

Enhancement of germination, early growth, biochemicals, and antioxidant enzymes in drought-sensitive Iraqi rice through seed nano-priming with SiO₂ nanoparticles (SiO₂NPs) under drought stress

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ABSTRACT

Rice (*Oryza sativa* L.), a major staple providing nearly 20% of global dietary energy, is highly vulnerable to drought, threatening productivity and food security in Iraq. This study aims to evaluate seed nano-priming with SiO₂NPs for improving germination, seedling growth, biochemical traits, and antioxidant activities in three drought-sensitive Iraqi rice genotypes, 'Amber', 'Furat', and 'Yasmine'. The SiO₂NPs was synthesized using sodium silicate and sulfuric acid methods and characterized by scanning electron microscopy, transmission electron microscopy, and X-ray diffraction analyses. Seeds were primed with different SiO₂NPs concentrations (2.5%, 3.0%, 3.5%, and 4.0%) for 8 h before being grown under drought condition in a completely randomized block design with five replicates. Germination traits of primed seed increased as germination percentage (100%) in all genotypes compared with 83.33% in unprimed 'Amber'. Shoot fresh weight increased up to 5-fold in 'Amber' (0.82 mg), while seedling dry weight increased up to 23-fold at 2.5% (0.21 mg). Shoot length increased 2-fold at 2.5%-3.0% (47.33 mg) compared to unprimed seeds (23.00 mg). Biochemical traits improved significantly ($P < 0.05$) particularly total sugar increased to 118.95 mg mL⁻¹ in 'Amber' (4.0%) and 149.09 mg mL⁻¹ in 'Yasmine' (3.0%); while malondialdehyde decreased with the highest (6.4-fold) in 'Yasmine' (59.82 μmol g⁻¹ fwt) compared to unprimed (382.47 μmol g⁻¹ fwt). Catalase activity increased nearly 4-fold (1.17 and 1.35 U g⁻¹ fwt) in 'Amber' and 'Yasmine', respectively, while ascorbate peroxidase increased > 2-fold in both genotypes. As conclusion, SiO₂NPs nano-priming demonstrating potential for sustainable rice production in drought-prone Iraq.

Key words: Antioxidant enzymes, drought tolerance indices, germination traits, Iraqi rice genotypes, nanoparticle solution (NPs), seed nano-priming.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the staple crops in Iraq, which has been significant in terms of ensuring food security as well as economic stability. Genotypes that have been cultivated fit well with the local conditions include 'Amber', 'Furat', and 'Yasmine'. These genotypes are used as staple food, and they are part of the culture of the region. Although they are significant, drought stress is a growing threat to rice production, as it reduces the rates of germination and the overall yield (Kshash and Oda, 2022). Drought stress is an extreme abiotic factor that reduces the availability of water and affects the growth of plants. Such stress has an adverse impact on the germination of rice seeds, leading to decreased crop establishment and productivity (Zhang et

al., 2018). This is important to understand the impacts of drought on the germination of rice as an important step to coming up with effective measures.

Nanotechnology represents a rapidly advancing frontier with significant potential to enhance agricultural resilience, particularly under abiotic stress conditions. In this context, the application of engineered nanomaterials has emerged as a promising strategy to improve crop performance and stress tolerance. Among these, silicon dioxide nanoparticles (SiO_2NPs) have gained considerable attention as an effective form of nano-silica due to their unique physicochemical properties, including high surface area and reactivity. These nanoparticles can modulate various physiological and biochemical processes in plants, such as enhancing antioxidant defense systems, improving water-use efficiency, and maintaining cellular integrity under water-deficit conditions. Consequently, the use of SiO_2NPs has been increasingly explored as a viable approach to mitigate the adverse effects of drought stress and to promote sustainable crop productivity (Singh and Patel, 2022; Kang et al., 2025). Recent studies further substantiate that Si-based nanoparticles play a multifaceted role in enhancing plant performance under stress conditions. These nanoparticles improve water uptake, strengthen oxidative stress resistance, and facilitate nutrient absorption, thereby promoting higher germination rates and improved seedling growth. Mechanistically, their effectiveness is attributed to enhanced water retention capacity around the seed microenvironment, activation of antioxidant defense systems (such as superoxide dismutase, catalase, and peroxidase), and increased bioavailability of essential nutrients (Khan et al., 2020). In addition to Si-based nanomaterials, nano-formulated biostimulants have also showed potential for improving plant performance under drought stress. Alowaiesh et al. (2024) reported that nanoparticles of ascorbic acid, benzoic acid, and salicylic acid significantly improved plantlet length, leaf number, root development, lateral branching, and root length in potato subjected to polyethylene glycol (PEG)-induced drought stress. Combination nano-biostimulant applications were more effective than individual treatments, with ascorbic acid and benzoic acid producing particularly strong responses. The study also demonstrated significant differences between potato cultivars, indicating that the effectiveness of nano-based treatments may depend on genotype-specific responses. In addition, nano silica has been shown to form a thin, protective coating on seed surfaces, which reduces transpiration water loss and improves water-use efficiency during early developmental stages (Liu et al., 2019). More recent investigations have reinforced these findings, highlighting that SiO_2NPs can regulate gene expression associated with stress tolerance, improve osmotic adjustment, and maintain cellular homeostasis under drought stress conditions. Furthermore, SiO_2NPs have been reported to enhance root system architecture, enabling better soil moisture exploration and nutrient uptake, which collectively contribute to improved plant vigor under limited water availability.

Although the application of nanoparticles has demonstrated promising outcomes in improving resilience in major crops, including rice, significant knowledge gaps remain. There is still limited study on the response of Iraqi rice genotypes to nanoparticle-based seed priming under drought stress conditions, highlighting the need for genotype-specific investigations to optimize this emerging technology (El-Saadony et al., 2022; Wahab et al., 2023). The present study aims to evaluate the effects of seed priming with nano-silica (SiO_2) nanoparticles on germination and early growth of Iraqi rice genotypes 'Amber', 'Furat', and 'Yasmine' under drought stress conditions. Seed priming is a pre-sowing technique that gained considerable attention in modern agriculture because it improves seed performance, enhances stress tolerance, and promotes better crop establishment under both normal and adverse environmental conditions. This study also investigates the biochemical traits, and antioxidant enzyme activities to elucidate the underlying mechanisms of stress tolerance. The findings are expected to contribute to the development of sustainable strategies for improving rice production in Iraq in water-limited environments.

MATERIALS AND METHODS

Preparation of nano-silica (SiO_2NPs)

Nano-silica (silicon dioxide nanoparticles, SiO_2NPs) was prepared by Gama Tec Center in Babylon, Iraq. Nanoparticles of silica were prepared according to the method described by Jafari and Allahverdi (2014). The production of nano-silica from sodium silicate was carried out by the synthesis sodium silicate and sulfuric acid (H_2SO_4 , 95%-97%) of analytical quality, and polyvinyl alcohol (PVA) with an average molecular weight of 88 000 g mol^{-1} . Silicate was prepared by dissolving it in distilled water containing 1% PVA and stirring until fully

dissolved. A few drops of sulfuric acid were added to a mixture of sodium silicate and PVA while the mixture was agitated. The silica gel solution was continuously mixed until it thickened and became translucent, ensuring its pH 12. The mechanism of silica formation is presented in Equation 1.



The sodium silicate solution was stirred continuously while strong sulfuric acid solution was added gradually to form silica gel. The precipitated silica gel was thoroughly washed with distilled water. The gel was dried at 100 °C at least 24 h and ground using a mortar to produce silica nanoparticles as white powder. The characterization of SiO₂NPs was carried out using scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), and Fourier-transform infrared spectroscopy (FTIR).

Source of rice seed

The Iraqi rice (*Oryza sativa* L.) seeds ‘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.

Seed priming and measurement of germination and early seedling traits

Different concentrations of SiO₂NPs (2.5%, 3.0%, 3.5%, and 4.0%) 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 SiO₂NPs solutions for 8 h and incubated in a laboratory growth room at 25 °C in complete darkness. After priming, 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 unprimed (as a control) seeds were then placed in glass jars lined with moistened filter paper containing 8 mL -0.6 MPa polyethylene glycol (PEG6000) solution to stimulate 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):

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

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):

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

Mean germination time (MGT) was calculated according to the formula (Ellis and Roberts, 1980):

$$\text{MGT} = \frac{\sum Dn}{\sum n}$$

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, Columbus, Ohio, 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 vigor (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 (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, Nicosia, Cyprus) at 470, 649, and 665 nm.

$$\text{Chlorophyll } a \text{ (C}_a\text{)} = 13.95 \text{ (A}_{665}\text{)} - 6.88 \text{ (A}_{649}\text{)}$$

$$\text{Chlorophyll } b \text{ (C}_b\text{)} = 24.96 \text{ (A}_{649}\text{)} - 7.32 \text{ (A}_{665}\text{)}$$

$$\text{Carotenoid (C}_{x+c}\text{)} = (1000 \text{ (A}_{470}\text{)} - 2.05\text{C}_a - 114.8\text{C}_b)/245$$

$$\text{Total chlorophyll} = 20.29 \text{ (A}_{649}\text{)} + 8.05 \text{ (A}_{665}\text{)}$$

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 method 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 and Srivastava (2001) with slight modifications. Leaf samples (100 mg) were cut and added to test tubes containing 10 mL de-ionized water. The test tubes were placed in a water bath maintained at a temperature of 40 °C and incubated for 30 min. The electrical conductivity meter (HI2300-02, EC/TDS/NaCl/°C Meter, Auto Range; HANNA, Selangor, Malaysia) measured electrical conductivity (EC) of samples in the first instance (EC1). The samples were then treated in a water bath maintained at 100 °C for 10 min to ensure that cell tissues were lysed and all electrolytes were released. The final EC (EC2) in the samples was determined when cooled to room temperature. The EL is expressed as follows: $EL = EC1/EC2$, where EC1 is used to measure the solutions' initial EC; EC2 is used to measure the solutions' final EC.

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 20000 × 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 and measured spectrophotometrically at 595 nm. 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.

Malondialdehyde. Buege and Aust (1978) analyzed lipid peroxidation in rice seedlings to determine malondialdehyde (MDA) content. The level of lipid peroxidation in plants can be assessed by measuring MDA concentration. Leaf sample (0.5 g) was ground with chilled mortar with 10 mL 80% ethanol. The resultant mixture was centrifuged for 6 min at 4 °C and 13000 g and supernatant was taken. Supernatant was mixed with thiobarbituric acid (TBA)-trichloroacetic acid (TCA)-HCl solution in a 1:1:1 ratio. The mixture was placed in a

boiling water bath for a total of 15 min and cooled at room temperature. The sample was centrifuged for a total of 15 min at 2000 rpm. The sample's absorbance was determined using a spectrophotometer at 532 nm.

Measurements of antioxidant enzymes

Catalase activity (CAT). Reaction mixture (1 mL) was prepared containing 100 μ L enzyme extract, 100 μ L 15 mM hydrogen peroxide, 800 μ L 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., 2018).

Ascorbate peroxidase (APX) activity. One milliliter (1 mL) reaction mixture consisting of 200 μ L enzyme extract, 200 μ L 0.5 mM ascorbic acid, 200 μ L 0.1 mM H_2O_2 , and 400 μ L 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 (SOD) activity. Superoxide dismutase activity was determined according to the method described by Zheng et al. (2016). The decrease of Nitroblue Tetrazolium (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 25; IBM, Armonk, New York, USA) to evaluate the effects of priming treatments, followed by Duncan's multiple range test (DMRT) for mean comparison.

RESULTS

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 nano-silica (silicon dioxide nanoparticles, SiO_2 NPs) significantly enhanced (ANOVA, $P < 0.05$) germination across all three rice genotypes (Table 1).

Table 1. Effects of seed priming with nano-silica (silicon dioxide nanoparticles, SiO_2 NPs) on the germination attributes of three Iraqi rice genotypes under drought stress. Values are mean and standard errors ($n = 5$). Different letters within the means of each column indicate significant difference among treatments (DMRT, $P \leq 0.05$). GP: Germination percentage; GI: germination index; MGT: mean germination time.

Rice	SiO ₂ NPs priming treatment	GP	GI	MGT
	%	%	%	d
Amber	Control	83.33 $\pm$ 9.52 ^{ab}	4.33 $\pm$ 0.33 ^g	10.67 $\pm$ 0.33 ^a
	2.5	100.00 $\pm$ 0.00 ^a	7.33 $\pm$ 0.33 ^{abc}	9.67 $\pm$ 0.33 ^{bcd}
	3.0	100.00 $\pm$ 0.00 ^a	6.00 $\pm$ 0.00 ^{def}	9.60 $\pm$ 0.00 ^{bc}
	3.5	100.00 $\pm$ 0.00 ^a	6.33 $\pm$ 0.33 ^{cde}	9.67 $\pm$ 0.33 ^{bcd}
	4.0	100.00 $\pm$ 0.00 ^a	7.67 $\pm$ 0.33 ^{ab}	9.00 $\pm$ 0.00 ^e
Yasmine	Control	88.88 $\pm$ 5.55 ^{bc}	5.52 $\pm$ 0.81 ^{ef}	9.85 $\pm$ 0.43 ^{bcd}
	2.5	100.00 $\pm$ 0.00 ^a	6.35 $\pm$ 0.42 ^{cde}	9.77 $\pm$ 0.17 ^{bcd}
	3.0	100.00 $\pm$ 0.00 ^a	6.25 $\pm$ 0.30 ^{cde}	9.79 $\pm$ 0.12 ^{bcd}
	3.5	100.00 $\pm$ 0.00 ^a	6.16 $\pm$ 0.30 ^{cde}	9.84 $\pm$ 0.12 ^{bcd}
	4.0	100.00 $\pm$ 0.00 ^a	6.56 $\pm$ 0.30 ^{bcd}	9.68 $\pm$ 0.11 ^{bcd}
Furat	Control	94.44 $\pm$ 5.55 ^b	5.00 $\pm$ 0.42 ^{fg}	10.25 $\pm$ 0.09 ^{ab}
	2.5	100.00 $\pm$ 0.00 ^a	7.76 $\pm$ 0.29 ^a	9.24 $\pm$ 0.10 ^{de}
	3.0	100.00 $\pm$ 0.00 ^a	6.99 $\pm$ 0.22 ^{abcd}	9.51 $\pm$ 0.08 ^{cde}
	3.5	100.00 $\pm$ 0.00 ^a	7.01 $\pm$ 0.26 ^{abcd}	9.51 $\pm$ 0.09 ^{cde}
	4.0	100.00 $\pm$ 0.00 ^a	7.59 $\pm$ 0.25 ^{ab}	9.30 $\pm$ 0.08 ^{de}

Germination percentage (GP) and germination index (GI) increased in all concentrations of SiO₂NPs compared to unprimed as shown in Table 1. The GI in 'Amber' and 'Furat' increased more than 1X fold compared to unprimed. Meanwhile, lower mean germination time (MGT) of 'Amber' and 'Furat' in all concentrations; however, nonsignificant effect in 'Yasmine'.

Enhancement rice early growth

Seed nano-priming with different concentrations of SiO₂NPs also significantly enhanced (ANOVA, $P < 0.05$) the early growth of Iraqi rice genotypes as tabulated in Table 2. Shoot fresh weight (SFW) of 'Amber' found the highest which is nearly 5-fold in 2.5% of SiO₂NPs while 2.55-fold and 3-fold in 2.5% 'Yasmine' and 'Furat' primed in 3.0% of SiO₂NPs. The highest seedling dry weight (SDW) which is 23X higher found in 'Amber' primed with 3.0% and 3.5% SiO₂NPs. In addition, 'Furat' primed with 4.0% have the highest SDW. The findings show that shoot length of primed seed in 'Amber' a found 2X higher and number of leaves more than 1X higher in all concentrations of SiO₂NPs compared to unprimed seed.

Table 2. Effects of seed priming with nano-silica (silicon dioxide nanoparticles, SiO₂NPs) on the early seedling growth of three Iraqi rice genotypes under drought stress. Values are mean and standard errors ($n = 5$). Different letters within the means of each column indicate significant difference among treatments (DMRT, $P \leq 0.05$). SFW: Shoot fresh weight; SDW: seedling dry weight; SHL: shoot length.

Rice	SiO ₂ NPs priming treatment	SFW	SDW	SHL	Number of leaves
	%	mg	mg	cm	
Amber	Control	0.24 ± 0.01 ^f	0.02 ± 0.00 ⁱ	23.00 ± 0.58 ^f	3.00 ± 0.00 ^d
	2.5	1.17 ± 0.01 ^a	0.21 ± 0.00 ^b	47.33 ± 2.60 ^a	5.33 ± 0.33 ^{abc}
	3.0	0.99 ± 0.01 ^b	0.23 ± 0.00 ^a	47.33 ± 0.88 ^a	5.67 ± 0.33 ^b
	3.5	0.51 ± 0.01 ^d	0.08 ± 0.00 ^d	35.67 ± 1.33 ^c	5.00 ± 0.00 ^{abc}
	4.0	0.82 ± 0.01 ^c	0.16 ± 0.00 ^c	42.33 ± 0.88 ^b	5.33 ± 0.33 ^{abc}
Yasmine	Control	0.10 ± 0.01 ⁱ	0.016 ± 0.00 ⁱ	23.00 ± 0.58 ^f	3.00 ± 0.00 ^d
	2.5	0.25 ± 0.01 ^f	0.03 ± 0.00 ^g	28.00 ± 0.58 ^{de}	6.00 ± 0.00 ^a
	3.0	0.18 ± 0.01 ^g	0.01 ± 0.00 ^h	28.33 ± 1.67 ^{de}	5.00 ± 0.58 ^{abc}
	3.5	0.15 ± 0.01 ^h	0.03 ± 0.00 ^{fg}	28.33 ± 0.67 ^{de}	4.67 ± 0.33 ^{bc}
	4.0 %	0.25 ± 0.01 ^f	0.03 ± 0.00 ^{fg}	27.67 ± 0.33 ^e	4.67 ± 0.33 ^{bc}
Furat	Control	0.16 ± 0.01 ^h	0.02 ± 0.00 ⁱ	21.67 ± 1.45 ^f	2.67 ± 0.33 ^d
	2.5	0.20 ± 0.01 ^g	0.05 ± 0.00 ^e	25.00 ± 1.00 ^{ef}	5.33 ± 0.33 ^{abc}
	3.0	0.32 ± 0.01 ^e	0.03 ± 0.00 ^{fg}	24.00 ± 1.15 ^f	4.33 ± 0.33 ^c
	3.5	0.25 ± 0.01 ^f	0.04 ± 0.00 ^f	31.67 ± 0.88 ^d	5.67 ± 0.33 ^{ab}
	4.0	0.31 ± 0.01 ^e	0.05 ± 0.00 ^e	28.67 ± 1.33 ^{de}	4.67 ± 0.33 ^{bc}

Enhancement of rice biochemicals attributes

Nano-priming with different concentrations of SiO₂NPs showed significantly different (ANOVA, $P < 0.05$) on biochemicals of three Iraqi rice genotypes as presented in Table 3. Total chlorophyll in nano-primed of 'Amber' and 'Yasmine' found more than two-fold higher than unprimed seed in all concentration except in 'Furat' which increased only in 3.5% and 4.0%.

Total sugar, carbohydrate, and total protein increased in primed seed compared to unprimed seed. Total sugar highest in 4.0% in 'Amber' (118.95 mg mL⁻¹), 3.0% in 'Yasmine' (149.09 mg mL⁻¹), and 3.0% and 4.0% in 'Furat' (102.00 mg mL⁻¹) which is nearly two-fold higher than unprimed seed. Total carbohydrate and proline content increased in all primed seed, while 'Yasmine' primed with 3.0% shows two-fold higher in total carbohydrate and 'Furat' primed with 4.0% compared to unprimed seed. Total protein found increased in 2.5%-3.5% in 'Amber'; however, nonsignificant increase in 'Yasmine' and 'Furat'. Electrolyte leakage (EL) decreased in 'Amber' primed with 3.5% SiO₂NPs; while in 'Furat', EL significantly decreased in primed with 3.0%-3.5%, which is 2X lower than unprimed.

Table 3. Effects of seed priming with silicon dioxide nanoparticles (SiO₂NPs) on the total chlorophyll content, carbohydrate, total sugar, electrolyte leakage (EL), protein, and malondialdehyde (MDA) of three Iraqi rice genotypes under drought stress. Values are mean and standard errors (*n* = 5). Different letters within the means of each column indicate significant difference among treatments (DMRT, *P* ≤ 0.05).

Rice	SiO ₂ NPs priming treatment	Total Chl.	Carbohydrate	Total sugar
	%	mg mL ⁻¹	mg g ⁻¹ fwt	mg mL ⁻¹
Amber	Control	51.62 ± 29.16 ^g	20.10 ± 0.56 ^{cde}	69.40 ± 4.10 ^{cde}
	2.5	116.08 ± 33.08 ^{fg}	24.80 ± 0.40 ^{bcd}	79.99 ± 23.05 ^{bcd}
	3.0	666.89 ± 30.32 ^b	29.25 ± 7.83 ^{abc}	74.31 ± 22.68 ^{cde}
	3.5	378.93 ± 36.75 ^d	20.55 ± 3.51 ^{cde}	59.69 ± 14.87 ^{de}
	4.0	752.49 ± 23.39 ^a	23.70 ± 0.04 ^{bcd}	118.95 ± 27.02 ^{abc}
Yasmine	Control	175.25 ± 33.12 ^f	17.30 ± 0.90 ^{de}	34.99 ± 12.85 ^{de}
	2.5	316.58 ± 30.50 ^{de}	26.90 ± 0.65 ^{abcd}	110.17 ± 6.62 ^{abcd}
	3.0	594.72 ± 34.37 ^{bc}	35.20 ± 6.07 ^a	149.09 ± 25.63 ^a
	3.5	396.91 ± 20.99 ^d	33.70 ± 5.67 ^{ab}	92.24 ± 4.75 ^{bcd}
	4.0	524.25 ± 27.60 ^c	25.70 ± 0.06 ^{abcd}	128.37 ± 9.27 ^{ab}
Furat	Control	279.54 ± 14.48 ^e	13.60 ± 1.16 ^e	65.12 ± 14.14 ^e
	2.5	266.86 ± 21.75 ^e	23.70 ± 0.09 ^{bcd}	74.50 ± 11.21 ^{cde}
	3.0	156.89 ± 24.87 ^f	22.45 ± 0.67 ^{cde}	102.00 ± 3.88 ^{abcd}
	3.5	388.38 ± 23.05 ^d	21.90 ± 1.94 ^{cde}	82.56 ± 8.22 ^{bcd}
	4.0	379.02 ± 22.87 ^d	23.70 ± 0.56 ^{bcd}	102.00 ± 5.29 ^{abcd}
		EL	Protein	MDA
		dSm ⁻¹	mg g ⁻¹ fwt	μmol g ⁻¹ fwt
Amber	Control	78.00 ± 6.65 ^{ab}	26.33 ± 6.76 ^{ab}	444.44 ± 60.28 ^a
	2.5	65.33 ± 2.72 ^{def}	47.83 ± 5.19 ^a	111.11 ± 29.91 ^{ef}
	3.0	70.66 ± 2.84 ^{bcd}	40.33 ± 7.12 ^{ab}	117.52 ± 23.50 ^{ef}
	3.5	64.66 ± 2.40 ^{def}	37.23 ± 3.65 ^{ab}	126.06 ± 10.68 ^b
	4.0	66.66 ± 1.85 ^{cde}	22.98 ± 5.95 ^b	198.71 ± 51.28 ^{bcd}
Yasmine	Control	59.00 ± 0.57 ^{ef}	23.58 ± 6.69 ^b	382.47 ± 5.65 ^a
	2.5	84.66 ± 3.92 ^a	21.93 ± 5.41 ^b	123.93 ± 23.50 ^{ef}
	3.0	69.66 ± 3.38 ^{bcd}	20.88 ± 5.04 ^b	145.29 ± 10.68 ^{def}
	3.5	64.00 ± 4.04 ^{def}	24.83 ± 7.79 ^{ab}	177.35 ± 8.54 ^{cde}
	4.0	58.66 ± 3.38 ^{ef}	23.73 ± 6.56 ^b	59.82 ± 8.54 ^f
Furat	Control	68.38 ± 5.08 ^{bcd}	27.13 ± 8.69 ^{ab}	416.66 ± 9.79 ^a
	2.5	54.56 ± 3.53 ^f	21.33 ± 6.22 ^b	151.70 ± 4.27 ^{def}
	3.0	35.05 ± 2.75 ^g	27.03 ± 10.58 ^{ab}	250.00 ± 70.51 ^{bc}
	3.5	33.49 ± 1.51 ^g	25.53 ± 8.47 ^{ab}	286.32 ± 4.27 ^{bcd}
	4.0	77.12 ± 2.57 ^{abc}	28.08 ± 8.08 ^{ab}	241.45 ± 4.27 ^{bcd}

Results show that nano-priming with SiO₂NPs decreased malondialdehyde (MDA) compared to unprimed. The MDA decreased 4X in 'Amber' primed with 2.5%, 6.4X in 'Yasmine' primed with 4.0%, and 2.7X in 'Furat' primed with 2.5% compared to control. The EL decreased in 'Amber' primed with 5.0% SiO₂NPs. While in 'Furat', EL significantly decreased in primed with 3.0%-3.5% which is 2X lower than unprimed.

Enhancement of antioxidant enzymes

The antioxidant activities of primed seed significantly increased (ANOVA, *P* < 0.05) compared to unprimed seed (Table 4). The catalase (CAT) activities in primed seeds show nearly four-fold higher than unprimed seed which is the highest at 2.5% and 4.0% in 'Amber' (1.17 U g⁻¹ fwt) and 'Yasmine' (1.35 U g⁻¹ fwt) respectively. The ascorbate peroxidase (APX) activities of 'Amber' and 'Yasmine' increased (more than two times) in all treatments, while in 'Furat' SiO₂NPs showed a significant increased only in 2.5% compared to unprimed.

Table 4. Effects of seed priming with silicon dioxide nanoparticles (SiO₂NPs) on catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX) of three Iraqi rice genotypes under drought stress. Values are mean and standard errors ($n = 5$). Different letters within the means of each column indicate significant difference among treatments (DMRT, $P \leq 0.05$).

Rice	SiO ₂ NPs priming treatment	U g ⁻¹ fwt		
		CAT	SOD	APX
Amber	Control	0.15 ± 0.01 ^d	8.96 ± 0.21 ^{bc}	0.07 ± 0.00 ^e
	2.5	1.17 ± 0.22 ^{ab}	10.49 ± 0.13 ^{ab}	0.18 ± 0.03 ^{bc}
	3.0	0.87 ± 0.29 ^{abc}	10.04 ± 0.62 ^{abc}	0.21 ± 0.04 ^{ab}
	3.5	0.56 ± 0.04 ^{cd}	9.70 ± 0.22 ^{abc}	0.21 ± 0.00 ^{ab}
	4.0	0.58 ± 0.01 ^{cd}	9.51 ± 0.07 ^{abc}	0.24 ± 0.00 ^a
Yasmine	Control	0.21 ± 0.02 ^d	9.06 ± 0.34 ^{abc}	0.08 ± 0.01 ^e
	2.5	0.60 ± 0.03 ^{cd}	9.72 ± 0.01 ^{abc}	0.14 ± 0.01 ^{cd}
	3.0	0.61 ± 0.00 ^{cd}	9.60 ± 0.02 ^{abc}	0.16 ± 0.01 ^{bc}
	3.5	0.54 ± 0.03 ^{cd}	9.61 ± 0.00 ^{abc}	0.17 ± 0.01 ^{bc}
	4.0	1.35 ± 0.35 ^a	10.68 ± 0.83 ^a	0.17 ± 0.00 ^{bc}
Furat	Control	0.26 ± 0.01 ^d	8.40 ± 0.23 ^c	0.07 ± 0.01 ^e
	2.5	0.53 ± 0.00 ^{cd}	8.99 ± 0.15 ^{bc}	0.16 ± 0.01 ^{bc}
	3.0	0.64 ± 0.23 ^{bcd}	9.81 ± 0.32 ^{abc}	0.09 ± 0.01 ^{de}
	3.5	0.61 ± 0.37 ^{cd}	9.99 ± 1.47 ^{abc}	0.08 ± 0.01 ^e
	4.0	0.31 ± 0.05 ^{cd}	8.69 ± 0.17 ^c	0.08 ± 0.01 ^e

DISCUSSION

Rice is one of the essential crops for more than 50% of the world's population; however, drought disasters pose an increasing threat to this crop growth and production. Drought stress significantly inhibits rice germination by restricting the essential uptake of water during the imbibition phase, leading to delayed and uneven seed activation and weaker seedling establishment. It reduced the activation of hydrolytic enzymes such as α -amylase which is responsible for starch degradation and energy release for radicle emergence. As a result, germination percentage (GP) and germination index (GI) decline, while mean germination time increases under drought conditions (Choi et al., 2025). Moreover, drought stress reduces cell division and cell elongation due to loss of turgor pressure, which is essential for cell expansion and meristematic activity in crops such as rice. Reduced mitotic activity in root and shoot apices leads to stunted plant height, decreased leaf area, and impaired root architecture.

This current study used seed nano-priming approach to improve the germination, early growth, and antioxidant enzymes of three Iraqi rice genotypes. This study found that combination of seed priming and silicon dioxide nanoparticles (SiO₂NPs) exhibited higher GP, GI, lower mean germination time (MGT), and high seedling vigor under water stress compared to unprimed indicating enhanced stress resilience. In addition, this study revealed that the optimal concentration of SiO₂NPs that enhanced the studied traits were different on all three Iraqi rice genotypes. Seed priming with nanoparticles (nano-priming) enhances germination performance by improving seed coat permeability and facilitating more efficient water and nutrient uptake during the early imbibition phase. Due to their ultra-small size and high surface area-to-volume ratio, nanoparticles can penetrate seeds coat micro-pores, increase hydration efficiency and stimulate pre-germinative metabolic activity (Ye et al., 2026). This improved water imbibition under drought conditions promotes faster radicle protrusion and uniform seedling establishment of nano-primed seeds. The improvement in germination and early seedling growth in primed SiO₂NPs is consistent with the recent findings of Alwan et al. (2026) that proved that CaSiO₃NPs priming improved germination performance and seedling growth of the similar Iraqi rice genotypes under drought stress. Their study showed that the effects strongly dependent on genotype and nanoparticle concentration. For example, 'Furat' achieved 90% germination and a GI of 9.35 at 0.03% CaSiO₃NPs, whereas 'Yasmine' showed 96.66% germination and a GI of 11.42 at 0.02%. Similarly, seedling

growth was enhanced, with 'Yasmine' exhibiting a four-fold increase in root length at 0.02% CaSiO₃NPs compared with the control (unprimed). These findings support the present observation that Si-based nanoparticles can alleviate the inhibitory effects of drought during the critical germination and seedling establishment stages. The variation among genotypes further suggests that the effectiveness of nano-priming is not uniform and may depend on genotype-specific physiological responses and the concentration of nanoparticles applied.

Nano-priming regulates hydration by activating early metabolic processes prior to radicle emergence. It accelerates the mobilization of seed reserves through enhanced activity of hydrolytic enzymes such as α -amylase, cellulase, and xylanase, which facilitate starch degradation and energy supply for embryo growth. Recent studies demonstrate that silica (SiO₂NPs), zinc oxide (ZnONPs), and Ca-based nanoparticles stimulate enzyme activation, mitochondrial respiration, and antioxidant defense systems during germination under drought stress (Nile et al., 2022; Janah et al., 2025). Ahmad and Hassim (2024) reported that rice seeds primed with SiO₂ nanoparticles under simulated drought conditions showed accelerated cell division in meristematic tissues, improved radicle elongation, and significantly higher germination rates compared to untreated seeds. In addition, Huang et al. (2024) reported that improvement in shoot length and fresh and dry biomass of *Musa* spp. treated with different concentrations of SiO₂NPs.

Drought stress interferes with the chlorophyll synthesis process by affecting the enzyme activity and causing oxidative damage to the chloroplast (Zahra et al., 2023). This study found that Iraqi rice seeds primed with SiO₂NPs and grown under drought stress had significantly higher of the physio-biochemical attributes by increasing the photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids), total soluble sugars, total carbohydrates, total soluble proteins, proline, decrease electrolyte leakage (EL) and upregulating key antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) while exhibiting lower levels of malondialdehyde (MDA). These enhancements indicate that nano-priming alters early metabolic and stress-adaptive pathways that are critical under drought conditions. The increased photosynthesis pigments content suggests improved chloroplast integrity and light-harvesting efficiency, which can mitigate drought-induced photosynthetic inhibition. Elevated soluble sugars and carbohydrates reflect enhanced reserve mobilization and osmotic adjustment essential for sustaining energy supply and cellular turgor under water deficit, while increased soluble proteins likely include stress-responsive enzymes and protective proteins that stabilize cellular structures. Proline accumulation and boosted antioxidant activities further imply a strengthened redox buffering system that alleviates reactive oxygen species (ROS) accumulation and lipid peroxidation under stress. These nano-priming effects are consistent with recent evidence showing that seed nano-priming improves physiological and biochemical resilience in crops by enhancing osmolyte accumulation and antioxidant defense, thereby facilitating better establishment under abiotic stress (e.g., enhanced biochemical performance in nano-primed seedlings reported in rice, tomato, and other species). For examples, previous study found that priming 'FARO44' rice seed with SiO₂ significantly increased carbohydrate and total soluble sugar under drought stress (Ali et al., 2020). Zahedi et al. (2023) found both SiO₂ and SiO₂NPs mitigating the detrimental effects of drought stress on strawberry plants. Both SiO₂ and SiO₂NPs at 50 mg L⁻¹ enhanced morphological traits, including the number of leaves and leaf stalk length, improved osmolytes, such as proline and total soluble sugars, and chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids under drought stress. Under drought stress, plants store soluble solutes, thus enhancing the antioxidant activity to remove the excess ROS, leading to oxidative stress and lipid peroxidation. The increased of carbohydrates, total soluble sugars, and total chlorophyll is essential to osmotic adjustment and to enhance photosynthetic activity when in a drought (Abid et al., 2018). EL is one important parameter in determining the extent of cell membrane injuries due to stressors such as salinity and drought. Under drought stress, plant cells experience severe water deficit, which disrupts membrane integrity and increases the production of ROS such as superoxide radicals (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radicals (•OH). These ROS attack membrane lipids through lipid peroxidation, causing damage to phospholipid bilayers and resulting in increased membrane permeability. Consequently, electrolytes and other essential cellular constituents leak from the cells, leading to higher EL, which is commonly used as an indicator of membrane injury and cellular damage under stress conditions (Pamungkas and Farid, 2022). In the present study, seed nano-priming reduced membrane injury, as evidenced by lower EL in nano-primed rice seeds compared to unprimed seeds under drought stress. This protective effect may be associated with the role of SiO₂NPs in enhancing the plant antioxidant defense system and stabilizing membrane structures. Silicon nanoparticles can stimulate antioxidant enzymes such as SOD, CAT, and

peroxidase (POD), which scavenge excess ROS and minimize oxidative damage to membrane lipids. Reduced ROS accumulation subsequently decreases lipid peroxidation and preserves membrane stability. In addition, SiO₂NPs may enhance the accumulation of osmoprotectants such as proline and soluble sugars, which help maintain cell turgor and membrane integrity during dehydration conditions. Silicon deposition in cell walls and membranes also improves structural rigidity and reduces water loss, thereby protecting cellular components from drought-induced injury. Similarly, Daler et al. (2024) reported that SiO₂NPs improved drought tolerance in grapevines by reducing membrane damage and lipid peroxidation. Their findings found that lower MDA content and EL in nano-treated plants, indicating improved membrane stability under water deficit conditions. Comparable findings were also reported in rice, wheat, and maize, where Si nanoparticle priming enhanced antioxidant activity, reduced oxidative stress, and maintained membrane integrity under drought stress conditions.

Primed seeds with SiO₂NPs elevated CAT, SOD, and APX activities of Iraqi rice genotypes under drought stress conditions. The enhancement of these antioxidant enzymes suggests that silicon dioxide nanoparticles play an important role in strengthening the plant defense system against oxidative stress induced by water deficit. Increased activities of these enzymes in nano-primed seeds indicate a greater capacity to scavenge ROS and protect plant tissues from drought-induced oxidative damage. Furthermore, SiO₂NPs may enhance antioxidant activity through improved nutrient uptake, modulation of stress-responsive genes, and stimulation of signaling pathways associated with stress tolerance. Silicon nanoparticles are also known to improve osmotic adjustment, maintain membrane stability, and reduce lipid peroxidation, leading to lower MDA accumulation in stressed plants. The reduced MDA content indicates decreased membrane lipid degradation and enhanced membrane integrity under drought stress. Previous studies on *Brassica napus* L. demonstrated that seed priming with different concentrations of SiO₂NPs significantly enhanced antioxidant enzyme activities while reducing MDA levels, indicating improved oxidative stress tolerance (Wang et al., 2022). Abd-El-Aty et al. (2024) reported that SiO₂NPs application enhanced ROS-scavenging enzymes, photosynthetic efficiency, water-use efficiency, and agronomic performance of rice under water deficit conditions. These studies suggested that nano-Si improves drought tolerance by activating enzymatic and non-enzymatic antioxidant defense systems, which minimize oxidative damage and sustain physiological processes during stress. In addition, Ahmad and Hassim (2024) found that SiO₂NPs application at 600 mg L⁻¹ significantly promoted seedling growth, root vigor, biomass accumulation, and antioxidant enzyme production in two rice cultivars, UMTR and MR219. Enhanced root development may improve water and nutrient absorption under drought conditions, while elevated antioxidant enzyme activities contribute to better stress adaptation and survival. These findings indicate that SiO₂NPs-mediated seed nano-priming is an effective strategy for enhancing drought tolerance in rice through the activation of antioxidant defense mechanisms and maintenance of cellular stability.

CONCLUSIONS

Seed nano-priming with silicon dioxide nanoparticles (SiO₂NPs) improves the germination, early seedling growth, physio-biochemicals, and defense system of three Iraqi rice genotypes under drought stress. Enhancement of these parameters indicates the efficiency of seed nano-priming as an easy, viable, and sustainable approach that can be implemented to help the establishment of improving rice production in drought-affected areas in Iraq.

Author contribution

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

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