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Influence of seed priming with zinc oxide nanoparticles on germination physiology of maize (*Zea mays* L.)

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Abstract

What role can nanotechnology play in overcoming poor seed germination in tropical maize? This research investigated the effects of seed priming with zinc oxide nanoparticles (ZnO NPs) on germination physiology of maize (*Zea mays* L.) under controlled conditions. Seeds of variety Suwan-3 were primed with five concentrations of ZnO NPs (0, 25, 50, 75, and 100 mg/L) for 12 hours at 25 °C. Germination percentage, shoot length, root length, seedling vigor index, and α -amylase activity were recorded after 7 days. Priming at 50 mg/L produced the highest germination rate (86.9%), shoot length (13.7 cm), and seedling vigor index (1842.3), all significantly greater than the control at $p < 0.05$. The α -amylase activity peaked at 50 mg/L (3.47 U/mg protein), indicating enhanced starch mobilization. Concentrations above 75 mg/L appeared to cause mild phytotoxicity, as germination dropped to 68.7% at 100 mg/L. The findings suggest that ZnO NP priming at moderate doses can serve as a practical seed enhancement technique for maize.

Keywords: Seed priming, zinc oxide nanoparticles, maize, germination physiology, seedling vigor, α -amylase, *Zea mays*, nanotechnology, seed enhancement, phytotoxicity

Introduction

Can nanoparticle-based seed treatments offer a cost-effective way to boost germination in staple crops? Maize (*Zea mays* L.) ranks among the most widely cultivated cereals worldwide, and its early establishment phase often determines final yield outcomes. In tropical regions, farmers regularly encounter uneven germination and weak seedling stands, particularly when soil moisture is erratic or seed quality has declined during storage ^[1]. Conventional priming methods hydropriming, osmopriming, and hormonal priming have been shown to improve germination speed and uniformity ^[2]. Yet the search for more effective priming agents hasn't stopped.

Zinc oxide nanoparticles (ZnO NPs) have drawn attention because zinc is an essential micronutrient involved in enzyme activation, protein synthesis, and membrane integrity ^[3]. When delivered as nanoparticles, zinc can penetrate seed coat barriers more easily than bulk zinc salts, reaching embryonic tissues at lower applied concentrations ^[4]. Several reports indicate that ZnO NPs at moderate doses stimulate germination, enhance root elongation, and activate hydrolytic enzymes like α -amylase ^[5, 6].

That said, the dose-response relationship isn't always straightforward. High nanoparticle concentrations can trigger reactive oxygen species production, leading to lipid peroxidation and cell damage ^[7]. The threshold between beneficial and harmful doses depends on nanoparticle size, exposure duration, and plant species ^[8]. This research was designed to evaluate how different ZnO NP concentrations affect germination parameters and α -amylase activity in maize seeds, aiming to identify the concentration range that maximizes germination while avoiding phytotoxic effects ^[9, 10].

Research area description

The laboratory work was carried out at the Seed Science and Technology Laboratory, Faculty of Agriculture, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia (2°58'N, 101°43'E). UPM sits in the humid tropical belt of Peninsular Malaysia at about 31 m elevation, with a

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tropical rainforest climate (Köppen Af), mean annual temperatures of 25-32 °C, and average annual rainfall exceeding 2,400 mm. The laboratory maintained controlled conditions: 25 ± 1 °C, 65 ± 5% relative humidity, and a 12-hour photoperiod at approximately 2,000 lux. Maize seeds of variety Suwan-3 were sourced from the Malaysian Agricultural Research and Development Institute (MARDI). ZnO nanoparticles (30-40 nm, purity >99%) were procured commercially and characterized using transmission electron microscopy and X-ray diffraction before use [11].

Materials and methods

Materials

The research was conducted between March 2023 and August 2023 at the Seed Science and Technology Laboratory, Universiti Putra Malaysia, Serdang, Malaysia. Certified seeds of maize variety Suwan-3 were obtained from MARDI. Seeds were visually sorted for uniformity, and damaged or discolored seeds were discarded. ZnO nanoparticles (30-40 nm, spherical, >99% purity) were purchased from Sigma-Aldrich. Stock suspensions were prepared by dispersing ZnO NPs in deionized water and sonicating for 30 minutes at 40 kHz. Working solutions at 25, 50, 75, and 100 mg/L were prepared through serial dilution from

a 1,000 mg/L stock [12]. Standard Petri dishes (90 mm), Whatman No. 1 filter papers, and a Memmert ICH260L germination chamber were used throughout.

Methods

A completely randomized design with five treatments and four replications was used. Each replication consisted of 25 seeds in a Petri dish lined with double-layered filter paper moistened with 10 mL of the respective ZnO NP solution. Control seeds received deionized water. Seeds were soaked for 12 hours, then surface-dried under laminar airflow for 30 minutes before placement in the germination chamber at 25 °C with a 12-h photoperiod. Germination was recorded daily for 7 days; a seed was considered germinated when the radicle emerged to at least 2 mm. At day 7, shoot length, root length, and seedling fresh weight were measured. The seedling vigor index (SVI) was calculated as: SVI = Germination (%) × [Shoot length + Root length]. The α-amylase activity was determined by the dinitrosalicylic acid method [13] using 48-h-old germinating seed extracts, expressed as U/mg protein. Data were analysed by one-way ANOVA with means compared by Duncan’s Multiple Range Test at *p* < 0.05 using SPSS v26.0 [14].

Results

Table 1: Effect of ZnO nanoparticle concentrations on germination parameters of maize at 7 days after priming

Treatment	Germination (%)	Shoot length (cm)	Root Length (cm)	SVI	α-Amylase (U/mg)
T ₀ (Control)	72.3c	8.4c	6.2c	1056.0d	2.14d
T ₁ (25 mg/L)	78.6b	10.2b	7.8b	1414.1c	2.78c
T ₂ (50 mg/L)	86.9a	13.7a	9.5a	1842.3a	3.47a
T ₃ (75 mg/L)	83.1ab	12.1ab	8.9ab	1747.4b	3.21b
T ₄ (100 mg/L)	68.7d	7.9c	5.8c	941.1e	1.93e

Means followed by different lowercase letters within a column differ significantly at *p* < 0.05 (DMRT). SVI = Seedling Vigor Inde

Seeds primed at 50 mg/L ZnO NPs (T₂) achieved the highest germination rate of 86.9%, significantly greater than the control (72.3%) and the 100 mg/L treatment (68.7%). A similar trend appeared for shoot and root length, with T₂ producing the

longest shoots (13.7 cm) and roots (9.5 cm). The seedling vigor index peaked at 1842.3 under T₂, a 74.5% increase over the control. T₄ (100 mg/L) depressed germination below control levels to 68.7% with the lowest SVI of 941.1.

Table 2. Correlation matrix among germination parameters and α-amylase activity

Parameter	Germination%	Shoot length	Root length	α-amylase
Germination%	1.000	0.934**	0.891**	0.912**
Shoot length		1.000	0.947**	0.878**
Root length			1.000	0.863**
α-amylase				1.000

** Significant at *p* < 0.01

Correlation analysis revealed strong positive associations among all parameters. Germination showed the strongest correlation with shoot length (*r* = 0.934, *p* < 0.01), followed by α-amylase

activity (*r* = 0.912, *p* < 0.01), suggesting enzyme activation during priming directly supported seedling growth.

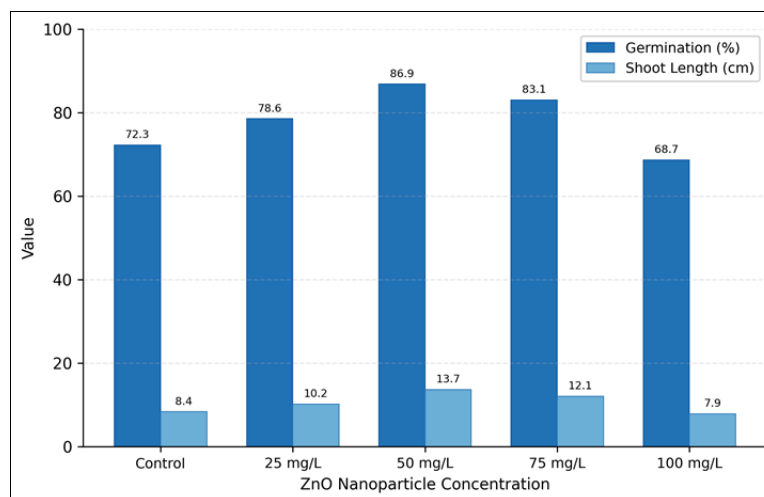


Fig 1: Effect of ZnO nanoparticle concentrations on germination percentage and shoot length of maize at 7 days after priming.

Figure 1 visually confirms the dose-dependent response. Both germination and shoot length increased from the control through 50 mg/L, then declined. The drop was sharper at 100 mg/L, where shoot length fell to 7.9 cm below the unprimed control.

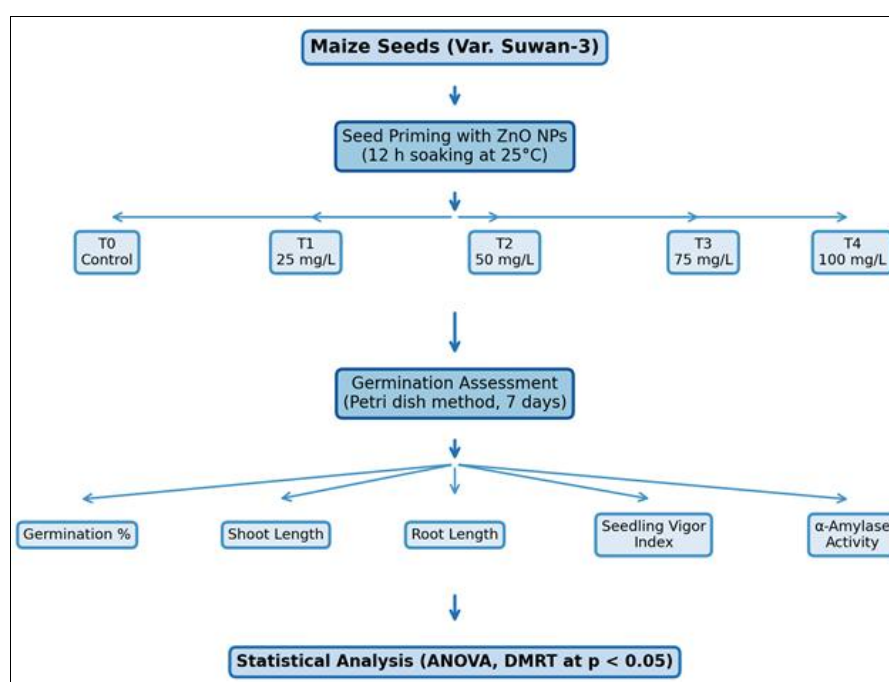


Fig 2: Schematic flowchart of the experimental protocol for ZnO nanoparticle seed priming and germination assessment in maize

Comprehensive interpretation

The data indicate a clear bell-shaped dose-response curve. Moderate ZnO NP concentrations (25–75 mg/L) consistently improved germination metrics, while 100 mg/L suppressed them. The strongest effects appeared at 50 mg/L, where every parameter reached its maximum. Tight correlations between α -amylase activity and seedling growth suggest enzyme-mediated starch hydrolysis is a key mechanism by which ZnO NPs enhance early vigor in maize.

Discussion

These results align with previous reports showing moderate ZnO NP concentrations improve cereal germination. Mahajan *et al.* [3] observed a similar bell-shaped response in wheat, where 50 mg/L maximized growth while 200 mg/L caused tissue damage. The mechanism appears to involve zinc's role as a cofactor for hydrolytic enzymes. At 50 mg/L, ZnO NPs likely provided optimal zinc to activate α -amylase, converting endosperm starch into sugars that fueled seedling growth [5].

The phytotoxic response at 100 mg/L deserves attention. High nanoparticle concentrations can generate excessive reactive oxygen species within seed tissues, damaging membranes and disrupting mitochondrial function [7]. It's worth noting that the germination drop at 100 mg/L wasn't dramatic only about 3.6 points below control suggesting maize seeds possess some tolerance to nano-zinc stress through endogenous antioxidant defenses.

Compared to conventional zinc sulfate priming, ZnO NPs seem to work at lower effective concentrations, likely because of their higher surface area-to-volume ratio and better seed coat penetration [4]. This has practical implications: lower concentrations mean reduced costs and lower environmental zinc loading. That said, the long-term fate of ZnO NPs in soil after planted seeds decompose remains an open question [16].

Conclusion

This research demonstrated that seed priming with ZnO nanoparticles at 50 mg/L significantly improved germination

percentage, shoot and root elongation, seedling vigor index, and α -amylase activity in maize variety Suwan-3. The response followed a bell-shaped curve, with moderate concentrations (25-75 mg/L) being beneficial and 100 mg/L causing mild inhibition. ZnO NP priming at 50 mg/L is a viable seed enhancement technique for tropical maize production. Future research should examine these responses under field conditions, test additional varieties, and evaluate environmental persistence of ZnO NPs post-germination.

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