

Original Article

Increasing drought stress tolerance of maize seedlings through nano seed coating using ZnO, Fe₃O₄, and SiO₂

Widia Sri Fatma¹, Satriyas Ilyas^{2*}, Sri Wilarso Budi³, and Ladiyani Retno Widowati⁴

¹ *Seed Science and Technology Study Program, Graduate School, Bogor Agricultural University, Bogor, West Java, 16680 Indonesia*

² *Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University, Bogor, West Java, 16680 Indonesia*

³ *Department of Silviculture, Faculty of Forestry and Environment, Bogor Agricultural University, Bogor, West Java, 16680 Indonesia*

⁴ *Center for Engineering and Modernization of Food Crop, Bogor, West Java, 16111 Indonesia*

Received: 27 March 2024; Revised: 8 July 2025; Accepted: 6 October 2025

Abstract

Drought stress negatively impacts maize growth and yield. This study evaluated the use of nano seed coating with ZnO (15, 25, 50 mg L⁻¹), Fe₃O₄ (100, 200, 300 mg L⁻¹), and SiO₂ (300, 600, 900 mg L⁻¹) to enhance maize drought tolerance during germination. Seeds were tested under osmotic stresses of 0, -0.15, and -0.30 MPa using PEG 6000. Fe₃O₄ at 300 mg L⁻¹ significantly increased germination rate, first count germination, and speed of germination compared to the control at -0.30 MPa. ZnO at 50 mg L⁻¹ produced the highest radicle emergence (43%) under severe stress. Additionally, Fe₃O₄ (300 mg L⁻¹) and SiO₂ (600 mg L⁻¹) coatings reduced malondialdehyde (MDA) content, indicating a decrease in oxidative damage. These results highlight the potential of nano seed coating, especially with Fe₃O₄ and ZnO, to enhance maize drought tolerance during early growth. Further field validation is recommended.

Keywords: malondialdehyde, nanoparticles, seed coating, seed viability, seed vigor

1. Introduction

Drought is a significant challenge for most countries regarding agricultural availability and production (Chukwuneme, Babalola, Kutu, & Ojuederie, 2020). Altered rainfall patterns reduce water availability, limiting crop options and increasing the risk of drought and crop failure, a phenomenon already observed in Indonesia, where climate change has impacted the agricultural sector (Ruminta,

Handoko, & Nurmala, 2018; Sukarman, Mulyani, & Purwanto, 2018). Rahmah, Ilyas, and Setiawan (2020) showed that climate change worsened soil quality and water availability. Continuous water shortages can thus induce drought stress in plants.

Drought stress during early maize growth induces oxidative stress that inhibits plant growth, causes necrosis, and may lead to plant death. Supplying essential micro- and macronutrients can mitigate these effects (Matlok, Piechowiak, Krolikowski, & Balawejder, 2022). Nanotechnology enhances fertilizer efficiency because nanoparticles (≈1–100 nm) are highly reactive, target-specific, and require smaller doses (Widowati, Husnain, & Hartatik, 2011). Metal-oxide nanoparticles (NPs) such as zinc oxide

*Corresponding author

Email address: satriyas_ilyas@apps.ipb.ac.id

(ZnO), magnetite (Fe₃O₄), and silicon dioxide (SiO₂) reduce cellular damage under drought by lowering reactive oxygen species (ROS) and boosting antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and guaiacol peroxidase (POD) (Guha, Ravikumar, Mukherjee, Mukherjee, & Kundu, 2018).

Seed invigoration is a promising strategy to improve maize productivity under drought stress (Estevez-Geffraud, Vicente, Vergara-Diaz, Reinaldo, & Trillas, 2020). It includes techniques such as seed coating, priming, and conditioning (Ilyas, 2012). Seed coating involves applying carriers containing protective agents, microorganisms, micronutrients, or growth regulators. This method enhances seed quality, protects seedlings from biotic and abiotic stresses (Palupi, Ilyas, Machmud, & Widajati, 2017; Rehman *et al.*, 2020), and delivers micronutrients that support early growth and yield (Skrzypczak *et al.*, 2021).

Previous studies have emphasized nano seed coating as an effective strategy for micronutrient delivery, particularly in improving seed germination and seedling vigor. ZnO NPs enhance maize germination and early growth by increasing zinc uptake and stimulating indole-3-acetic acid synthesis (Adhikari, Kundu, & Rao, 2016). Fe₃O₄ NPs improve seedling development by facilitating iron translocation (Lau *et al.*, 2020), while SiO₂ NPs boost antioxidant enzyme activity and reduce malondialdehyde accumulation, preserving seed quality during storage (Tian *et al.*, 2019).

Limited information is available on the effectiveness of ZnO, Fe₃O₄, and SiO₂ nano-seed coatings in enhancing maize drought resilience, particularly under Indonesian agro-environmental conditions. Therefore, this study aimed to evaluate the effectiveness of these NPs in improving maize germination and early seedling growth under polyethylene glycol (PEG)-induced drought stress.

2. Materials and Methods

This research was conducted from June to October 2023 at the Seed Science and Technology Laboratory, Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University, Indonesia. Lamuru maize seeds were sourced from the Indonesian Cereals Research Institute (ICERI), Maros, South Sulawesi. The seeds, harvested on June 20, 2022, and quality-tested on July 25, 2022, had 90% germination and 11.6% moisture content. They were stored at 20 ± 1 °C and 50% relative humidity and used in July 2023.

The experiment was conducted using a split-plot design based on a randomized complete block design (RCBD). The main plot factor was osmotic stress at three levels: 0, -0.15, and -0.30 MPa. The subplot factor consisted of ten seed film coating treatments with NPs: an untreated control; ZnO at 15, 25, and 50 mg L⁻¹; Fe₃O₄ at 100, 200, and 300 mg L⁻¹; and SiO₂ at 300, 600, and 900 mg L⁻¹. The 30 treatment combinations were replicated three times, with each replicate containing 50 seeds, totaling 90 experimental units.

2.1 Nano-particles (NPs)

Analytical grade ZnO, Fe₃O₄, and SiO₂ NPs for seed coating were procured from Nano Research Company NRE Lab, ITNANO™ (Medan, Indonesia). Characterization data

showed ZnO NPs had a particle size of 57 nm, crystallite size of 15–18 nm, molecular weight of 81.38 g mol⁻¹, and 99% purity. Fe₃O₄ NPs exhibited a particle size of approximately 58 nm, a crystallite size of 16 nm, a molecular weight of 231.53 g mol⁻¹, and 99% purity. SiO₂ NPs had an 8.6 nm crystallite size, molecular weight of 60.08 g mol⁻¹, and 98% purity. Particle sizes were measured using scanning electron microscopy (SEM), while crystallite sizes were determined by X-ray diffraction (XRD).

2.2 Seed coating

Seeds were surface-sterilized in 0.5% sodium hypochlorite for 10 min, rinsed three times with distilled water, and once with deionized water. Coating treatments included 2.5% polyvinyl alcohol (PVA) and 5% tapioca polymer (Marwanto *et al.*, 2020), combined with ZnO NPs (15, 25, and 50 mg L⁻¹), Fe₃O₄ NPs (100, 200, and 300 mg L⁻¹), and SiO₂ NPs (300, 600, and 900 mg L⁻¹). The polymer solution was prepared by dissolving the polymer in 100 mL of deionized water. Seeds were coated by handshaking with the nanoparticle-polymer solution in a closed container for 15 min and air-dried to approximately 11% moisture content.

2.3 Drought stress simulation and determination of seed germination indices

Germination tests were conducted using sterilized sand as the medium, sieved through a 2 mm mesh (ISTA, 2018). Each plastic box (16 × 11 × 5 cm) contained 250 g of sand and 25 seeds, incubated at 25 ± 1 °C with ~60% relative humidity, monitored using an HTC-2 digital thermohygrometer. PEG 6000 was used to simulate osmotic potentials of 0, -0.15, and -0.30 MPa. Treatments were maintained from sowing until day 7. Watering was done with distilled water for the control and PEG solutions for drought treatments, maintaining field capacity at 37 mL per container. Sand moisture was monitored daily, with rewatering as needed using the respective solutions.

The in-sand method was used to assess germination rate (GR), first count germination (FCG), speed of germination (SG), dry weight of normal seedlings (DW), and seedling growth rate (SGR). Radicle emergence (RE) was assessed using the between-paper (BP) method, where seeds were placed between layers of kitchen paper towels inside plastic boxes. Three layers were placed below and two above the seeds, moistened with ~16 mL of distilled water or PEG solution according to the treatment. Paper towels were kept moist throughout without adding extra solution. Tests were performed under ambient laboratory lighting without artificial illumination.

To evaluate drought stress effects, germination and seedling growth parameters were measured. GR was calculated as the percentage of normal seedlings at the first count (day 4) and final count (day 7), following ISTA (2018) guidelines.

$$\text{GR (\%)} = \frac{\text{Number of normal seedling at final count}}{\text{Total number of seeds tested}} \times 100\%$$

FCG was calculated based on the percentage of normal seedlings in the first count of the GR test.

$$FCG (\%) = \frac{\text{Number of normal seedling at first count}}{\text{Total number of seeds tested}} \times 100\%$$

SG was observed based on the emergence of normal seedlings every 24 h (etmal) during the germination test (seven days) (Sadjad, 1994).

$$SG (\%NS \text{ etmal}^{-1}) = \sum(\%ns/\text{etmal})$$

DW was measured from normal seedlings at the final count after oven-drying at 80 °C for 24 hours (ISTA, 1995), and expressed as total dry weight per replicate. RE was calculated as the percentage of seeds with radicles at least 2 mm long within 51 ± 15 minutes at 25 ± 1 °C (Khusna, Zamzami, & Ilyas, 2021). SGR was determined as the ratio of the total dry weight of normal seedlings (ISTA, 1995).

$$SGR (\text{mg}/\text{seedling}) = \frac{\text{Seedling dry weight (mg)}}{\text{Total number of normal seedlings}}$$

The malondialdehyde (MDA) content was measured on day 7 using the thiobarbituric acid method (Liu *et al.*, 2019).

2.4 Statistical analysis

Data were analyzed using R Studio version 2023.06.1 via analysis of variance (ANOVA). Treatment means were compared using Duncan's multiple range test (DMRT) at a significance level of $\alpha = 0.05$.

3. Results

The results indicated that drought stress reduced GR in untreated seeds (control), whereas nano seed coatings with ZnO (15 and 25 mg L⁻¹) and Fe₃O₄ (300 mg L⁻¹) maintained GR levels. At -0.15 MPa, ZnO and Fe₃O₄ coatings significantly improved GR compared to the control. Under more severe drought (-0.30 MPa), all nano seed coating treatments resulted in higher GR than the control (Table 1).

The effect of nano seed coating on FCG under drought stress is presented in Table 2. The FCG of control seeds declined from 35% to 17% and 15% at -0.15 MPa and -0.30 MPa, respectively. At -0.15 MPa, FCG was significantly higher than the control in seeds coated with Fe₃O₄ (200 and 300 mg L⁻¹) and SiO₂ (600 and 900 mg L⁻¹). Under -0.30 MPa drought stress, ZnO 25 mg L⁻¹ and all tested Fe₃O₄ concentrations significantly increased FCG compared to the control.

Higher SG values indicate more vigorous seeds, as they germinate faster than less vigorous ones. Table 3 shows that nano seed coating influenced germination speed under different drought stress levels. SG of control seeds decreased from 17.6 %NS etmal⁻¹ at 0 MPa to 11.2 %NS etmal⁻¹ at -0.30 MPa. Treatments with ZnO at 50 mg L⁻¹ and Fe₃O₄ of 300 mg L⁻¹ maintained SG across drought levels. Under -0.30 MPa stress, all nano seed coating treatments, except ZnO 25 mg L⁻¹ and SiO₂ 900 mg L⁻¹, significantly increased SG compared to the control.

Table 1. Effect of nano-seed coating and drought stress on the germination rate (%) of maize seeds

Nano seed coating (mg L ⁻¹)	Drought stress (MPa)		
	0	-0.15	-0.30
Control	83.0 CDa	75.3 Eb	49.0 Cc
ZnO 15	88.0 ABCa	84.0 ABCa	80.0 Aa
ZnO 25	77.3 Da	84.7 ABa	76.0 Aa
ZnO 50	88.0 ABCa	87.0 ABab	80.0 Ab
Fe ₃ O ₄ 100	88.0 ABCa	90.7 Aa	78.0 Ab
Fe ₃ O ₄ 200	94.0 Aa	86.0 ABb	81.0 Ac
Fe ₃ O ₄ 300	83.3 BCDa	83.3 ABCDa	83.3 Aa
SiO ₂ 300	81.3 CDa	76.0 DEb	79.0 Aab
SiO ₂ 600	91.3 ABCa	82.0 BCDEb	75.0 ABb
SiO ₂ 900	93.3 ABa	77.0 CDEb	68.0 Bb

Note: Numbers followed by the same capital letter in the same column or the same small letter in the same row indicate that these values are not significantly different based on DMRT at the $\alpha = 5\%$.

Table 2. Effect of nano-seed coating and drought stress on the first count germination (%) of maize seeds

Nano seed coating (mg L ⁻¹)	Drought stress (MPa)		
	0	-0.15	-0.30
Control	35.0 Ca	17.0 Eb	15.0 DEb
ZnO 15	34.0 Ca	18.0 Eb	20.0 CDb
ZnO 25	17.0 Eb	18.0 Eb	33.0 Aa
ZnO 50	25.0 Da	22.0 DEa	20.0 CDa
Fe ₃ O ₄ 100	24.0 Db	25.0 CDEb	28.0 ABa
Fe ₃ O ₄ 200	36.0 Ca	40.0 Aa	24.0 BCb
Fe ₃ O ₄ 300	45.0 Ba	29.0 ABCDb	30.0 ABb
SiO ₂ 300	24.0 Dab	27.0 BCDEa	15.3 DEb
SiO ₂ 600	57.0 Aa	37.0 ABb	13.0 Ec
SiO ₂ 900	29.0 CDa	35.0 ABCa	20.0 CDa

Note: Numbers followed by the same capital letter in the same column or the same small letter in the same row indicate that these values are not significantly different based on DMRT at the $\alpha = 5\%$.

Table 3. Effect of nano-seed coating and drought stress on the speed of germination (%NS etmal⁻¹) of maize seeds

Nano seed coating (mg L ⁻¹)	Drought stress (MPa)		
	0	-0.15	-0.30
Control	17.6 Ba	20.0 ABa	11.2 Db
ZnO 15	11.5 Cc	13.8 Db	16.1 Ba
ZnO 25	12.3 Cb	17.7 BCa	10.5 Dc
ZnO 50	18.5 Ba	16.1 Ca	16.7 ABa
Fe ₃ O ₄ 100	18.7 Bab	20.1 Aa	17.2 ABb
Fe ₃ O ₄ 200	13.2 Cb	19.0 ABa	18.1 ABa
Fe ₃ O ₄ 300	18.9 Ba	18.7 ABa	18.7 Aa
SiO ₂ 300	18.5 Ba	17.6 BCa	16.0 Bb
SiO ₂ 600	21.0 Aa	18.8 ABb	13.5 Cc
SiO ₂ 900	12.2 Cb	18.5 ABCa	11.0 Db

Note: Numbers followed by the same capital letter in the same column or the same small letter in the same row indicate that these values are not significantly different based on DMRT at the $\alpha = 5\%$.

Based on Table 4, drought stress significantly reduced the percentage of radicle RE across all treatments, including the control. In untreated seeds, RE decreased markedly from 71.3% at 0 MPa to 45% at -0.15 MPa and further to 17% at -0.30 MPa. However, nano seed coatings with ZnO at 25 and 50 mg L⁻¹, and Fe₃O₄ at 100 and 200 mg L⁻¹, maintained RE levels under increasing drought stress, showing no significant reduction from -0.15 MPa to -0.30 MPa. At -0.30 MPa, all nano-coated seeds exhibited higher RE percentages than the control, with ZnO 50 mg L⁻¹ achieving the highest RE under this stress level.

Table 5 shows that under non-stress conditions (0 MPa), the SiO₂ nano-seed coating at 900 mg L⁻¹ resulted in the highest DW of normal seedlings. Under -0.15 MPa drought stress, ZnO at 15 and 25 mg L⁻¹, as well as Fe₃O₄ at 300 mg L⁻¹, produced higher DW compared to other treatments. However, at -0.30 MPa, no significant differences in DW were observed among the nano seed coating treatments.

Table 6 shows that nano seed coating with ZnO at 15 and 25 mg L⁻¹, and Fe₃O₄ at 100 and 200 mg L⁻¹, did not reduce SGR under varying drought stress conditions. At -0.15 MPa, Fe₃O₄ at 300 mg L⁻¹ produced the highest SGR, significantly outperforming other treatments and the control. However, at 0 MPa and -0.30 MPa, no significant differences in SGR were observed among the nano seed coating treatments. These findings indicate that Fe₃O₄ at moderate concentrations may enhance seedling vigor under mild drought stress.

Our results showed that drought stress increased MDA content in maize seedlings (Table 7). At -0.15 MPa, nano seed coating with Fe₃O₄ at 300 mg L⁻¹ significantly reduced MDA levels compared to the control. Under more severe stress (-0.30 MPa), Fe₃O₄ at 300 mg L⁻¹ and SiO₂ at 600 mg L⁻¹ both significantly decreased MDA content relative to the control, indicating a potential role in mitigating oxidative damage under drought conditions.

4. Discussion

Nano seed coating proved beneficial in enhancing the viability and vigor of maize seeds under drought stress. In this study, all nano seed coating treatments exhibited a higher GR compared to the control under severe drought stress (-0.30 MPa). Zaim *et al.* (2023) reported that nanoparticle-based seed coatings improve GR by facilitating water uptake during imbibition and minimizing nutrient loss through leaching, thereby enhancing nutrient availability for early growth. Similarly, at -0.15 MPa, nano seed coatings with ZnO and Fe₃O₄ significantly improved germination percentages relative to the control. Raju and Rai (2017) observed that seed polymer coating with Fe NPs at 25 ppm significantly enhanced GR, SG, seedling dry weight, and FCG in pigeon peas.

Our results demonstrated that nano-seed coatings with ZnO 25 mg L⁻¹ and all concentrations of Fe₃O₄ NPs increased FCG under -0.30 MPa drought stress compared to the control. Itroutwar, Kasivelu, Ragumaram, Malaichamy, and Sevathapandian (2020) reported that nanopriming with ZnO at 100 mg L⁻¹ enhanced maize FCG, leaf length, and width. Similarly, Guha *et al.* (2018) found that nanopriming with 20 mg L⁻¹ zero-valent iron (nZVI) increased FCG by

Table 4. Effect of nano-seed coating and drought stress on radicle emergence (%) of maize seeds

Nano seed coating (mg L ⁻¹)	Drought stress (MPa)		
	0	-0.15	-0.30
Control	71.3 BCDA	45.0 ABb	17.0 Dc
ZnO 15	73.3 ABCDA	50.0 Ab	26.0 Cc
ZnO 25	68.0 Da	44.7 ABb	32.7 BCb
ZnO 50	75.3 ABCDA	36.7 Bb	43.0 Ab
Fe ₃ O ₄ 100	76.0 ABCDA	47.3 Ab	35.3 ABb
Fe ₃ O ₄ 200	78.7 ABA	36.0 Bb	34.0 BCb
Fe ₃ O ₄ 300	77.3 ABCa	47.0 Ab	33.3 BCc
SiO ₂ 300	69.3 CDA	41.0 ABb	28.0 BCc
SiO ₂ 600	75.3 ABCDA	49.0 Ab	29.0 BCc
SiO ₂ 900	80.7 Aa	44.0 ABb	26.0 Cc

Note: Numbers followed by the same capital letter in the same column or the same small letter in the same row indicate that these values are not significantly different based on DMRT at the $\alpha = 5\%$.

Table 5. Effect of nano seed coating and drought stress on the dry weight of normal seedlings (g) of maize seeds

Nano seed coating (mg L ⁻¹)	Drought stress (MPa)		
	0	-0.15	-0.30
Control	4.59 BCDA	3.73 Ba	2.76 Ab
ZnO 15	4.50 BCDEa	4.95 Aa	2.90 Ab
ZnO 25	4.50 BCDEa	5.21 Aa	3.50 Ab
ZnO 50	4.20 CDEa	3.90 Ba	3.25 Aa
Fe ₃ O ₄ 100	3.89 DEa	3.13 Ba	3.52 Aa
Fe ₃ O ₄ 200	3.81 Ea	3.59 Ba	3.39 Aa
Fe ₃ O ₄ 300	4.79 ABCb	5.45 Aa	3.57 Ac
SiO ₂ 300	4.96 ABA	3.35 Bb	2.79 Ab
SiO ₂ 600	4.30 BCDEa	3.78 Bab	3.12 Ab
SiO ₂ 900	5.35 Aa	3.62 Bb	3.13 Ab

Note: Numbers followed by the same capital letter in the same column or the same small letter in the same row indicate that these values are not significantly different based on DMRT at the $\alpha = 5\%$.

Table 6. Effect of nano-seed coating and drought stress on the seedling growth rate (mg/normal seedling) of maize seeds

Nano seed coating (mg L ⁻¹)	Drought stress (MPa)		
	0	-0.15	-0.30
Control	110 Aa	96 Bab	86 ABb
ZnO 15	114 Aa	86 Ba	91 ABa
ZnO 25	97 ABa	83 Ba	84 Ba
ZnO 50	109 Aa	91 Bb	82 Bb
Fe ₃ O ₄ 100	85 Aa	80 Ba	87 ABa
Fe ₃ O ₄ 200	103 Aa	83 Ba	102 Aa
Fe ₃ O ₄ 300	104 Aa	120 Aa	86 ABb
SiO ₂ 300	109 Aa	81 Bb	87 ABb
SiO ₂ 600	104 Aa	91 Bb	90 ABb
SiO ₂ 900	111 Aa	85 Bb	84 Bb

Note: Numbers followed by the same capital letter in the same column or the same small letter in the same row indicate that these values are not significantly different based on DMRT at the $\alpha = 5\%$.

Table 7. Effect of nano-seed coating and drought stress on MDA content ($\mu\text{mol g}^{-1}$) of maize seeds

Nano seed coating (mg L^{-1})	Drought stress (MPa)		
	0	-0.15	-0.30
Control	10.36 Ac	26.21 Ab	60.21 Aa
ZnO 15	9.21 Ab	22.86 Ab	48.11 ABa
Fe ₃ O ₄ 300	8.42 Ac	13.76 Bb	29.00 Ba
SiO ₂ 600	9.65 Ac	20.01 Ab	32.47 Ba

Note: Numbers followed by the same capital letter in the same column or the same small letter in the same row indicate that these values are not significantly different based on DMRT at the $\alpha = 5\%$.

1.95-fold and improved water uptake in rice. The improved germination may be linked to enhanced water uptake, triggering oxidative respiration, where accumulated H₂O₂ interacts with gibberellic acid, promoting α -amylase activity, starch hydrolysis, and ultimately, seedling vigor (Rai-Kalal, Tomar, & Jajoo, 2021).

The present study demonstrated that nano seed coatings of ZnO 50 mg L^{-1} and Fe₃O₄ 300 mg L^{-1} did not reduce SG under varying drought stress levels. Younes, Hassan, Elkady, Hamed, and Dawood (2020) attributed the acceleration of germination to the ability of NPs to penetrate the seed coat, activating embryo differentiation by triggering enzymes involved in breaking seed dormancy, thereby enhancing germination speed. Additionally, all nano seed coatings increased RE percentages compared to the control, with ZnO 50 mg L^{-1} showing the highest RE at -0.30 MPa. Similarly, Korishettar *et al.* (2016) reported that polymer seed coating with 500 ppm Zn and Fe nanoparticles enhanced hydrolytic enzyme activity during early germination stages in pigeon peas, improving SG and early emergence.

Although drought stress reduces maize seedling dry weight (DW), nano-seed coatings with ZnO (15 and 25 mg L^{-1}) and Fe₃O₄ (300 mg L^{-1}) significantly improved DW under -0.15 MPa stress. Similarly, Korishettar *et al.* (2017) reported that seed polymer coatings containing Fe and Zn nanoparticles (500 ppm and 750 ppm, respectively) enhanced DW in pigeon peas, alongside increased enzymatic activities related to respiration and nutrient mobilization. The improved dry matter accumulation in maize seedlings likely results from enhanced efficiency in utilizing seed reserves (Andrade, Coelho, & Padilha, 2019).

The highest SGR at -0.15 MPa drought stress was observed with Fe₃O₄ nano-seed coating at 300 mg L^{-1} . Li *et al.* (2021) reported that Fe₃O₄ NPs at 50 mg L^{-1} increased chlorophyll content in rice by 26.9% and mitigated stress by reducing oxidative damage and lowering concentrations of stress-related phytohormones such as gibberellin and indole-3-acetic acid. Similarly, iron oxide nanoparticles (IO-NPs) have been shown to enhance maize growth, chlorophyll content, and stem and leaf length. Acting as phytoferritin cores within plants, IO-NPs are not fully dissolved and may serve as a slow-release iron source. Therefore, IO-NPs have the potential as a novel iron fertilizer for crop improvement (Pariona, Martínez, Hdz-García, Cruz, & Hernández-Valdes, 2017).

The elevated MDA levels observed under drought stress indicate oxidative damage caused by lipid peroxidation. MDA serves as a key marker of membrane damage resulting

from excessive accumulation of ROS. Mazhar *et al.* (2022a) reported that drought stress disrupts the lipid bilayer structure of membranes, leading to increased MDA levels. In this study, nano seed coatings with Fe₃O₄ at 300 mg L^{-1} and SiO₂ at 600 mg L^{-1} significantly reduced MDA content under -0.30 MPa osmotic stress, suggesting a protective effect against oxidative damage.

The mechanisms by which these NPs reduce oxidative stress vary. Fe₃O₄ NPs act as nano-catalysts by enhancing α -amylase activity, accelerating starch breakdown, and supporting early seedling metabolism (Afzal, Sharma, & Singh, 2021). They also serve as essential cofactors for antioxidant enzymes such as SOD, POD, and CAT, thereby enhancing ROS detoxification (Alharby & Ali, 2022; Elanchezian *et al.*, 2017). Additionally, Fe₃O₄ nanoparticles regulate respiration, osmoregulation, and secondary metabolism under drought stress conditions (Moradi & Siosemardeh, 2023; Rezayian, Niknam, & Arabloo, 2023).

In contrast, SiO₂ NPs protect membrane stability by suppressing ROS-induced aquaporin overactivity and enhancing antioxidant defenses (Sharf-Eldin *et al.*, 2023). They also facilitate osmotic adjustment through the accumulation of soluble sugars and amino acids, as well as by promoting K⁺ translocation (Zargar, Mahajan, Bhat, Nazir, & Deshmukh, 2019). These biochemical processes contribute to maintaining cellular homeostasis under stress conditions. The observed reduction in MDA thus not only serves as a biochemical marker of stress alleviation but also correlates with improved seedling vigor. This finding aligns with previous studies showing that Fe and Si nanoparticle priming decreased MDA levels and enhanced drought tolerance in wheat and flax (Mazhar *et al.*, 2022b; Raza *et al.*, 2023).

5. Conclusions

This study demonstrated that nano seed coating is a promising strategy to enhance maize seedling performance under drought stress. Among the treatments, ZnO at 50 mg L^{-1} resulted in the highest radicle emergence (43%) under -0.30 MPa, indicating superior effectiveness compared to other formulations and the control. Fe₃O₄ at 300 mg L^{-1} improved seed viability, vigor, and seedling growth, while Fe₃O₄ and SiO₂ coatings reduced oxidative stress by lowering MDA levels. These findings suggest that nano seed coating, particularly ZnO at 50 mg L^{-1} , could be integrated into seed enhancement protocols to improve maize establishment in drought-prone areas. However, further large-scale field trials and environmental impact assessments are necessary before widespread adoption in sustainable agriculture.

Acknowledgements

This study was fully funded by the Ministry of Education, Culture, Research and Technology of the Republic of Indonesia (Grant number 18924/IT3.D10/PT.01.03/P/B/2023).

Author Contributions

Widia Sri Fatma: Investigation, Methodology, Data curation, Data analysis, Writing – original draft. Satriyas Ilyas: Conceptualization, Methodology, Supervision, Writing

– review and editing, Funding acquisition. Sri Wilarso Budi: Conceptualization, Methodology, Supervision. Ladiyani Retno Widowati: Conceptualization, Methodology, Supervision.

References

- Adhikari, T., Kundu, S., & Rao, A. S. (2016). Zinc delivery to plants through seed coating with nano-zinc oxide particles. *Journal of Plant Nutrition*, 39(1), 136-146. Retrieved from <https://doi.org/10.1080/01904167.2015.1087562>
- Afzal, S., Sharma, D., & Singh, N. K. (2021). Eco-friendly synthesis of phytochemical-capped iron oxide nanoparticles as nano-priming agent for boosting seed germination in rice (*Oryza sativa* L.). *Environmental Science and Pollution Research*, 28(30), 40275-40287. Retrieved from <https://doi.org/10.1007/s11356-020-12056-5>.
- Andrade, G. C., Coelho, C. M. M., & Padilha, M. S. (2019). Seed reserves reduction rate and reserves mobilization to the seedling explain the vigour of maize seeds. *Journal of Seed Science*, 41(4), 488-497. doi:10.1590/2317-1545v41n4227354
- Alharby, H. F., & Ali, S. (2022). Combined role of Fe nanoparticles (Fe NPs) and *Staphylococcus aureus* L. in the alleviation of chromium stress in rice plants. *Life*, 12(3), 338. Retrieved from <https://doi.org/10.3390/life12030338>.
- Chukwuneme, C. F., Babalola, O. O., Kutu, F. R., & Ojuederie, O. B. (2020). Characterization of actinomycetes isolates for plant growth promoting traits and their effects on drought tolerance in maize. *Journal of Plant Interactions*, 15(1), 93-105. doi:10.1080/17429145.2020.1752833
- Elanchezian, R., Kumar, D., Ramesh, K., Biswas, A. K., Guhey, A., & Patra, A. K. (2017). Morphophysiological and biochemical response of maize (*Zea mays* L.) plants fertilized with nano-iron (Fe₃O₄) micronutrient. *Journal of Plant Nutrition*, 40(14), 1969-1977. doi:10.1080/01904167.2016.1270320.
- Estevez-Geffriaud, V., Vicente, R., Vergara-Diaz, O., Reinaldo, J. J. N., & Trillas, M. I. (2020). Application of *Trichoderma asperellum* T34 on maize (*Zea mays*) seeds protects against drought stress. *Planta*, 252(8), 1-12. doi:10.1007/s00425-020-03404-3
- Guha, T., Ravikumar, K. V. G., Mukherjee, A., Mukherjee, A., & Kundu, R. (2018). Nanopriming with zero-valent iron (nZVI) enhances germination and growth in aromatic rice cultivar (*Oryza sativa* cv. Gobindabhog L.). *Plant Physiol Biochem*, 127, 403-413. doi:10.1016/j.plaphy.2018.04.014
- Ilyas, S. (2012). *Ilmu dan teknologi benih teori dan hasil-hasil penelitian*. Bogor, Indonesia: IPB Press.
- International Seed Testing Association [ISTA]. (1995). *Handbook of vigour test methods* (3rd ed.). Zurich, Switzerland: International Seed Testing Association.
- International Seed Testing Association [ISTA]. (2018). *International Rules for Seed Testing* (2018 ed.). Battersdorf, Switzerland: International Seed Testing Association.
- Itrotwar, P. D., Kasivelu, G., Raguraman, V., Malaichamy, K., & Sevathapandian, S. K. (2020). Effects of biogenic zinc oxide nanoparticles on seed germination and seedling vigor of maize (*Zea mays*). *Biocatalysis and Agricultural Biotechnology*, 29, 101778. doi:10.1016/j.bcab.2020.101778
- Khusna, A. U., Zamzami, A., & Ilyas, S. (2021). Modifikasi suhu uji pemunculan radikula untuk mempersingkat pengujian vigor benih jagung. *Indonesian Journal of Agronomy*, 49(3), 266-272. doi:10.24831/jaiv49i3.39053.
- Korishettar, P., Vasudevan, S. N., Shakuntala, N. M., Doddagoudar, S. R., Hiregoudar, S., & Kisan, B. (2016). Seed polymer coating with Zn and Fe nanoparticles: An innovative seed quality enhancement technique in pigeon pea. *Journal of Applied and Natural Science*, 8(1), 445-450.
- Korishettar, P., Vasudevan, S. N., Shakuntala, N. M., Doddagoudar, S. R., Hiregoudar, S., & Kisan, B. (2017). Influence of seed polymer coating with Zn and Fe nanoparticles on storage potential of pigeonpea seeds under ambient conditions. *Journal of Applied and Natural Science*, 9(1), 186-191.
- Lau, E. C. H. T., Carvalho, L. B., Pereira, A. E. S., Montanha, G. S., Correa, C. G., Carvalho, H. W. P., . . . Yiu, H. H. P. (2020). Localization of coated iron oxide (Fe₃O₄) nanoparticles on tomato seeds and their effects on growth. *ACS Applied Bio Materials*, 3(7), 4109-4117. Retrieved from <https://pubs.acs.org/doi/10.1021/acsabm.0c00216>
- Li, M., Zhang, P., Adeel, M., Guo, Z., Chetwynd, A. J., Ma, C., . . . Rui, Y. (2021). Physiological impacts of zero-valent iron, Fe₃O₄ and Fe₂O₃ nanoparticles in rice plants and their potential as Fe fertilizers. *Environmental Pollution*, 269, 116134. Retrieved from <https://doi.org/10.1016/j.envpol.2020.116134>
- Liu S., Zenda T., Dong A., Yang Y., Liu X., Wang Y., . . . Duan H. (2019). Comparative proteomic and morpho-physiological analyses of maize wild-type vp16 and mutant vp16 germinating seed responses to PEG-induced drought stress. *International Journal of Molecular Sciences*, 20(22), 5586. doi:10.3390/ijms20225586
- Marwanto, M., Bustaman, H., Handajingsih, M., Supanjani, S., Murcitra, B. G., & Salamah, U. (2020). Delivery of arbuscular mycorrhiza fungus spores via seed coating with biodegradable binders for enhancement of the spore viability and their beneficial properties in maize. *Akta Agrosia*, 23(1), 1-10. doi:10.31186/aa.23.1.1-10
- Matlok, N., Piechowiak, T., Krolkowski, K., & Balawejder, M. (2022). Mechanism of reduction of drought-induced oxidative stress in maize plants by fertilizer seed coating. *Agriculture*, 12(662), 1-12. doi:10.3390/agriculture12050662

- Mazhar, M. W., Ishtiaq, M., Hussain, I., Parveen, A., Bhatti, K. H., Azeem, M., . . . Nasir, N. (2022a). Seed nano-priming with zinc oxide nanoparticles in rice mitigates drought and enhances agronomic profile. *PLOS ONE*, *17*(3), e0264967. doi:10.1371/journal.pone.0264967
- Mazhar, M. W., Ishtiaq, M., Maqbool, M., Akram, R., Shahid, A., Shokralla, S., . . . Elansary, H. O. (2022b). Seed priming with iron oxide nanoparticles raises biomass production and agronomic profile of water-stressed flax plants. *Agronomy*, *12*, 982. Retrieved from <https://doi.org/10.3390/agronomy12050982>
- Moradi, L., & Siosemardeh, A. (2023). Combination of seed priming and nutrient foliar application improved physiological attributes, grain yield, and biofortification of rainfed wheat. *Frontiers in Plant Science*, *14*, 1287677. doi: 10.3389/fpls.2023.1287677.
- Palupi, T., Ilyas, S., Machmud, M., & Widajati, E. (2017). Effect of seed coating with biological agents on seed quality of rice. *Biodiversitas*, *18*(2), 727-732. doi:10.13057/biodiv/d180241
- Pariona, N., Martinez, A. I., Hdz-Gracia, H. M., Cruz, L. A., & Hernandez-Valdes, A. (2017). Effects of hematite and ferrihydrite nanoparticles on germination and growth of maize seedlings. *Saudi Journal of Biological Sciences*, *24*, 1547–1554. doi:10.1016/j.sjbs.2016.06.004
- Rahmah, N. I., Ilyas, S., & Setiawan, A. (2020). Evaluation of bambara groundnut (*Vigna subterranean* L. Verdc.) genotypes for drought tolerance at germination stage. *SABRAO Journal of Breeding and Genetics*, *52*(1), 45-63.
- Rai-Kalal, P., Tomar, R. S., & Jajoo, A. (2021). H₂O₂ signaling regulates seed germination in ZnO nanoprimed wheat (*Triticum aestivum* L.) seeds for improving plant performance under drought stress. *Environmental and Experimental Botany*, *189* (104561). doi:10.1016/j.envexpbot.2021.104561
- Raju, B. B., & Rai, P. K. (2017). Studies on effect of polymer seed coating, nanoparticles and hydro priming on seedling characters of pigeon pea (*Cajanus cajan* L.) seed. *Journal of Pharmacognosy and Phytochemistry*, *6*(4), 140-145.
- Raza, M. A. S., Zulfqar, B., Iqbal, R., Muzamil, M. N., Aslam, M. U., Muhammad, F., . . . Rahman, M. H. (2023). Morpho-physiological and biochemical response of wheat to various treatments of silicon nano-particles under drought stress conditions. *Scientific Reports*, *13*, 2700. doi:10.1038/s41598-023-29784-6
- Rehman, A., Qamar, R., Rehman, A., Wasaya, A., Farooq, O., Sarwar, N., . . . Ahmad, S. (2020). Silicon coating on maize seed mitigates saline stress in Yermosols of Southern Punjab. *Silicon*, *13*, 4293-4303. doi:10.1007/s12633-020-00737-2
- Rezayian, M., Niknam, V., & Arabloo, M. (2023). Iron nanoparticles regulate succinate dehydrogenase activity in canola plants under drought stress. *Scientific Reports*, *13*, 9628. Retrieved from <https://doi.org/10.1038/s41598-023-36105-4>.
- Ruminta, Handoko, & Nurmala, T. (2018). Indikasi perubahan iklim dan dampaknya terhadap produksi padi di Indonesia (studi kasus: Sumatera Selatan dan Malang Raya). *Jurnal Agro*, *5*(1), 48-60. doi:10.15575/1607.
- Sadjad, S. (1994) *Kuantifikasi metabolisme benih*. Jakarta, Indonesia: PT Gramedia Widiasarana Indonesia.
- Sharf-Eldin, A. A., Alwutayd, K. M., El-Yazied, A. A., El-Beltagi, H. S., Alharbi, B. M., Eisa, M. A., Alqurashi, M., Sharaf, M., Al-Harbi, N. A., Al-Qahtani, S. M., Ibrahim, M. F. (2023). Response of maize seedlings to silicon dioxide nanoparticles (SiO₂ NPs) under drought stress. *Plants*, *12*(14), 2592. Retrieved from <https://doi.org/10.3390/plants12142592>
- Skrzypczak, D., Jarzembowski, L., Izydorczyk, G., Miluka, K., Hoppe, V., Mielko, K. A., Malik, N. P., Mlynarz, P., Chojnacka, K., & Krowiak, A. W. (2021). Hydrogel alginate seed coating as an innovative method for delivering nutrients at the early stages of plant growth. *Polymers*, *13*(23), 4233. doi:10.3390/polym13234233
- Sukarman, Mulyani, A., & Purwanto, S. (2018). Modifikasi metode evaluasi lahan berorientasi perubahan iklim. *Jurnal Sumberdaya Lahan*. *12*(1), 1-11.
- Tian, F., Chen, W., Wu, C., Kou, X., Fan, G., Li, T., & Wu, Z. (2019). Preservation of *Ginkgo biloba* seeds by coating with chitosan/nano-TiO₂ and chitosan/nano-SiO₂ films. *International Journal of Biological Macromolecules*, *126*, 917-925. Retrieved from <https://doi.org/10.1016/j.ijbiomac.2018.12.177>
- Widowati, L. R., Husnain, & Hartatik, W. (2011). *Peluang Formulasi Pupuk Berteknologi Nano*. Bogor, Indonesia: Indonesian Institute of Soil and Fertilizer Standardization.
- Younes, N. A., Hassan, H. S., Elkady, M. F., Hamed, A. M., & Dawood, M. F. A. (2020). Impact of synthesized metal oxide nanomaterials on seedlings production of three *Solanaceae* crops. *Heliyon*. (6) e03188. doi:10.1016/j.heliyon.2020.e03188
- Zaim, N. S. H. B. H., Tan, H. L., Rahman, S. M. A., Bakar, N. F. A., Osman, M. S., Thakur, V. K., & Radacsi, N. (2023). Recent advances in seed coating treatment using nanoparticles and nanofibers for enhanced seed germination and protection. *Journal of Plant Growth Regulation*, *42*, 7374-7402. doi:10.1007/s00344-023-11038-4
- Zargar, S. M., Mahajan, R., Bhat, J. A., Nazir, M., & Deshmukh, R. (2019). Role of silicon in plant stress tolerance: opportunities to achieve a sustainable cropping system. *3 Biotech*, *9*(73), 1-16. Retrieved from <https://doi.org/10.1007/s13205-019-1613-z>.