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RESEARCH ARTICLE

Nano-priming with calcium silicate nanoparticles ($CaSiO_3$NPs) promotes germination, seedling growth, and antioxidant enzyme activity in Iraqi rice genotypes under drought stress

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ABSTRACT

Drought is a major abiotic stress limiting rice (*Oryza sativa* L.) cultivation in Iraq. This study aimed to enhance germination, early seedling growth, biochemicals, and antioxidant enzymes activity in Iraqi rice genotypes under drought stress through nano-priming with calcium silicate nanoparticles ($CaSiO_3$NPs). Seeds of three rice genotypes: 'Amber', 'Furat', and 'Yasmine' were primed with $CaSiO_3$NPs at 0.01%, 0.02%, 0.03%, and 0.05%, alongside a non-primed control. Results showed that primed seeds significantly improved, though differed among rice genotypes. 'Furat' showed the highest germination percentage (GP; 90%) and germination index (GI; 9.35 and 9.05) at 0.03%-0.05%, compared to control (GP 63.2% and GI 5.93). Shoot length increased in all genotypes, with 'Furat' showing more than a two-fold higher (8.78 cm and 9.08 cm) at 0.02%-0.03% compared to control (3.82 cm). 'Yasmine' showed the highest root length and seedling length at 0.02% (8.68 cm- four-fold that control 1.92 cm) and at 0.02% (18.79 cm *vs*. 11.24 cm in the control). Seed vigour was the highest in 'Furat' at 0.03%, showing 2.6-fold increase (12.0 *vs*. 4.6 in the control). Compared to control, total chlorophyll highest in 'Yasmine' (10.49 mg mL^{-1}) at 0.03%, total sugar in 'Furat' (74.56 mg mL^{-1}) at 0.05% showed more than one-fold higher while total protein three-fold higher in 'Amber' (21.36 mg g^{-1} fwt) at 0.03%. Catalase activity increased nearly two-fold in 'Yasmine' (0.42 U g^{-1} FW) at 0.03%. The ascorbate peroxidase activity increased in 'Amber' (0.32 U g^{-1} FW) and 'Furat' (0.34 U g^{-1} FW) at 0.01%. In conclusion, $CaSiO_3$NPs nano-priming is a promising strategy to enhance rice cultivation in Iraq.

Key words: Drought stress tolerance, early seedling growth traits, germination traits, Iraqi rice genotypes, seed nano-priming.

INTRODUCTION

Rice (*Oryza sativa* L.) is the primary grain consumed by more than half of the world's population, particularly in Asia and Africa. Throughout their lifespan, crops such as rice, wheat, and maize are exposed to various abiotic stresses such as cold, drought, heat, heavy metals, and salinity, which drastically reduce yield, productivity, threatening global food security, economies, and ecosystem (Ahmad et al., 2023; Wang and Ren, 2025). Depending on the crop species and stage of maturity, drought stress degrades plant metabolism and physiological processes, resulting in decreased growth and yield losses that can range from 30% to 90%. Seed germination and seedling emergence are the most important and vulnerable phases of the crop cycle. In the early stages of crops, drought leads to delays and uneven establishment and emergence of seedlings that lead to unbalanced growth, delayed panicle initiation, and ultimately lower yield. Drought induces several

modifications in plants, such as the capacity for division and growth, H_2O_2 level, nutrient uptake, distribution mechanisms, and nutrient transport inside the plant. It modifies chlorophyll content and function, hinders enzymes' activity, and produces significant quantities of reactive oxygen species (ROS), reducing sweetness due to decreased sink capacity and DM accumulation. This is because about 80%-95% of the fresh biomass of the plant body is comprised of water, which plays a vital role in various physiological processes including many aspects of plant growth, development, and metabolism (Qiao et al., 2024; Park et al., 2025).

Currently, several strategies are being employed to enhance the capability of different plant species to withstand environmental stress including both conventional and cutting-edge methods (Khan et al., 2025). One of these approaches is seed priming, which is a straightforward, dependable, cost-effective, short-term and commercially successful pragmatic practice for promoting seed germination, facilitating accelerated and consistent seedling emergence, improved seedling vigour, and increased yield in various crops, particularly in challenging abiotic circumstances such as drought and salinity and biotic stressors (Kunwar et al., 2025). Seed priming is a pre-sowing procedure which soaks seeds in a solution containing hormones, chemicals, and organic salts that modifies the seed's physiological mechanisms which leads to germinating more quickly. It is mainly because enzymes are activated, the time it takes to absorb water is sped up, metabolites are made that help with germination, metabolism recovers during water absorption, and seeds adapt to osmotic conditions that make them grow quickly and consistently. Seeds can be made less dormant by soaking them in solutions with different types of salt such as NaCl and KCl (halo-priming), water (hydro-priming), osmotic agents such as polyethylene glycol (PEG) (osmo-priming), valuable microbe solutions such as *Trichoderma harzianum* and *Pseudomonas fluorescens* (bio-priming), a magnetic field such as static magnetic field (SMF) and pulsed magnetic field (PMF) (magneto-priming), a solid carrier solution which is a mixed with solid materials of organic or inorganic such as calcium silicate with known water proportion (matrix-conditioning), and nanoparticles (NPs) such as metal oxide (Zn, Ti, and Ag) NPs (Yadav et al., 2023; 2024). Additionally, many priming agents have a range of unique qualities and potential applications, therefore, they should be tailored for each crop species.

For generating practicable agricultural yields, nano-priming is more effective than conventional priming techniques. Many types of nano-priming have been reported to stimulate seed germination and seedling vigour in various crops such as calcium phosphate, Si, Zn and zinc oxide, $ZnSO_4$, Fe, titanium oxide, and Ag nanoparticles (Rhaman et al., 2022). Seed priming with nanoparticles increased penetration through the seed coat that enhanced the seed's ability to absorb nutrients and water, which is the mechanism behind that improved seed germination. In agricultural sectors, the type of nanoparticles, priming time, size, shape, doses, coating, self-aggregate tendency, and stability, and the carrier should be considered before practices (Satya et al., 2024). Several studies reported NPs improved grain production, total biomass, and seed germination of different crops for examples, applying SiO_2NPs in sandy loam soil has enhanced the stem height, leaf area, and shoot and root growth of maize seedlings (Sharf-Eldin et al., 2023). In addition, Sorahinobar et al. (2023) revealed that adding various levels of ZnO nanoparticles enhanced Zn content of mung bean seedlings. Their study also found that 10 and 20 ppm ZnO nanoparticles increased growth and photosynthetic pigments. In contrast, higher concentrations cause oxidative stress, negatively impacting growth and antioxidant responses. The ideal concentration for biofortification was suggested to be 20 ppm, highlighting the effectiveness of ZnO nanoparticles in enhancing plant nutrition and resilience against environmental stress. Nanoparticles are also increasingly utilized in crop biofortification strategies. For example, the application of nano-silica-based organic fertilizers has been shown to enhance both yield and nutritional quality in rice. Haruni et al. (2024) reported that nano-silica treatment significantly improved grain production as well as the glycemic index of black and red rice varieties, indicating its potential role in improving functional food attributes and overall crop value.

Drought regarded as significant global issue that significantly affects rice growth and productivity in most parts of the world, making it a significant environmental problem affecting global agricultural activity and output (Khan et al., 2025). In Iraq, rice is the third most cultivated crop, after wheat and barley, based on both areas planted and total production. Despite this, Iraq imports rice, as its domestic production is insufficient to satisfy the demands of its population (Ewaid et al., 2020). Rice cultivation in Iraq is divided into two regions: The northern region mainly relies on dams for upstream water supply, with the water levels in the rivers declining by over 60% in the last two decades, while the southern and middle regions depend on the banks of the Tigris and Euphrates rivers. Iraq recently experienced a shortage of water resources due to its semi-arid climate, limited precipitation, and ineffective administration. Moreover, the effects of climate change, being

drought the most common, and water management practices in Iraq's neighbouring nations in the Tigris and Euphrates basins have worsened the problem (Yousif, 2024). Both farmed areas (from 79 300 to 5425 ha) and output levels (from 403 000 to 18 200 t) significantly decreased between 2014 and 2018. The average rice output has declined dramatically due to water shortages and adverse climate impacts (Kshash and Oda, 2022). Due to its small roots, Iraqis rice varieties suffer significantly from drought. The reduction in water potential due to drought induces stomatal closure, subsequently suppressing photosynthetic activity, which reduce photosynthesis, changes in glucose metabolism, and the accumulation of osmolytes. During the flowering stage and in the vicinity of rice cultivation, a lack of water disrupts the initiation of florets, leading to the formation of sterile spikelet, and consequently produce empty grains. Hence, drought stress has primarily limited rice production, which is incredibly disruptive in areas reliant on rivers (Tigris and Euphrates). Although seed priming is beneficial in the mitigating of abiotic challenges, lack of study on seed priming with nanoparticles that specifically focuses on enhancing the germination and establishment of Iraqi rice varieties. Therefore, this study was conducted to improve the germination, early seedling growth, biochemical traits, and antioxidant enzymes of three Iraqi rice genotypes 'Amber', 'Furat', and 'Yasmine' with calcium silicate nanoparticles ($CaSiO_3$NPs) priming under drought stress.

MATERIALS AND METHODS

Preparation of calcium silicate nanoparticles ($CaSiO_3$NPs)

Calcium silicate nanoparticles ($CaSiO_3$NPs) were prepared by Gama Tec Center in Babylon, Iraq. The nano-structured calcium silicate (NCS) was synthesized by following the steps given by Borrmann et al. (2008); 10 mol of sodium silicate was combined with 10 mol calcium and stirred for 6 h to form a white solid precipitate. After filtration, it was heated at 100 °C for 24 h. The characterization of $CaSiO_3$NPs as carried out using scanning electron microscope (SEM), transmission electron microscope (TEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR).

Source of rice seed

The Iraqi rice (*Oryza sativa* L.) seed genotypes: 'Amber' (accession no. KF0307), 'Furat' (accession no. KF03060), and 'Yasmine' (accession no. KF03055) were obtained from the Al-Mishqab Rice Research Station (AMRS) in Najaf Province, Iraq.

Optimization of the duration and concentration of the priming chemical compound

To ensure accurate and reliable results, initial priming experiments involved soaking rice (5 g) seeds in varying concentrations of calcium silicate nanoparticles ($CaSiO_3$NPs) prior to determining the optimal priming duration and concentration. The priming duration was standardized at 8 h based on preliminary trials. Four concentrations of $CaSiO_3$NPs (0.01%, 0.02%, 0.03%, and 0.05% were tested following the method described by Hyun-Hwoi et al. (2020).

Seed priming treatments and measurement of germination and early seedling traits

Different concentrations of $CaSiO_3$NPs (0.01%, 0.02%, 0.03%, and 0.05%) were prepared according to the protocol described by Hyun-Hwoi et al. (2020). Rice seeds (5 g) were sterilized using the flotation method, which involved soaking in autoclaved distilled water (Portable Pressure Steam Sterilizer DSX-280B, Hinotek Lab, Ningbo, China), followed by immersion in 70% ethanol for 30 min, and then rinsing three times with autoclaved distilled water (Ali et al., 2020). Following sterilization, seeds were primed in the prepared $CaSiO_3$NPs solutions for 8 h and incubated in a laboratory growth room at 25 °C in complete darkness. After priming, the seeds were air-dried at 25 °C for 48 h under 50%-70% relative humidity to restore their initial moisture content before germination (Ali et al., 2020). Primed and non-primed (as control) seeds were then placed in glass jars lined with moistened filter paper containing 8 mL -0.6 MPa polyethylene glycol (PEG6000) solution to simulate moderate osmotic stress. The PEG6000 induces osmotic and oxidative stress while simulating anoxic conditions in seeds. The growth chamber was maintained at 25 ± 1 °C, with a relative humidity of 50%-70% and 12:12 h photoperiod. The experiment was laid out in a completely randomized design (CRD) with five replicates as described by Ali et al. (2020). Seeds were considered germinated when the radicle reached approximately 2

mm in length. Germination was monitored daily for 14 d, and germination traits were assessed using five randomly selected seedlings per treatment using the following formulas:

Germination percentage (% GP) (Abdul-Baki and Anderson, 1970):

$$\%\ \text{GP} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

The germination index (GI) was calculated by determining the total number of seeds that germinated each day; GI was calculated using (Abdul-Baki and Anderson, 1970):

$$\text{GI} = \frac{\text{Number of germinated seeds}}{\text{Days of first count} - \text{Number of seeds}\dfrac{\text{germinated}}{\text{day}}\text{of final count}}$$

Mean germination time (MGT) was calculated according to the formula (Ellis and Roberts, 1981):

$$\text{MGT} = \sum Dn \Big/ \sum n$$

where n indicates the number of seeds that have germinated on day D, and D represents the number of days since the start of germination.

Biomass of the seedlings, both fresh and dry weight was measured using an electronic balance (Mettler Toledo, Mettler-Toledo, LLC, Columbus, USA). Dry weight was weighed at 80 °C in a hot air oven until constant weight was obtained (Ali et al., 2020).

Rice seedling length, shoot, and root length were measured using a ruler (Abdul-Baki and Anderson, 1970).

Seedling vigour (SV) index was calculated using the equation (Abdul-Baki and Anderson, 1970):

$$\text{SV} = \text{Seedling length} \times \text{Germination percentage}$$

Biochemical measurements

Total chlorophyll. Total chlorophyll content was measured according to the method of Lichtenthaler and Wellburn (1983), with slight modifications. Leaf sample at seedling stage (300 mg) was extracted in 1 mL 80% ethanol using a dry water bath at 70 °C for 20 min. The initial extract was collected, and the leaf material was re-extracted until the leaves turned white, indicating complete pigment extraction. The combined extracts were measured spectrophotometrically using a UV9200 spectrophotometer (Biotech Engineering Management Co. Ltd., Nicosia, Cyprus) at 470, 649, and 665 nm.

Chlorophyll *a* (C_a) = 13.95 (A_{665}) – 6.88 (A_{649})

Chlorophyll *b* (C_b) = 24.96 (A_{649}) – 7.32 (A_{665})

Carotenoid (C_{x+c}) = (1000 (A_{470}) – 2.05C_a – 114.8C_b)/245

Total chlorophyll = 20.29 (A_{649}) + 8.05 (A_{665})

Total carbohydrates. Total carbohydrates content was determined following the method reported by Nielsen (2024). Fresh leaf tissue (250 mg) was homogenized in 2 mL 80% ethanol and centrifuged, and the supernatant was collected. Sulfuric acid and phenol were then added to the wells of a 96-well microplate. Absorbance was measured spectrophotometrically at 490 nm. Total carbohydrate content was calculated using a standard curve generated from a series of known glucose concentrations.

Total soluble sugar. Total soluble sugar content in rice seedlings was measured following the methods of Masuko et al. (2005). The assay is based on the picric acid test, which detects reducing sugars by reacting them with picric acid to form picramic acid. This reaction occurs with all sugars containing a free aldehyde or ketone group. Sodium carbonate is added to make the solution alkaline, facilitating the reduction of picric acid to picramic acid. Leaf samples (250 mg) were ground in 2 mL 80% ethanol, centrifuged, and the supernatant collected. Picric acid was then added to the supernatant, and the mixture was heated in a boiling water bath. Absorbance was measured at 490 nm. Total soluble sugar content was calculated using a glucose standard curve prepared from known concentrations.

Cell membrane stability. Cell membrane integrity is essential for maintaining cellular homeostasis, and electrolyte leakage (EL) serves as an indicator of membrane stability. Increased EL reflects membrane damage, which can disrupt ion balance and ultimately lead to cell death. Electrolyte leakage in rice seedlings was measured following the method reported by Sairam et al. (2001) with slight modifications. Seedling samples

were cut into fragments and immersed in 10 mL deionized water within test tubes. The tubes were incubated in a water bath at 40 °C for 30 min. Initial electrical conductivity (EC1) of the solution was then measured using an electric conductivity meter (HI2300-02, EC/TDS/NaCl/°C Meter, Auto Range, Hanna Instruments, Washington Hwy. Smithfield, Rhode Island, USA). Subsequently, the samples were heated at 100 °C for 10 min to destroy the tissues and release all electrolytes. After cooling to room temperature, the final electrical conductivity (EC2) was recorded. Electrolyte leakage was calculated using the formula: EL = EC1/EC2.

Total soluble protein. Total soluble protein content was determined using the Bradford assay (Bradford, 1976). Rice seedling leaf samples (500 mg) were ground in liquid nitrogen and homogenized in protein extraction buffer. The homogenate was centrifuged at 20 000 × g at 4 °C for 30 min. The resulting supernatant was mixed with protein reagent (Bradford Reagent, Sigma-Aldrich, St. Louis, Missouri, USA) and incubated at room temperature for 10 min. Absorbance was measured spectrophotometrically at 595 nm, and protein concentration was calculated using a standard curve prepared with bovine serum albumin (BSA). The supernatant was then stored at -80 °C for subsequent assays of catalase and ascorbate peroxidase activities.

Measurements of antioxidant enzymes

Catalase activity (CAT). For CAT activity determination, a reaction mixture (1 mL) was prepared containing 100 µL enzyme extract, 100 µL 15 mM hydrogen peroxide, 800 µL 50 mM potassium phosphate buffer (pH 7.0). Absorbance was measured at 240 nm every 15 s to determine CAT activity. Absorbance changes result from the decomposition of H_2O_2 (Zhang et al., 2015).

Ascorbate peroxidase (APX) activity. One millilitre (1 mL) reaction mixture consisting of 200 µL enzyme extract, 200 µL 0.5 mM ascorbic acid, 200 µL 0.1 mM H_2O_2, and 400 µL 50 mM potassium phosphate buffer (pH 7.0) was prepared. The reaction was commenced by applying H_2O_2, and APX activity was detected at a wavelength of 290 nm every 15 s for 1 min using the slightly modified procedure of Nakano and Asada (1987).

Superoxide dismutase activity (SOD). Superoxide dismutase activity was determined according to the method described by Zheng et al. (2016). The decrease of NBT was evaluated by reading the absorbance change at 560 nm with a spectrophotometer (Model-Hitachi U-1900, Tokyo, Japan), and the activity of SOD was expressed as U mg^{-1} FW.

Statistical analysis

The normality of the data was assessed using the Kolmogorov-Smirnov test. A two-way ANOVA at $P \leq 0.05$ was performed using SPSS (window version 23; IBM, Armonk, New York, USA) to evaluate the effects of priming treatments, followed by Duncan's multiple range test (DMRT) for mean comparison.

RESULTS AND DISCUSSION

Enhancement of rice germination

Seed germination is the beginning of crop production and is critical for agricultural success. Poor germination and seedling establishment in rice can result in substantial yield losses. This study found that priming with calcium silicate nanoparticles ($CaSiO_3$NPs) significantly improved germination attributes across all three rice genotypes (Table 1).

Germination percentage (GP), a key indicator of seedling emergence, was found to decrease with increasing stress levels. In contrast, the germination index (GI), which reflects the speed and uniformity of germination, improved with $CaSiO_3$NPs treatment. A higher GI indicates better germination performance.

'Amber' exhibited higher GP and GI at 0.01%-0.02% $CaSiO_3$NPs, while 'Furat' showed increased GP and GI at all tested concentrations. 'Yasmine' recorded the highest GP and GI at 0.02% $CaSiO_3$NPs. Mean germination time (MGT), which represents the time from imbibition to radicle emergence, is inversely related to germination speed which is lower MGT indicates faster germination. In this study, 'Amber' and 'Furat' displayed lower MGT at all $CaSiO_3$NPs concentrations, whereas 'Yasmine' showed reduced MGT at 0.02% and 0.03%.

Table 1. Effects of seed priming with calcium silicate nanoparticles ($CaSiO_3$NPs) on the germination attributes of three Iraqi rice genotypes under drought stress. Values are mean and standard errors (n = 5). Superscripts within the means of each column with different letters indicated significantly different among treatment (DMRT, $P \leq 0.05$).

Rice genotype	Priming treatment	Germination percentage	Germination index	Mean germination time
		%		D
Amber	Control	80.00 ± 6.15^{abc}	7.60 ± 0.24^{abc}	9.00 ± 0.00^{a}
	0.01% $CaSiO_3$NPs	93.20 ± 4.16^{a}	10.60 ± 0.75^{a}	8.00 ± 0.00^{b}
	0.02% $CaSiO_3$NPs	83.20 ± 9.13^{ab}	8.20 ± 1.16^{abc}	8.60 ± 0.40^{ab}
	0.03% $CaSiO_3$NPs	73.40 ± 2.51^{abcd}	7.20 ± 1.02^{bc}	8.40 ± 0.24^{ab}
	0.05% $CaSiO_3$NPs	50.00 ± 11.74^{bc}	5.20 ± 1.24^{c}	8.40 ± 0.24^{ab}
Furat	Control	63.20 ± 6.21^{bc}	5.93 ± 0.27^{c}	8.91 ± 0.075^{a}
	0.01% $CaSiO_3$NPs	86.66 ± 6.23^{ab}	8.04 ± 0.60^{abc}	8.81 ± 0.11^{a}
	0.02%$CaSiO_3$NPs	80.00 ± 9.72^{abc}	7.75 ± 1.12^{abc}	8.75 ± 0.13^{a}
	0.03% $CaSiO_3$NPs	90.00 ± 6.67^{a}	9.35 ± 0.70^{a}	8.57 ± 0.08^{a}
	0.05% $CaSiO_3$NPs	90.00 ± 6.66^{a}	9.09 ± 0.71^{ab}	8.63 ± 0.13^{a}
Yasmine	Control	76.67 ± 11.30^{abc}	6.63 ± 0.70^{bcd}	8.93 ± 0.12^{ab}
	0.01% $CaSiO_3$NPs	60.00 ± 8.49^{bc}	4.79 ± 0.64^{d}	9.19 ± 0.19^{a}
	0.02% $CaSiO_3$NPs	96.66 ± 3.33^{a}	11.42 ± 0.83^{a}	8.31 ± 0.10^{b}
	0.03% $CaSiO_3$NPs	50.00 ± 5.27^{c}	5.07 ± 0.63^{d}	8.65 ± 0.15^{ab}
	0.05% $CaSiO_3$NPs	60.00 ± 10.00^{bc}	5.29 ± 0.99^{d}	9.00 ± 0.23^{ab}

Enhancement rice early growth

Drought stress significantly reduced early seedling growth; however, priming with $CaSiO_3$NPs improved these traits (Table 2). The effects of $CaSiO_3$NPs application varied significantly among the different rice genotypes.

'Amber', 'Furat', and 'Yasmine' exhibited the highest shoot length (SHL), with increases of more than one-fold compared to the control, following treatment with 0.02% and 0.03% $CaSiO_3$NPs, respectively. Root length (RL) was highest in 'Amber' at 0.03%, in 'Furat' at 0.03%-0.05%, and in 'Yasmine' at 0.03%.

The highest seedling length (SL) was observed at 0.05% in 'Amber', and at 0.02% in both 'Furat' and 'Yasmine'. This study also found that priming with 0.03%-0.05% $CaSiO_3$NPs increased seedling vigour (SV) in 'Amber', while all tested concentrations enhanced SV in 'Furat' and 'Yasmine'.

Table 2. Effects of seed priming with calcium silicate nanoparticles ($CaSiO_3$NPs) on the early seedling growth of three Iraqi rice genotypes under drought stress. Values are mean and standard errors (n = 5). Superscripts within the means of each column with different letters indicate significant differences among the treatment (DMRT Range Test, $P \leq 0.05$).

Rice genotype	Priming treatment	Shoot length	Root length	Seedling length	Seedling vigour
		cm	cm	cm	
Amber	Control	6.84 ± 0.32^{d}	1.76 ± 0.02^{d}	10.88 ± 0.95^{bc}	7.20 ± 0.49^{bcd}
	0.01% $CaSiO_3$NPs	9.78 ± 0.47^{bcd}	2.46 ± 0.25^{bcd}	12.83 ± 1.20^{bc}	11.40 ± 0.51^{a}
	0.02% $CaSiO_3$NPs	10.12 ± 1.03^{abcd}	2.72 ± 0.24^{bcd}	12.31 ± 2.00^{bc}	10.60 ± 1.29^{abc}
	0.03% $CaSiO_3$NPs	9.18 ± 1.32^{bcd}	3.18 ± 0.69^{ab}	12.72 ± 1.79^{bc}	9.20 ± 2.03^{abcd}
	0.05% $CaSiO_3$NPs	9.66 ± 1.75^{bcd}	3.08 ± 0.24^{abc}	13.18 ± 1.91^{b}	5.60 ± 0.98^{d}
Furat	Control	3.82 ± 0.32^{d}	2.78 ± 0.04^{c}	13.42 ± 0.39^{a}	4.60 ± 0.67^{e}
	0.01% $CaSiO_3$NPs	6.42 ± 0.36^{abc}	4.54 ± 0.43^{ab}	12.76 ± 1.02^{a}	9.40 ± 0.75^{abcd}
	0.02% $CaSiO_3$NPs	8.78 ± 0.73^{ab}	3.98 ± 0.44^{abc}	13.32 ± 0.90^{a}	10.40 ± 1.50^{abc}
	0.03% $CaSiO_3$NPs	9.08 ± 0.71^{a}	4.24 ± 0.36^{abc}	12.14 ± 0.91^{a}	12.00 ± 0.95^{ab}
	0.05% $CaSiO_3$NPs	7.90 ± 0.87^{abc}	4.24 ± 0.25^{abc}	11.89 ± 1.64^{a}	11.20 ± 1.32^{ab}
Yasmine	Control	6.66 ± 0.22^{c}	1.92 ± 0.05^{b}	11.24 ± 0.97^{bc}	6.40 ± 0.98^{d}
	0.01% $CaSiO_3$NPs	9.06 ± 0.89^{abc}	3.96 ± 0.52^{b}	14.25 ± 0.28^{ab}	7.40 ± 0.75^{bcd}
	0.02% $CaSiO_3$NPs	9.94 ± 0.39^{a}	4.32 ± 0.29^{b}	18.79 ± 4.87^{a}	13.60 ± 0.51^{a}
	0.03% $CaSiO_3$NPs	10.14 ± 0.94^{a}	8.68 ± 4.37^{a}	13.99 ± 0.88^{abc}	9.20 ± 2.49^{bcd}
	0.05% $CaSiO_3$NPs	9.90 ± 0.79^{a}	4.10 ± 0.33^{b}	12.36 ± 0.69^{bc}	8.40 ± 1.25^{bcd}

Enhancement of rice biochemicals attributes

The results show that biochemical traits varied significantly among Iraqi rice genotypes in response to different concentrations of $CaSiO_3$NPs priming (Table 3). Priming with 0.03% $CaSiO_3$NPs significantly increased chlorophyll content in 'Amber' and 'Furat' genotypes, while concentrations between 0.01%-0.03% led to a significant increase in 'Yasmine'. However, carbohydrate content did not significantly differ among the three genotypes.

Membrane stability, as indicated by reduced electrolyte leakage (EL), improved in 'Amber' and 'Yasmine' at all $CaSiO_3$NPs concentrations compared to unprimed seeds, though nonsignificant difference was observed in 'Furat'.

For protein content and total sugars, the highest levels were observed in 'Amber' at 0.03% and 0.05% $CaSiO_3$NPs, and in 'Furat' at 0.05%. In contrast, total protein content did not significantly differ in 'Yasmine'. Total sugar content in 'Furat' significantly increased at all $CaSiO_3$NPs concentrations, whereas in 'Yasmine', significant increases were observed at 0.1% and 0.05%.

Table 3. Effects of nano-priming with calcium silicate nanoparticles ($CaSiO_3$NPs) on the total chlorophyll content, carbohydrate, total sugar, electrolyte leakage, and protein of three Iraqi rice genotypes primed with $CaSiO_3$NPs under drought stress. Values are mean and standard errors (n = 5). Superscripts within the means of each column with different letters indicate significant differences among the treatment (DMRT, $P \leq 0.05$).

Rice genotype	Priming treatment	Total chlorophyll	Carbohydrates	Total sugar	Electrolyte leakage	Protein
		mg mL^{-1}	mg g-1 FW	mg mL^{-1}	dS m^{-1}	mg g^{-1} FW
Amber	Control	5.48 ± 0.78^{ab}	26.82 ± 1.32^{abc}	38.96 ± 1.27^{b}	89.60 ± 1.40^{ab}	6.86 ± 0.16^{b}
	0.01% $CaSiO_3$NPs	3.42 ± 0.63^{bc}	28.09 ± 1.39^{a}	49.53 ± 4.35^{b}	83.20 ± 3.88^{abc}	7.00 ± 0.93^{b}
	0.02% $CaSiO_3$NPs	5.20 ± 1.26^{ab}	27.33 ± 1.12^{abc}	40.68 ± 2.74^{b}	74.20 ± 6.34^{bc}	6.86 ± 1.17^{b}
	0.03% $CaSiO_3$NPs	6.11 ± 0.47^{a}	25.79 ± 0.69^{abc}	62.67 ± 5.60^{ab}	85.00 ± 3.52^{abc}	21.36 ± 4.70^{a}
	0.05% $CaSiO_3$NPs	5.17 ± 1.08^{ab}	25.02 ± 0.34^{abc}	67.03 ± 4.44^{ab}	80.60 ± 8.41^{abc}	14.86 ± 3.96^{ab}
Furat	Control	5.48 ± 0.78^{a}	37.31 ± 0.86^{a}	42.00 ± 1.65^{c}	84.40 ± 0.40^{c}	7.14 ± 0.32^{ab}
	0.01% $CaSiO_3$NPs	3.42 ± 0.63^{abc}	27.52 ± 0.68^{bc}	69.84 ± 11.26^{ab}	96.40 ± 0.92^{a}	9.20 ± 0.70^{a}
	0.02%$CaSiO_3$NPs	5.20 ± 1.27^{abc}	24.83 ± 1.18^{bc}	59.36 ± 7.27^{bc}	91.40 ± 2.25^{abc}	6.64 ± 0.97^{ab}
	0.03% $CaSiO_3$NPs	6.11 ± 0.47^{ab}	28.10 ± 2.80^{bc}	65.52 ± 5.07^{abc}	86.00 ± 2.45^{bc}	6.34 ± 1.88^{ab}
	0.05% $CaSiO_3$NPs	5.17 ± 1.08^{ab}	29.63 ± 1.55^{bc}	71.36 ± 9.93^{ab}	94.40 ± 0.40^{a}	8.02 ± 0.87^{ab}
Yasmine	Control	10.30 ± 0.37^{ab}	26.11 ± 2.27^{ab}	45.60 ± 2.07^{bcde}	98.20 ± 2.78^{ab}	7.72 ± 0.29^{a}
	0.01% $CaSiO_3$NPs	8.68 ± 2.97^{bcd}	23.94 ± 0.88^{ab}	74.56 ± 3.32^{a}	90.80 ± 3.36^{ab}	7.42 ± 0.89^{a}
	0.02% $CaSiO_3$NPs	10.63 ± 0.54^{bcd}	25.09 ± 1.40^{ab}	51.23 ± 3.42^{bcde}	88.80 ± 3.07^{ab}	6.90 ± 1.19^{a}
	0.03% $CaSiO_3$NPs	14.09 ± 2.81^{d}	21.76 ± 0.78^{b}	30.17 ± 8.76^{e}	84.80 ± 3.10^{a}	8.80 ± 1.76^{a}
	0.05% $CaSiO_3$NPs	4.51 ± 0.79^{bcd}	27.65 ± 1.23^{ab}	67.39 ± 6.18^{ab}	84.60 ± 7.78^{b}	7.90 ± 0.35^{a}

Enhancement of antioxidant enzymes

This study found that $CaSiO_3$NPs had nonsignificant effect on CAT activity in the 'Amber' and 'Furat' genotypes; however, CAT activity significantly increased in 'Yasmine' at 0.03%. Meanwhile, the activity of SOD did not significantly increase in any of the rice genotypes. The APX activity in 'Amber' and 'Furat' increased at 0.01% $CaSiO_3$NPs; however, no increase was observed in 'Yasmine' (Table 4).

Table 4. Catalase, superoxide dismutase, and ascorbate peroxidase of Iraqi rice genotypes primed with calcium silicate nanoparticles ($CaSiO_3$NPs) under drought stress. Values are mean and standard errors (n = 5). Superscripts within the means of each column with different letters indicate significant differences among the treatment (DMRT, $P \leq 0.05$).

Rice genotype	Priming treatment	CAT	SOD	APX
		U g^{-1} fwt		
Amber	Control	0.45 ± 0.14^{ab}	12.00 ± 0.30^{a}	0.21 ± 0.00^{b}
	0.01% $CaSiO_3$NPs	0.39 ± 0.09^{a}	6.71 ± 0.09^{c}	0.32 ± 0.04^{a}
	0.02%$CaSiO_3$NPs	0.32 ± 0.03^{a}	7.16 ± 0.32^{c}	0.19 ± 0.01^{b}
	0.03% $CaSiO_3$NPs	0.49 ± 0.08^{a}	6.92 ± 0.45^{c}	0.18 ± 0.02^{b}
	0.05% $CaSiO_3$NPs	0.33 ± 0.02^{a}	7.52 ± 0.30^{c}	0.22 ± 0.04^{b}
Furat	Control	0.48 ± 0.10^{a}	11.08 ± 0.08^{a}	0.25 ± 0.01^{abc}
	0.01% $CaSiO_3$NPs	0.34 ± 0.01^{a}	10.64 ± 0.22^{a}	0.34 ± 0.02^{a}
	0.02%$CaSiO_3$NPs	0.52 ± 0.12^{a}	9.23 ± 0.59^{b}	0.22 ± 0.01^{bc}
	0.03% $CaSiO_3$NPs	0.53 ± 0.13^{a}	10.28 ± 0.60^{ab}	0.24 ± 0.012^{abc}
	0.05% $CaSiO_3$NPs	0.33 ± 0.01^{a}	10.68 ± 0.45^{a}	0.28 ± 0.05^{abc}
Yasmine	Control	0.29 ± 0.01^{abcd}	10.83 ± 0.25^{a}	0.26 ± 0.01^{bcd}
	0.01% $CaSiO_3$NPs	0.37 ± 0.12^{abcd}	8.77 ± 0.49^{b}	0.20 ± 0.03^{cd}
	0.02%$CaSiO_3$NPs	0.29 ± 0.03^{abcd}	8.62 ± 0.55^{b}	0.24 ± 0.02^{cd}
	0.03% $CaSiO_3$NPs	0.42 ± 0.10^{abc}	8.19 ± 0.18^{b}	0.20 ± 0.04^{d}
	0.05% $CaSiO_3$NPs	0.17 ± 0.05^{d}	8.30 ± 0.31^{b}	0.19 ± 0.05^{d}

Drought is a common environmental constraint which caused water deficiency and serves as limiting and challenging factor in major crops such as maize, wheat, and rice. Integrated management approaches had been introduced to mitigate this problem such as improvement of the irrigation system, production of tolerant varieties through breeding program (conventional, molecular, tissue culture and genetic engineering approaches), and the application of various kind of fertilizers (organic and inorganic fertilizer, including nanoparticles). The application of nanotechnology has held a promising position for alleviating the challenges imposed by biotic and abiotic stresses to ensure sustainability and climate-smart agriculture in recent years (Yadav et al., 2024). Various types of nanoparticles (NPs) with differences in size, shape, surface charge and surface chemistry have been developed through nanotechnology. This study found that combination of seed priming and $CaSiO_3$NPs improved the germination attributes and early seedling growth of drought-stressed Iraqi rice genotypes compared to non-primed seed. The optimal concentration of $CaSiO_3$NPs that enhanced the germination and early seedling growth was found different on all three Iraqi rice genotypes. Nano-primed Iraqi rice seeds emerge faster and grow more vigorously in drought conditions. The germination process of primed seeds is more efficient, which results in higher germination rates and uniformity compared to non-primed seeds. Seed priming control hydration in seeds by stimulating pre-germination metabolic processes including increasing water imbibition, improves the ability of seeds to absorb water, develop electron exchange and enhanced surface reaction capabilities associated with various components of plant cells and tissues accelerating activating the mobilization of the hydrolytic enzymes such as amylase, cellulase and xylanase in seeds and cell division (Ali et al., 2020; Janah et al., 2025). Thus, the small size of $CaSiO_3$NPs used in this study penetrates and enter rice seed inducing germination and controlling water imbalance and finally accelerating germination. Nanoparticles (NPs) having a diameter of 5-20 nm might penetrate through the cell wall and reach the plasma membrane with ease. After accumulation and translocation of NPs, it produces changes in different cellular and physiological functions of the plant. In addition, $CaSiO_3$NPs contain Si, the most abundant on the earth surface, could be used as a mineral nutrient to increase plant resistance to various levels and abiotic stresses. Silicon increased catalytic enzymes activities which are α-amylase and protease, resulted in higher adequate supply of glucose and amino acids for seedling growth. Silicon has improved stress management components in agronomic crops, introducing morphological and biochemical adjustments without compromising the final crop field (Rachappanavar et al., 2024). Silicon can be considered an economically viable water-management strategy for conserving limited water resources in semi-arid regions. The findings by Celik et al. (2025) demonstrated that Si application significantly enhanced yield-related traits in cauliflower grown

under water-limited conditions by mitigating the adverse physiological effects associated with drought stress. Our findings corroborate with previous studies indicating that seed priming with $CaSiO_3$NPs has been shown to induce germination, early seedling growth and enhance antioxidant defence mechanisms in plants. Studies by Yadav et al. (2023) confirmed that nanoparticles have a high surface area-to-volume ratio, making them ideal for holding and releasing nutrients. Moreover, it can be used to control the release of nutrients, which ensures the nutrients are gradually released over an extended period, thus providing a steady supply of essential elements to the plants. Nano-priming induces starch degradation via the stimulation of amylase, which results in the stimulation of seed germination (Nile et al., 2022). The controlled-release system is more efficient than traditional fertilizers, as it reduces the need for frequent application and the amount of fertilizer. Another study found that $CaSiO_3$NPs has increased root development, and physiological activities under drought-stress conditions. These mechanisms include reduced water loss and enhanced efficiency in utilizing root-absorbed water, making rice plants more tolerant to drought conditions (Hossain et al., 2022). The intrinsic mechanisms of nano-priming-based stimulation of seed germination are still ambiguous. However, few mechanisms related to it were clearly suggested, including the generation of nanopores for increased intake of water, bootstrapping reactive oxygen species (ROS)/antioxidant network in seeds, hydroxyl radical's generation for slackening the cell wall, and stimulant for hastening the breakdown of starch.

In this study, priming seeds of Iraqi rice genotypes with $CaSiO_3$NPs effectively induced systemic acquired resistance and enhanced the plants' ability to withstand drought stress. This was reflected in significantly increased chlorophyll content, total carbohydrates, total sugars, and total proteins, along with reduced electrolyte leakage and elevated activity of protective enzymes under drought conditions, ultimately improving overall performance of Iraqi rice seedlings. These improvements suggested that combination of seed priming and nanoparticles may modulate the expression of genes involved in stress responses, leading to the activation of physiological pathways that mitigate the detrimental effects of drought. The NP induces ROS when it enters the seed coat and stimulates various cascades of downstream events. Chlorophyll is an essential pigment that plays a critical role in determining the effectiveness of photosynthesis in plants such as rice. These current results indicated that this approach improved the photosynthetic activity, development, and vigour in Iraqi rice seedlings. Previous studies have demonstrated that treating seeds with Ca, and silicon dioxide (SiO_2) improved the antioxidant activities in rice when exposed to various stressful circumstances. These priming compounds have been discovered to enhance the functions of antioxidant enzymes such as catalase, ascorbate peroxidase, superoxide dismutase, and glutathione reductase. Additionally, they decrease the buildup of ROS and lipid peroxidation levels. In addition, it has been discovered that seed priming treatments can stimulate or sustain the antioxidant defence system in rice seedlings, resulting in enhanced stress tolerance, and plant growth (Ali et al., 2021; Wang et al., 2023). Previous studies by Farman et al. (2022) indicated that Si improved the plant anti-oxidative defence system through detoxification of free radicals, limiting lipid peroxidation in cells, reducing malondialdehyde (MDA) levels and regulating cellular homeostasis. Silicon application to wheat increased the photosynthesis rate, and stomatal conductance compared to plants with no Si application. Nano-priming induces the formation of nanopores that helps in the uptake of water absorption, activates reactive oxygen species (ROS)/antioxidant mechanisms in seeds (Nile et al., 2022). Another finding reported that the application of SiO_2 and SiO_2NPs during drought stress enhanced the activation of antioxidant enzymes such as CAT, APX, SOD, and glutathione peroxidase in wheat (Zahedi et al., 2023). Silicon plays an important role in maintaining cellular homeostasis by enhancing plant structural stability, regulating membrane permeability, and improving cell membrane integrity. Moreover, Si increased plant tolerance to stresses largely due to its ability to strengthen physical barriers, modulate antioxidant defence systems, and support more efficient metabolic regulation.

CONCLUSIONS

All Iraqi rice seed genotypes primed with calcium silicate nanoparticles ($CaSiO_3$NPs) responded differently in improving germination traits, early seedling growth, biochemicals traits, and antioxidant enzymes under drought stress. Increase in these parameters in Iraqi rice genotypes indicates enhanced drought tolerance, which may be linked to the potential of $CaSiO_3$NPs priming.

The results also indicated that $CaSiO_3$NP nano-priming was more effective in 'Furat' and 'Yasmine'. The findings of this study validated the effectiveness and reliability of seed nano-priming as a simple, practical, and cost-efficient technique that can be readily adopted to enhance plant resilience under challenging environmental stress conditions.

Author contribution

Conceptualization: A.I.J.A., R.N., R.G., M.H.H. Methodology: A.I.J.A., R.N., M.H.H. Software: A.I.J.A., R.N. Validation: R.N, M.H.H. Formal analysis: A.I.J.A., R.N., M.H.H. Investigation: A.I.J.A. Resources: A.I.J.A., R.N., M.H.H. Data Curation: R.N., M.H.H., R.G. Writing-original draft: A.I.J.A. Writing-review & editing: A.I.J.A., R.N., R.G. Visualization: A.I.J.A., R.N. Supervision: R.N., R.G. Funding acquisition: A.I.J.A., R.N. All co-authors reviewed the final version and approved the manuscript before submission.

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