

Green synthesis of silica nanoparticles using chia seeds boosts rice germination and physiological responses

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Title page**Green synthesis of silica nanoparticles using chia seeds boosts rice germination and physiological responses****Shaimaa Mohamed Nagy Tourky^{1*}, Amr Mohamed Abdelghany^{2, 3}, Eman Mohammed Elghareeb¹**¹Botany Department, Faculty of Science, Mansoura University, Mansoura, 35516, Egypt²Spectroscopy Department, Physics Research Institute, National Research Centre, 33 Elbehouth St., Giza, 12311, Egypt³Basic and Applied Science Department, Horus University, International Coastal Rd, New Damietta, Kafr Saad, 34511, Damietta Governorate, Egyptshaimaa_tourky@mans.edu.eg; a.m_abdelghany@yahoo.com;
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ORCIDDr Shaimaa M. N. Tourky, <https://orcid.org/0000-0003-1510-7540>Dr Amr M. Abdelghany, <https://orcid.org/0000-0003-0119-9253>Dr Eman M. Elghareeb, <https://orcid.org/0000-0003-4524-8272>**Main Conclusion**

This article highlights the impact of various methods for synthesizing silicon nanoparticles, whether chemically or biologically. It also discusses using chia seed extract to improve the germination of rice seeds as a sustainable approach to improving the quality of rice seedlings.

Abstract

This study aimed to synthesize silicon nanoparticles (SiNPs) using two different approaches: chemical (Chem-SiNPs) and biological (Bio-SiNPs) methods, utilizing chia (*Salvia hispanica* L.) seed extract as a capping agent and bulk silica (SiO₂) as a precursor (Bulk-Si). Both routes were employed to compare the conventional chemical method with an eco-friendly, plant-based approach, enabling a comprehensive evaluation of how the fabrication technique influences the physicochemical properties and biological effectiveness of the resulting SiNPs. The synthesized SiNPs were characterized using UV- spectrophotometer, TEM, FTIR, XRD, and zeta potential analysis. Also, the impact of nano-priming with two concentrations (30 and 100 ppm) of Bulk-Si, Chem-, and Bio-SiNPs on the germination and key physiological mechanisms of *Oryza sativa* L. var. Sakha 108 seedlings were investigated. Characterization results showed that Bio-SiNPs surpassed Chem-SiNPs, exhibiting greater stability and a higher surface area, likely due to the available bioactive phytochemicals in the chia extract. This phytochemical capping layer enhances the stability and bioactivity of Bio-SiNPs, thereby activating key metabolic and physiological responses in rice seedlings. Bio-SiNPs at 100 ppm exhibited the highest improvement in germination rate (97.3%), germination index (65.3%), germination rate index (130.73), seedling vigour index (18.68), seedling biomass (0.033 g), and water uptake (31.53%) compared to Chem-SiNPs and Bulk-Si treatments. Likewise, Bio-SiNPs at 100 ppm concentration significantly increased total soluble sugars, α -amylase, radicle dehydrogenase activities, and Si content in rice seedlings. All SiNPs treatments induced significant changes in proline, malondialdehyde, hydrogen peroxide accumulation, glutathione level, and the antioxidant enzyme activities compared to the bulk material. This research reveals substantial variations in many critical physiological and biochemical parameters, which explain the varying responses of rice seedlings to various nano-synthesis protocols. It illustrates the efficacy of using chia seed extract as a green capping agent for nano priming as a sustainable approach to enhance germination and boost future rice productivity.

Keywords: Rice, chia, silicon nanoparticles, green synthesis, germination, antioxidants

Author Contributions

SMN and EME contributed to the study's conception and design. SMNT, EME, and AMA performed material preparation, data collection, and analysis. SMNT, EME, and AMA wrote the first draft of the manuscript, and all authors commented on previous versions. All authors read and approved the final manuscript.

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Declarations**Competing interests**

The authors have no relevant financial or non-financial interests to disclose.

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Introduction

In recent decades, climate change and an expanding population have posed unprecedented challenges to the global agriculture sector, further exacerbated by rising demands for food and agricultural output [1]. As a result, sustainable agrarian practices prioritizing the judicious and effective use of resources have gained prominence. Agriculture

is fundamental in supporting the three pillars of sustainability: environmental, social, and economic [2]. Rice, a staple crop consumed by over 95 countries, is crucial to global food security. As the global population is expected to surpass 10 billion by 2050, the demand for rice is expected to increase considerably [3]. The application of cost-effective physiological practices, such as seed priming with diverse priming agents, has been shown to enhance seed germination and seedling vigour, even in high-yielding rice varieties. Such a technique enables plants to counteract multiple abiotic stresses in the field conditions [4].

Nanotechnology has contributed significantly to green chemistry, with nanoscale materials increasingly utilized across diverse fields owing to their unique properties, including enhanced biological activity, improved targeted therapy, and diminished toxicity [5]. Nanoparticles (NPs), characterized by their ultrafine size (1–100 nm) and high surface-to-volume ratio, have distinctive physical and chemical features. To ensure sustainable agricultural application, NPs must be cost-effective, environmentally safe, biocompatible, and non-toxic, which are achieved through the synthesis of biogenic NPs [6,7]. Therefore, green nanotechnology provides a direct pathway to sustainable agriculture strategy by reducing hazardous chemical inputs and environmental contamination, and by improving soil health, resource use efficiency, and crop productivity through safer, bio-based NPs [8,9]. Among the several approaches, biological synthesis is particularly efficient for fabricating NPs using plant-derived materials such as algae, leaf extracts, or flowers. These biological methods offer significant advantages over physical and chemical synthesis, as they are cost-effective, eco-friendly, and do not require toxic chemicals [10,11]. Furthermore, the phytochemicals present in plant materials act as natural reducing and capping agents, forming a biocompatible halo around the nanoparticles that enhances their stability and biological efficacy, making them a better alternative for long-term agricultural sustainability [12].

Non-metallic nanoparticles, widely used as Nutri-priming agents, have significantly improved agricultural innovation by leveraging their physiochemical properties [13,14]. These properties trigger key physiological and metabolic processes and enhance abiotic stress tolerance in plants, thereby increasing agricultural productivity [15]. Various studies demonstrated that nano-priming application improves seed germination and seedling growth and positively mediates plant interactions with the environment at multiple levels, making it a promising tool in sustainable agriculture [16].

Silicon (Si) is the second most prevalent element in Earth's crust and soil [17]. While not a requisite for most plants, Si positively influences plant growth and productivity [18]. Silica nanoparticles (SiNPs) have emerged as an innovative source of Si, which confers plant

growth and stress resilience [19]. Various studies have shown the beneficial effects of SiNPs on seed germination, seedling vigour, plant growth, photosynthesis, uptake of essential nutrients, synthesis of primary cellular metabolites, antioxidant metabolism, and overall productivity across numerous plant species [20,21,22,23,24]. The efficacy of SiNPs depends on their morphology, dimensions, and surface features, which affect plants' potential application and responses [25]. Recently, cost-effective green synthesis of SiNPs has become accessible to small-scale farmers, fostering wider adoption and economic advantages [5]. The green synthesis of SiNPs using agricultural wastes such as rice straw and husk [23,26], and maize stalk [27], shows significant advantages over physical and chemical synthesis approaches. SiNPs are biodegradable and eco-friendly, making them ideal candidates for long-term agricultural applications [28].

Among the versatile plant-based sources, chia seed extract offers a promising, eco-friendly, and sustainable approach for the green synthesis of metallic nanoparticles with potential applications in agriculture [29, 30]. Chia (*Salvia hispanica* L.) is an annual herbaceous plant from the Lamiaceae family. It is unique in its phytochemical profile, including oil, protein, fibre, minerals, tocopherols, phytosterols, carotenoids, and phenolic compounds, all of which can improve human health [31]. It can be consumed as oilseed, flour, whole seeds, or mucilage. These bioactive compounds can function as powerful agents for the synthesis of nanoparticles [32]. Despite the growing interest in green synthesis of SiNPs, there remains a research gap regarding the use of innovative, waste-free sources and the direct comparison of their efficacy with chemically synthesized counterparts. Accordingly, this study aimed to directly address this gap by (1) developing a pioneer eco-friendly approach for synthesizing SiNPs using chia seed extract; (2) presenting a comprehensive, side-by-side comparison of these novel Bio-SiNPs with their chemically synthesized analogs and bulk material; (3) illustrating how the synthesis method affects the physicochemical characteristic and biological performance of SiNPs; and (4) evaluating their dose-dependent effects on the rice germination efficiency and key physiological and metabolic processes including carbohydrate metabolism, α -amylase and dehydrogenase activities, water content, Si uptake, and oxidative stress responses (MDA, H_2O_2 , proline, antioxidant enzymes). Our findings thus contribute to the development of sustainable nano-priming agents to enhance rice productivity and resistance against climate change.

Materials and methods

Chemicals and plant materials

Silica (SiO_2) was obtained from local chemical suppliers in Egypt, while chia (*Salvia Hispanica*) seeds were purchased from a local market in Mansoura City, Egypt. Seeds of rice

cultivar Sakha 108 were procured from the Research and Training Centre Sakha, Kafer El-Sheikh, Egypt. All the glassware used in the experiment underwent thorough cleansing with detergent, followed by at least two rinses with sterile distilled water. All aqueous solutions utilized in the synthesis process were prepared with deionized water.

Preparation and phytochemical characterization of aqueous chia seed extract

About 10 g of clean chia seeds were ground into a fine powder using a mortar and pestle. About 100 mL of boiled deionized water was added to the fine seed powder and stirred continuously for 30 min. After cooling, the milky-colored extract was filtered using Whatman No.1 filter paper to eliminate undesired residue before being stored at 4 °C as a reducing and stabilizing agent [31]. Total phenolics and flavonoids of chia seed extract were analyzed according to the methods of [33,34].

Chemical synthesis of silica nanoparticles (Chem-SiNPs)

Silica (SiO_2) nanomaterials were synthesized using the sol-gel method. One mole of tetraethoxysilane (TEOS) was initially dissolved in 10 mL of ethanol and 35 mL of deionized water. After stirring for 10 min, 1 mole of HCl was added dropwise to the mixture and then stirred magnetically at 60 °C for 50 min until a white, transparent homogeneous solution formed.

For the gelation process, the prepared solution was kept for about 2 h at room temperature. The suspension was then dried in an oven for 6 h at 110 °C to obtain crystallized SiO_2 particles, which were then ball-milled to form white SiO_2 powder. The obtained powder was calcined in a furnace at 500 °C for 1 h for further characterization (Fig. 1).

Biological synthesis of silica nanoparticles (Bio-SiNPs)

Silica nanoparticles were synthesized via a hydrothermal method using tetraethyl orthosilicate (TEOS) as the silicon precursor and chia seed extract as the bio-reducing and capping agent. The synthesis protocol is illustrated in Figure 1. The synthesis was conducted in a 100 mL Teflon-lined stainless-steel autoclave (high-pressure reactor). About 22.329 mL of TEOS was mixed with 376.71 mL of the prepared chia seed extract with a weight ratio of approximately 1:17. The autoclave was sealed and heated to 200°C for 12 h. These elevated temperatures and pressures facilitate the hydrolysis of TEOS and subsequent condensation reactions that form a siloxane network (Si-O-Si), leading to the fabrication of SiNPs. The organic compounds present in the chia seed extract act as capping agents, stabilizing SiNPs, and preventing their agglomeration. After the hydrothermal reaction, the autoclave can cool down to room temperature. The resulting milky-white suspension containing the Bio-SiNPs is collected and centrifuged (10,000 rpm for 10 min) to separate the nanoparticles from the

reaction mixture. The supernatant was discarded, and the pellet was re-washed in deionized water and centrifuged. This process was repeated 3 times to remove any unreacted precursors or byproducts. Finally, the obtained Bio-SiNPs were dried overnight at 80 °C for 24 h and stored under room conditions for further characterization and applications [35].

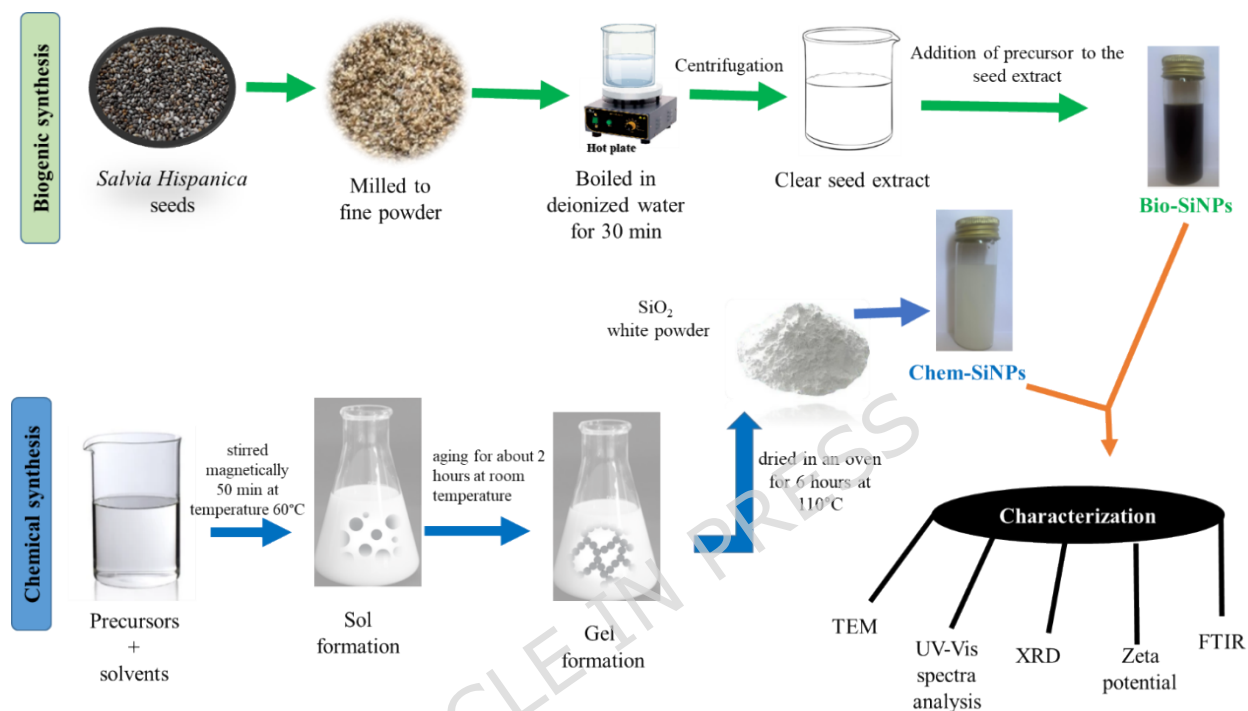


Fig. 1

Characterization of chemically and biologically synthesized SiNPs

Various techniques have been employed to characterize synthesized SiNPs. The optical properties of both Chem- and Bio-SiNPs were investigated using UV-Vis spectroscopy (Jasco V570, Japan). The size and morphology of both Chem- and Bio-SiNPs were measured through Transmission Electron Microscopy (JEOL JEM-2100, U.S.A) using a carbon-coated grid (Type G 200, 3.05 µm diameter, TAAP, U.S.A) following procedures adopted by Wang et al. [36]. The X-ray diffraction (XRD, Bruker Co., D8 Discover, Germany) using Cu Kα (1.54Å) radiation with the X-ray generator operating at 40 kV and 40 mA was employed to determine the crystalline structure of the obtained powder of SiNPs. The crystallite size was calculated using XRD data based on Scherrer's equation:

$$D = \frac{K \lambda}{\beta \cos \theta}$$

Where: D is the mean crystallite size, K is the shape factor usually used as 0.9 for spherical particles, λ is the wavelength of X-ray used 1.54 Å, β is the full width at half maximum (FWHM) of the diffraction peak (in radians), and θ is the Bragg diffraction angle. The stability of synthesized SiNPs was detected utilizing a Zeta Potential Analyzer (Zetasizer ver. 7.01; Malvern Instruments, Westborough, MA, USA). Specifically, 5 mg of SiNPs were combined with 5 mL of ultrapure water and stirred at room temperature for 30 min. Zeta potential was measured using an electron microscope unit at Mansoura University, Egypt, at a 90° detection angle. Fourier Transform Infra-Red (FTIR) (BRUKER ALPHA II, Germany) in the spectral range 400–4000 cm^{-1} and spectral resolution 2 cm^{-1} was carried out for the determination of the functional groups responsible for the biosynthesis and stabilization of silica nanoparticles.

Preparation of priming solutions and seed priming

Seed priming was conducted using two freshly-prepared solutions of Chem- and Bio-synthesized SiNPs at concentrations of 30 and 100 ppm, designated Chem-SiNPs₃₀, Chem-SiNPs₁₀₀, Bio-SiNPs₃₀, and Bio-SiNPs₁₀₀. The SiNPs solutions were dispersed in deionized water via ultrasonic vibration (100 W, 40 kHz) for 30 min. Similarly, SiO₂ at 30 and 100 ppm, labelled Bulk-Si₃₀ and Bulk-Si₁₀₀, were prepared in deionized water and stored in a dark bottle. These nano- and bulk materials concentrations (30 and 100 ppm) were chosen based on the preliminary experiment results in which 3 concentrations (30, 60, and 100 ppm) were evaluated. The results showed that 30 ppm represented the lowest effective concentration, whereas 100 ppm provided the highest enhancements in seedlings' germination and growth traits. Therefore, selection of these two concentrations allowed us to show biological effects of clear dose-dependent response of the SiNPs treatments on the seed priming (Supplementary Table S1).

Seed germination assay

Homogenized and healthy rice seeds, cv. Sakha 108 with Sakha 101/HR5824-B-3-2-1 pedigree [37] were sterilized with 2% (w/v) NaClO solution for 20 min and rinsed three times with distilled water. Seeds were divided into 7 sets; the first set was primed in deionized water (hydro priming control; HP), while the second to the seventh sets were primed in Bulk-Si₃₀, Bulk-Si₁₀₀, Chem-SiNPs₃₀, Chem-SiNPs₁₀₀, Bio-SiNPs₃₀, and Bio-SiNPs₁₀₀ solutions, respectively. The seed-to-priming solution ratio was 1:5 (w/v). Priming was carried out at 28 °C and 65% relative humidity in the dark for 24 h with aeration by an air pump to prevent anaerobic respiration. Then, primed seeds were allocated into 21 germination boxes (22×17×9 cm) lined with sterile Whatman No.1 filter paper saturated with fresh tap water (0.55 mS/cm). Three plastic boxes were arranged for each treatment, containing 15 primed

seeds per box, corresponding to the priming treatments. The germination boxes were later moved to a digital incubator at 27 ± 30 °C in the dark and maintained under ideal conditions for germination. After 6 days of germination, nine seedlings were randomly assigned and utilized to evaluate germination kinetics and seedling growth characteristics. Another set of nine seedlings was then dried to estimate silicon content and carbohydrate fractions (total soluble sugars and starch). The remaining fresh seedlings were equally split into three sets, and each of these sets was pooled to form a composite biological replicate for physiological and biochemical investigations, including enzyme activities (α -amylase, dehydrogenase, and antioxidant enzymes), antioxidant compounds (GSH), osmoprotectants (proline), and stress biomarkers (H_2O_2 and malondialdehyde).

Germination indices

Germination data were evaluated via surveillance of the following parameters:

Parameters	Equations
Germination potential (GP)	The number of normally germinated seeds on the 6th day / the total number of tested seeds $\times 100$
Germination rate (GR)	The number of normally germinated seeds / the total number of tested seeds $\times 100$
Germination index (GI)	$\sum N_i/T$, where N_i is the number of seeds that emerged on the i^{th} day, and T is the day counted [38]. $G_1/1 + G_2/2 + \dots + G_x/x$,
Germination rate index (GRI)	where, G_1 = germination rate at first day after sowing; G_2 = germination percentage at second day after sowing; G_x = germination percentage at x day after sowing [39].
Seedling vigour index (SVI)	Seedling length $\times$ Germination percentage / 100 [40].

Seedling growth

Whole seedling length and fresh and dry weight of the 6-day-old seedlings were recorded. The seedling's dry weight was determined following a 24 h drying period at 60 °C. After drying, the samples were weighed, finely ground, and stored until further analysis. Some fresh seedlings were frozen directly in liquid nitrogen and stored at -80 °C for the biochemical assays.

Water uptake

Water uptake (WU) by rice seeds during imbibition was assessed in triplicate, following the method of Bhardwaj et al. [41]. Weighed seeds were placed in petri dishes lined with water or different Si treatments saturated cotton, and incubated at 28 °C. After 12 and 24 h intervals, all germinated seedlings were removed, blotted dry, and weighed. Weight changes resulting from imbibition were quantified as the volume of water absorbed per unit of seed dry weight and calculated using the formula provided by

$$\text{WU (\%)} = ((\text{fresh weight of seed} - \text{dry weight of seed}) / \text{Dry weight of seed}) \times 100$$

Silicon (Si) content

Approximately 0.1 g of dried seedlings were digested in 10 mL HNO₃: H₂O₂ (1:1 v/v) at a temperature below 120 °C. After cooling, the liquid was diluted to a final volume of 10 mL with deionized water, and filtered. Si concentration was determined with SpectroCirosCCD ICP-OES (Spectro Analytical Instruments, Kleve, Germany) following ionic analysis [42], and the results were expressed as parts per million (ppm) on a dry weight basis.

Biochemical analyses

α-Amylase activity assay

To determine α-amylase activity, 0.3 g of frozen tissue was homogenized in a phosphate buffer (20 mM, pH 6.9). Following centrifugation at 12,000 xg for 20 min, the supernatant was collected. The activity was assessed using the 3,5-dinitrosalicylic acid (DNS) method, quantifying maltose released after starch hydrolysis [43]. The reaction mixture's absorption was measured at 560 nm, and the activity was calculated and expressed as μg maltose min⁻¹ g⁻¹ FWT.

Radicle's dehydrogenase activity assay

Radicle dehydrogenase activity, an indicator of vitality, was assessed using the triphenyl tetrazolium chloride (TTC) test [44]. Freshly germinated young white radicles were blotted dry with tissue paper before determining their fresh mass. A total of 0.5 g of radicles was incubated in a solution containing 5 mL of 0.4% TTC and 5 mL of 0.06 M phosphate buffer (pH 7.0) at 37 °C for 3 h. The resulting extract was transferred to the test tube, and the volume was adjusted to 10 mL with ethyl acetate. Absorbance was recorded at 485 nm using

the UV-Vis spectrophotometer (Shimadzu model UV-160A). Dehydrogenase activity was expressed as $\mu\text{g TTF g}^{-1} \text{ h}^{-1} \text{ FW}$, representing the rate of TTF reduction.

Estimation of total soluble sugars (TSS) and starch

TSS were quantified using the method established by Yemm and Willis [45]. The intensity of the resulting blue green color was measured at 630 nm relative to a reagent blank. TSS were calculated from a standard curve using glucose as a standard. After repeated extractions of sugars with 80% ethanol, starch was estimated in the residue according to the Thayumanavan and Sadasivam [46] method. The developed color was recorded at 630 nm, and starch content was then calculated using a standard glucose curve.

Measurement of proline, hydrogen peroxide (H_2O_2), and lipid peroxidation

For proline analysis, 0.1 g of frozen seedlings was thoroughly mixed with 5 mL of 3% aqueous sulphosalicylic acid. The solution was centrifuged at 10,000 $\times g$ for 15 min, and 1 mL was transferred into a test tube containing 1 mL of acidic ninhydrin and 1 mL of glacial acetic acid. The residual steps were completed according to the method of Bates et al. [47]. The free proline concentration was quantified using a standard curve at 520 nm using a Shimadzu spectrophotometer (model UV-160A) and reported as $\mu\text{mol g}^{-1} \text{ FWT}$. H_2O_2 was quantified spectrophotometrically following its reaction with KI, as reported by Alexieva et al. [48]. The quantity of H_2O_2 was approximated using a standard curve generated from known H_2O_2 values. The thiobarbituric acid (TBA) method quantified malonaldehyde (MDA), a marker of lipid peroxidation [49]. MDA concentration was expressed as $\mu\text{mol g}^{-1} \text{ FWT}$ by subtracting A_{600} readings from A_{532} readings using an extension coefficient of $155 \times 10^{-3} \mu\text{M}^{-1} \text{ cm}^{-1}$.

Determination of antioxidant enzymes and glutathione (GSH)

The antioxidant enzymes were extracted as adopted by [50]. Catalase (CAT) activity was measured by adding 0.5 mL enzyme extract to 1 mL phosphate buffer (pH 7, 0.01 M), 0.40 mL H_2O , and 0.50 mL H_2O_2 (0.2 M) at 25 °C for 1 min, as described by Sinha [51]. The reaction was halted by adding 2 mL of acid reagent (a 5% dichromate/acetic acid mixture in a 1:3 v/v ratio) and heating for 10 min. The absorbance was measured at 610 nm and reported in $\text{mmol H}_2\text{O}_2 \text{ min}^{-1} \text{ g}^{-1} \text{ FWT}$. Peroxidase (POD) and polyphenol oxidase (PPO) activities were measured spectrophotometrically at 420 nm according to Devi's [52] method. One POD or PPO unit was expressed as $\text{U min}^{-1} \text{ g}^{-1} \text{ FWT}$. Following the manufacturer's instructions, glutathione reductase (GR) was measured using a GR test kit purchased from Biodiagonistic (HP 25, Giza, Egypt). Glutathione (GSH) was extracted by homogenizing 0.5 g of frozen

seedling in 5 mL of cold potassium phosphate buffer (50 mM potassium phosphate, pH 7.5, 1 mM EDTA). The homogenate was subsequently centrifuged at 4000 xg and 4 °C for 15 min, and utilized for GSH assessment using the Biodiagnostic kit (GR 2511) per the manufacturer's instructions.

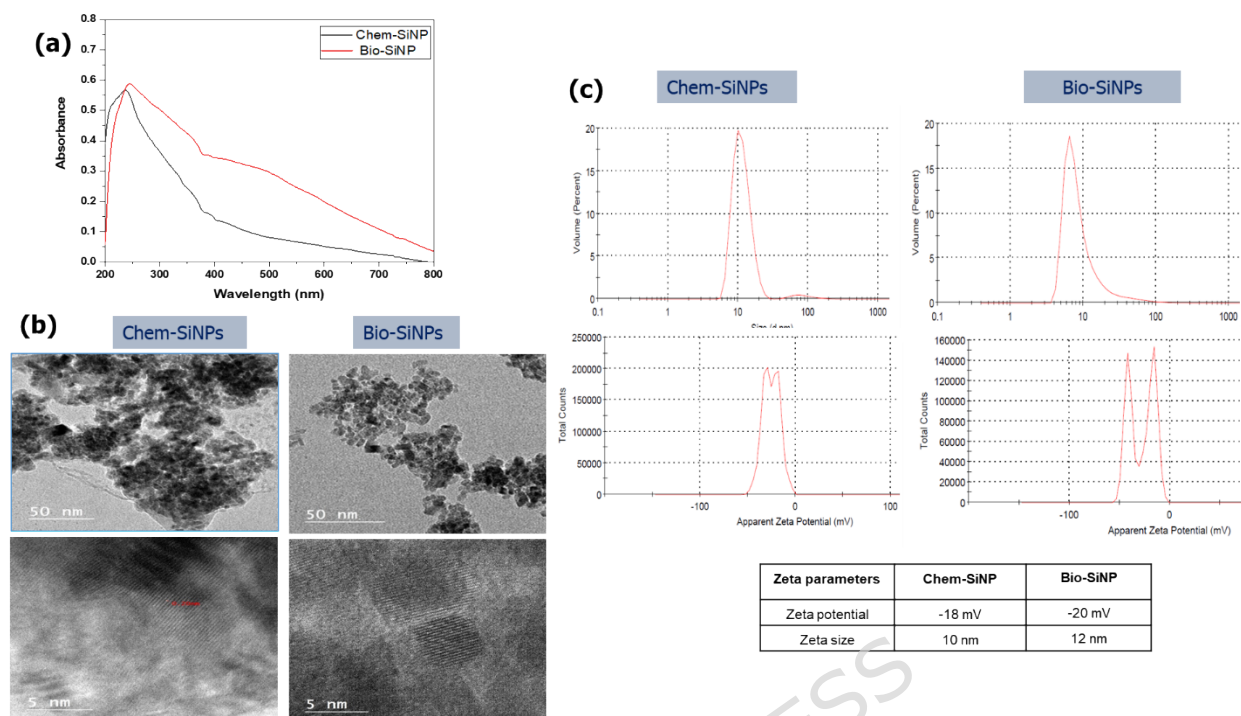
Statistical analysis

The experiment was performed using a completely randomized design (CRD) with six Si treatments along with a control one. Each treatment is replicated three times. Each replicate consists of 15 primed seeds and is used for data collection, including germination indices, seedling growth, Si content, and biochemical investigations. For the statistical analyses, data was subjected to one-way analysis of variance (ANOVA) to assess the effects of different SiNPs treatments using *XL-STAT* software (2016.02.28451). Fisher's Least Significant Difference (LSD) test was used to compare the means of different treatments at a significant level of $p \leq 0.05$. All data are presented as the averages of three replicates $\pm$ SE. The Graphs shown were created using the GraphPad Prism version 9.0.2 (GraphPad Software, Inc., LA Jolla, CA, USA). The heatmap and correlation matrix between the measured germination indices, seedling traits, and the physiochemical metrics of SiNPs-primed seedlings were constructed using the Metaboanalyst 5.0 analysis software.

Experimental Results

Phytochemical characteristics of chia seed extract and characterization of nano solutions

The content of total phenols and flavonoids in the aqueous extract of chia seeds was 9.2 and 12 mg/g, respectively (Supplementary Table S2). Spectra analysis of both Chem- and Bio-SiNPs was performed in the 200–800 nm (Fig. 2a). The Chem-SiNPs exhibited an absorption peak at 237 nm, while Bio-SiNPs showed it at 245 nm. The TEM study indicated that the Bio-SiNPs were monodispersed and spherical with an average diameter of 10-12 nm (Fig. 2b). On the other hand, Chem-SiNPs showed a multi-dispersed spherical morphology with a smaller average diameter range of 6 to 10 nm. The surface charge of the freshly prepared Bio- and Chem-SiNPs was determined immediately using the zeta potential technique. The Bio-SiNPs displayed a zeta potential of -20, while Chem-SiNPs showed a slightly less negative value of -18 mV. Moreover, the mean size of particles was around 12 nm and 10 nm for Bio- and Chem-SiNPs, respectively (Fig. 2c).

**Fig. 2**

The crystalline structure of both Bio- and Chem-synthesized SiNPs in Figure 3a was measured by XRD analysis. For both Bio- and Chem-SiNPs, a small broad peak at around $2\theta = 22^\circ$ in the XRD spectrum of SiNPs was observed, which is attributed to the (010) plane of a specific crystalline phase of silica (tridymite). Following Scherrer's equation, the crystallite size was calculated from XRD data using the most intense peak at about 24° . The estimated size was 8 nm, which agrees with that previously observed from the TEM image. The FTIR spectra of the chia seed extract and the synthesized SiNPs are presented in Figures 3b and 3c. The extract spectrum exhibited a broad band at 3295 cm^{-1} corresponding to O-H stretching vibrations of cell-wall polysaccharides, while a small band at 3010 cm^{-1} indicates =C-H stretching from unsaturated fatty acids present in chia seeds. Peaks observed at 2963 and 2853 cm^{-1} are assigned asymmetric and symmetric C-H stretching of $-\text{CH}_3/-\text{CH}_2-$ groups typical of fatty acids. The prominent sharp band at 1742 cm^{-1} reflects ester C=O stretching of lipids, which are the main storage compounds in chia seeds. The 1654 cm^{-1} band corresponds to protein amide I and water bending. In addition, the 1460 cm^{-1} band indicates C-H bending vibrations in methylene groups, while the 1160 cm^{-1} band arises from C-O-C vibrations in polysaccharides and esters. Peaks at 720 cm^{-1} and $602-532\text{ cm}^{-1}$ indicate CH_2 rocking of long-chain hydrocarbons and out-of-plane bending =C-H groups. FTIR spectral data of the studied silica (SiO_2) (Fig. 3c) showed significant similarities to the chia seed extract spectrum. Notably, the peaks corresponding to O-H ($\sim 3300\text{ cm}^{-1}$), C-H ($\sim 2920\text{ cm}^{-1}$),

C=O/C=C ($\sim 1630\text{ cm}^{-1}$), and C-O ($\sim 1100\text{ cm}^{-1}$) were also present in the SiNPs spectrum, indicating that proteins, polyphenols, and/or polysaccharides from the extract are capping the NPs. Most significantly, the most intense peak at 1100 cm^{-1} is characteristic of the asymmetric stretching vibration of Si-O-Si bonds, showing formation of the silica network.

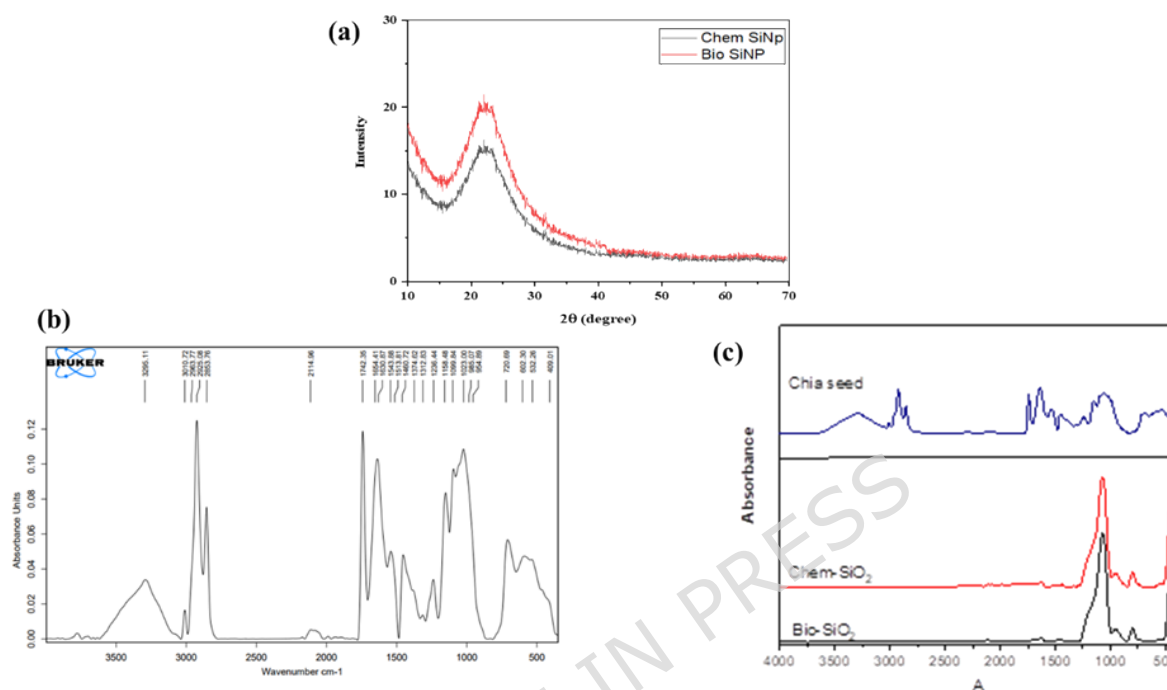


Fig. 3

Effect of Si nano-priming on seed germination indices

To directly evaluate the effectiveness of nano-priming with SiNPs in promoting early rice seed emergence, seed germination traits, including GR, GI, GRI, and SVI were measured as primary indicators of germination. Results in Table 1 showed that nano-silica treatments significantly ($p \leq 0.05$) enhanced seed germination and seedling vigour indices compared to Bulk-Si and control treatments. Among them, Bio-SiNPs₁₀₀ exhibited the highest GR (97.33%), GI (65.37%), GRI (130.73), and SVI (18.68), surpassing other nano-treatments. However, GP wasn't significantly affected by different Si treatments. All other Si treatments exhibited intermediate values, with Bulk-Si₃₀ showing the lowest GR (88%), GI (59.71%), GRI (119.41), and SVI (12.11). Bio-SiNPs₃₀ and Chem-SiNPs₁₀₀ followed a similar pattern with notable improvements of 94.67%, 64.54%, 127.08%, and 16.24% in GR, GI, GRI, and SVI, respectively, over the control. To evaluate the effect of priming agents on seed imbibition and initiating metabolism during the early stages of germination, seed water uptake was monitored at 12 and 24 h of imbibition under different Si treatments. Significant variations

at $p \leq 0.05$ were observed among Si treatments, with higher concentrations of 100 ppm of all Si treatments exhibiting higher water uptake than 30 ppm. Notably, nano-silica treatments enhanced water uptake relative to Bulk-Si and hydro-primed peers. At both time points (at 12 and 24 h), Bio-SiNPs₁₀₀ demonstrated the highest water uptake (30.82 and 32.25%), while Bulk-Si₃₀ exhibited the lowest values (25.28 and 27.91%), respectively.

Table 1. Effect of silica and silica nanoparticles priming at 30 and 100 ppm concentrations on rice seedlings' germination criteria and water uptake. Data are presented as means $\pm$ SE. Significant differences in the mean value of each treatment are represented by different lowercase letter (s) based on Fisher's test ($p \leq 0.05$, $n = 3$). HP: hydro priming, Bulk-Si: silica, Bio-SiNPs: biologically synthesized silica nanoparticles, Chem-SiNPs: chemically synthesized silica nanoparticles.

Treatments	Germination percentage (GP) (%)	Germination rate (GR) (%)	Germination Index (GI)	Germination rate index (GRI)	Seedling vigour index (SVI)	Water uptake (WU) (%)	
						12 h	24 h
HP	73.33 ^{ab} $\pm$ 0.66	88.67 ^{cd} $\pm$ 0.67	55.04 ^c $\pm$ 0.19	110.09 ^c $\pm$ 0.3	10.44 ^e $\pm$ 0.34	25.31 ^c $\pm$ 0.26	28.39 ^{bc} $\pm$ 0.65
Bulk-Si ₃₀	71.33 ^b $\pm$ 4.05	88.00 ^d $\pm$ 1.15	59.71 ^b $\pm$ 1.66	119.41 ^b $\pm$ 3.1	12.11 ^{de} $\pm$ 0.1	25.28 ^c $\pm$ 0.68	27.91 ^c $\pm$ 0.60
Bulk-Si ₁₀₀	75.33 ^{ab} $\pm$ 0.66	90.00 ^{cd} $\pm$ 1.15	60.96 ^b $\pm$ 1.51	121.90 ^b $\pm$ 3.1	13.93 ^{cd} $\pm$ 0.7	26.85 ^{bc} $\pm$ 0.81	30.44 ^{abc} $\pm$ 0.18
Chem-SiNPs ₃₀	74.66 ^{ab} $\pm$ 1.33	92.00 ^{bc} $\pm$ 1.15	61.76 ^{ab} $\pm$ 0.9	123.52 ^{ab} $\pm$ 1.9	13.72 ^{cd} $\pm$ 0.6	28.32 ^b $\pm$ 0.20	30.02 ^{abc} $\pm$ 0.57
Chem-SiNPs ₁₀	76.00 ^{ab} $\pm$ 1.15	94.67 ^{ab} $\pm$ 0.67	63.19 ^{ab} $\pm$ 0.6	126.38 ^{ab} $\pm$ 1.2	15.72 ^{bc} $\pm$ 0.5	28.69 ^{ab} $\pm$ 0.10	31.17 ^{ab} $\pm$ 0.43
Bio-SiNPs ₃₀	76.00 ^{ab} $\pm$ 1.15	94.67 ^{ab} $\pm$ 0.67	63.89 ^{ab} $\pm$ 0.8	127.78 ^{ab} $\pm$ 1.6	16.76 ^{ab} $\pm$ 0.4	28.37 ^b $\pm$ 0.80	31.52 ^{ab} $\pm$ 1.06
Bio-SiNPs ₁₀₀	79.33 ^a $\pm$ 0.66	97.33 ^a $\pm$ 0.67	65.37 ^a $\pm$ 0.82	130.73 ^a $\pm$ 1.6	18.68 ^a $\pm$ 0.61	30.82 ^a $\pm$ 0.41	32.25 ^a $\pm$ 1.16
Significance at	0.143	< 0.0001	0.0001	0.0001	< 0.0001	0.003	< 0.0001

$p \leq 0.05$

Effect of Si nano-priming on rice seedlings' growth

In this present study, seedling growth and biomass were measured to show how SiNPs application influences early vigour and overall seedling establishment. Figure 4 showed the significant ($p \leq 0.05$) positive effects of SiNPs application on growth traits of rice seedlings compared to Bulk-Si and hydro-primed peers. Seedling length, fresh, and dry weight showed notable improvement across Si treatments, with Bio-SiNPs₁₀₀ resulting in the highest

seedling length (19.20 cm), fresh weight (0.138 g), and dry weight (0.033 g), outperforming Bulk-Si and Chem-SiNPs, which showed intermediate values compared to control peers.

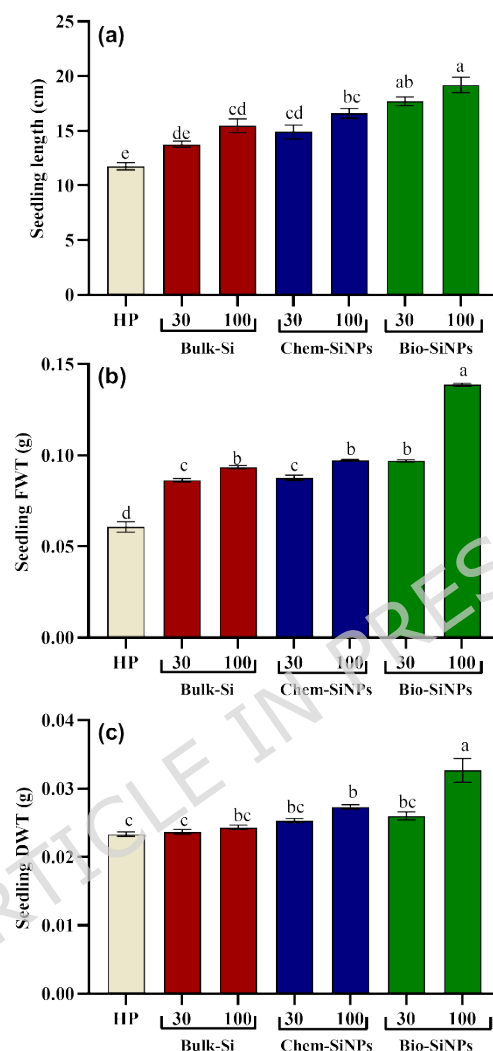


Fig. 4

Effect of Si nano-priming on Si accumulation in rice seedlings

Silicon content was quantified to determine the impacts of SiNPs treatments on Si absorption and bioavailability in rice seedlings. As illustrated in Figure 5, application of SiNPs significantly ($p \leq 0.05$) increased Si content in rice seedlings compared to Bulk-Si and control treatments. Bulk-Si treatments, particularly at 30 ppm, showed the lowest Si content (2.85 ppm), while SiNPs at 100 ppm had considerably higher Si content than those at 30 ppm. Notably, Bio-SiNPs treatments outperformed Chem-SiNPs analogues, with 100 ppm Bio-SiNPs yielding 25.50 ppm Si content in seedlings, compared to 11.20 ppm with Chem-SiNPs.

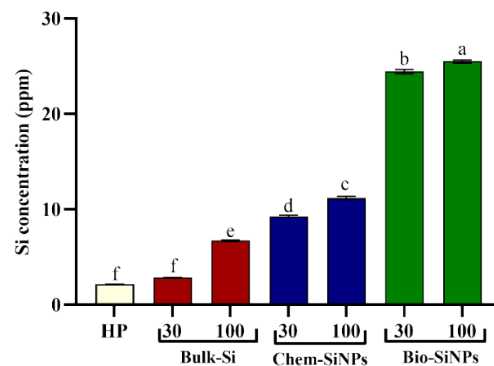


Fig. 5

Effect of Si nano-priming on α -amylase and dehydrogenase enzyme activities of rice seedlings

To evaluate key enzymatic processes involved in starch mobilization and radicle metabolism during germination, affecting the seed's metabolic activity, α -amylase and radicle dehydrogenase activities were assessed. Nano-priming with Si-based agents significantly ($p \leq 0.05$) enhanced α -amylase activity in rice seedlings compared to Bulk-Si and hydro-primed peers (Fig. 6a). Bulk-Si at both 30 and 100 ppm led to a relative rise in α -amylase activity, with the 100 ppm concentration exhibiting a higher activity (11.37 mg maltose g⁻¹ FWT min⁻¹) than the 30 ppm treatment (10 mg maltose g⁻¹ FWT min⁻¹). Bio-SiNPs seedlings demonstrated the highest α -amylase activity (19.24 mg g⁻¹ FWT min⁻¹), surpassing Chem-SiNPs peers (13.47 mg g⁻¹ FWT min⁻¹), particularly at 100 ppm concentration. Regarding dehydrogenase activity, all Si treatments significantly enhanced dehydrogenase activity compared to the control treatment (Fig. 6b). The highest dehydrogenase activity was displayed by Bio-SiNPs₁₀₀ (13.25 μ g TTF g⁻¹ h⁻¹ FWT), followed by Chem-SiNPs₁₀₀ (11.15 μ g TTF g⁻¹ h⁻¹ FWT). Both Bio-SiNPs₃₀ and Chem-SiNPs₃₀ showed similar intermediate activity of the dehydrogenase enzyme ($\sim 9.85 \mu$ gTTF g⁻¹ h⁻¹ FWT), which was higher than Bulk-Si at both 30 (6.90 μ g TTF g⁻¹ h⁻¹ FWT) and 100 ppm (8 μ g TTF g⁻¹ h⁻¹ FWT).

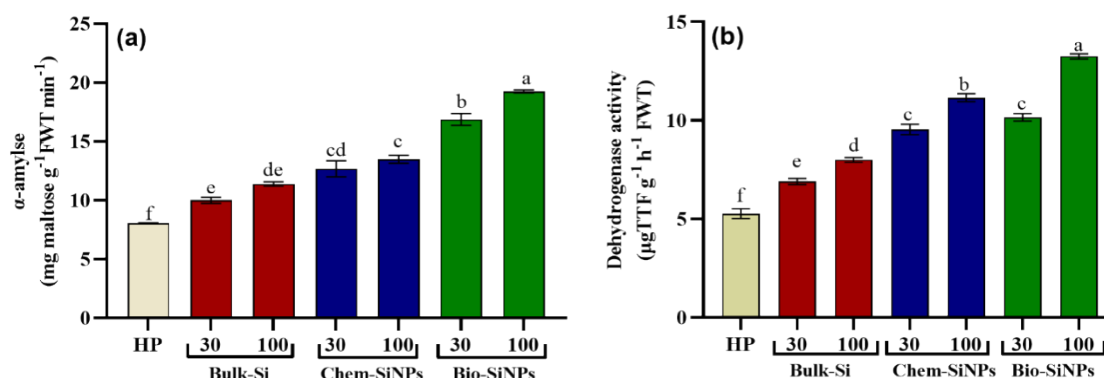


Fig. 6

Effect of Si nano-priming on TSS and starch content of rice seedlings

To comprehensively link energy and sugar availability to metabolic activity and growth under nano-priming treatments, we estimated TSS and starch content in rice seedlings. As presented in Figure 7a, Bulk-Si treatment at both concentrations and Chem- and Bio-SiNPs treatments at 30 ppm exhibited relatively close values of TSS compared to the hydro-primed treatment. Bio-SiNPs₁₀₀ seedlings showed the highest TSS content (63.69 mg g⁻¹ DWT) compared to Chem-SiNPs₁₀₀ (58.54 mg g⁻¹ DWT). Whilst the inverse relationship between starch and TSS levels is depicted in Figure 7b. Hydro-primed control exhibited the highest starch content (242.55 mg g⁻¹ DWT) with non-major differences compared to Si treatments at 30 ppm (~237.3 mg g⁻¹ DWT). However, at 100 ppm, Si treatments significantly ($p \leq 0.05$) reduced starch content in rice seedlings. Bio-SiNPs₁₀₀ seedlings recorded the lowest starch content (138.19 mg g⁻¹ DWT), while Bulk-Si₁₀₀, at par with Chem-SiNPs₁₀₀, showed intermediate values (208.55 mg g⁻¹ DWT).

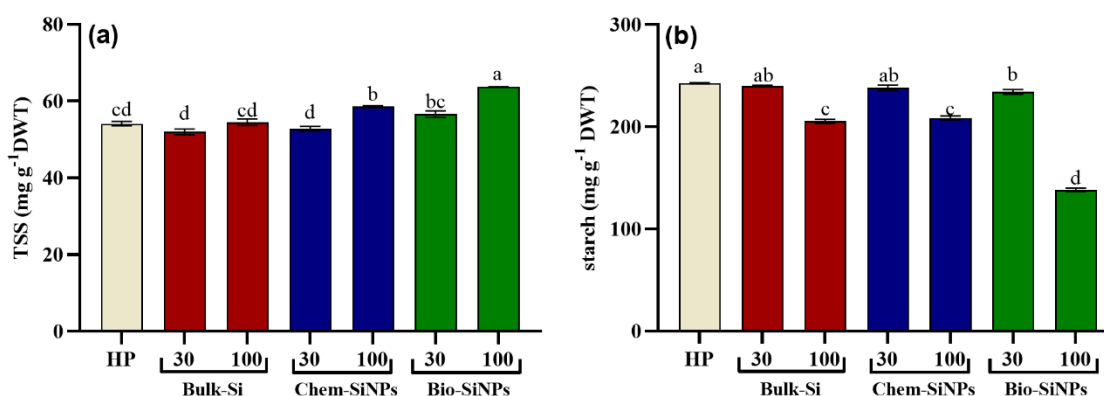


Fig. 7**Effect of Si nano-priming on oxidative stress markers in content of rice seedlings**

As direct indicators to oxidative damage and osmotic adjustment, we monitored H_2O_2 , MDA, and proline levels in rice seedlings primed with SiNPs. Application of various Si treatments resulted in differential effects on MDA and H_2O_2 levels in rice seedlings. Among them, Bulk-Si at 100 ppm induced the highest MDA value ($13.22 \mu\text{mole g}^{-1}$ FWT), exceeding both Chem-SiNPs ($9.57 \mu\text{mole g}^{-1}$ FWT) and Bio-SiNPs ($9.01 \mu\text{mole g}^{-1}$ FWT) at the same concentration (Fig. 8a). In contrast, at 30 ppm, Bio-SiNPs yielded the lowest MDA level ($5.49 \mu\text{mole g}^{-1}$ FWT), and Bulk and Chem-SiNPs showed intermediate values. Regarding H_2O_2 content, all 100 ppm Si treatments significantly elevated H_2O_2 content in rice seedlings, with Bulk-Si and Chem-SiNPs exhibiting the highest values ($\sim 2.71 \mu\text{mole g}^{-1}$ FWT) (Fig. 8b). While Bio-SiNPs at the 30 ppm treatments generally resulted in lower H_2O_2 levels ($1.30 \mu\text{mole g}^{-1}$ FWT), differences among the other 30 ppm treatments were non-significant. Proline accumulation in rice seedlings showed a substantial variation among different Si treatments (Fig. 8c), with Bulk-Si treatments recording the highest levels ($\sim 3.22 \mu\text{g g}^{-1}$ FWT). Conversely, the SiNPs treatments showcased differing values based on concentration and synthesis. For example, at 100 ppm, Chem-SiNPs resulted in a relative increase in proline level ($1.91 \mu\text{g g}^{-1}$ FWT) comparable to Bio-SiNPs ($1.36 \mu\text{g g}^{-1}$ FWT). Meanwhile, Bio-SiNPs at 30 ppm showed the lowest proline accumulation ($0.96 \mu\text{g g}^{-1}$ FWT) among all treatments.

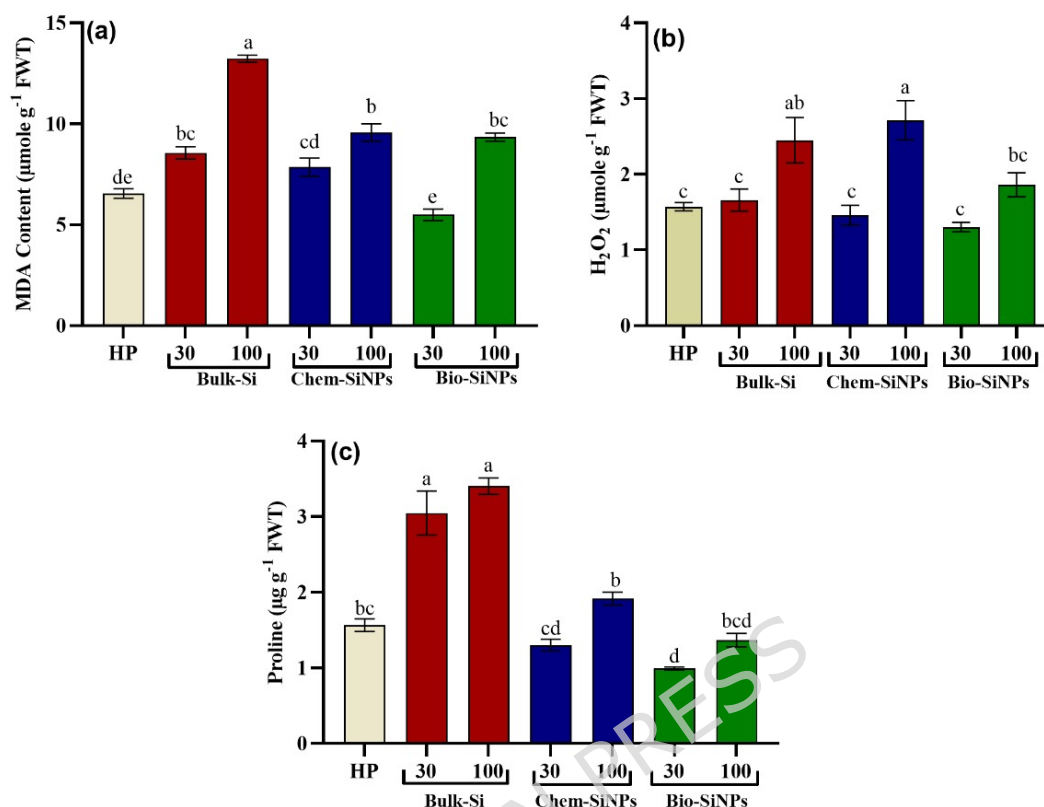


Fig. 8

Effect of Si nano-priming on the activity of antioxidant enzymes and GSH content of rice seedlings

In the present study, antioxidant enzymes and GSH were investigated to evaluate the capacity of the seedling's defense mechanisms to scavenge ROS (reactive oxygen species) induced during germination. The varying impacts of various Si treatments on the activity of antioxidant enzymes (CAT, POD, PPO, and GR) and GSH content in rice seedlings are illustrated in Figure 9. Chem-SiNPs and Bio-SiNPs treatments at 30 ppm substantially induced the highest CAT activity (~ 5.7 mmole H₂O₂ min⁻¹ g⁻¹ FWT), followed by Bulk-Si₃₀ treatment (3.16 mmole H₂O₂ min⁻¹ g⁻¹ FWT). However, other Si-treatments at 100 ppm showed convergent activity to the control peers (Fig. 9a). Likewise, POD activity increased considerably at 30 ppm for all Si treatments, culminating at Bio-SiNPs₃₀ (3.26 U min⁻¹ g⁻¹ FWT) and all other Si treatments were comparable to hydro-primed treatment (Fig. 9b). For PPO, Bio-SiNPs₃₀ resulted in the highest PPO activity (8.21 U min⁻¹ g⁻¹ FWT), surpassing Chem-SiNPs and Bulk-Si (7.37 and 6.30 U min⁻¹ g⁻¹ FWT) at the same concentration (Fig. 9c). For 100 ppm concentration, Bio-SiNPs seedlings showed higher PPO activity than other 100

ppm treatments but maintained lower than hydro-primed control. For GR, all Si treatments resulted in relatively small increases in GR activity relative to control seedlings, except Bio-SiNPs₁₀₀ seedlings, which exhibited the highest GR activity (30.21 U min⁻¹ g⁻¹ FWT) (Fig. 9d). Consistent with GR activity, Bio-SiNPs₁₀₀ seedlings had the highest GSH content (15.64 mmol g⁻¹ FWT), exceeding Bulk-Si₁₀₀ and Chem-SiNPs₁₀₀ (~ 13.35 mmol g⁻¹ FWT) and the hydro-primed peers (12.01 mmol g⁻¹ FWT). In addition, Bio-SiNPs₃₀ seedlings recorded higher GSH levels (13.11 mmol g⁻¹ FWT) compared to Bulk-Si₃₀ and Chem-SiNPs₃₀ (~ 12.60 mmol g⁻¹ FWT) (Fig. 9e).

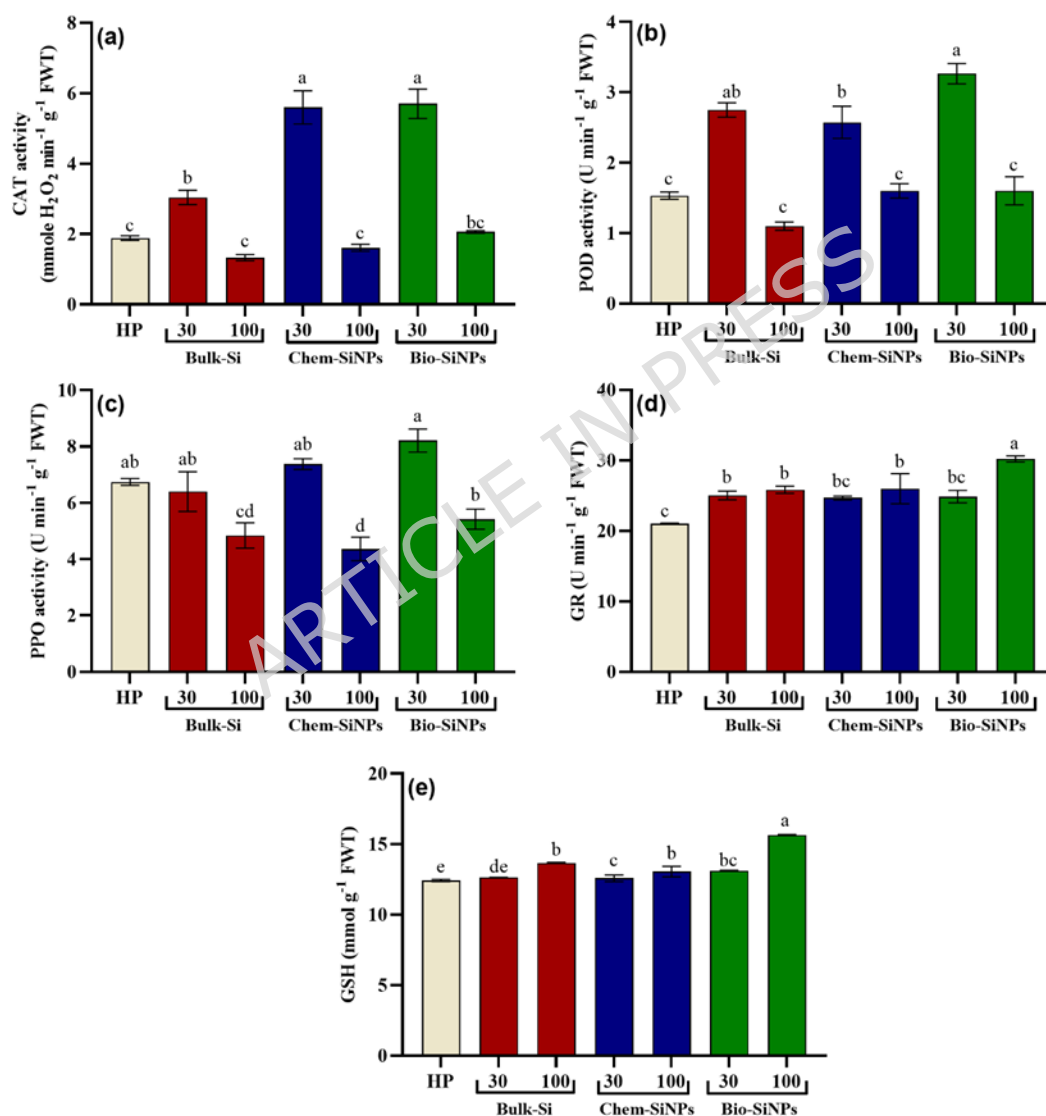


Fig.

Correlation analysis

The correlation matrix in Figure 10a revealed significant positive correlations among various germination indices, seedling growth traits, and several biochemical traits (TSS, GSH, GR, dehydrogenase, and amylase activity). These correlations underscore the coordinated role of these traits in enhancing rice seedlings development. In contrast, the growth indices were negatively correlated with starch, proline, and PPO activity, reflecting the complex interplay between the growth process and stress imposed by various Si priming agents. Also, as illustrated in the heatmap (Fig. 10b), various priming treatments differentially influence the investigated traits, with Bio-SiNPs emerged as particularly effective in enhancing all germination features, growth-related traits, hydrolytic enzymes, as well as TSS, GSH, and GR activity.

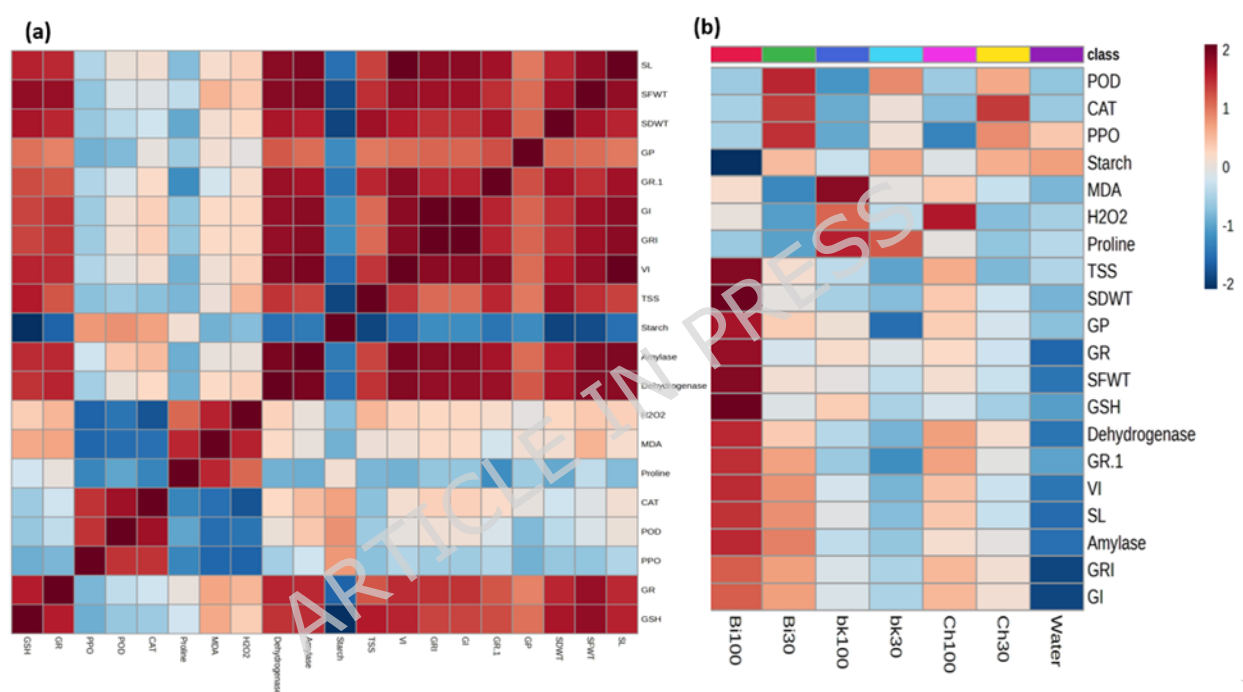


Fig. 10

Discussion

Our comparative results revealed significant differences between Bio-SiNPs and Chem-SiNPs in their physicochemical characteristics, underscoring the influence of the synthesis method on NPs' properties. The UV-vis spectra showed that Bio-SiNPs exhibited a broader peak at 245 nm, while chemical synthesis displayed a narrow absorption peak at 237 nm. These findings corroborate those of Banerjee et al. [53] and Meftah et al. [54], suggesting that peak variations result from differences in the NP's size, morphology, surface chemistry, and defect concentrations [55]. Notably, the broader peak in Bio-SiNPs arises from active phytochemicals (e.g., flavonoids, phenolic compounds (supplementary Table S2), and other

antioxidants) in chia seed extract, which increase its diversity and organic residues [28]. In contrast, chemical synthesis provides greater control over NPs' properties, yielding a narrower peak at a shorter wavelength.

TEM investigations further revealed the structural differences, where Bio-SiNPs were spherical with an average diameter of 10-12 nm, and Chem-SiNPs had a multi-dispersed spherical shape (6-10 nm) (Fig. 2b). This Bio-SiNPs' spherical shape conveys greater stability in diverse media, which could be helpful in agriculture applications as it influences interaction with the plant and subsequent uptake efficiency. These observations corroborate with recent reports of Ijaz et al. [23] on rice-husk-derived SiNPs, albeit in different sizes, highlighting the role of the biological entity and its components in shaping NPs properties. Notably, the high phytochemical content of chia seeds may contribute to the observed stability and shape of Bio-SiNPs [56].

Zeta potential results indicate that both types of SiNPs possess a negative charge, with Bio-SiNPs displaying a zeta potential of -20 mV and Chem-SiNPs showing -18 mV. This negative charge contributes to their colloidal stability by providing sufficient electrostatic repulsion between particles, preventing aggregation [57]. Moreover, slight differences in zeta potential values between the two types of SiNPs may be attributed to variations in functional capping groups on the nanoparticle surface, as Saad et al. [58] suggested. These capping groups can significantly affect NP interaction with biological systems, which is crucial for agricultural uses. For example, the strong electronegativity of Si-OH groups on the surface of the nano Si supports the role of surface chemistry in impacting the physicochemical properties of SiNPs [59].

In addition to zeta potential, the XRD pattern confirmed the amorphous nature of both Chem- and Bio-SiNPs with the broad peak at $2\theta = 22^\circ$ (101), aligning with prior results of Larkunthod et al. [60] and Rahimzadeh et al. [61]. This amorphous structure is pivotal in obtaining for achieving a higher surface area and facilitating interaction with plant cells. The calculated crystallite size of 8 nm is consistent with TEM observations, reinforcing the effectiveness of our synthesis methods in formulating SiNPs. FTIR analysis provides critical insights into the chemical characteristics and functionalities of synthesized SiNPs. The prominent peak at 1100 corresponds to the asymmetric stretching vibrations of the Si-O-Si bonds, which are the backbone of the Si matrix [62]. Moreover, the band between 3000-3500 is characteristic of the OH stretching vibrations of silanol groups, indicating the presence of hydroxyl functional groups on the surface of Bio-SiNPs. This finding corroborates the characterization of bioactive compounds present in phenolic compounds, flavonoids, alkaloids, terpenes, and tannins [63]. In addition, the spectral region between 1700-750 cm^{-1} band corresponds to the C=O stretching vibrations associated with ketones, carboxylic acids,

and esters of bioactive compounds [64]. The 1200 cm^{-1} band is characteristic of the C–O stretching of the polysaccharides and phenols. The presence of these functional groups aligns with those from chia seed extract (O–H, C=O, C–O) (Fig. 3b), confirming that biomolecules such as proteins, lipids, and polysaccharides are involved in the synthesis process, acting as effective capping and stabilizing agents on the surface of Bio-SiNPs.

These findings are consistent with Rahimzadeh et al. [61], who reported similar functional groups in SiNPs synthesized from *Rhus coriaria* L. and rice straw-based plant extracts. The ability to encapsulate and deliver these compounds to biological systems by Bio-SiNPs could improve plant growth, nutrient uptake, and defense mechanisms. Thus, these biomolecules with a more uniform shape, charge distribution, and greater crystallinity and biocompatibility contribute to the bigger size of Bio-SiNPs than their chemically synthesized counterparts [65]. Collectively, these physicochemical characteristics will likely play an effective role in determining NPs performance and their subsequent impacts on rice seeds during germination.

Seed germination, seedling establishment, and water uptake

Our results show that SiNPs application significantly improved various germination indices (e.g, GR, GRI, GI, and VI) and seedling growth traits (seedling length, fresh, and dry weight) compared to Bulk-Si treatment. This is in collaboration with studies of Xiong et al. [19], who reported similar positive effects of SiNPs on rice germination and growth. Bio-SiNPs enhanced these indices more than Chem-SiNPs (Table 1), particularly at 100 ppm concentration. This dose-dependent effect of SiNPs is consistent with the findings of Raza et al. [66] in wheat plants. Our optimal concentration aligns with Jiang et al. [67] in rice and Adress et al. [68] in wheat. This emphasizes the significance of NPs concentration and fabrication method in predicting plant response [69]. Moreover, Bio-SiNPs significantly enhanced water uptake and radicle dehydrogenase activity of rice seeds compared to Chem-SiNPs and Bulk-Si treatments (Fig. 6b). These observed differences in germination and seedling physiology between Chem- and Bio-SiNPs can be directly related to their distinct physiochemical properties. Both Chem- and Bio-SiNPs types displayed a negative surface charge, as indicated by their zeta potential values (-20 mV for Bio-SiNPs and -18 mV for Chem-SiNPs), which contributes to colloidal stability by preventing NPs aggregation. However, the higher negative charge of Bio-SiNPs enhanced their colloidal stability and dispersion in priming solution, thereby increasing their interaction with the seed surface during imbibition [61; 70]. In addition to surface charge, the availability of bioactive functional groups on Bio-SiNPs, conferred by chia seed extract, may facilitate seed coat interactions through hydrogen bonding. Moreover, the higher surface area of Bio-SiNPs increases reactive sites for biochemical interactions, which promote rapid water uptake, and

enhance enzymatic activity and nutrient uptake, accelerating seedling development [71, 72, 73].

Si content in rice seedlings

Our findings demonstrated that Bio-SiNPs played a significant role in improving Si accumulation in rice seedlings in a dose-dependent manner compared to other Si treatments (Fig. 5). The presence of surface Si-OH and functional groups originating from chia seed extract increased surface reactivity, biocompatibility, seed coat interactions, which may facilitate Si uptake, thereby minimizing precipitation and promoting seedling growth [74]. Similar findings have been found in wheat and barley [75]. At the molecular level, Bio-SiNPs could upregulate Si transporter genes such as *Lsi1* and *Lsi2*, which are crucial for rice uptake [76]. Physiologically, Si uptake in rice involves passive diffusion and energy-dependent transport, which may further enhance with Bio-SiNPs [77].

α -amylase activity, starch, and TSS content in rice seedlings

In the current study, Bio-SiNPs at 100 ppm boosted the highest α -amylase activity and TSS content, while reducing starch content in rice seedlings (Fig. 6a and 7), revealing the efficient role of Bio-SiNPs in inducing carbohydrate metabolism at the initial phases of rice germination. This is reinforced by strong positive correlations among germination indices, α -amylase activity, and TSS content (Figure 10). On the other hand, the negative correlation with starch levels suggests an efficient role of α -amylase in starch hydrolysis, supporting energy supply for early germination and promoting seedling vigour. The elevated α -amylase activity observed is closely linked to increased water uptake, which will drive ongoing metabolic processes during germination [78]. Moreover, the higher bioavailability of Si from Bio-SiNPs may act as a nano-catalyst, triggering starch degradation by enhancing α -amylase activity. These biochemical responses can be mediated by physiochemical characteristics of Bio-SiNPs, particularly their higher surface area and negative charge, which promote the seed coat interactions during early germination. These advantages were reflected in the greater Si content, enhanced water uptake, α -amylase activity, starch hydrolysis, and TSS accumulation, providing the energy required for higher seedling development. Similar induction of α -amylase activity, sugar accumulation, and germination performance has been reported in maize-primed with biogenic SiNPs [79]. These findings highlight role of surface characteristics of eco-friendly synthesized NPs in modulating initial germinating metabolism.

H₂O₂, MDA, and proline in rice seedlings

Our study indicates that high concentrations of Si treatments, particularly Bio-SiNPs₁₀₀, enhanced germination indices, although concomitant increases in H₂O₂ and MDA levels (Fig.

8a, b). This suggests that, at high concentration, the positive effects on germination may outweigh the oxidative damage. On the other hand, low-concentration Bio-SiNPs₃₀ may prioritize stress mitigation over than growth-related processes, resulting in suboptimal germination but - reduced oxidative stress [80]. Generally, Bio-SiNPs at both concentrations were the most effective in reducing H₂O₂ and MDA levels, indicating their the ability to activate their ability to activate the antioxidant system, which maintains a balance between ROS generation and scavenging during germination [21]. These observations align with studies of Ijaz et al. [23] and Hussain et al. [81], emphasizing the role of Bio-SiNPs in modulating oxidative stress in rice plants. In addition, the notable elevation in MDA and H₂O₂ levels in Bulk-Si and Chem-SiNPs treatments was accompanied by increased proline level (Fig. 8c). Proline acts as a protective osmolyte under stress conditions, with its synthesis being induced in response to oxidative stress [82]. In contrast, Bio-SiNPs alleviated oxidative stress, thereby minimizing the necessity for proline accumulation while promoting germination and seedling vigour. This corroborates with Larkunthod et al. [60] results, suggesting that Bio-SiNPs application can effectively reduce proline content in rice under salinity stress, further highlighting the role of Bio-SiNPs in stress tolerance.

Antioxidant defense system in rice seedlings

The present study reveals the potential of SiNPs to enhance antioxidant activity, reinforcing the role of Si in mitigating oxidative stress during germination [83]. This antioxidant response was concentration and type-dependent, as Bio-SiNPs at 30 ppm showed the highest CAT, POD, and PPO activity, indicating that Bio-SiNPs have a higher ability for scavenging generated oxidative stress, consistent with the lowest H₂O₂ and MDA levels at 30 ppm compared to 100 ppm (see Figure 8a, b). Sharma et al. [84] also demonstrated that rice seedlings treated with different concentrations of molybdenum NPs (α -MoO₃ or MoS₂ NPs) show altered activities of antioxidant enzymes (SOD and CAT). Conversely to CAT, PPO, and POD activities, GR and GSH levels increased at 100 ppm compared to 30 ppm for all priming agents. This shift in the antioxidant system suggests that high concentrations, particularly those of Bio-SiNPs, activate the ascorbate-glutathione cycle, which is crucial -for eliminating ROS [85]. This cycle may compensate for the reduction in CAT and POD activities via engaging other oxidative stress detoxification pathways. Such observed inhibition in CAT, POD, and PPO activities in response to specific stress conditions with SiNPs application indicates less need for higher CAT, PPO, and POD activities [86]. Furthermore, physicochemical features of Bio-SiNPs modulate redox signaling and defense mechanisms during early germination. Altogether, these findings highlight that NPs surface area, stability, and chemistry are key factors for nano-priming efficiency and demonstrated the superior efficiency of Bio-SiNPs compared to Chem-SiNPs and Bulk-Si. A detailed

investigation into the molecular mechanisms by which Bio-SiNPs can mediate the expression of key antioxidant enzyme-related genes is essential to elucidate their mode of action and broaden their potential applicability in agriculture.

Although this study showed the efficacy of seed priming, other application methods merit consideration for different growth stages and large-scale agriculture. For instance, foliar spray could enhance defense at key stages, while soil drenches may bolster rhizosphere health and Si availability to the plant, potentially benefiting growth throughout the life cycle. Thus, future studies should evaluate -delivery methods in field settings to identify the most appropriate and cost-effective formulation for maximizing rice productivity. This current study also elucidates the beneficial effects of SiNPs seed priming on rice performance; however, the environmental implications of SiNPs, including their long-term stability, accumulation, degradation behaviour, and potential ecotoxicity, , remain insufficiently considered.

Conclusion

This study illustrates a sustainable eco-friendly approach for the biogenic synthesis of silica nanoparticles using *Salvia hispanica* (chia) seed extract as an effective bio-reducing, stabilizing, and capping agent. The various characterization techniques showed that Bio-SiNPs were spherical, nano-sized, colloidal stability, and functional-group rich, compared to Chem-SiNPs. Nano priming with Bio-SiNPs at 100 ppm significantly enhanced rice seed germination indices and subsequent seedling establishment. This enhancement is attributed to improved α -amylase and radicle dehydrogenase activities, increased soluble sugars and starch hydrolysis, and elevated Si content. Furthermore, Bio-SiNPs enhanced GR activity and GSH levels, thereby improving stress tolerance. While Chem-SiNPs and Bulk-Si, particularly at 100 ppm, induced oxidative stress markers and proline accumulation, application of Bio-SiNPs at 30 ppm resulted in higher activities of antioxidant enzymes (CAT, POD, and PPO). These results highlight the outstanding performance of Bio-SiNPs in promoting rice seedling growth and the underlying physiochemical mechanisms compared to conventional approaches. Hence, our findings provide new insights for the potential application of biogenic SiNPs in agriculture and open new avenues for enhancing crop productivity and stress resilience in sustainable agriculture systems.

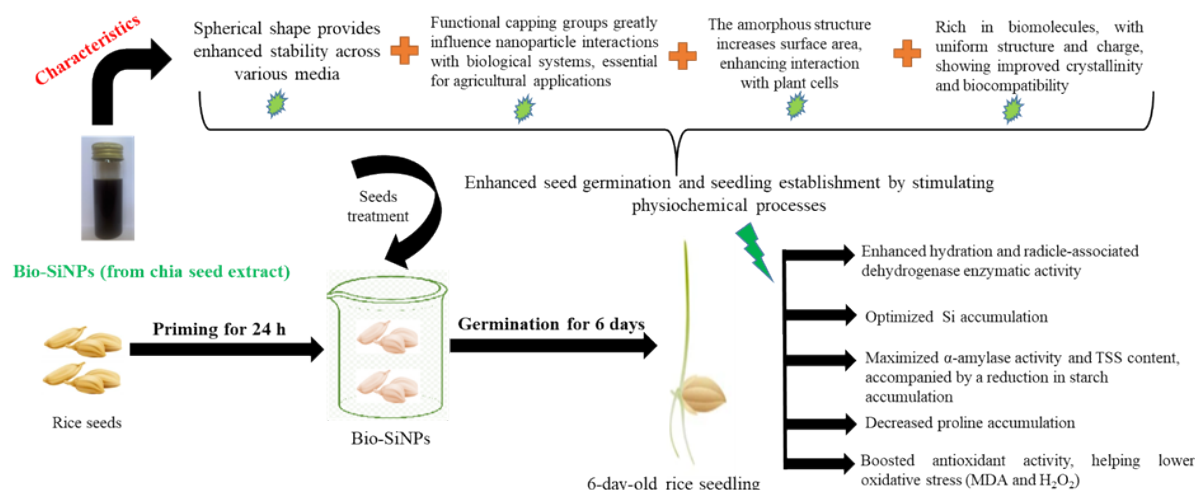


Fig. 11

Data availability

All data generated or analyzed during this study are included in this manuscript.

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Captions

Fig. 1 Preparation of silica nanoparticles (Chemical and biogenic synthesis).

Fig. 2 The **(a)** UV-visible spectrum, **(b)** TEM image, and **(c)** Zeta potential analysis of SiNPs synthesized chemically (Chem) and biologically (Bio) from chia seeds extract.

Fig. 3 The **(a)** XRD analysis and **(b)** FTIR of chia seeds extract and **(c)** FTIR of SiNPs synthesized chemically (Chem) and biologically (Bio) from chia seeds extract.

Fig. 4 Effect of silica and silica nanoparticles priming at 30 and 100 ppm concentrations on **(a)** seedling length, **(b)** seedling fresh weight, and **(c)** seedling dry weight of 6-day-old rice seedlings. Data are presented as means $\pm$ SE. Significant differences in the mean value of each treatment are represented by different lowercase letter (s) based on Fisher's test ($p \leq 0.05$, $n = 3$). HP: hydro priming, Bulk-Si: silica, Bio-SiNP: biologically synthesized silica nanoparticles, Chem-SiNP: chemically synthesized silica nanoparticles.

Fig. 5 Effect of silica and silica nanoparticles priming at 30 and 100 ppm concentrations on the silicon (Si) content of 6-day-old rice seedlings. Data are presented as means $\pm$ SE. Significant differences in the mean value of each treatment are represented by different lowercase letter (s) based on Fisher's test ($p \leq 0.05$, $n = 3$). HP: hydro priming, Bulk-Si: silica, Bio-SiNP: biologically synthesized silica nanoparticles, Chem-SiNP: chemically synthesized silica nanoparticles.

Fig. 6 Effect of silica and silica nanoparticles priming at 30 and 100 ppm concentrations on **(a)** α -amylase, and **(b)** dehydrogenase activities of 6-day-old rice seedlings. Data are presented as means $\pm$ SE. Significant differences in the mean value of each treatment are represented by different lowercase letter (s) based on Fisher's test ($p \leq 0.05$, $n = 3$). HP: hydro priming, Bulk-Si: silica, Bio-SiNP: biologically synthesized silica nanoparticles, Chem-SiNP: chemically synthesized silica nanoparticles.

Fig. 7 Effect of silica and silica nanoparticles priming at 30 and 100 ppm concentrations on **(a)** total soluble sugars (TSS), and **(b)** starch of 6-day-old rice seedlings. Data are presented as means $\pm$ SE. Significant differences in the mean value of each treatment are represented by different lowercase letter (s) based on Fisher's test ($p \leq 0.05$, $n = 3$). HP: hydro priming, Bulk-Si: silica, Bio-SiNPs: biologically synthesized silica nanoparticles, Chem-SiNPs: chemically synthesized silica nanoparticles.

Fig. 8 Effect of silica and silica nanoparticles priming at 30 and 100 ppm concentrations on **(a)** malondialdehyde (MDA), **(b)** hydrogen peroxide (H_2O_2), and **(c)** proline of 6-day-old rice seedlings. Data are presented as means $\pm$ SE. Significant differences in the mean value of each treatment are represented by different lowercase letter (s) based on Fisher's test ($p \leq 0.05$, $n = 3$). HP: hydro priming, Bulk-Si: silica, Bio-SiNPs: biologically synthesized silica nanoparticles, Chem-SiNPs: chemically synthesized silica nanoparticles.

Fig. 9 Effect of silica and silica nanoparticles priming at 30 and 100 ppm concentrations on the activity of **(a)** CAT, **(b)** POD, **(c)** PPO, **(d)** GR, and the content of **(e)** GSH of 6-day-old rice seedlings. Data are presented as means $\pm$ SE. Significant differences in the mean value of each treatment are represented by different lowercase letter (s) based on Fisher's test ($p \leq 0.05$, $n = 3$). HP: hydro priming, Bulk-Si: silica, Bio-SiNPs: biologically synthesized silica nanoparticles, Chem-SiNPs: chemically synthesized silica nanoparticles.

Fig. 10 Correlation matrix **(a)**, and Heatmap **(b)** of germination and physiochemical-related traits of rice seedlings primed with different silica and silicon nanoparticles across two concentrations, 30 and 100 ppm. Abbreviations: **GP**; germination percentage, **GR.1**; germination rate, **GI**; germination index, **GRI.1**; germination rate index, **VI**; vigour index, **SDWT**; seedling dry weight, **SFWT**; seedling fresh weight, **SL**; seedling length, **RD**; root dehydrogenase, **TSS**; total soluble sugars, **MDA**; malondialdehyde, **H₂O₂**; hydrogen peroxide, **CAT**; catalase, **POD**; peroxidase, **PPO**; polyphenol oxidase, **GR**; glutathione reductase, **GSH**; reduced glutathione.

Fig. 11 Summary of the ameliorative effects of Biogenic SiNPs from chia seed extract in enhancing rice germination and seedling establishment at 6-day-old age.