International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 05-02-2026, Accepted: 08-03-2026, Published: 23-03-2026
Volume 11, Issue 1, 2026, Page No. 335-343

Enhancement of growth and biochemical responses of *in vitro* raised Indian mustard (*Brassica juncea* L. Czernj. Cosson) seedlings via silica (SiO_2) nano-priming

Pratibha Dwivedi, Vitthal Chhipa, Shamshadul Haq, Ashutosh Pathakand, Sumita Kachhwaha*
Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India
Corresponding Author: Sumita Kachhwaha

Abstract
Nanoparticle (NPs) based seed-priming enhances plant growth by modulating key metabolic pathways, improving physiological performance and crop productivity.Silicon-based nano-priming has emerged as a promising strategy to enhance early crop establishment through modulation of growth and antioxidant defense mechanisms. This study assessed concentration-dependent effects of SiO_2 NP seed-priming (25-100 mg L^{-1}) on germination, growth, photosynthetic pigments, secondary-metabolites, and antioxidant defense in *in-vitro* cultured *Brassica juncea* seedlings grown on half-strength MS-medium. SiO_2 NP-priming significantly enhanced germination percentage, seedling vigor index (SVI), elongation, growth, biomass, and tissue moisture content, with 100 mg L^{-1}emerging as the most effective concentration. Shoot and root lengths were maximized at 50-100mg L^{-1}, while fresh-dry biomass and moisture content peaked at 100 mg L^{-1}, indicating improved assimilate production and water relations. Photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids) were markedly enhanced at 75-100 mg L^{-1}, reflecting improved chloroplast development and photosynthetic efficiency. Secondary metabolites, including total phenolics and flavonoids, along with DPPH radical scavenging activity, increased progressively at higher concentrations, suggesting stimulation of non-enzymatic antioxidant systems. Enzymatic antioxidants (SOD, CAT, and GPX) exhibited strong up-regulation, particularly at 100 mg L^{-1}, indicating enhanced reactive oxygen species detoxification and redox-homeostasis. In contrast, 25 mg L^{-1} appeared sub-optimal, resulting in comparatively reduced physiological and biochemical responses. Overall, SiO_2 NP priming at 75-100 mg L^{-1} significantly improved growth-dynamics, photosynthetic capacity, secondary metabolism, and antioxidant defense, proving its potential as a sustainable approach to strengthen early seedling establishment and physiological resilience in Indian mustard.

Keywords: Nano-priming, indian mustard (*Brassica juncea* L. Czernj. Cosson), DPPH Inhibition percentage, total phenolics, total flavonoids, redox-enzymes

Introduction

Nanotechnology has become a powerful approach in sustainable crop management, providing advanced solutions to enhance plant growth and stress adaptability. Among various engineered nanoparticles, silicon dioxide nanoparticles (SiO_2 NPs) have gained significant interest for their potential to improve seed germination, seedling vigor, and tolerance to environmental challenges. These NPs influence plant physiology through diverse mechanisms, such as enhancing nutrient absorption, escalating photosynthetic efficiency, strengthening antioxidant defense systems, and triggering stress-responsive signaling pathways. Furthermore, SiO_2 NPs stimulate root growth, thereby improving water and nutrient uptake, and alleviate the detrimental effects of abiotic stresses-including drought, salinity, and heavy metal toxicity,by modulating critical physiological and biochemical processes (Dhingra *et al.,* 2022; Hasanaklou *et al.,* 2023) [8, 16].

Seed priming, a controlled pre-sowing hydration treatment, is a well-established approach to enhance germination performance and early seedling establishment. By initiating pre-germinative metabolic activities without radicle protrusion, priming accelerates the lag phase, enhances enzymatic activation, supports cellular repair mechanisms, and promotes metabolite accumulation. Consequently, primed seeds exhibit improved germination uniformity, vigor, and stress tolerance, particularly under suboptimal environmental conditions. The integration of nanotechnology with seed priming is termed nano-priming, represents a promising advancement, combining the physiological benefits of priming with the unique physicochemical properties of NMs to enhance early plant establishment and productivity (Nile *et al.,* 2022; Pathak *et al.,* 2023). *Brassica juncea* (L.) [21, 22] is one of the most important oilseed crops in India, occupying approximately 9.18 million hectares and contributing over 85% of the country's rapeseed-mustard acreage and nearly one-third of total edible oil production. The crop is valued for its high oil content (35-45%), balanced fatty acid composition, tocopherols, and antioxidant compounds. Its by-products serve as protein-rich animal feed, while crop residues contribute to green manure and sustainable farming systems. Despite its substantial agronomic, nutritional, and industrial importance, Indian mustard productivity is significantly constrained by diverse abiotic and biotic stresses. Enhancing yield stability and stress resilience is therefore imperative under increasing climate variability and rising global food demand (Shekhawat *et al.*, 2012; Sharma *et al.*, 2024) [25, 26].

In this context, the present study focuses on nano-priming as a targeted pre-sowing intervention to improve early-stage performance of the Indian mustard variety NRCDR-2. Specifically, the research investigates the effects of SiO_2 NPs-mediated seed priming on germination behavior, seedling vigor, and associated physiological and biochemical responses. By leveraging the high surface reactivity and bioavailability of SiO_2 NPs, nano-priming is hypothesized to stimulate germination metabolism, enhance antioxidant capacity, and improve nutrient mobilization during early growth. This study aims to elucidate the mechanistic basis of SiO_2 nano-priming–induced

improvements in regeneration efficiency and seedling establishment, thereby contributing to the development of resilient and high-performing mustard production systems.

Materials and methods

Seeds procurement and preparation of SiO_2 NP's suspensions

Seeds of *Brassica juncea* (L.) Czern. and Coss. var. NRCDR-2 were procured from ICAR-Directorate of Rapeseed-Mustard Research (ICAR-DRMR), Bharatpur, Rajasthan, India. Seeds were initially surface-sterilized with 20% (v/v) Extran (Merck, India) for 20 min and thoroughly rinsed under running tap water to remove detergent residues, followed by repeated washing with distilled water. Under aseptic conditions in a laminar airflow cabinet, seeds were further sterilized with 0.1% (w/v) $HgCl_2$ (HiMedia, India) for 3 min and rinsed three times with autoclaved distilled water to eliminate residual traces of $HgCl_2$. SiO_2 nano powder (SRL, India) was dispersed in distilled water to obtain final concentrations of 10, 20, 40, and 80 mg L^{-1}. Suspensions were autoclaved and subsequently sonicated for 2-3 h using an ultrasonicator (Labman, India) to ensure uniform dispersion. These freshly prepared suspensions were used for seed nano-priming treatments.

Seed nano-priming and *in vitro* culture

Surface sterilized seeds were primed in SiO_2 NP suspensions at concentrations of 0 (control), 10, 20, 40, and 80 mg L^{-1} under aseptic conditions. Approximately 150 seeds per treatment were placed in Erlenmeyer flasks and incubated on a rotary shaker for 18-20 h. After priming, the seeds were transferred to autoclaved half-strength MS-medium (Murashige and Skoog 1962) [20] (supplemented with 1% (w/v) sucrose (HiMedia, India) and the pH were adjusted to 5.77 ± 0.08. The cultures were maintained for 14 days under controlled conditions of 25 ± 2 °C, a 16 h photoperiod, 85% relative humidity, and a light intensity of 100 µmol m^{-2} s^{-1}. Fourteen-day-old seedlings were then collected for morphological and physio-biochemical evaluations.

Growth andMorphological parameters

Seedlings were gently removed after 14 days and washed to eliminate residual medium. Morphological parameters, including shoot length, root length, number of leaves, and number of nodes, were recorded. Biomass accumulation was determined as fresh weight (FW) and dry weight (DW). For dry weight estimation, seedlings were oven-dried at 65 °C until constant weight. Moisture content (MC%) (Reeb *et al.,* 1999) [24], germination percentage, and Seedling vigor index (SVI) were calculated using standard formulae (Abdul-Baki and Anderson, 1973) [1].

Physio-biochemical analysis

Chlorophyll (chl a, chl b and Total chl) and total carotenoid content (Lichtenthaler and Wellburn, 1983) [19] were estimated using the DMSO (Rankem, India) extraction method (Hiscox and Israelstam, 1979) [18]. Fresh leaf tissue (100 mg) was homogenized in 2 mL DMSO and incubated in the dark for 24 h, followed by heating at 65 °C for 1 h. After centrifugation (10,000 rpm, 20 min), the absorbance of the supernatant was measured at 480, 645, and 663 nm using a UV-visible spectrophotometer (Shimadzu UV-1900, Japan) against DMSO as a blank. Pigment concentrations were calculated according to standard equations (Wellburn, 1994) [31] and expressed as mg g^{-1} FW.

Total phenolic content (TPC), total flavonoid content (TFC), and DPPH radical scavenging activity were quantified to assess the effect of SiO_2 NP priming on non-enzymatic antioxidant capacity in shoot and root tissues of Indian mustard (Haq *et al.,* 2022) [13]. Methanolic extracts were prepared by homogenizing 200 mg of fresh tissue in 80% methanol, followed by 24 h incubation at 4 °C in the dark and subsequent sonication. The extracts were centrifuged, and the supernatant was used for analysis. TPC was determined using the Folin-Ciocalteu reagent, with absorbance recorded at 760 nm and results expressed as µg gallic acid equivalents (GAE) g^{-1} FW. TFC was estimated by the aluminum chloride colorimetric method, measuring absorbance at 510 nm and expressing values as µg quercetin equivalents (QE) g^{-1} FW. Antioxidant capacity was further evaluated using the DPPH assay, in which the methanolic extract was mixed with 25 µM DPPH solution (HiMedia, India), incubated in the dark for 45 min, and absorbance was recorded at 517 nm. Radical scavenging activity was calculated according to the method described by Bemani *et al.* (2012) [7].

Antioxidant enzyme assays

Fresh plant tissue (200 mg) was homogenized in chilled extraction buffer containing 50 mM phosphate buffer (pH 7.8), 2 mM EDTA(HiMedia, India), 5 mM PMSF(HiMedia, India), 0.5% Triton X-100(Sigma, Switzerland), and 5% PVP(HiMedia, India). After sonication and centrifugation (10,000 rpm, 20 min, 4 °C), the supernatant was used for the enzyme assays (Haq *et al.,* 2021) [14].

Superoxide Dismutase (SOD; EC 1.15.1.1)

SOD activity was assayed by measuring 50% inhibition of nitroblue tetrazolium (NBT) photoreduction following (Beauchamp and Fridovich, 1971) [6]. The reaction mixture contained 50 mM phosphate buffer (pH 7.8), riboflavin(HiMedia, India), NBT(HiMedia, India), DL-methionine(HiMedia, India), EDTA(HiMedia, India), and 50 µL enzyme extract. After 30 min illumination at 25 °C, absorbance was recorded at 560 nm, with non-illuminated tubes as blanks and buffer-only tubes as controls. One unit of SOD activity was defined as the amount of enzyme causing 50% inhibition of NBT reduction and expressed as U g^{-1} fresh weight.

Catalase (CAT; EC 1.11.1.6)

Catalase (CAT) activity was determined at 25 °C by monitoring the decrease in H_2O_2 absorbance at 240 nm for 5 min (Aebi, 1984) [3]. The reaction mixture contained 50 µL enzyme extract, 50 mM phosphate buffer (pH 7.0), and 6.5 mM H_2O_2, with buffer alone as a blank. Activity was calculated using an extinction coefficient of 43.6 $M^{-1}cm^{-1}$ and expressed as µmol H_2O_2 decomposed $min^{-1}g^{-1}$fresh weight.

Guaiacol Peroxidase (GPX; EC 1.11.1.7)

Guaiacol peroxidase (GPX) activity was measured at 25 °C by monitoring the increase in absorbance at 470 nm due to H_2O_2-mediated oxidation of guaiacol (Fernández-García *et al.,* 2004) [11]. The reaction mixture contained 50 µL enzyme extract, 50 mM phosphate buffer (pH 7.0), and 5 mM guaiacol, initiated with 6.5 mM H_2O_2. Absorbance was recorded for 5 min at 30-second intervals, with buffer plus guaiacol (without enzyme) as a blank.Activity was calculated using an extinction coefficient of 26.6 $mM^{-1}cm^{-1}$ and expressed as µmol guaiacol oxidized $min^{-1}g^{-1}$ fresh weight.

Statistical analysis

All biochemical analyses were performed in triplicate for each treatment, and the results are presented as mean ± standard error in the figures. Data were subjected to one-way analysis of variance (ANOVA) to evaluate significant differences among treatment means. When significant effects were detected, post hoc comparisons were conducted using the Tukey–Kramer multiple comparison test to determine differences between individual groups (Hammer *et al.,* 2001) [12]. Principal component analysis (PCA) and correlation analysis were performed to identify the patterns of variation, underlying relationships, and the degree of association among the studied physiological and biochemical parameters, thereby elucidating the overall impact of treatments and the interdependence of measured traits (Haq *et al.,* 2019; Verma *et al.,* 2025) [15, 30].

Results and Discussion

Seed priming with SiO_2 NPs exerted significant, concentration-dependent effects on early growth, vigor, and biomass of *Brassica juncea* seedlings (Fig. 1). Shoot length increased markedly at 50 and 100 mg L^{-1} (7.89 cm), compared with the control (6.43 cm), while root length was maximized at 50 mg L^{-1} (8.16 cm). 75-100 mg L^{-1}treatments maintained superior root growth relative to control, indicating improved water and nutrient acquisition. Leaf number showed a slight increase at higher concentrations, whereas the node number remained largely unchanged, suggesting that SiO_2 NP priming predominantly stimulated elongation growth rather than meristematic differentiation.Germination percentage improved progressively with increasing concentration, peaking at 100 mg L^{-1} (97.53%) compared to 90% in the control. 25 mg L^{-1} reduced germination (81.25%), while higher concentrations restored and enhanced performance. Accordingly, seedling vigor index (SVI) was significantly elevated at 50 and 100 mg L^{-1}, confirming enhanced establishment potential (Table 1).Biomass accumulation mirrored these trends. The highest fresh and dry weights were recorded at 100 mg L^{-1} (0.62 g and 0.16 g, respectively), indicating enhanced assimilate production and growth efficiency (Table 2). Moisture content also increased with concentration, reaching 87.25% at 100 mg L^{-1}, reflecting improved tissue hydration and osmotic balance. In contrast, 25 mg L^{-1} resulted in comparatively lower biomass, suggesting that suboptimal dosing may be insufficient to trigger beneficial physiological responses.Overall, SiO_2 NP priming at 50-100 mg L^{-1} significantly enhanced germination, seedling vigor, growth, biomass, and tissue moisture, with 100 mg L^{-1} emerging as the most effective concentration. These results highlight the potential of silicon-based nano-priming as a sustainable approach to improve early crop establishment through enhanced growth dynamics and water relations (Alsaeedi *et al.,* 2019; Dhingra *et al.,* 2022; Aboellail *et al.,* 2023; Al-Tabbal *et al.,* 2024) [2, 4, 5, 8].

Table 1: Morphological response at various levels of SiO_2 NPs treatment in 14-day old Indian mustard seedlings

Treatment	Concentration (mg L^{-1})	Shoot length (cm) (Mean ± SE)	Root length (cm) (Mean ± SE)	Number of Leaves (Mean ± SE)	Number of Nodes (Mean ± SE)	Germination percentage	Seedling Vigor index (Mean ± SE)
SiO_2 NP	0	6.43 ± 0.20^{b}	6.42 ± 0.20^{c}	4.33 ± 0.21^{b}	2.33 ± 0.14^{a}	90	1158.22 ± 33.45^{b}
	25	7.05 ± 0.4^{ab}	7.42 ± 0.5^{ab}	4.54 ± 0.25^{b}	2.08 ± 0.15^{b}	81.25	1176.31 ± 49.40^{b}
	50	7.89 ± 0.33^{a}	8.16 ± 0.29^{a}	4.64 ± 0.25^{ab}	2.32 ± 0.15^{a}	91.66	1473.14 ± 35.49^{a}
	75	7.51 ± 0.32^{ab}	7.33 ± 0.41^{b}	4.50 ± 0.16^{b}	2.14 ± 0.1^{ab}	93.66	1389.91 ± 41.27^{ab}
	100	7.89 ± 0.38^{a}	7.44 ± 0.47^{ab}	4.72 ± 0.24^{a}	2.26 ± 0.13^{a}	97.53	1495.14 ± 45.66^{a}

Different letters in superscript indicate differential significance within a column at $P \leq 0.05$ based on the post hoc multiple comparison Tukey-Kramer tests.

Table 2: Biomass accumulation response at various levels of SiO_2 NPs treatment in 14-day old Indian mustard seedlings

Treatment	Concentration (mg L^{-1})	Fresh Weight (FW) (g) (Mean ± SE)	Dry Weight (DW) (g) (Mean ± SE)	Percentage Moisture Content (MC %) (Mean ± SE)
SiO_2 NP	0	0.57 ± 0.06^{ab}	0.15 ± 0.10^{a}	69.31 ± 16.65^{b}
	25	0.49 ± 0.06^{b}	0.09 ± 0.04^{b}	71.02 ± 11.41^{b}
	50	0.53 ± 0.03^{ab}	0.12 ± 0.01^{ab}	81.67 ± 3.06^{ab}
	75	0.43 ± 0.04^{b}	0.11 ± 0.01^{ab}	84.96 ± 1.76^{a}
	100	0.62 ± 0.04^{a}	0.16 ± 0.01^{a}	87.25 ± 0.83^{a}

Different letters in superscript indicate differential significance within a column at $P \leq 0.05$ based on the post hoc multiple comparison Tukey-Kramer tests

Fig 1: Morphological effect of silica nano-treatments on seedlings of *Brassica juncea* (L.) Czernj. Cosson var. NRCDR-2.

SiO_2 NP seed priming significantly modulated photosynthetic pigments in *Brassica juncea* in a concentration-dependent manner (Fig. 2). Chl a, chl b, and total chlorophyll decreased at 25 mg L^{-1} but increased markedly at 75 and 100 mg L^{-1}, with the highest values recorded at 100 mg L^{-1}. Total carotenoids followed a similar trend, showing significant enhancement at higher concentrations.The increased pigment accumulation at 75-100 mg L^{-1} indicates improved chloroplast development, photosynthetic efficiency, and photoprotection, likely mediated by enhanced nutrient assimilation and membrane stabilization. In contrast, the reduced pigment content at 25 mg L^{-1} suggests that suboptimal dosing is insufficient to stimulate pigment biosynthesis. Overall, 100 mg L^{-1} emerged as the most effective concentration for enhancing photosynthetic capacity (Hatami *et al.,* 2021; Rai-Kalal *et al.,* 2021; Simko *et al.,* 2026) [17, 23, 28].

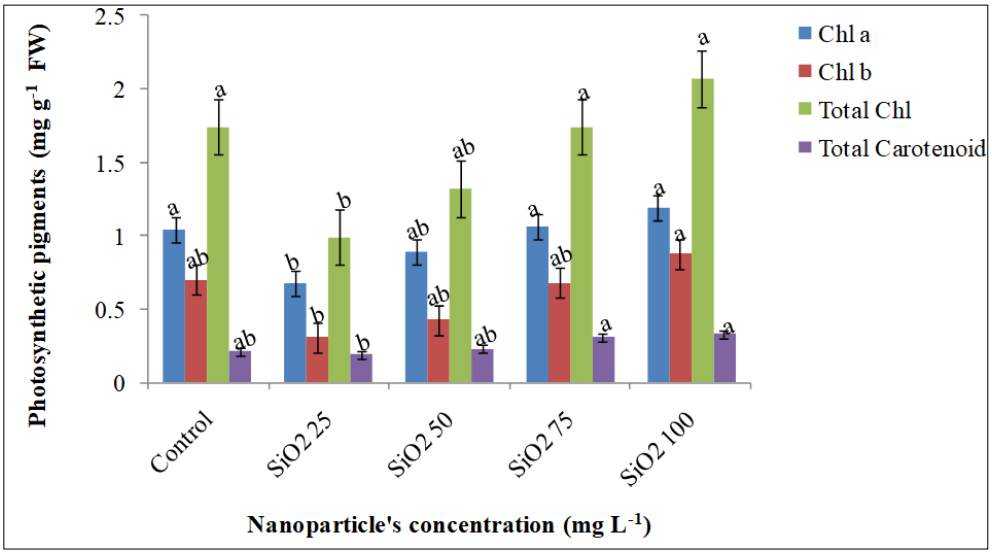
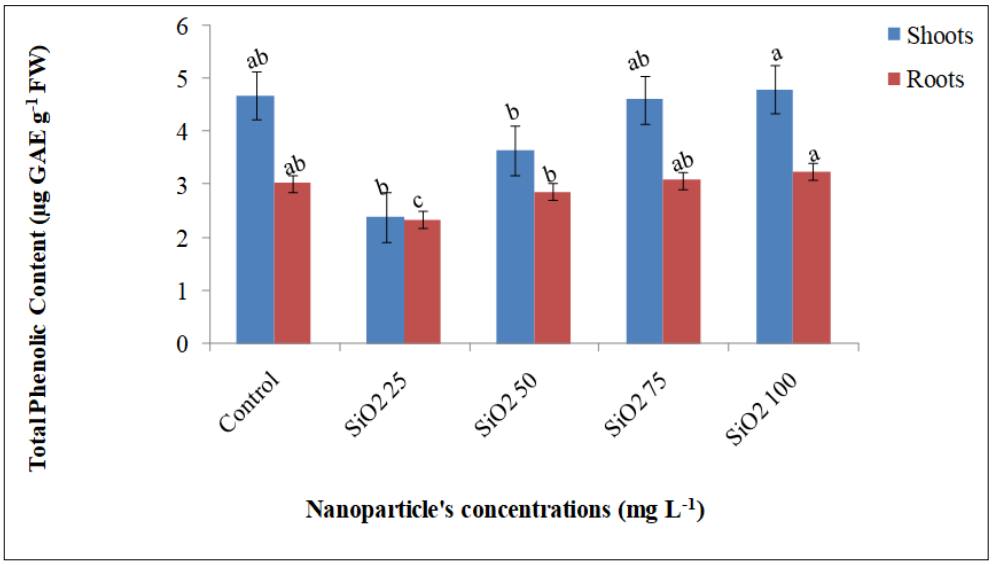


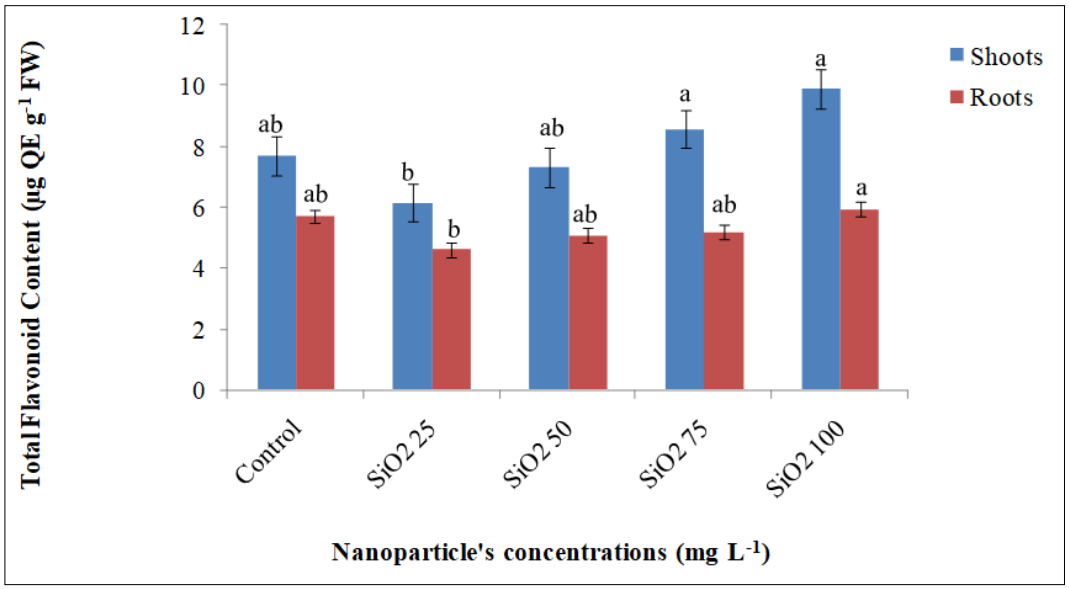
Fig 2: Effect of silica nano-treatmentson photosynthetic pigments (chlorophyll and total carotenoid content) in shoots of 14 days grown Indian mustard seedling

The data represented as mean ± SE and letters above the mean bar indicate differential significance at $P \leq 0.05$, as determined by the Tukey–Kramer post hoc multiple comparison test.

SiO_2 NP priming differentially modulated secondary metabolites and antioxidant activity in *Brassica juncea*in a dose-dependent way. Total phenolics and flavonoids (Fig. 3) declined at 25 mg L^{-1} but increased progressively at 50-100 mg L^{-1}, with maximum accumulation at 100 mg L^{-1} in both shoots and roots.DPPH radical scavenging activity (Fig. 4) similarly peaked at 100 mg L^{-1}, indicating enhanced antioxidant capacity. Overall, higher concentrations (75-100 mg L^{-1}) stimulated secondary metabolism and redox defense, whereas 25 mg L^{-1} was suboptimal.Overall, optimal SiO_2 NP concentrations (75-100 mg L^{-1}) enhanced phenolic and flavonoid biosynthesis along with antioxidant potential, suggesting stimulation of the plant's redox defense system. In contrast, 25 mg L^{-1} appeared suboptimal, leading to reduced metabolite accumulation. These findings indicate that higher SiO_2 NP doses promote secondary metabolism and antioxidative competence, contributing to improved physiological resilience and seedling vigor (Hasanaklou *et al.,* 2023; Zahedi *et al.,* 2023; Dudi *et al.,* 2025) [9, 16, 32].



(A)



(B)

Fig 3: Effect of SiO_2 NPs on (A) total phenolic content and (B) total flavonoid content in shoots and roots of 14 days grown Indian mustard seedling

The data represented as mean ± SE and letters above the mean bar indicate differential significance at $P \leq 0.05$, as determined by the Tukey–Kramer post hoc multiple comparison test.

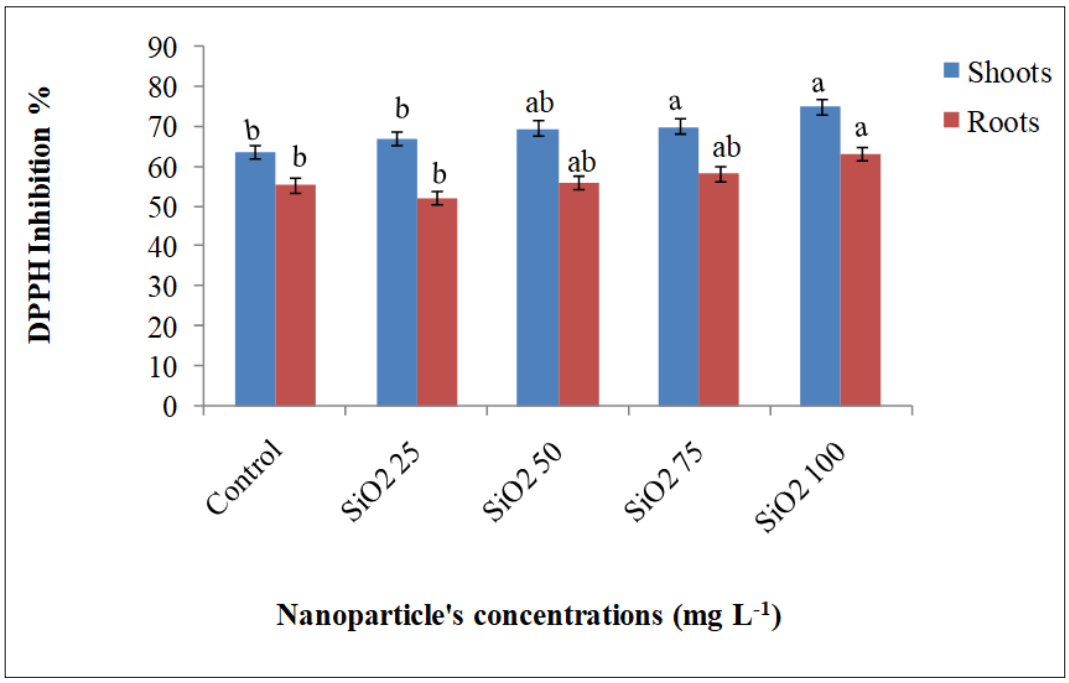


Fig 4: Effect of SiO_2 NPs on DPPH free radical scavenging efficiency in shoots and roots of 14 days grown Indian mustard seedling

The data represented as mean ± SE and letters above the mean bar indicate differential significance at $P \leq 0.05$, as determined by the Tukey–Kramer post hoc multiple comparison test.

SiO_2NP priming significantly altered antioxidant enzyme activities in *Brassica juncea*, indicating a strong concentration-dependent response in both shoots and roots (Table 3). SOD activity increased progressively with concentration, reaching a maximum at 100 mg L^{-1} (601.28 units g^{-1} FW in shoots; 702.56 units g^{-1}FW in roots), significantly higher than the control. Moderate enhancement was observed at 50 mg L^{-1}, while 75 mg L^{-1} showed comparatively lower root activity.CAT activity exhibited substantial stimulation at 100 mg L^{-1}, with shoot and root values (263.88 and 288.45 µmol g^{-1} FW min^{-1}, respectively) markedly exceeding control levels. Lower concentrations(25-75 mg L^{-1}) produced moderate increases, but none matched the response at 100 mg L^{-1}.GPX activity also followed an increasing trend, peaking at 100 mg L^{-1} (9.58 in shoots; 9.08 µmol g^{-1} FW min^{-1} in roots). Although 50 mg L^{-1} enhanced GPX activity, maximal induction was clearly observed at the highest concentration.Overall, SiO_2 NP priming at 100 mg L^{-1} strongly up-regulated key antioxidant enzymes (SOD-CAT-GPX cascade), indicating enhanced reactive oxygen species (ROS) detoxification and improved redox homeostasis. Lower concentrations induced partial activation, whereas optimal dosing (100 mg L^{-1}) provided the most pronounced enhancement of enzymatic

defense mechanisms, contributing to improved physiological performance and stress resilience (Emamverdian *et al.,* 2020; Siddiqui *et al.,* 2020; Vanitha *et al.,* 2024) [10, 27, 29].

Table 3: Impact of SiO_2NPs treatment on SOD activity, catalase activity, and GPX activity in shoots and roots of 14-day old Indian mustard seedlings

Treatment	Concentration (mg L^{-1})	SOD Activity (Units g^{-1} FW) (Mean ± SE)		CAT Activity (µmol per g FW min^{-1}) (Mean ± SE)		GPX Activity (µmol per g FW min^{-1}) (Mean ± SE)	
		Shoot	Root	Shoot	Root	Shoot	Root
SiO_2 NPs	0	421.16 ± 1.03^{b}	536.20 ± 3.67^{bc}	113.16 ± 2.95^{c}	157.11 ± 9.50^{b}	0.74 ± 0.06^{c}	1.96 ± 0.03^{c}
	25	439.14 ± 2.33^{b}	559.13 ± 4.31^{bc}	138.43 ± 5.66^{b}	178.60 ± 5.44^{b}	1.47 ± 0.03^{c}	6.34 ± 0.03^{b}
	50	597.21 ± 1.19^{ab}	578.75 ± 2.16^{c}	147.08 ± 6.45^{c}	238.15 ± 4.36^{b}	6.79 ± 0.10^{c}	8.47 ± 0.25^{b}
	75	498.39 ± 2.43^{ab}	419.63 ± 4.34^{b}	136.17 ± 7.18^{ab}	187.68 ± 13.3^{ab}	4.04 ± 0.07^{b}	5.31 ± 0.05^{a}
	100	601.28 ± 1.77^{a}	702.56 ± 7.33^{a}	263.88 ± 3.58^{a}	288.45 ± 17.75^{a}	9.58 ± 0.26^{a}	9.08 ± 0.07^{a}

Different letters in superscript indicate differential significance within a column at $P \leq 0.05$ based on the post hoc multiple comparison Tukey–Kramer tests.

Principal component analysis (PCA) revealed clear associations among antioxidant enzymes, DPPH activity, and SVI in both shoots and roots under SiO_2 NP priming (Fig. 5). In shoots, PC1 explained 98.81% of the total variance (PC2: 5.15%), with SOD, GPX, DPPH, and total phenolics clustering strongly along PC1, while CAT loaded more on PC2, indicating a partially independent role; SVI aligned positively with PC1, confirming that enhanced antioxidant capacity was closely linked to improved vigor. In roots, PC1 (77.43%) and PC2 (22.41%) showed greater dispersion, with SOD contributing most strongly to variability, followed by CAT and GPX; SVI was positively associated with PC1 but negatively with PC2, suggesting root vigor was mainly driven by antioxidant enhancement along PC1. Overall, SiO_2 NP priming promoted a coordinated antioxidative response correlated with seedling performance, with shoots exhibiting more synchronized defense regulation and roots showing greater tissue-specific variability.

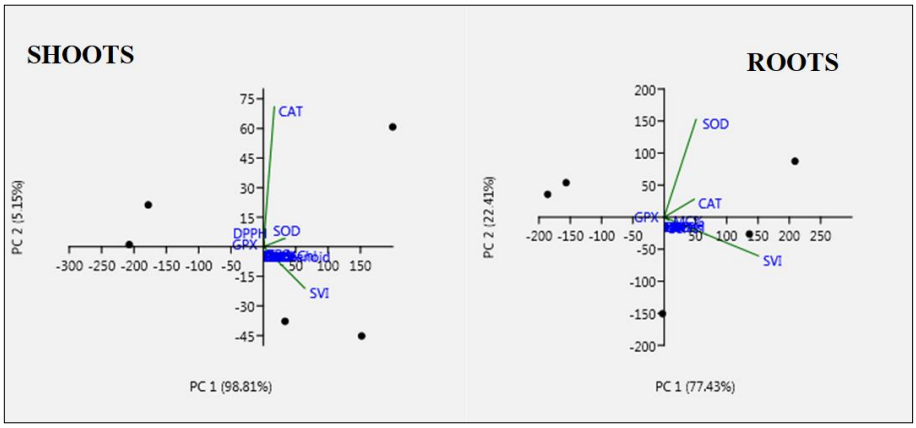


Fig 5: Principal Component Analysis (PCA) of biochemical datasets in nano-treated shoots and roots of Indian mustard seedlings

The correlation analysis of shoot and root attributes under SiO_2 NP priming (Fig. 6) revealed a highly coordinated relationship among growth traits, photosynthetic pigments, secondary metabolites, and antioxidant defense systems, indicating an integrated physio-biochemical response.Across both tissues, SVI showed strong positive correlations with growth parameters as shoot length (SL), root length (RL), moisture content (MC%), and antioxidant enzymes (SOD, CAT, GPX). The association was particularly strong with MC% ($r \approx 0.95$ in both tissues) and with SOD/CAT activities, demonstrating that improved hydration status and enhanced enzymatic antioxidant defense were major contributors to overall seedling performance. Growth parameters (SL, RL, and leaf numbers; LN) were strongly interrelated ($r > 0.80$), confirming synchronized seedling development. In both shoots and roots, GPX exhibited strong correlations with growth traits (especially LN and RL), indicating its central role in regulating oxidative balance during tissue expansion. CAT and SOD also showed positive intercorrelations, reflecting coordinated enzymatic detoxification of reactive oxygen species (ROS).Photosynthetic pigments (chl a, chl b, total chl, and carotenoids) were highly positively correlated ($r > 0.90$), particularly in shoots, confirming synchronized regulation of the photosynthetic apparatus. These pigments also showed strong associations with total phenolic content (TPC) and total flavonoid content (TFC), suggesting that enhanced secondary metabolism paralleled improvements in photosynthetic capacity.Non-enzymatic antioxidant parameters (TPC, TFC, and DPPH activity) were strongly correlated with each other ($r \approx 0.75–0.95$) in both shoots and roots. DPPH activity also showed positive associations with enzymatic antioxidants, especially GPX, demonstrating a tightly linked enzymatic and non-enzymatic defense network.Comparatively, shoots exhibited more compact and stronger intercorrelations among pigments and antioxidant traits, indicating a highly synchronized metabolic adjustment. Roots, while also strongly correlated, displayed slightly greater variability, suggesting tissue-specific modulation of antioxidant enzymes, particularly CAT and GPX. Overall, the correlation analysis clearly indicated that SiO_2 NP priming promotes an integrated antioxidative-

growth network in both shoots and roots. Enhanced ROS-scavenging capacity (SOD, CAT, GPX), elevated phenolic metabolism (TPC, TFC), improved radical scavenging activity (DPPH), and better moisture retention collectively contributed to enhanced photosynthetic efficiency and biomass accumulation. These strong positive inter-relationships confirm that oxidative stress mitigation and metabolic coordination are the principal mechanisms underlying improved seedling growth under silica nano-priming.

SHOOTS

	SL	RL	LN	Nodes	Ger %	SVI	FW	DW	MC%	Chl a	Chl b	Total Chl	Carotenoid	TPC	TFC	DPPH	SOD	CAT	GPX
SL	1																		
RL	0.855563	1																	
LN	0.916722	0.798238	1																
Nodes	0.012581	-0.0944	-0.04365	1															
Ger %	0.54298	0.088192	0.376846	0.511204	1														
SVI	0.94751	0.696644	0.809621	0.257527	0.771932	1													
FW	0.043236	-0.16525	0.274192	0.66724	0.379988	0.174921	1												
DW	0.017871	-0.36877	0.065555	0.758066	0.721626	0.280095	0.839943	1											
MC%	0.892258	0.554236	0.731779	0.048363	0.802615	0.946157	0.032032	0.237229	1										
Chl a	0.232355	-0.27394	0.114576	0.482449	0.930501	0.498864	0.443618	0.830093	0.591559	1									
Chl b	0.118595	-0.40273	0.068065	0.388403	0.845244	0.367742	0.493248	0.841396	0.49272	0.976622	1								
Total Chl	0.172029	-0.34538	0.090026	0.434278	0.889722	0.430685	0.47313	0.841086	0.541428	0.993183	0.995022	1							
Carotenoid	0.624582	0.147233	0.494865	0.0101	0.861734	0.742949	0.112253	0.429078	0.898868	0.806578	0.77479	0.794067	1						
TPC	0.075868	-0.38452	-0.09758	0.545056	0.875277	0.374155	0.347948	0.798059	0.463118	0.969937	0.930738	0.954365	0.691303	1					
TFC	0.455582	-0.0668	0.380463	0.301812	0.93597	0.652417	0.422854	0.731732	0.76099	0.949844	0.936252	0.948039	0.928907	0.850974	1				
DPPH	0.880451	0.549153	0.890822	-0.05062	0.665873	0.867644	0.258474	0.274409	0.908349	0.488537	0.457876	0.474773	0.830563	0.285347	0.727464	1			
SOD	0.93335	0.743273	0.876288	0.353907	0.682367	0.964071	0.349208	0.324712	0.840952	0.397597	0.27668	0.334358	0.595961	0.254402	0.556563	0.838795	1		
CAT	0.640851	0.280743	0.798639	0.099786	0.599812	0.657045	0.616951	0.53087	0.669774	0.532616	0.570026	0.556023	0.694996	0.31319	0.735523	0.894768	0.70652	1	
GPX	0.900995	0.612562	0.890501	0.291292	0.748018	0.94376	0.440053	0.435028	0.875489	0.523864	0.445114	0.48423	0.722246	0.350319	0.702748	0.92838	0.968014	0.85307	1

ROOTS

	SL	RL	LN	Nodes	Ger%	SVI	FW	DW	MC%	TPC	TFC	DPPH	SOD	CAT	GPX
SL	1														
RL	0.855563	1													
LN	0.916722	0.798238	1												
Nodes	0.012581	-0.0944	-0.04365	1											
Ger%	0.54298	0.088192	0.376846	0.511204	1										
SVI	0.94751	0.696644	0.809621	0.257527	0.771932	1									
FW	0.043236	-0.16525	0.274192	0.66724	0.379988	0.174921	1								
DW	0.017871	-0.36877	0.065555	0.758066	0.721626	0.280095	0.839943	1							
MC%	0.892258	0.554236	0.731779	0.048363	0.802615	0.946157	0.032032	0.237229	1						
TPC	0.299704	-0.17001	0.130916	0.570603	0.96373	0.576629	0.401591	0.804492	0.631596	1					
TFC	0.023511	-0.42444	0.030785	0.655447	0.785575	0.296506	0.731956	0.978808	0.316926	0.877739	1				
DPPH	0.600732	0.107508	0.55299	0.294472	0.928474	0.760622	0.463668	0.693222	0.832315	0.86047	0.763222	1			
SOD	0.345183	0.211114	0.636208	0.362736	0.245983	0.338042	0.878901	0.552415	0.189089	0.1528	0.435879	0.443801	1		
CAT	0.850858	0.588916	0.920945	0.267884	0.648545	0.864523	0.545548	0.444931	0.779388	0.45821	0.410382	0.779413	0.75233	1	
GPX	0.92061	0.862883	0.992309	-0.05823	0.303755	0.793003	0.209278	-0.02525	0.693885	0.047462	-0.07121	0.460908	0.591048	0.880272	1

Fig 6: Correlation analysis of morphological and biochemical traits in nano-treated shoots and roots of Indian mustard seedlings

Conclusion

Seed priming with SiO_2 NPs significantly enhanced growth, biomass, and physio-biochemical performance of *Brassica juncea* in a concentration-dependent manner, with 100 mg L^{-1}emergingas the most effective dose. Optimal treatments (50-100 mg L^{-1}) improved germination, seedling vigor, shoot and root elongation, fresh and dry weight, and tissue moisture content, indicating better early establishment and water relations. Higher concentrations (75-100 mg L^{-1}) also increased chlorophyll and carotenoid levels, stimulated phenolic and flavonoid accumulation, enhanced DPPH radical scavenging activity, and up-regulated antioxidant enzymes (SOD, CAT, GPX), reflecting strengthened photosynthetic efficiency and redox homeostasis. In contrast, 25 mg L^{-1} was suboptimal. Overall, SiO_2 NP priming at 100 mg L^{-1} effectively promoted growth, metabolic activation, and antioxidant defense, highlighting its potential as a sustainable nano-priming strategy for improved crop establishment and stress resilience. Principal component analysis (PCA) revealed that variation among treatments was primarily governed by the coordinated enhancement of antioxidant enzyme activities and seedling vigor, reflecting a dominant antioxidant–growth axis with comparatively limited root-specific influence. Correlation analysis confirmed strong positive linkages among growth, pigments, secondary metabolites, and ROS-scavenging systems. Overall, silica nano-priming enhanced seedling performance through integrated redox regulation and metabolic activation.

Acknowledgements

Pratibha Dwivedi (Project Assistant), Shamshadul Haq (Post-Doctoral Fellow), and Ashutosh Pathak (Post-Doctoral Fellow) sincerely express their gratitude to the Ministry of Human Resource Development, Department of Higher Education, Government of India, for providing financial support under RUSA 2.0, Thematic Project III, sanctioned to Prof. Sumita Kachhwaha (F.35-532/PP/2021/1203). The authors are grateful to ICAR–Directorate of Rapeseed-Mustard Research (DRMR), Bharatpur, Rajasthan, for providing mustard seeds. The authors further acknowledge the facilities provided by the Department of Botany, University of Rajasthan, Jaipur, which were instrumental for the smooth conduct of the research.

Declaration of competing interest

The authors declare no competing financial interests or personal relationships that could have influenced the work presented in this paper.

References

1. Abdul-Baki AA, Anderson JD. Vigor determination in soybean seed by multiple criteria 1. Crop science,1973:13:630-633. https://doi.org/10.2135/cropsci1973.0011183X001300060013x
2. Aboellail G, Mahdy A, Badr Eldin RM. Use of silicon nanoparticles as a seed-priming solution for increasing the germination and growth parameters of faba bean (*Vicia faba* L.) seedling under salinity stress. Alexandria Journal of Agricultural Sciences,2023:68:273-287. https://doi.org/10.21608/alexja.2023.237309.1046
3. Aebi H. Catalase *in vitro*. Methods in Enzymology,1984:105:121–126. https://doi.org/10.1016/s0076-6879(84)05016-3

4. Al-Tabbal J, Al-Harahsheh M, Al-Zou'by J, Al-Zboon K, Al-Bakour Al-Rawashda K. Silica nanoparticle: eco-friendly waste having potential for seed germination of wheat (*Triticum turgidum* L. var. Sham) under salt stress conditions. Waste and Biomass Valorization,2024:15:2973-2987. https://doi.org/10.1007/s12649-023-02338-7
5. Alsaeedi A, Elgarawany M, El-Ramady H, Alshaal T, Al-Otaibi A. Application of silica nanoparticles induces seed germination and growth of cucumber (*Cucumis sativus*). Met Environ Arid Land Agric Sci,2019:28:57-68. https://doi.org/10.4197/Met.28-1.6
6. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry,1971:44:276-287. https://doi.org/10.1016/0003-2697(71)90370-8
7. Bemani E, Ghanati F, Boroujeni LY, Khatami F. Antioxidant activity, total phenolics and taxol contents response of hazel (*Corylus avellana* L.) cells to benzoic acid and cinnamic acid. Notulae Botanicae Horti Agrobotanici Cluj-Napoca,2012:40:69-73. https://doi.org/10.15835/nbha4017404
8. Dhingra P, Sharma S, Singh KH, Kushwaha HS, Barupal JK, Haq S, *et al*. Seed priming with carbon nanotubes and silicon dioxide nanoparticles influence agronomic traits of Indian mustard (*Brassica juncea*) in field experiments. Journal of King Saud University-Science,2022:34:102067. https://doi.org/10.1016/j.jksus.2022.102067
9. Dudi P, Verma L, Pathak A, Haq S, Kachhwaha S. Enhancing agronomic traits of finger millet (*Eleusine coracana* (L.) Gaertn.) through seed nano-priming using silicon dioxide nanoparticles (SiO_2 NPs). Journal of Stored Products Research,2025:112:102654. https://doi.org/10.1016/j.jspr.2025.102654
10. Emamverdian A, Ding Y, Mokhberdoran F, Xie Y, Zheng X, Wang Y. Silicon dioxide nanoparticles improve plant growth by enhancing antioxidant enzyme capacity in bamboo (*Pleioblastus pygmaeus*) under lead toxicity. Trees,2020:34:469-481. https://doi.org/10.1007/s00468-019-01929-z
11. Fernández-García N, Carvajal M, Olmos E. Graft union formation in tomato plants: peroxidase and catalase involvement. Annals of Botany,2004:93:53-60. https://doi.org/10.1093/aob/mch014
12. Hammer Ø, Harper DA, Ryan PD. PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica,2001:4:1-9.
13. Haq S, Kumari D, Dhingra P, Kothari SL, Kachhwaha S. Display of distinct endogenous phenolic contents in different rice ecotypes grown under normal conditions: a potent biomarker system for sensing growth, stress and adaptability. Research Journal of Biotechnology,2022:17:15-26.
14. Haq S, Kumari D, Dhingra P, Kothari S, Kachhwaha S. Variant biochemical responses: intrinsic and adaptive system for ecologically different rice varieties. Journal of Crop Science and Biotechnology,2021:24:279-292. https://doi.org/10.1007/s12892-020-00076-z
15. Haq S, Verma R, Poonia R, Dhingra P, Kothari S, Kachhwaha S. Effect of multiwalled carbon nanotube (MWCNT) on rice (Pusa Basmati 1121): insight into their physiological and biochemical parameters during growth and development. Int J Pure Appl Biosci,2019:7:423–433. https://doi.org/10.18782/2320-7051.7419
16. Hasanaklou NT, Mohagheghi V, Hasanaklou HT, Ma'mani L, Malekmohammadi M, Moradi F, *et al*. Seed nano-priming using silica nanoparticles: effects in seed germination and physiological properties of *Stevia rebaudiana* Bertoni. Chemical and Biological Technologies in Agriculture,2023:10:96. https://doi.org/10.1186/s40538-023-00445-0
17. Hatami M, Khanizadeh P, Bovand F, Aghaee A. Silicon nanoparticle-mediated seed priming and *Pseudomonas* spp. inoculation augment growth, physiology and antioxidant metabolic status in *Melissa officinalis* L. plants. Industrial Crops and Products,2021:162:113238. https://doi.org/10.1016/j.indcrop.2021.113238
18. Hiscox JD, Israelstam GF. A method for the extraction of chlorophyll from leaf tissue without maceration. Canadian Journal of Botany,1979:57:1332-1334. https://doi.org/10.1139/b79-163
19. Lichtenthaler HK, Wellburn AR. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochemical Society Transactions,1983:11:591-592. https://doi.org/10.1042/bst0110591
20. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant,1962:15:473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x
21. Nile SH, Thiruvengadam M, Wang Y, Samynathan R, Shariati MA, Rebezov M, *et al*. Nano-priming as emerging seed priming technology for sustainable agriculture-recent developments and future perspectives. Journal of Nanobiotechnology,2022:20:254. https://doi.org/10.1186/s12951-022-01423-8
22. Pathak A, Haq S, Meena N, Dwivedi P, Kothari SL, Kachhwaha S. Multifaceted role of nanomaterials in modulating *in vitro* seed germination, plant morphogenesis, metabolism and genetic engineering. Plants,2023:12:3126. https://doi.org/10.3390/plants12173126
23. Rai-Kalal P, Tomar RS, Jajoo A. Seed nanopriming by silicon oxide improves drought stress alleviation potential in wheat plants. Functional Plant Biology,2021:48:905-915. https://doi.org/10.1071/FP21079
24. Reeb JE, Milota MR. Moisture content by the oven-dry method for industrial testing, 1999. http://hdl.handle.net/1957/5190
25. Sharma A, Garg M, Sharma HK, Rai P. Mustard and its products. In: Handbook of Spices in India: 75 Years of Research and Development. Springer, 2024, 2385-2451. https://doi.org/10.1007/978-981-19-3728-6_33
26. Shekhawat K, Rathore S, Premi O, Kandpal B, Chauhan J. Advances in agronomic management of Indian mustard (*Brassica juncea* (L.) Czernj. & Cosson): an overview. International Journal of Agronomy,2012:2012:408284. https://doi.org/10.1155/2012/408284
27. Siddiqui H, Ahmed KBM, Sami F, Hayat S. Silicon nanoparticles and plants: current knowledge and future perspectives. Sustainable Agriculture Reviews,2020:41:129-142. https://doi.org/10.1007/978-3-030-33996-8_7

28. Simko I, Peng H, Hasegawa DK. Foliar application of silicon dioxide improves lettuce growth parameters, disease resistance, post-harvest quality, and modifies mineral composition. Journal of Soil Science and Plant Nutrition, 2026, 1-17. https://doi.org/10.1007/s42729-025-03000-y
29. Vanitha C, Kathiravan M, Umarani R, Sathiya K, Menaka C, Yuvaraj M, *et al*. Seed priming with nano silica alleviates drought stress through regulating antioxidant defense system and osmotic adjustment in soybean (*Glycine max* L.). Silicon,2024:16:2157-2170. https://doi.org/10.1007/s12633-023-02826-4
30. Verma A, Choudhary R, Singh VJ, Kachhwaha S, Kothari SL, Jain R. Assessment of the effect of multi-walled carbon nanotubes (-OH functionalized) on growth characteristics and biochemical profile of *Brassica juncea* (L.) Czern. & Coss. Environmental Science and Pollution Research,2025:32:1345-1360. https://doi.org/10.1007/s11356-024-35681-w
31. Wellburn AR. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. Journal of Plant Physiology,1994:144:307-313. https://doi.org/10.1016/S0176-1617(11)81192-2
32. Zahedi SM, Hosseini MS, Hoveizeh NF, Kadkhodaei S, Vaculík M. Comparative morphological, physiological and molecular analyses of drought-stressed strawberry plants affected by SiO2 and SiO2-NPs foliar spray. Scientia Horticulturae,2023:309:111686. https://doi.org/10.1016/j.scienta.2022.111686