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Effects of TiO_2 nano-priming and field capacity levels on germination and growth of cayenne pepper (*Capsicum frutescens*)

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Abstract. *Rahmawati NA, Solichatun, Pitoyo A. 2026. Effects of TiO_2 nano-priming and field capacity levels on germination and growth of cayenne pepper (*Capsicum frutescens*). Asian J Agric 10 (1): g100112. https://doi.org/10.13057/asianjagric/g100112. Capsicum frutescens* is a vital crop in Indonesia but suffers from seasonal yield instability due to water stress. This study explored nano-priming with titanium dioxide nanoparticles (TiO_2NPs) to improve seedling resilience. The experiment was conducted in two stages. In the germination stage, four TiO_2NP concentrations (0%, 2%, 4%, and 8%) were tested on seed germination using a Completely Randomized Design (CRD). In the growth stage, seedlings were subjected to a 4 × 3 factorial CRD combining the same TiO_2NP concentrations with three levels of water availability (100%, 75%, and 50% field capacity), with three replicated. Data were analyzed using two-way ANOVA and Duncan's Multiple Range Test ($p < 0.05$). During germination, nano-priming with 4% TiO_2NP accelareted germination rate (5.3 days) and enhanced sprout length (6.68 cm), whereas the control exhibited slower germination (9.03 days) and shorter sprouts (2.60 cm). In the subsequent growth phase, TiO_2NPs significantly influenced seedling height, shoot-to-root ratio, and proline content. The tallest seedling was observed at 8% TiO_2NP, particularly under 75% field capacity. In contrast, optimal shoot-to-root ratio and elevated proline accumulation were associated with 2% TiO_2NP under 100% and 75% field capacity, respectively. These finding demonstrated that TiO_2NP nano-priming exerts stage-dependent effects, with distinct concentrations optimizing germination performance and drought-related physiological responses during early seedling growth, highlighting its potential as a scalable approach for improving *C. frutescens* cultivation under water-limited conditions.

Keywords: *Capsicum frutescens*, drought, nano-priming, seedling

INTRODUCTION

Cayenne pepper (*Capsicum frutescens* L.) is widely recognized as an important agricultural commodity in Indonesia, yet its production remains inconsistent. While the plant is capable of growing vigorously across elevations up to 1000 meters, its cultivation success depends not only on adequate land availability and favorable conditions but also on managing abiotic stressors and disease challenges. Water stress, encompassing both drought and waterlogging, is a major limiting factor, as imbalance in water supply can disrupts plant growth, development, and yield (Shao et al. 2008; Farooq et al. 2009; Xu et al. 2010; Zhou et al. 2020; Chen et al. 2022; Wu et al. 2022). Understanding the physiological adaptability underlying *C. frustescens* response to water stress is therefore assensial for improving yield stability.

Plants adapt to abiotic stress through coordinated morphological, anatomical, and physiological adjustments, particulary via regulationof Reactive Oxygen Species (ROS) (Shao et al. 2008). Excessive ROS accumulation cause cellular damage, yet ROS also function as critical signaling molecules in stress responses (Hasanuzzaman et al. 2020; Wang et al. 2024). To maintain redox balance, plant activate antioxidant defense systems comprising enzymatic and non-enzymatic components (Hasanuzzaman et al. 2020). Among non-enzymatic antioxidants, proline plays central role as both a ROS scavenger and osmolyte, contributing to osmotic adjustment and cellular turgor maintenance under water stress (Sahitya et al. 2018; Kijowska-Oberc et al. 2024; Luan et al. 2024).

Titanium dioxide nanoparticles (TiO_2NPs) have emerged as a promising agent for enhancing plant tolerance to abiotic stress due to their dual function in oxidative stress mitigation and osmotic regulation. TiO2NPs reduce ROS accumulation by stimulating antioxidant enzymes such as superoxide dismutase and catalase (Katiyar et al. 2020), while simultaneously promoting osmotic adjustment through increased accumulation of osmolytes, including proline and glycine betaine (Iqbal et al. 2021). These properties have driven the increasing application of TiO_2NPs in agricultural systems. TiO_2NPs can also enhance synchronized seed germination through a two-phase mechanisms. Initially, they increase seed coat permeability and upregulate aquaporin gene expression, facilitating water uptake. Subsequently, they activate metabolic pathways, particularly mitochondrial respiration, ROS-mediated signaling, and hydrolytic enzymes (e.g.,α-amylase), enabling efficient reserve mobilization (Basahi 2021). Empirical studies have demonstrated dose-dependent improvements in germination rate and seedling vigor in wheat (Bovand et al. 2023) and enhances antioxidant enzyme activity during germination in camphor tree seeds (Zhou et al. 2022).

Seed priming has gained significant attention as an effective strategy for enhancing plant stress tolerance by exposing seeds to controlled environmental conditions or

specific agents, enabling them to "pre-adapt" to potential challenges (Jarrar et al. 2024). In *C. frutescens*, osmopriming with polyethylene glycol (PEG) has been shown effective in improving plant resilience under salinity stress (Rachmawati et al. 2023). Integrating nanoparticle application with seed priming offers a synergistic approach to improve germination, early vigor, and stress resilience. Accordingly, TiO_2NP nano-priming represents a promising strategy to enhance early growth performance and water-stress tolerance in *C. frutescens* cultivation.

MATERIALS AND METHODS

Plant materials and seed preparation

Seeds of *C. frutescens* (Magelang accession), commonly cultivated by local farmers in Magelang District, Central Java, Indonesia, were used in this study. Fully ripe fruits with a uniform red color (Munsell Color Chart at 10R 5/10) were selected. Each fruit had an average weight of 2.9 g at harvest. The seeds were extracted from these fruits, ensuring homogeneous selection for the experiment. The seeds are prepared for planting by drying them under indirect sunlight. Subsequently, the desiccated seeds are isolated from the placenta, and only those with uniform size are chosen for further use.

Grow media preparation

Plastic polybags were used as the growth medium containers, each filled with 2 kg of a soil-compost mixture in a 1:1 ratio by volume. The medium was thoroughly mixed to ensure a uniform distribution of nutrients before planting.

Design of experiment

The experiment was conducted in two stages. First, four TiO_2NP concentrations (0%, 2%, 4%, and 8%) were tested on seed germination using a Completely Randomized Design (CRD). In the second stage, seedlings were subjected to a 4 × 3 factorial CRD combining the same TiO_2NP concentrations with three levels of water availability (100%, 75%, and 50% field capacity), replicated three times.

Nano-priming treatment

Titanium dioxide nanoparticles (TiO_2NPs) used in this study were obtained from NRE-Lab with a reported purity of 99%, molecular weight of 79.87 g/mol, and classified as research-grade quality. The nano-priming solution was prepared by dispersing TiO_2NPs in 50 mL of aquabides at concentrations of 0%, 2%, 4%, and 8%. The nanoparticles were thoroughly mixed in the aquabides using an ultrasonic homogenizer for 15 to 20 minutes. Afterward, *C. frutescens* seeds were immersed in the solution for 24 hours. Once the soaking process was complete, the seeds were rinsed and allowed to dry naturally for another 24 hours. One week later, the seeds were evaluated to assess their viability and ability to germinate.

Seed viability test

Seed viability tests were performed using a tetrazolium solution both before and after the nano-priming treatment to evaluate the effects of nano-priming on seed viability. The experimental procedure began with the preparation of a phosphate buffer solution. This was accomplished by dissolving 9.08 g of KH_2PO_4 in 1 L of distilled water to create solution I and dissolving 11.88 g $Na_2HPO_4 \cdot 2H_2O$ in 1 L of distilled water to make solution II. To create the final phosphate buffer, 400 mL of solution I and 600 mL of solution II were mixed in a 2:3 (v/v) ratio, followed by thorough homogenization. A tetrazolium solution was prepared by dissolving 10.00 g of tetrazolium salt (2,3,5-triphenyl tetrazolium chloride) in 1L of the phosphate buffer, following the method described by Wang et al. (2023). This 1% tetrazolium (w/v) solution was used to assess seed viability by staining living tissue.

A total of thirty *C. frutescens* seeds were subjected to a tetrazolium test by soaking them in distilled water for 24 hours. Following this, the seeds were bisected and immersed in a 1% tetrazolium solution for another 24 hours at a temperature of 28°C in the absence of light. According to Kusumawardana et al. (2018), viable seeds are identified by embryos and cotyledons exhibiting pink to dark red coloration. The tetrazolium test was repeated three times to ensure accuracy. The percentage of seed viability was calculated using the formula:

$$\text{Seed viability (\%)} = \frac{\text{Amount of viable seed}}{\text{Total number of seeds}} \text{x100\%}$$

Germination test

Seeds that have been subjected to nano-priming are cultivated on filter paper media to initiate germination. Over 24 hours, a total of 30 *C. frutescens* seeds were submerged in distilled water. The seeds undergo germination in a petri dish. Each treatment holds a total of 30 seeds, with 10 seeds being repeated three times. Each day, the filter paper was dampened with 5 mL of distilled water in the morning and evening. The process of germination occurred over two weeks under normal room temperature conditions. On a daily basis, data was gathered on the timing of sprout emergence, and the germination % and rate were computed. Below is the equation for calculating the proportion of seeds that have germinated and the pace at which germination occurs.

$$\text{Germinate percentage (\%)} = \frac{\text{Amount of seed germinate}}{\text{Total number of seeds}} \text{x100\%}$$

$$\text{Germinate rate} = \frac{\text{N1T1} + \text{N2T2} = \cdots + \text{NxTx}}{\text{Total number of germinate}}$$

Where, N: The number of seeds that germinate in a certain period, T: The time interval between the commencement of the test and the finish of specific observations.

Treatment of variations in water availability

The seedling treatment was conducted for 9 weeks. During the initial 3 weeks, seeds were sown and maintained under 100% Field Capacity (FC) to ensure uniform

establishment. Subsequently, water availability was adjusted to 100%, 75%, and 50% FC for the remaining 6 weeks. The planting medium consisted of a 1:1 (v/v) mixture of soil and compost.

Seeds subjected to nano-priming were planted in the prepared medium and irrigated at 100% FC during the early growth stage. After seedlings reached uniform size, they were transferred to polybags and exposed to the designated FC treatments. Field capacity was determined gravimetrically by first measuring the initial dry weight of the growing medium (Wi), followed by saturating the medium with water until drainage ceased. After 24 h, the final weight (Wf) was recorded. Water content at 100% FC (Wc) was calculated as Wc = Wf − Wi (Özbek and Kaman 2014). To establish 75% and 50% FC, the applied water volume was adjusted proportionally based on Wc. Water content to be added :

$$Wa = FC \times W_c$$

Where, Wa: Water content tobe added (g), FC represents the target Field Capacity (either 75% or 50%), W_c is the water content at 100% field capacity, calculated by subtracting the initial weight from the final weight after saturation.

Seedling growth parameters

Seedling growth was monitored at 9 weeks after planting (WAP). The observed parameters included plant height, fresh weight of the seedlings, shoot-to-root ratio, leaf area, and the number of leaves.

Physiological parameters

The physiological parameters tested included the levels of proline, chlorophyll, and carotenoids.

Measurement of leaf proline levels. Proline concentration in leaf tissue was determined using a modified method of Bates et al. (1973). Fresh leaf samples (0.5 g), collected from the second and third shoots of 9-week-old plants, were thoroughly washed and homogenized in 10 mL of 3% (w/v) sulfosalicylic acid. The homogenate was filtered through Whatman No. 42 filter paper. An aliquot of 2 mL filtrate was mixed with 2 mL of 0.14 M ninhydrin acid solution (containing 1.25 g ninhydrin) and 2 mL of glacial acetic acid. The reaction mixture was incubated in a water bath at 100 °C for 60 min, then immediately cooled in an ice bath for 5 min. Subsequently, 4 mL of toluene (99.5%) was added, and the mixture was vortexed for 15-20 s until two distinct phases formed. The chromophore-containing toluene phase (red layer) was carefully separated, and absorbance was measured at 520 nm using a UV-Vis spectrophotometer.

A standard curve was prepared using L-proline (Sigma) dissolved in 3% (w/v) sulfosalicylic acid, with concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 mM. Each standard solution was processed following the same procedure as the samples. Proline content was calculated using the following equation:

$$Proline\ content\ (\mu mol\ g^{-1}FW) = \frac{C \times V}{M_r \times W}$$

Where, C: Proline concentration from standard curve (μg mL^{-1}), V: Toluene volume (mL), W: Fresh weight sample, Mr (Relative molecular mass) proline: 115.13

Measurement of total chlorophyll and carotenoid levels. Chlorophyll and carotenoid contents were determined in 8-week-old plants. Leaf samples were collected from the third fully expanded leaf, rinsed with distilled water, blotted dry, weighed, and finely ground. Pigments were extracted by homogenizing the tissue in 10 mL of 80% (v/v) acetone, followed by thorough mixing to ensure complete pigment release. The extract was filtered through Whatman No. 1 filter paper. An aliquot of up to 3 mL of the filtrate was transferred into a quartz cuvette, and absorbance was measured using a UV-Vis spectrophotometer at 480, 645, and 663 nm, with 80% acetone used as the blank. This procedure was applied uniformly to all treatment samples.

Data analysis

Data from the two-stage experiment were analyzed separately. In the first stage, germination data from four TiO_2NP concentrations (0%, 2%, 4%, and 8%) were analyzed using one-way ANOVA under a Completely Randomized Design (CRD) with three replicates. In the second stage, a 4 × 3 factorial CRD was used to assess the combined effects of TiO_2NP concentration and water availability (100%, 75%, and 50% field capacity) on seedling growth. Two-way ANOVA was used to test main and interaction effects. Significant results were followed by Duncan's Multiple Range Test (DMRT) at a 5% significance level. Data were checked for normality and homogeneity before analysis.

RESULTS AND DISCUSSION

Viability assay

A tetrazolium test was performed on each set of 30 *C. frutescens* seeds both before and after the priming treatment. This assay relies on the enzymatic activity of dehydrogenase during mitochondrial respiration. The enzyme malate dehydrogenase catalyzes the reduction of 2,3,5-triphenyl tetrazolium chloride (TTC) in living tissue. The process involves immersing the seeds in a 1% TTC solution, which halts the reduction process, enabling the tetrazolium salt to bind with hydrogen ions produced by the enzyme. As a result, triphenyl formazan, a stable red compound forms, indicating viable (living) seed tissue (França-Neto and Krzyzanowski 2019).

Seed viability was consistently 100% across all nano-priming concentrations, as shown in Figure 1. The red coloration observed in all cotyledons and seed embryos indicates high viability, reflecting a strong potential for successful germination and seedling development. According to Kusumawardana et al. (2018), seed viability is categorized into four levels based on tetrazolium staining: high, medium, low, and non-viable. The results of this study confirm that TiO_2 nanoparticles (TiO_2NPs) used in nano-priming do not negatively impact seed viability, preserving high viability rates across all treatments. Although the

viability test in the present study revealed no significant differences among all tested TiO_2 NP concentrations, many authors have previously warned about the importance of both concentration range and particle size (Larue et al. 2012; Feizi et al. 2013). For example, research on wheat (*Triticum aestivum* L.) has shown that higher TiO_2NP concentrations and smaller particle sizes can inhibit seed germination and reduce seedling vigor. Consequently, the absence of observable effects in this study does not rule out potential risks, underscoring the need for further investigation across diverse plant species and nanoparticle properties.

Germination assay

Soaking *C. frutescens* seeds in water for 24 hours likely activates aleurone cells and effectively breaks seed dormancy. Radicle emergence of at least 2 mm is the criterion for germination (Yuniati et al. 2023). In this study, nano-priming with 4% titanium dioxide nanoparticles (TiO_2NPs) resulted in a germination percentage of 93.3%, lower than the 100% observed in the control treatment (Table 1). Despite this, 4% TiO_2NPs-treated seeds produced the longest average shoot length of 6.68 cm, compared to the shortest shoot length of 1.08 cm observed in seeds treated with 2% TiO_2NPs.

In this study, nano-priming with 4% TiO_2NPs yielded the most favorable outcomes among the treatments, with a germination percentage of 93.3%, a significantly longer sprout length of 6.68 cm, and the shortest germination time of 5.3 days ($p < 0.05$). While the control group (0%) showed the highest germination percentage (100%), it had significantly longer time to germinate (9.03 days) and shorter sprouts (2.60 cm). These results suggest that 4% TiO_2NPs improve both speed and vigor of seedling emergence compare to untreated seeds. This highlight the potential of nano-priming not only to enhance seed germination but also to synchronize the process, leading to more uniform and predictable harvests. Such consistency is critical for optimizing agricultural productivity and reducing yield variability.

Previous research has demonstrated similar trends; for instance, nano-priming with TiO_2NPs improved germination percentages, accelerated germination rates, and enhanced seedling growth in *Zea mays* L. (Shah et al. 2021). This may be due to nanoparticles enhancing water absorption, facilitating carbohydrate metabolism, weakening cell walls, and promoting faster radicle and root hair development as reviewed by Rhaman et al. (2022). Moreover, Mahakham et al. (2017) previously suggested that seedlings subjected to nano-priming absorb water more efficiently than those treated with hydro-priming, leading to higher germination rates. Additionally, Dileep et al. (2020) proposed that the observed increase in antioxidant enzyme activity and potential reinforcement of seed membranes following nano-priming contribute to improved seed vigor and germination quality.

Seedling growth

Growth parameters of seedlings

Figure 2 illustrates the visual appearance of the seedlings under the different treatment conditions. The seedlings subjected to water stress at 50% field capacity (FC) exhibit stunted growth and reduced vigor compared to those under optimal conditions. Notably, seedlings treated with TiO_2 nanoparticles under the same water-stress conditions appear healthier, with improved growth and development, suggesting a mitigating effect of the nanoparticles on water stress.

Table 1. Germination percentage, average sprout length, and average germination rate of *Capsicum frutescens* seeds after nano-priming treatment with TiO_2

TiO_2 Variance	Germinate percentage (%)	Sprouts length (cm)	time to germinate (days)
0%	100	2.60 ± 0.89^{ab}	9.03 ± 0.11^{b}
2%	36.60	1.08 ± 0.79^{a}	6.25 ± 0.19^{a}
4%	93.30	6.68 ± 0.88^{b}	5.30 ± 0.11^{a}
8%	83.30	3.47 ± 0.76^{c}	8.12 ± 0.11^{b}

Note: According to the 5% DMRT Test, values within the same column that are marked with the same letter indicate no significant difference (P>0.05)

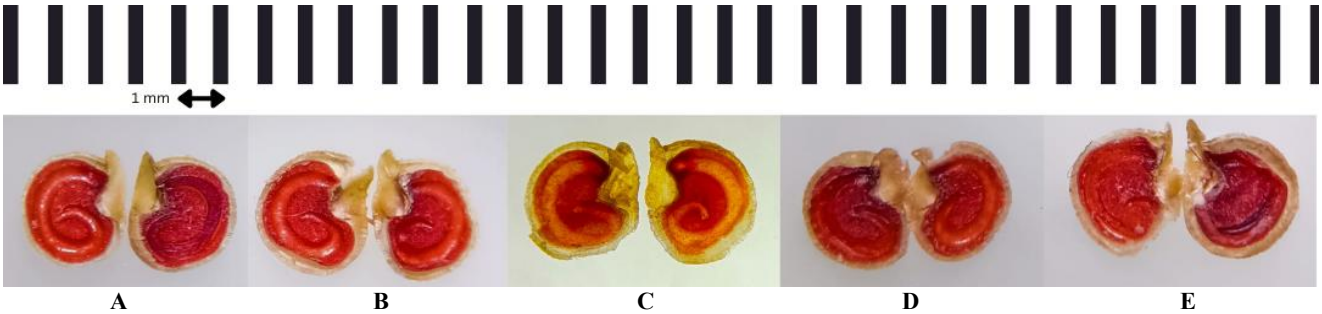

Figure 1. Longitudinal sectional morphology of *Capsicum frutescens* seeds observed under a stereo microscope, highlighting changes in cotyledon coloration and the red embryo across different treatments. A. Before priming, B. Nano-priming concentration 0%, C. Nano-priming concentration 2%, D. Nano-priming concentration 4%, E. Nano-priming concentration 8%

The growth data of *C. frutescens* seedlings was evaluated using several parameters, including plant height, shoot-to-root ratio, fresh weight, leaf area, and leaf count, as summarized in Table 2. Despite variations in seedling height among treatments, all plants exhibited continuous growth over time, with no signs of growth inhibition or decline across replications. Seedlings subjected to the 75% Field Capacity (FC) treatment exhibited the greatest average height of 57.67 cm, indicating that *C. frutescens* achieves optimal growth under these conditions. At 75% field capacity, the soil is likely to provide an optimal balance of moisture and aeration, which may support superior growth and development in plants treated with TiO_2NPs. This condition is expected to facilitate efficient water uptake and retention by promoting the upregulation of aquaporins and the accumulation of osmolytes like proline (Ghorbani et al. 2023). These combined effects could enhance osmotic adjustment and water balance, suggesting the potential effectiveness of TiO_2NPs in improving plant resilience and growth under moderate water availability (Kandhol et al. 2022).

The study also demonstrated that plants exposed to 100% Field Capacity (FC) exhibited a decrease in height, likely due to their adaptation to stressful environmental conditions. Similarly, at 50% FC, a decrease in both leaf area and seedling height was observed, indicating that limited water availability impairs cell growth by reducing cell division and expansion. The results also revealed that the use of 8% TiO_2NPs nano-priming led to the most significant variation in average plant height among treatments, suggesting its potential to enhance seedling adaptation to drought stress conditions and promote optimal growth. These findings can be explained by the interaction between plant metabolism and environmental conditions, which drives variations in plant morphology, such as height and leaf area.

Figure 2. Seedling *Capsicum frutescens* aged 9 weeks after planting (WAP)

Table 2. Growth of *Capsicum frutescens* given nano-priming treatment and variations in field capacity

Variation of nano-priming TiO_2NPs concentration (%)	Variance of field capacity (%)	Plant height (cm)	Shoot to root ratio	Wet weight seedling (g)	Leaf area (cm^2)	Number of leaves
0	50	30.67±15.94^{a}	7.79±1.08^{c}	42.93±4.59^{b}	5.39±2.57^{ab}	22.67±3.21^{bc}
	75	58.33±18.00^{c}	7.93±0.79^{c}	51.73±7.27^{b}	8.64±4.69^{b}	25.67±7.02^{c}
	100	51.33±14.36^{bc}	6.96±0.20^{abc}	42.91±16.25^{b}	5.72±3.95^{ab}	22.33±3.215^{bc}
2	50	39.67±2.31^{ab}	9.16±2.41^{cd}	17.18±1.29^{a}	1.24±2.02^{a}	15.67±0.58^{ab}
	75	50.67±1.15^{bc}	7.76±1.36^{c}	50.95±7.70^{b}	8.26±3.04^{b}	23.00±4.58^{bc}
	100	45.67±3.05^{abc}	11.15±1.59^{d}	42.20±6.51^{b}	3.78±1.43^{ab}	22.00±5.20^{bc}
4	50	54.33±1.53^{bc}	6.17±9.31^{ab}	34.73±9.311^{ab}	5.18±2.39^{ab}	22.00±2.00^{bc}
	75	60.33±0.58^{c}	6.29±0.64^{ab}	39.85±5.46^{b}	5.22±2.73^{ab}	20.67±3.51^{bc}
	100	51.00±7.21^{bc}	6.95±2.11^{abc}	35.15±7.24^{ab}	4.13±2.44^{ab}	21.67±1.53^{bc}
8	50	58.67±2.08^{c}	5.87±0.15^{ab}	36.93±1.41^{ab}	4.43±0.82^{ab}	20.67±1.15^{bc}
	75	61.33±1.53^{c}	6.65±1.67^{abc}	50.71±20.88^{b}	7.70±6.08^{b}	21.00±4.36^{bc}
	100	52.67±15.01^{bc}	4.47±0.84^{a}	18.39±19.78^{a}	1.26±1.74^{a}	21.00±4.36^{bc}

Note: According to the 5% DMRT Test, numbers indicated with the same letter in the same column show no significant difference ($P>0.05$)

Under flooding-induced stress at 100% FC, oxygen availability decreases, resulting in a slowdown of metabolism and reduced plant growth (Zhou et al. 2020). Additionally, the ABA (abscisic acid) hormone, crucial for plant adaptation to unfavorable conditions, induces stomatal closure, leading to a reduction in photosynthesis and respiration. This decline in activity hampers cell division and expansion, ultimately diminishing plant morphological traits such as height and leaf area. However, the application of TiO_2NPs nano-priming has shown potential to mitigate these adverse effects by enhancing seedlings' ability to respond effectively to drought stress, thereby improving their growth and resilience.

The results indicate that TiO_2NPs nano-priming treatment had a significance impact on the shoot-to-root ratio of *C. frutescens* seedlings, as shown in Table 2. Among the treatments, the application of a 2% concentration of TiO_2NPs nano-priming resulted in the highest shoot-to-root ratio. In comparison, the 8% concentration yielded the lowest ratio. Higher concentrations of TiO_2NPs nano-priming (4% and 8%) generally showed a reduction in the shoot-to-root ratio, suggesting that plant adaptation processes vary with the concentration of the treatment. The treatment combining 2% TiO_2NPs nano-priming with 100% FC, produced the largest shoot-to-root ratio. This likely reflects a plant adaptation mechanism in which the number of roots is reduced to limit water uptake, aligning with stress conditions at full canopy closure. The distinct shoot-to-root ratio under these conditions demonstrates an adaptive strategy to environmental stress, which also led to a reduction in proline production compared to other treatments. Based on these findings, it is anticipated that applying 2% TiO_2NPs nano-priming can enhance plant tolerance by promoting an optimal shoot-to-root ratio under 100% FC conditions, enabling effective stress adaptation.

The data presented in Table 2 also highlights the effects of different filed capacity levels on the wet weight of *C. frutescens* seeds. Among the treatments, the 75% FC condition yielded the highest seed wet weight at 48 g, showcasing its superiority in supporting optimal seed hydration and development. In comparison, seeds grown under 50% and 100% FC conditions demonstrated significantly lower wet weights of 33 g and 35 g, respectively, indicating reductions of 13-15 g from the optimal 75% FC. These findings emphasize the importance of maintaining moderate soil moisture levels, as 75% FC appears to provide the most favorable conditions for the growth of the Magelang accession of *C. frutescens*.

The wet weight of plant seedlings is influenced by various factors related to plant growth and development, including the number of leaves, plant height, and the outcomes of photosynthesis that are transferred to the plant's vegetative organs. Leaf number and leaf area significantly affect seedling wet weight, as larger leaves contribute to greater photosynthate production, thereby increasing biomass accumulation. In this study, nono-priming with TiO_2NPs did not have significant impact on leaf number and leaf area, which in turn influenced the wet weight of the seedlings. Nevertheless, an increase in seedling wet weight was observed in treatments with higher leaf numbers, particularly in nano-priming TiO_2NPs treatments at 0% and 75% FC, 2% concentration at 75% FC, and 8% concentration at 75% FC.

The treatment with 75% FC produced relatively higher leaf area in all level of TiO_2NPs (Table 2). This result aligns with the understanding that plants tend to perform optimally when maintained at 75% of their Field Capacity (FC). In contrast, under condition of waterlogging or drought stress, such as 50% FC, the leaf area significantly reduced, with drought stress causing up to a 50% decrease. Interestingly, while drought stress does not impact the formation of leaf buds in *C. frutescens,* it does adversely affect leaf area development. Applying a 2% concentration of TiO_2NPs nano-priming at 50% FC was associated with a reduction in leaf area. Conversely, using an 8% concentration of TiO_2NPs nano-priming at 100% FC increased leaf area. The observed reduction in leaf area during drought stress, at 50% FC, may be attributed to cell shrinkage and a decrease in surplus water content, likely due to the inability of TiO_2NPs nano-priming to sustain the

plant's adaptive responses under such conditions fully. This cell shrinkage directly impacts the overall leaf area. Plants generally achieve optimal physiological and metabolic performance when they have sufficient access to water, highlighting the importance of balanced moisture availability for maintaining growth and development.

Plants exhibit various responses to unfavorable environmental conditions, such as waterlogging and drought stress, which often result in a reduction in leaf area. Under arid or otherwise limiting conditions, cellular activity is inhibited, reducing leaf surface area to limit transpiration and help prevent oxygen depletion in plant tissues (Pamungkas et al. 2022). In this study, TiO_2NPs nano-priming did not enhance priming memory via increased antioxidant activity under stress, indicating the need for further research; accordingly, cayenne pepper (*C. frutescens*) showed no improved stress adaptation, as reflected by reduced leaf area.

The control treatment (0% TiO_2NPs) showed that the highest number of leaves was observed when the water availability was at 75% Field Capacity (FC), with a decrease in leaf number at 50% and 100% FC. This indicates that the ideal water supply for *C. frutescens* plants under typical circumstances is 75% of Field Capacity (FC). It is believed that plant growth is hindered when the soil moisture reaches 100% Field Capacity (FC) because the water blocks the pores, reducing oxygen availability. This leads to a disruption of physiological processes. The majority of leaves were observed in the control treatment. These findings indicate that the application of nano-priming TiO_2NPs at concentrations of 2%, 4%, and 8% led to a reduction in leaf count. This study shows that the application of TiO_2NPs nano-priming does not affect the leaf number characteristics in *C. frutescens* plants, which are considered a type of stress response.

Table 3. Chlorophyll, carotenoid, and proline levels after nano-priming treatment and variations in water availability

Variation of nano-priming TiO_2NPs concentration (%)	Variance of field capacity (%)	Chlorophyll content (mg/g wet mass)	Carotenoid content (mg/g wet mass)	Proline content (mg/g wet mass)
0	50	4.29±1.27^{ab}	1.16±0.34^{a}	1.17±0.62^{bc}
	75	4.20±0.62^{ab}	1.11±0.12^{a}	1.44±0.71^{b}
	100	3.80±1.33^{ab}	1.01±0.36^{a}	0.63±0.16^{a}
2	50	4.59±0.14^{ab}	1.27±0.04^{a}	2.12±0.11^{c}
	75	4.90±0.43^{b}	1.27±0.13^{d}	4.25±0.11^{d}
	100	3.16±0.21^{a}	0.90±0.10^{a}	0.67±0.04^{a}
4	50	4.09±0.28^{ab}	1.09±0.09^{a}	1.07±0.09^{bc}
	75	4.29±0.98^{ab}	1.15±0.24^{a}	0.95±0.07^{bc}
	100	4.28±0.99^{ab}	1.13±0.28^{a}	0.68±0.23^{a}
8	50	4.54±0.75^{ab}	1.21±0.11^{a}	0.75±0.19^{a}
	75	4.29±0.71^{ab}	1.13±0.19^{a}	0.69±0.22^{a}
	100	4.13±0.33^{ab}	1.08±0.13^{a}	0.66±0.20^{a}

Note: According to the 5% DMRT Test, numbers indicated with the same letter in the same column show no significant difference (P>0.05)

Physiology parameters

Proline accumulation varied across combinations of nano-priming concentration and water availability, indicating a treatment-dependent physiological response (Table 3). Under 100% field capacity, proline levels remained consistently low across all TiO_2NP concentrations (0-8%), suggesting minimal osmotic stress under well-watered conditions. At 50% field capacity, proline content was also relatively low and showed no consistent increasing trend with higher TiO_2NP concentrations, indicating that severe water limitation may constrain proline accumulation regardless of nano-priming treatment. In contrast, under 75% field capacity, a marked differentiation among treatments was observed. Proline content increased at 2% TiO_2NP, reaching the highest value among all treatments, while higher concentrations (4% and 8%) did not further enhance proline accumulation and instead exhibited levels comparable to the control. This pattern suggests that moderate water stress combined with an optimal nano-priming dose promotes proline accumulation, whereas higher TiO_2NP concentrations may not confer additional physiological benefits. Overall, these results indicate that proline accumulation in *C. frutescens* is regulated by the interaction between water availability and nano-priming concentration, rather than by nano-priming alone. These data reflect the critical role of proline in plant stress responses. Under water deficit (e.g., 75% field capacity), plants experience osmotic stress, leading to the accumulation of Reactive Oxygen Species (ROS) and disruption of cellular homeostasis. Proline acts as an osmoprotectant and antioxidant, stabilizing proteins, membranes, and other macromolecules while mitigating ROS damage. Furthermore, the significant enhancement of proline content occurred in such water-deficit conditions, i.e., 75% FC, particularly when combined with TiO_2NPs priming. The synergistic effect of TiO_2NPs priming under water deficit likely amplifies these protective mechanisms by improving nutrient uptake, enhancing water-use efficiency, and stimulating stress-related pathways. While at 100% field capacity, the plants experience optimal water availability, minimizing osmotic stress and the need for stress-induced metabolic adjustments like proline accumulation. TiO_2NPs nano-priming under these conditions do not trigger significant proline-related stress responses, as the plant is not under substantial environmental pressure. Hence, the benefits of TiO_2NPs priming become less pronounced, as the primary drivers for proline synthesis, osmotic adjustment and stress signaling are not activated. The significant improvement at 2% TiO_2NPs nano-priming suggests this concentration effectively balances the activation of stress-adaptive mechanisms without inducing toxicity or metabolic burdens. Conversely, concentrations above 2% may lead to nanoparticle aggregation or excessive ROS generation, which could offset the benefits of proline accumulation or even cause mild toxicity, reducing the effectiveness of the treatment. These findings align with the morphological data, such as leaf area, plant height, and wet weight of seedlings, which indicated that the most favorable conditions for plant growth were observed at 75% Field Capacity (FC). Therefore, TiO2NP at 2% represents

a threshold at which the *C. frutescens* can effectively activate its stress response, particularly the synthesis of osmoprotectants like proline. This trend suggests that while proline is typically upregulated under stress, excessive nanoparticle exposure may surpass the plant's capacity to respond adaptively, leading to a diminished proline response.

Interestingly, the application of nano-priming treatments of TiO_2NPs did not result in an enhancement of proline accumulation under the 50% FC treatment. The lack of proline accumulation under the 50% FC treatment, even with nano-priming TiO_2NPs, suggests that plants might prioritize alternative adaptive mechanisms, such as altering the shoot-to-root ratio, to cope with water stress in this specific scenario. This observation aligns with the hypothesis that proline accumulation is not the only or primary response to stress, and its role may be condition-dependent. In the S3A0 treatment, combining 2% TiO_2NPs nano-priming with 100% FC resulted in the highest proportion of plants with low proline levels and a notable adjustment in the root-shoot ratio, highlighting a shift in the plant's strategy to manage stress without heavily relying on proline synthesis. Previous studies support the variability in proline response based on stress type and severity. Sardar et al. (2021) demonstrated that TiO_2NPs increased proline levels under cadmium stress, indicating that the type of stressor plays a significant role in determining whether proline accumulation is activated. Similarly, Mustafa et al. (2022) found that TiO_2NPs enhance proline levels under salt stress by acting as an osmoprotectant, stabilizing cell membranes, and preventing enzyme denaturation. However, at 50% FC, the water deficit might not trigger the same biochemical stress signals as salinity or cadmium toxicity, leading to a different physiological response. Additionally, proline levels are regulated within a specific range to avoid detrimental effects. While low proline levels confer resistance to abiotic stress, excessive accumulation can disrupt cellular homeostasis, as noted by Dikilitas et al. (2020). This self-regulation mechanism might explain why plants under 50% FC stress with TiO_2NPs do not exhibit significant proline enhancement, instead relying on morphological changes like root-shoot ratio adjustments to optimize water uptake and maintain growth.

The study reveals that nano-priming treatments and varying water availability had no significant overall impact on leaf chlorophyll levels in *C. frutescens* seedlings, though specific treatments showed slight variations. The S3A1 treatment (2% nano-priming TiO_2NPs at 75% FC) produced the highest chlorophyll content (4.894 mg/g wet weight), whereas S3A0 (2% nano-priming TiO_2NPs at 100% FC) had the lowest (3.163 mg/g wet weight). The lack of significant differences across treatments, including with 8% nano-priming TiO_2NPs, indicates that the treatments did not induce stress levels sufficient to decrease chlorophyll content. The relative uniformity in chlorophyll levels across treatments suggests that the soil provided sufficient nutrients, particularly magnesium (Mg) and iron (Fe), essential for chlorophyll biosynthesis. Adequate nutrient availability may have mitigated the potential negative effects of water stress or excessive TiO_2NPs. Even with reduced water content, sufficient Mg and Fe binding likely supported chlorophyll formation, as supported by Khazaei et al. (2020), who found that soil nutrient solubility is critical under water stress conditions. TiO_2NPs are known to enhance the photochemical activity of photosystem II, as demonstrated by Mohammadi et al. (2016) in lavender plants, where nano-priming increased chlorophyll concentrations. However, in this study, the moderate application of TiO_2NPs (e.g., 2%) under optimal water availability (100% FC) may have reduced the need for additional stress-induced adaptations like increased chlorophyll synthesis. Conversely, under water deficits, adaptation mechanisms such as proline accumulation or root-shoot ratio adjustments may have compensated for potential declines in chlorophyll, preventing significant variations across treatments.

These findings align with Larue et al. (2012), who reported no significant changes in chlorophyll levels in wheat treated with nano-priming TiO_2NPs under environmental stress. Similarly, the 8% TiO_2NPs treatment in this study did not lead to significant changes at different FC levels, likely because it did not induce sufficient stress to disrupt chlorophyll stability.

The preservation of chlorophyll levels could also be linked to the action of antioxidant enzymes, which inhibit chlorophyllase activity and promote DNA synthesis to maintain chlorophyll integrity, as noted by Azimi et al. (2022). This mechanism likely complements the nutrient availability, further stabilizing chlorophyll levels.

The study indicates that carotenoid levels in *C. frutescens* seedlings remained relatively stable across water availability treatments and TiO_2NPs nano-priming, suggesting a resilient photosynthetic system under the experimental conditions. The highest carotenoid content was observed under moderate water availability (50% and 75% FC), averaging 1.27 mg/g wet weight. In comparison, the lowest was recorded with 2% TiO_2NPs nano-priming at 100% FC (0.893 mg/g wet weight). The relatively stable carotenoid levels observed suggest that the plants maintained sufficient photosynthetic and antioxidative capacity under most treatments. Carotenoids, essential for photosynthesis and ROS scavenging, may increase under stress to protect plants, as noted in studies linking carotenoid synthesis to stress-adaptive genes (Samadi et al. 2014; de Araújo et al. 2021). However, the lowest carotenoid levels at 100% FC with 2% TiO_2NPs may reflect a reduced stress response in optimal water conditions, where additional antioxidant activity was unnecessary. TiO_2NPs, known to enhance photosynthetic efficiency and stress resilience (Mohammadi et al. 2016), appeared to stabilize carotenoid levels without significant variation, consistent with findings by Larue et al. (2012) in wheat. Additionally, proline, another key stress response molecule, complements carotenoid functions by stabilizing macromolecules and mitigating cellular stress (Ramadan et al. 2022). Increased proline levels under stress conditions, as observed in this study, contribute to macromolecular stabilization and hydration (Ramadan et al. 2022), potentially complementing carotenoid functions in ROS detoxification. The interaction between proline and carotenoids allows plants to adapt to stress through multiple mechanisms.

The study demonstrates that TiO_2 nanoparticles (TiO_2NPs) applied as nano-priming agents significantly influence the germination and growth of *C. frutescens* seedlings under varying water availability conditions. A 4% TiO_2NPs nano-priming concentration accelerated germination by 5.3 days and increased seed sprout length to 6.7 cm. TiO_2NPs primarily affected seedling height and the shoot-to-root ratio but did not alter the number of leaves, leaf area, or wet weight of the seedlings. Underwater availability conditions, 8% TiO_2NPs nano-priming at 75% Field Capacity (FC) yielded the tallest seedlings. Conversely, at 100% water availability, 2% TiO_2NPs nano-priming produced the highest shoot-to-root ratio; that treatment enhanced plant stress resilience by elevating chlorophyll, carotenoid, and proline concentrations. Notably, the highest proline content was observed with 2% TiO_2NPs under 75% FC conditions, while 8% TiO_2NPs maintained consistent chlorophyll and carotenoid levels irrespective of water availability.

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