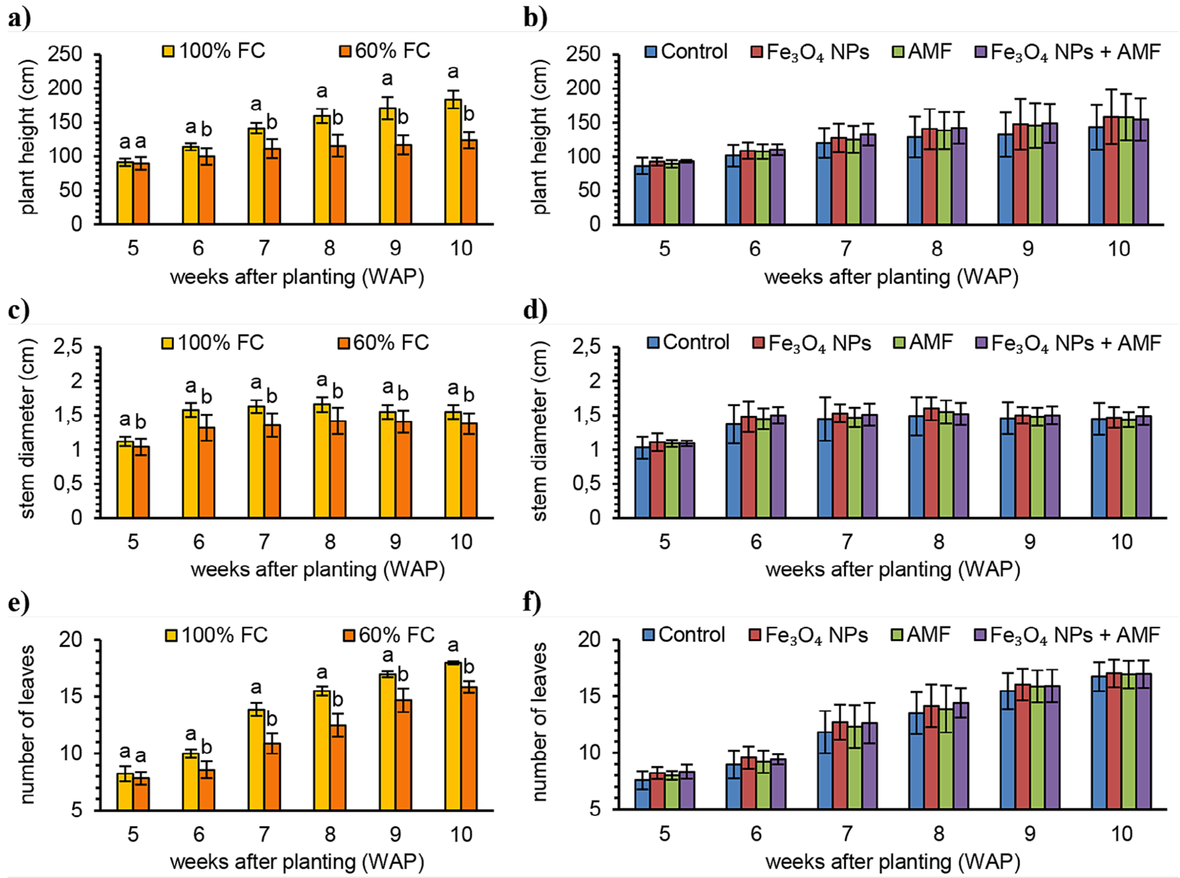

Figure 2. Effects of (a, c, e) field capacity (FC) and (b, d, f) seed coating treatments on (a, b) plant height, (c, d) stem diameter, and (e, f) number of leaves of maize across WAP. Different letters within the same measurement indicate significant differences between FC levels (DMRT, P <.05). Seed coating treatments showed no significant differences (P >.05)

and Fe_3O_4 NPs in enhancing nutrient uptake, water acquisition, and stress tolerance mechanisms (Nasution et al., 2014; Gholamhoseini et al., 2013; Wu and Zou, 2017). These mechanisms may require longer establishment periods or optimized conditions to manifest clearly. Thus, further research focusing on optimizing treatment protocols and stress management strategies is warranted to fully exploit the potential benefits of seed coating technologies in maize under drought stress.

The drought stress levels at 100% FC and 60% FC, with seed coating treatments, exhibited similar photosynthetic performance across these stress levels (Figure 3a). This study demonstrates that seed coating enhances the photosynthetic rate in maize leaves (Figure 3b). The consistent photosynthetic response under varying drought stress conditions suggests that seed coating may improve the efficiency of water and nutrient uptake, thus stabilizing photosynthetic function under stress. These findings are consistent with previous studies, which highlight the beneficial effects of seed coating, warranting further research into the underlying mechanisms. Feng et al. (2022) reported that Fe_3O_4 nano seed priming at 200 mg L^{-1} and 500 mg L^{-1} was non-toxic to wheat and led to an increase in the photosynthetic rate, Fe content, and P in the leaves. Similarly, Li et al. (2020) found that Fe_3O_4 NPs accelerate electron transport in the thylakoid membrane, acting as electron donors and acceptors, which enhances photosynthesis by binding to chloroplasts. Zhu et al. (2012) observed that AMF symbiosis under drought stress boosts photosynthesis in maize leaves. Begum et al. (2019) highlighted that AMF increases antioxidant levels, repairing oxidative damage to membrane function and photosynthesis, while also enhancing mineral uptake and providing protection under drought conditions. Additionally, Hu et al. (2020) demonstrated that AMF improves photosystem II efficiency and chlorophyll concentration, irrespective of water status.

Fe_3O_4 NPs enhance iron bioavailability and mobility within plant tissues, contributing to chlorophyll synthesis and redox regulation (Jalali et al., 2016; Pariona et al., 2017). Meanwhile, AMF enhances phosphorus uptake through their hyphal networks and by increasing the expression

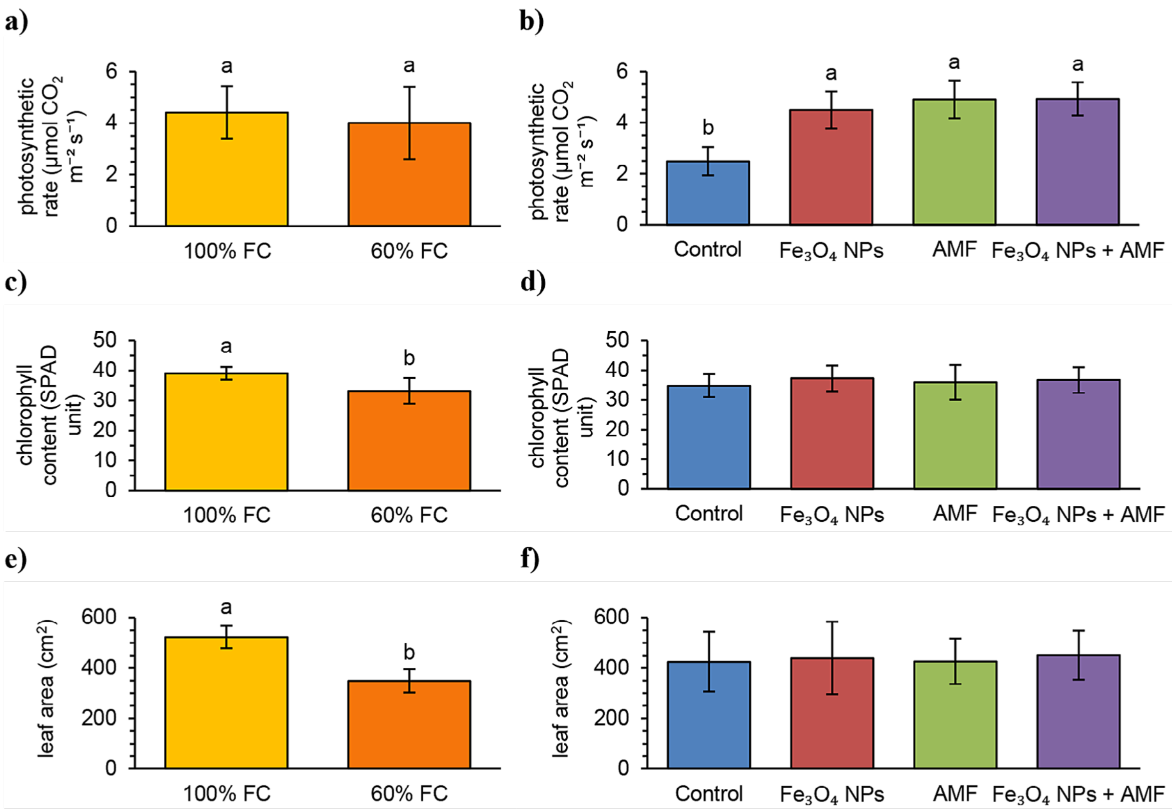

Figure 3. Effects of (a, c, e) field capacity (FC) and (b, d, f) seed coating treatments on (a, b) photosynthetic rate at 25 days after drought stress, (c, d) chlorophyll content at 21 days after drought stress, and (e, f) leaf area of maize at 10 WAP. Different letters indicate significant differences between FC levels (DMRT, P <.05). No significant effects of seed coating were found on chlorophyll content and leaf area (P >.05)

of phosphorus transporter genes (Liu et al., 2020), further improving photosynthetic efficiency under drought conditions. Notably, inoculation with *Glomus intraradices* has been shown to enhance phosphorus uptake more effectively than indigenous AMF species (Cozzolino et al., 2013). These findings underscore the synergistic benefits of combining AMF with Fe_3O_4 NPs.

Drought stress at 60% FC significantly reduced chlorophyll content and leaf area (Figure 3c and 3e). Leaf water potential, chlorophyll content, and chlorophyll fluorescence are important indicators of plant adaptation to drought stress (Chen et al., 2016). A decrease in chlorophyll content represents a protective mechanism to reduce photo-oxidative damage due to the inhibition of photosynthesis and excessive light excitation. Photosynthetic efficiency typically declines under drought stress, limiting carbohydrate accumulation in tissues and consequently impacting leaf expansion (Li et al., 2019). Huang et al. (2022) also reported that drought stress reduces maize leaf area. Drought stress during the seedling and vegetative stages significantly affects plant height, whereas, during the tasseling and seed-filling stages, it impacts the leaf area index. Continuous water deficits can also affect stem diameter.

In contrast, seed coating treatments did not significantly influence chlorophyll content or leaf area (Figure 3d and 3f). This suggests that under the drought stress conditions applied, the benefits of Fe_3O_4 NPs and AMF were insufficient to significantly enhance photosynthetic pigment retention or promote leaf expansion in maize. Factors such as drought severity, the physiological resilience of maize, or suboptimal concentrations and colonization efficiency of the applied treatments may have contributed to the lack of significant responses.

Drought stress at 60% FC significantly increased proline content compared to 100% FC (Figure 4a). Plant tolerance to drought is closely related to the accumulation of osmoprotectants such as proline and soluble sugars (Mohammadkhani and Heidari, 2008). Proline, a non-protein amino acid, acts as an osmoregulator that accumulates at high concentrations without disrupting cell structure or metabolism. It is also a reliable indicator of drought stress, as its levels rise more rapidly than other amino acids and decline quickly after stress ends (Zadehbagheri et al., 2014). In this study, seed coating with Fe_3O_4 NPs, AMF, and their combination significantly reduced proline content compared to the control. The Fe_3O_4 NPs+AMF treatment showed the greatest reduction at 60% FC, outperforming the individual treatments. This may be attributed to the antioxidant activity of iron NPs, which activate both enzymatic and non-enzymatic defense systems, thereby reducing lipid peroxidation and ROS accumulation under drought (Elanchezhian et al., 2017; Rezayian et al., 2023). Improved membrane stability and lower oxidative stress consequently led to reduced proline accumulation, supporting the synergistic role of the combined treatment in mitigating drought-induced oxidative damage.

Askary et al. (2017) reported that Fe_2O_3 NPs, acting as micronutrients, reduced lipid peroxidation, and proline content in mint under salinity stress, thereby alleviating abiotic stress. Similarly, Li et al. (2020) found that Fe_3O_4 NPs function like antioxidant enzymes, enhancing stress defense. Hameed et al. (2014) and Zou et al. (2013) showed that AMF inoculation improves drought tolerance by reducing proline accumulation, with *Funneliformis mosseae* notably lowering proline levels in *Poncirus trifoliata* and enhancing leaf

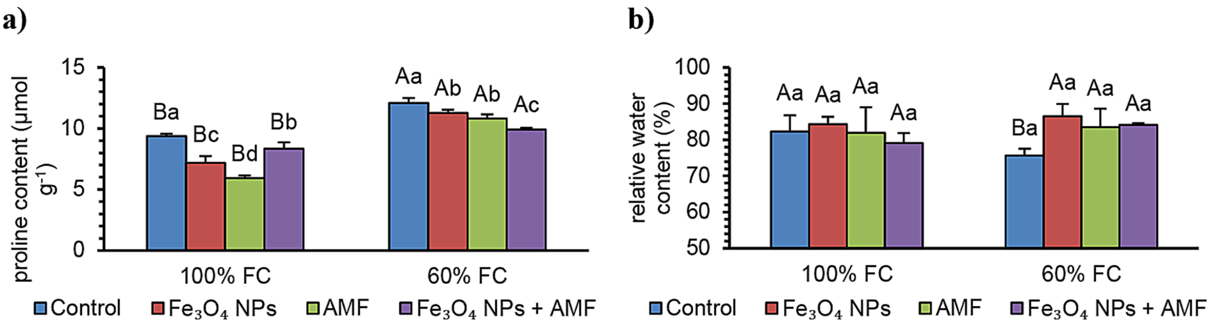

Figure 4. Effect of field capacity (FC) and seed coating treatments on (a) proline content of maize plants at 38 DAS and (b) relative water content of leaves at 7 WAP. Capital letters indicate significant differences between 100% FC and 60% FC within the same seed coating treatment, while lowercase letters indicate significant differences among seed coating treatments within the same FC levels (DMRT, P <.05)

water status and root volume. Hayat et al. (2012) explained that proline synthesis occurs via glutamate and ornithine pathways, with the glutamate pathway contributing more under osmotic stress. La et al. (2020) further reported that inhibiting glutamate synthesis reduced proline accumulation under both drought and non-stress conditions.

Leaf relative water content in the control treatment decreased at 60% FC drought stress. Seed coating treatment of Fe_3O_4 NPs and AMF, both singly and combined, maintained leaf relative water content at 60% FC (Figure 4b). According to Pirasteh-Anosheh et al. (2016), in the early stages of drought stress, stomatal closure occurs and decreases the amount of CO_2, which can inhibit photosynthesis. Aslam et al. (2022) stated that stomatal closure under drought stress is induced by abscisic acid (ABA) produced by the roots to prevent water loss in plants through a decrease in the transpiration rate. Ghahfarokhi et al. (2015) reported that the relative water content of the leaves significantly decreased under drought stress conditions and increased again after watering. Research by Song et al. (2021b) showed that when leaf relative water content is less than 75.4%, watering cannot restore the optimal photosynthetic ability of maize plants because the photosynthetic organs have been damaged.

Fe_3O_4 NPs regulate phytohormones like gibberellins (GA) and indole-3-acetic acid (IAA), enhancing plant growth and osmotic balance (Li et al., 2021). In maize roots colonized by AMF, higher indole-3-butyric acid (IBA) levels and auxin-related gene expression promote root development and water retention (Kaldorf and Ludwig-Müller, 2000; Liu et al., 2020). Although not directly measured here, AMF has been linked to increased ABA production, which regulates stomatal closure and improves water retention under drought stress (Bahadur et al., 2019). These findings highlight the potential synergy between AMF and Fe_3O_4 NPs treatments. Applying Fe_3O_4 NPs increases Fe uptake through roots and plant stability under water deficit conditions (Alabdallah et al., 2021). Fe NPs maintain water retention in soybean plant cells under drought-stress conditions (Linh et al., 2020). Roots absorb $NiFe_2O_4$ NPs, which are then transmitted by plants, increasing mineral uptake, chlorophyll content, soluble protein, and relative water content in barley plant leaves (Tombuloglu et al., 2019a). Applying TiO_2 NPs combined with AMF increased the leaf water content in mint plants under drought stress. Symbiosis of plants with AMF can increase the hydraulic conductivity of water into the roots, thus increasing the relative water content in plant cells (Ostadi et al., 2022b). Mycorrhizal roots have a more potential xylem network for transporting water from the soil to the plant (Budi et al., 2014). In wheat, AMF symbiosis increases leaf relative water content and soil moisture in the root zone, supported by enhanced root development and hyphal elongation for water uptake under drought (Mathur et al., 2019).

The percentage of AMF colonization on the roots was observed after 14 WAP. Drought stress treatment did not significantly differ in the percentage of AMF colonization. AMF seed coating and Fe_3O_4 NPs+AMF treatments showed a significantly higher percentage of AMF colonization (81.7% and 93.3%, respectively) compared to the control (46.7%) and Fe_3O_4 NPs seed coating (31.7%) (Figure 5). Metwally and Abdelhameed (2024) reported similar results, where $ZnFe_2O_4$ NPs reduced the percentage of AMF colonization (75% to 66.7%) in peas. The combination increased nutrient uptake (N, P, K, and Mg), enzyme activities (phosphatase, catalase, and peroxidase), and accumulation of primary metabolites (carbohydrates, proteins, and amino acids). Our study used mixed AMF spores, including *Glomus etunicatum*, *Glomus manihotis*, *Gigaspora* sp., and *Acaulospora* sp. Fasusi et al. (2021) reported that using mixed AMF spores increased the percentage of AMF colonization compared to single spores on maize roots. Rocha et al. (2019b) stated that using mixed AMF through seed coating significantly increased chickpea (*Cicer arietinum*

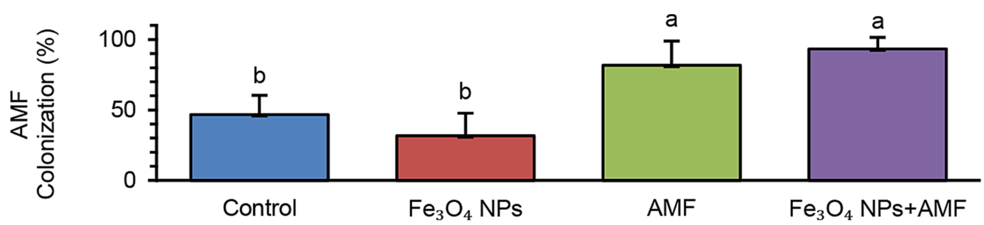

Figure 5. Effect of seed coating and drought stress on AMF colonization on roots at 14 WAP

L.) production compared to single isolate AMF. Oliveira et al. (2016) found that applying AMF through seed coating can accelerate growth and nutrient absorption and increase AMF colonization in wheat roots compared to direct application.

Low concentrations of Fe_3O_4 NPs support root elongation, potentially increasing the root surface area available for AMF colonization (Yan et al., 2020). Microelements such as Fe and Zn are also crucial for AMF spore germination and hyphal development (Tamayo et al., 2014). However, these interactions are sensitive to NP type, concentration, and environmental context (Dimkpa, 2014). The combination treatment may enhance root growth and colonization efficiency, improving drought resilience.

Root staining revealed that maize roots symbiotic with AMF contained vesicles, spores, and intra- (IH) and extraradical hyphae (EH) (Figure 6). Similar structures—including vesicles, spores, hyphae, and arbuscules—have also been reported in *Acacia gerrardii* roots colonized by AMF (Hashem et al., 2016). According to Souza (2015), hyphae are classified as IH, which spread within the root and form vesicles or branched structures called arbuscules, and EH, which extend into the surrounding soil. Vesicles function as storage organs for fungal reserves (Teotia et al., 2017), while arbuscules facilitate nutrient exchange between AMF and the host plant (Luginbuehl and Oldroyd, 2017). Spores, together with EH and colonized root fragments, act as propagules that enable AMF dissemination (Paz et al., 2020). EH typically form spores via intercalary hyphal swelling or auxiliary cells, whereas IH may produce spores from vesicles or arbuscule-like structures (Dodd et al., 2000; Marleau et al., 2011). IH obtain carbon from the host root, supporting the growth of EH (Lekberg et al., 2013). Both EH and root hairs absorb water and nutrients from the soil; however, under drought conditions, AMF can partially replace the role of root hairs by enhancing stress tolerance through an alternative water-uptake mechanism (Zou et al., 2019). AMF hyphae have a smaller diameter (3.5–4.8 µm) compared to maize root hairs (200–400 µm) (Costa et al., 2002; Drew et al., 2003). The extensive EH network enables the exploration of soil microsites inaccessible to plant roots, thereby enhancing water and nutrient absorption and mitigating drought stress (Saddique et al., 2018; Hamel, 2004).

Drought stress at 60% FC significantly reduced plant dry weights (Figure 7a), which is consistent with previous findings that drought stress hampers biomass accumulation. Shao et al. (2021) reported that 60% FC reduced plant dry weight by 61.4% at 54 DAS. Water deficit inhibits biomass accumulation, especially in leaves and maize cobs. Ali et al. (2011) showed that at 50% and 75%, FC decreased leaf area, height, cell membrane thermostability, osmotic potential, turgor, and dry weight of maize. The decrease in weight is an adaptation of plants to stress. According to Jiang et al. (2018), drought stress slows biomass formation, and the longer the stress period significantly reduces plant dry weight. Based on the research of Song et al. (2019), drought stress reduces the leaf area index and biomass of maize due to reduced intercepted photosynthetically active radiation (IPAR) and radiation-use efficiency (RUE) due to damage to the chloroplast membrane structure. According to Jain et al. (2019), plants adapt to drought by reducing stomatal conductance, leaf evaporation, and growth rate, leading to lower transpiration, early senescence, and ultimately decreased photosynthesis and dry weight.

Contrary to expectations based on previous studies, the seed coating treatments did not show

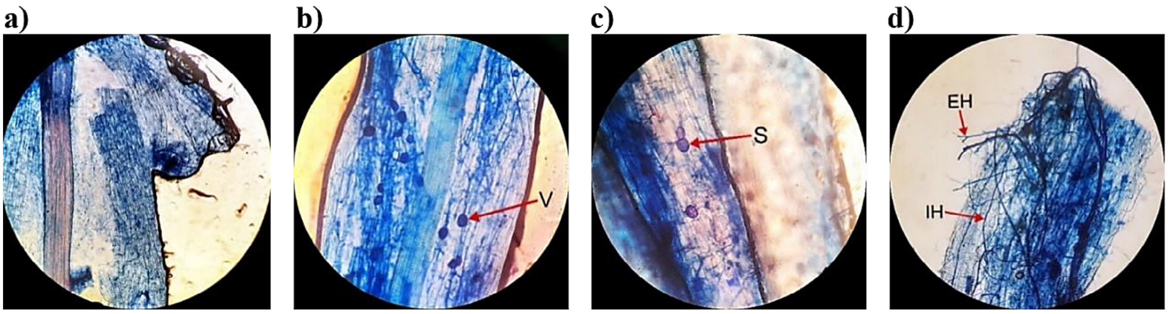

Figure 6. The presence of AMF on the root system of maize (a) non-colonized, (b) vesicles, (c) spores, (d) intraradical hyphae and extraradical hyphae (V – vesicles, IH – intraradical hyphae, EH – extraradical hyphae, S – intraradical spores, under 20× magnification)

any significant effect on plant dry weight (Figure 7b). The results indicate that under the drought conditions tested in this study, these treatments were not able to mitigate the negative effects of drought on plant biomass. This contrasts with previous research, such as that by Abdelhameed et al. (2021), who found that $ZnFe_2O_4$ NPs combined with AMF increased the shoot and root dry weights of pea plants compared to the single AMF treatment. Similarly, Jayarambabu et al. (2018) highlighted that iron, through its role in photosynthesis and the electron transport chain, can enhance plant growth.

Drought stress at 60% FC significantly reduced all yield parameters in this study, including the number of cobs (Figure 8a), cob weight (Figure 8c), kernel weight per cob (Figure 8e), cob length (Figure 8g), and cob diameter (Figure 8i). These findings align with previous studies showing that drought stress at 50% FC significantly reduced maize growth and yield components, including cob weight and seed quality (Ghazi, 2017). Rou et al. (2020) reported a 21% inhibition in cob formation under similar conditions, while Shashi et al. (2018) found that drought at 60% FC lowered photosynthetic rates, affecting kernel number and dry yield. Daryanto et al. (2016) emphasized that drought during the reproductive phase has a more severe impact on yield than during the vegetative phase. Similarly, Li et al. (2018) highlighted that water deficit during vegetative growth reduces seed number, and leaf dry weight and restricts leaf and seed development. Sah et al. (2020) further demonstrated that drought during the flowering and seed-filling phases substantially decreased maize yield. Representative visual differences between maize cobs grown under 60% and 100% FC are shown in Figure 9.

In contrast, seed coating treatments with Fe_3O_4 NPs, AMF, or their combination did not significantly affect maize yield parameters, including the number of cobs (Figure 8b), cob weight (Figure 8d), kernel weight per cob (Figure 8f), cob length (Figure 8h), and cob diameter (Figure 8j). These results differ from prior studies demonstrating beneficial effects of AMF and Fe-based NPs under abiotic stress. For instance, Alsamadany et al. (2024) reported that the application of Fe NPs significantly increased plant height, total dry weight, and maize grain weight under salinity stress. Similarly, Al-Shaheen et al. (2019) found that Fe NPs enhanced maize growth and productivity by improving respiration processes, activating stress-related enzymes, promoting stomatal function, and enhancing nutrient uptake.

The absence of significant improvements in this study may be attributed to extreme environmental conditions during the experimental period. Specifically, maize plants were exposed to high temperatures reaching up to 56°C during the seed-filling phase. According to Qi et al. (2022), combined drought and high-temperature stress can inhibit seed filling rates and durations, suppress the transport efficiency of photosynthetic assimilates into seeds, and reduce endosperm cell numbers crucial for starch synthesis. In addition, Ayub et al. (2021) showed that drought stress at 60% FC combined with high temperatures increased soluble sugars, proline, and free amino acids while decreasing soluble protein content and nitrate reductase activity, further limiting plant growth and productivity.

Moreover, high temperatures are known to exacerbate the adverse effects of drought stress. Chukwudi et al. (2021) observed that high temperatures significantly reduced cob weight (64%), kernel weight (73%), number of kernels (69%), and dry biomass (23%) in maize. Bheemanahalli et al. (2022) also noted that high temperatures (38 °C) stimulated stomatal opening to enhance leaf cooling through transpiration; however, prolonged exposure can impair water-use efficiency

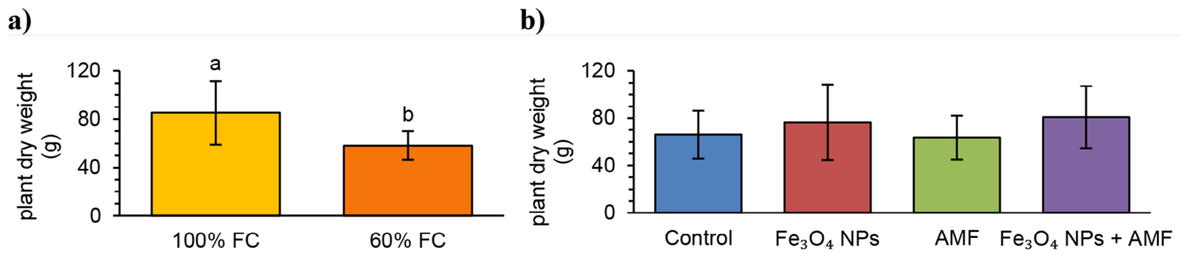

Figure 7. Effects of (a) field capacity (FC) and (b) seed coating treatments on plant dry weight of maize. Different letters within the same measurement indicate significant differences between FC levels (DMRT, $P < 0.05$). Seed coating treatments showed no significant differences ($P > 0.05$)

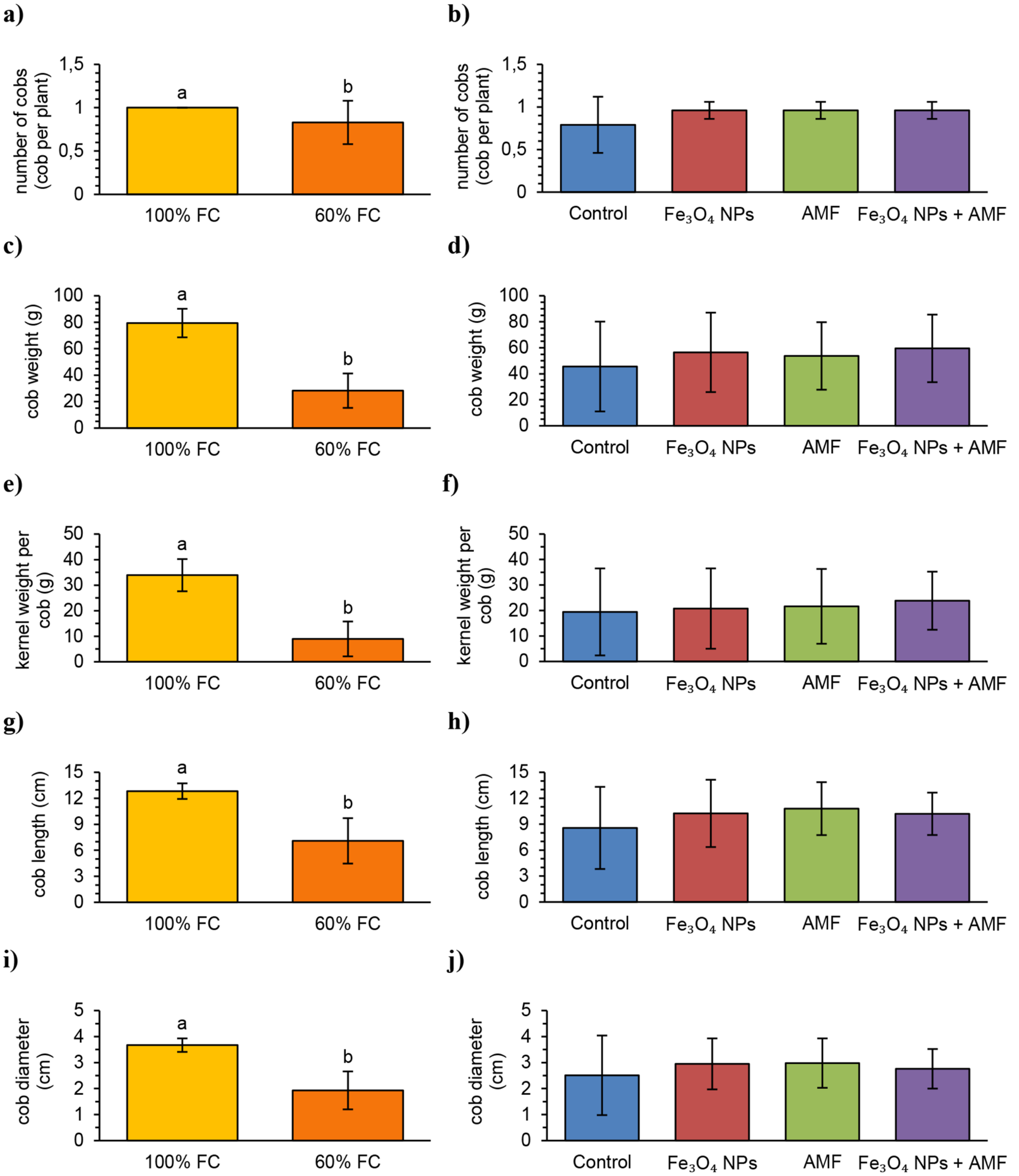

Figure 8. Effects of (a, c, e, g, i) field capacity (FC) and (b, d, f, h, j) seed coating treatments on (a, b) number of cobs, (c, d) cob weight, (e, f) kernel weight per cob, (g, h) cob length, and (i, j) cob diameter. Different letters within the same measurement indicate significant differences between FC levels (DMRT, $P < 0.05$). Seed coating treatments showed no significant differences in shoot and total dry weight ($P > 0.05$)

and photosynthetic performance, ultimately reducing yield. Another plausible explanation for the lack of significant effects could be related to the dosage and efficiency of Fe_3O_4 NPs and AMF applied. Although previous studies have demonstrated that NPs enhance plant resistance through increased reactivity and metabolic support, the optimal application rate and synergy with AMF under combined drought and heat stress conditions may not have been achieved in this study.

To further understand the interrelationships among the measured variables and their contributions to yield performance, a Pearson correlation analysis was conducted (Figure 10). Kernel

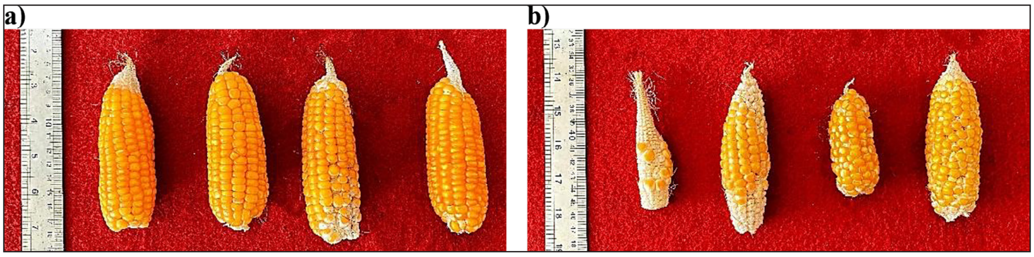

Figure 9. Maize cobs at (a) 100% FC, (b) 60% FC

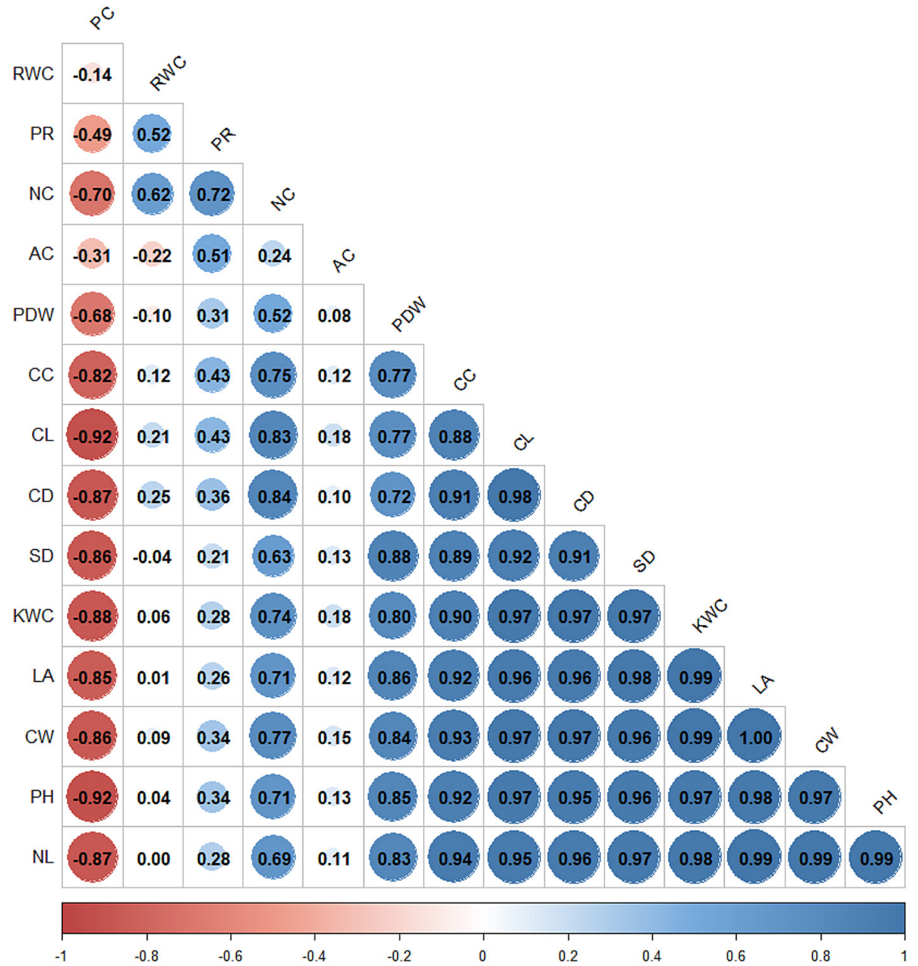

Figure 10. Pearson correlation matrix between variables. Positive correlations are shown in blue, and negative correlations in red. The strength of the correlation is indicated by the intensity of the color and the size of the circle, which is proportional to the correlation coefficient. PC = proline content, RWC = leaf relative water content, PR = photosynthetic rate, NC = number of cobs, AC = AMF colonization, CC = chlorophyll content, CL = cob length, CD = cob diameter, SD = stem diameter, KWC = kernel weight per cob, LA = leaf area, CW = cob weight, PH = plant height, NL = number of leaves, and PDW = plant dry weight

weight per cob showed a very strong positive correlation with plant height (r = 0.97), stem diameter (r = 0.97), leaf number (r = 0.98), leaf area (r = 0.99), chlorophyll content (r = 0.90), cob length (r = 0.97), cob diameter (r = 0.97), and cob weight (r = 0.99). Strong positive correlations were also observed between kernel weight per cob and number of cobs (r = 0.74), and plant dry weight (r = 0.80). These findings are consistent with previous studies. Malik et al. (2005) reported that plant height, leaf area, number of leaves, cob weight, and kernel weight per cob are strongly positively correlated with maize production. Similarly, Khan et al. (2018) found a strong association between the number of kernels per cob and plant height, while Ali et al. (2014) and Ali and Ahsan (2015) demonstrated that chlorophyll content, shoot dry weight, and plant dry weight are important contributors to increased maize yield.

Pearson correlation analysis showed that cob weight had a perfect positive correlation with leaf area (r = 1.00) and number of leaves (r = 0.99), as well as a strong positive correlation with chlorophyll content (r = 0.93) and kernel weight per cob (r = 0.99). This indicates that higher photosynthetic capacity, reflected by greater leaf area and chlorophyll content, supports biomass production and grain filling. Hokmalipour and Darbandi (2011) reported that increased leaf area enhances sunlight absorption and photosynthesis, ultimately improving maize yield, while Ghimire et al. (2015) found significant correlations between chlorophyll content and key yield components. Conversely, proline content was strongly negatively correlated with plant dry weight and kernel weight per cob (r = –0.88), suggesting that higher proline accumulation under drought stress is associated with reduced growth and productivity. Similarly, Kravić et al. (2013) linked drought-induced proline accumulation with stress severity, while Ndou et al. (2023) demonstrated that nano-priming with α-Fe_2O_3 reduced proline levels and improved sorghum growth under drought stress. Wu et al. (2017) reported that AMF reduced proline accumulation in *Poncirus trifoliata* by enhancing its catabolism via the glutamate pathway.

Although the seed coating treatments did not produce statistically significant differences in maize yield parameters, a positive trend was observed. Among the treatments, the combination of Fe_3O_4 NPs and AMF consistently showed higher mean values for the number of cobs, cob weight, kernel weight per cob, cob length, and cob diameter compared to the control and individual treatments. These trends suggest that the synergistic effect of Fe_3O_4 NPs and AMF inoculation through seed coating may enhance maize productivity under drought stress conditions. Previous research has demonstrated that the integration of NPs and AMF can improve plant tolerance to abiotic stress. Ostadi et al. (2022a) reported that TiO_2 NPs combined with AMF increased the productivity of sage under drought by promoting nutrient uptake necessary for chlorophyll and carotenoid biosynthesis and by inducing antioxidant activity. Similarly, Joel et al. (2023) highlighted that the co-application of NPs and AMF represents an emerging approach to enhancing plant resilience against abiotic stresses.

In addition, seed coating offers an efficient and targeted delivery method for AMF and NPs. Hussain et al. (2021) demonstrated that AMF application via seed coating improved maize growth more effectively than conventional inoculation methods. Almagrabi and Abdelmoneim (2012) also indicated that seed coating drastically reduced the required inoculum quantity, from 500 spores to approximately 50 spores per seed, without compromising plant performance. Therefore, although statistical significance was not achieved, the positive trends observed in this study align with the existing body of research supporting the synergistic benefits of NPs and AMF integration, particularly through efficient seed coating technology under stress conditions.

While molecular responses were not directly measured in this study, the observed physiological improvements may reflect underlying molecular mechanisms. At the molecular level, Fe_3O_4 NPs and AMF have been linked to enhanced drought tolerance by regulating stress-responsive genes and signaling pathways. Fe_3O_4 NPs promote H_2O_2 signaling and antioxidant enzyme activity, such as ascorbate peroxidase (APX) and guaiacol-dependent peroxidase (POD), contributing to the detoxification of ROS and the potential upregulation of stress-related genes (Tombuloglu et al., 2019b; Kreslavski et al., 2023). Meanwhile, AMF regulate genes associated with proline biosynthesis, ABA signaling, and micronutrient (Fe) transport, supporting both stress adaptation and nutrient homeostasis (Xu et al., 2018; Rajapitamahuni et al., 2023). These findings suggest that both agents act through complementary molecular mechanisms, warranting further gene-level investigation.

Taken together, the results of this study suggest that although extreme drought and heat stress conditions may have limited the full expression of the benefits of the seed coating treatments, the observed positive trends in yield parameters, supported by strong physiological correlations, highlight the potential of combining Fe_3O_4 NPs and AMF through seed coating as a promising strategy to enhance maize resilience under abiotic stress. The integration of Fe_3O_4 NPs and AMF through seed coating offers a simple, low-input approach particularly suitable for smallholder farmers, as it requires minimal changes to existing cropping practices. AMF inoculum can be produced locally using organic carriers such as zeolite or compost, and Fe_3O_4 NPs can be applied at relatively low concentrations (300 mg L^{-1}), helping to minimize input costs. However, practical scalability remains a challenge due to the current market availability and production cost of Fe_3O_4 NPs, alongside the need for standardized formulation and application protocols. Furthermore, while Fe_3O_4 NPs are generally regarded as biocompatible, the long-term impacts on soil microbiota and potential bioaccumulation in food chains must be carefully evaluated. Future research should focus on optimizing nanoparticle synthesis for affordability, refining seed coating formulations, and conducting field-scale validations under controlled and variable stress conditions to fully realize the potential of this technology.

CONCLUSIONS

In conclusion, seed coating with Fe_3O_4 NPs (300 mg L^{-1}) enriched with AMF improved physiological performance in maize under drought stress (60% FC), as evidenced by increased photosynthetic rate, higher leaf relative water content, enhanced AMF colonization, and reduced proline content. Although yield parameters such as cob weight and kernel weight per cob showed positive trends compared to the control, these improvements were not statistically significant. Pearson correlation analysis demonstrated strong positive correlations between kernel weight per cob and key growth and physiological parameters, alongside a negative correlation with proline accumulation. These results indicate that the combination of Fe_3O_4 NPs and AMF through seed coating may represent a promising low-input strategy to enhance maize drought tolerance. Future studies should focus on optimizing seed coating formulations and conducting large-scale field trials under varying environmental conditions to further validate and translate these findings into practical agricultural applications.

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