



RESEARCH ARTICLE

Seed priming with g-C₃N₄/SiO₂ nanocomposites enhances germination, seedling vigour and salt stress tolerance in rice (*Oryza sativa* L.)

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Received: 11 April 2026; Accepted: 27 July 2026; Available online: Version 1.0: 30 August 2026

Cite this article: Jayasoorya R, Krishnaprabu N, Jawahar V, Kumar TP. Seed priming with g-C₃N₄/SiO₂ nanocomposites enhances germination, seedling vigour and salt stress tolerance in rice (*Oryza sativa* L.). Plant Science Today. 2026; 13(sp5): 1-12. <https://doi.org/10.14719/pst.14955>

Abstract

Nanotechnology holds the potential to enhance agricultural production through various engineered nanomaterials. The use of nanoparticles in agriculture is prevalent for augmenting crop growth under abiotic stress conditions. However, metal and metal oxide nanoparticles are highly toxic due to their acute and chronic toxicity and their persistence in the environment. The metal-free alternatives such as g-C₃N₄ (graphitic carbon nitride) and SiO₂ (silicon dioxide) have been explored in various research fields, including agriculture. The g-C₃N₄ and SiO₂ were synthesised using thermal polymerisation and green synthesis methods, respectively. The comprehensive characterisation of the nanocomposites was carried out using X-ray diffraction (XRD), Fourier-transform infrared (FTIR) spectroscopy, ultraviolet-visible (UV-Vis) spectroscopy, scanning electron microscopy (SEM), field-emission scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (FESEM-EDS) and dynamic light scattering (DLS) analyses. Furthermore, we have focused on the nanopriming technique by using g-C₃N₄/SiO₂ to mitigate salt stress in *Oryza sativa* L. seeds. The treatments include an absolute control (no treatment), positive control (NaCl), 5, 10, 15, 20 and 25 ppm g-C₃N₄/SiO₂ nanocomposites. All treatments except the absolute control were maintained in saline conditions at 80 mM using NaCl. The germination parameters, such as germination percentage, mean germination time, germination rate index, coefficient of velocity of germination, vigour index, dry weight, root and shoot length, were influenced in treated seeds. Among the treatments, 20 ppm g-C₃N₄/SiO₂ nanocomposite seed priming resulted in the highest germination percentage (93.3 %), germination rate index (2.6), coefficient of velocity of germination (18.21), mean seedling length (34.56 cm), vigour index (3224), root length (22.55 cm), shoot length (12 cm), while reducing mean germination time (5.50 days). Overall, the metal-free nanocomposites were beneficial in mitigating salt stress in *O. sativa* seeds and their application may stimulate the plant defence mechanism.

Keywords: graphitic carbon nitride; metal free; nano priming; nanotechnology; saline stress; silicon dioxide

Introduction

Recently, the primary goal in agriculture has been to increase food production without compromising environmental safety (1). Since the Green Revolution, chemical utilisation in agriculture has been highly prevalent. While addressing global hunger and improving food security, it indirectly poses severe health disorders due to the injudicious use of synthetic fertilisers and pesticides (2). In addition, rising concerns about global agriculture are driven by the combined effects of a burgeoning population, climate change, shrinking arable land and soil degradation (3). Rice (*Oryza sativa* L.) is a widely cultivated cereal crop that contributes to the dietary requirements of more than half of the world's population. Specifically, more than 90 % of production and consumption was accounted for by various nations in Asia and it plays a critical role in caloric and protein consumption. However, the sustainability of rice production was hindered by environmental challenges, including drought, salinity and other climatic factors (4). Among the existing constraints in agriculture, salinity has emerged as a severe stress, causing poor

crop productivity. High concentrations of sodium and chloride ions induce salinity stress in soil and water, leading to osmotic stress, ion toxicity and nutrient imbalance (5).

In the modern world, there is a high chance of uncertainty in agriculture owing to climate change and reliance on huge external inputs. Nanotechnology-mediated novel agricultural inputs are promising in enhancing crop production and protection (6). Notably, nanomaterials can be used as an alternative to bulk chemical fertilisers in promoting plant growth (7). Nanomaterials fall in the nanoscale range, ranging from 1 to 100 nm and possess a high surface-to-volume ratio (8). Unlike conventional chemical fertilisers, which suffer from leaching, volatilisation and runoff, nanomaterials can improve nutrient delivery and plant uptake, thereby reducing chemical requirements. In this context, nanotechnology has emerged as a promising tool for mitigating salinity stress and efficient resource utilisation. Nanopriming is an emerging strategy that significantly improves germination capacity and enables stress resilience. Compared with conventional seed

priming techniques such as hydro-priming, hormonal priming, halo-priming and osmo-priming, nanoprimer provides significant results due to better penetration and interaction of nanomaterials with seed tissues (9, 10).

Nanomaterials, including metal/metal oxide and carbon-based materials, are widely used for enhancing seed germination and overall productivity under salinity stress conditions (11). In recent years, a wide range of nanomaterials, including gold (Au) (12), copper oxide (CuO) (13), silver (Ag) (14), iron (Fe) (15), cerium oxide (CeO₂) (16), zinc oxide (ZnO) (17), titanium dioxide (TiO₂) (18), magnesium oxide (MgO) (19), selenium (Se) (20), silicon dioxide (SiO₂) (21) and carbon-based nanomaterials (22), have been investigated for improving stress tolerance. Overall, these nanomaterials improve seed germination, seed vigour and photosynthetic performance by regulating nutrient availability and antioxidant enzyme activity (23).

Meanwhile, nanocomposite materials exhibit superior physicochemical properties and show excellent outcomes in plants compared to pristine nanomaterials (24). Nanocomposites are materials with multiple matrix phases, where at least one of the phases falls at the nanoscale with less than 100 nm (8). In addition, nanocomposites have unique intrinsic properties, including thermal, electrical, mechanical and chemical properties. Its application in agriculture can influence crop growth and development (24). Despite their advantages, metal and metal oxide-based nanomaterials, including metal-based nanocomposites, pose hazardous impacts on the ecosystem due to environmental toxicity (25). These materials pose a risk to living organisms, often associated with acute and chronic toxicity through bioaccumulation and long-term persistence (26).

To overcome these limitations, interest has been gained in metal-free nanomaterials. graphitic carbon nitride (g-C₃N₄) is a carbon and nitrogen-rich nanomaterial, ensuring a metal-free nature (27). It has emerged as a promising material because of its unique physicochemical properties, such as visible-light activity, non-metallic, thermal and chemical stability (28). In addition, it is synthesised from inexpensive nitrogen precursors such as melamine, urea, thiourea, cyanamide and dicyanamide, using a simple thermal polymerisation procedure (29). The g-C₃N₄ has been extensively studied in various fields, including photocatalysis, energy generation and environmental remediation, but in recent times it has gained interest in agriculture owing to its properties (28).

Furthermore, the addition of SiO₂, another non-metallic nanomaterial, to g-C₃N₄ enhances its functional properties and agricultural applicability. Silicon dioxide nanoparticles are widely used to improve crop growth, especially under abiotic stress conditions like salinity, drought and heavy metal toxicity (30). In this study, SiO₂ nanomaterials were synthesised via a green synthesis approach. Recently, the green synthesis method for producing nanoparticles has received great response among the scientific community (31). Green synthesis methods employ plant extracts and microbial metabolites that act as reducing agents, stabilisers and capping agents, thereby introducing functional groups on the nanoparticle surface to enhance stability (32). Plant extracts are promising for green synthesis of nanomaterials due to the availability of phytochemicals such as polyphenols, glycosides, flavonoids, proteins and terpenoids. Thus, the eco-friendly synthesis of SiO₂ nanomaterial ensures sustainable agricultural applicability (33, 34).

Studies have reported that nanocomposites elevate overall photosynthetic performance and antioxidant activity in plants (35). The application (4–32 µg mL⁻¹) of molybdenum disulfide (MoS₂) sheets loaded with copper (Cu) nanoparticles significantly enhanced rice growth, increased molybdenum (Mo) accumulation and chlorophyll content. Moreover, the nanocomposite activated key antioxidant enzymes, conferring resistance against abiotic stress. Even though the nanocomposite exhibited plant growth-promoting effects and effectively suppressed the phytopathogen *Xanthomonas oryzae* (36), its broad-spectrum activity raises concerns. Likewise, while *X. oryzae* is a disease-causing bacterium, its antimicrobial action may inadvertently affect non-target beneficial microorganisms, potentially disrupting ecological balance. Copper has been reported for its antimicrobial efficacy, but it poses risks of excessive soil accumulation when applied indiscriminately, leading to potential metal toxicity.

Therefore, selecting nanomaterials with less or zero toxicity ensures ecological safety. The g-C₃N₄/SiO₂ nanocomposites have previously been investigated as photocatalysts for environmental remediation (37). The synergistic effect of g-C₃N₄/SiO₂ results in a larger surface area and enhanced bioactivity. Despite the growing interest in metal-free nanomaterials for sustainable agriculture, the application of nano-metallic nanocomposites (g-C₃N₄/SiO₂) for nanoprimering of *Oryza sativa* L. seeds under salt stress remains unexplored. Therefore, we hypothesised that this nanocomposite could improve germination performance and seedling vigour while enhancing plant stress tolerance mechanisms.

Materials and Methods

Synthesis of graphitic carbon nitride

The thermal polymerisation method was adopted to prepare bulk g-C₃N₄. Likewise, 4 g of melamine was placed in a crucible with the closing lid. The crucible was kept in a muffle furnace for 5 hr at 550 °C (38). After complete polymerisation, the resultant powder was allowed to cool down to room temperature. The obtained product was completely ground using a pestle and mortar for 30 min.

Leaf extract preparation and silicon dioxide synthesis

Green-synthesised SiO₂ nanoparticles were prepared following previously reported methodologies with slight modification (33, 39). For green synthesis, *Mangifera indica* L. leaf was completely shade-dried and finely powdered. Then, 5 g of *M. indica* powder was heated with 100 mL of double-distilled water in a reflux condenser for 1 hr. The final mixture was filtered with Whatman No. 1 filter paper and the extract was tightly sealed and stored in the refrigerator for further use.

For SiO₂ synthesis, sodium metasilicate (Na₂SiO₃) was used as a precursor with a molecular weight of 122.06 g mol⁻¹. Notably, 2 mM Na₂SiO₃ was diluted in 100 mL of distilled water. Then 20 mL of leaf extract was added dropwise to the solution mixture while stirring at 450 rpm on the magnetic stirrer. Further, the solution was added with 0.1 M NaOH to maintain an alkaline pH. The solution with adjusted pH was kept under a reflux condenser for 4 hr. Finally, the mixture was dried completely and the material deposited in the beaker was collected separately. The obtained material was ground using a pestle and mortar. Centrifugation was performed at 8000 rpm for 10 min, repeated three times with distilled water and thrice with ethanol to remove all impurities present in the

nanoparticles. The supernatant was then collected and dried in a hot-air oven until completely dry. Furthermore, the material was calcined in a muffle furnace at 450 °C for 3 hr. Finally, the SiO₂ nanoparticles were stored in an air-tight container for future use.

Synthesis of nanocomposite

The g-C₃N₄/SiO₂ nanocomposite was prepared at a weight ratio of 1 : 1, respectively. The separate g-C₃N₄ and SiO₂ powders were crushed and thoroughly mixed in a pestle and mortar for 30 min. The mixture was kept in a silica crucible and calcinated in the muffle furnace at 400 °C for 3 hr to produce the nanocomposite. The produced nanocomposite was used for characterisation and germination experiments. For better understanding, we have illustrated the synthesis procedure for g-C₃N₄/SiO₂ nanocomposite synthesis in Fig. 1.

Characterisation

Different characterisation techniques were used to analyse the synthesised g-C₃N₄/SiO₂ nanocomposite. The optical absorption properties were investigated using UV-visible spectroscopy. X-ray diffraction (XRD) was used to determine the crystalline structure. Fourier transform infrared spectroscopy (FTIR) was used to determine the functional groups and chemical bonding. Scanning electron microscopy (SEM) and field-emission scanning electron microscopy (FESEM) with elemental analysis by energy-dispersive X-ray spectroscopy (EDS) were used to characterise surface morphology and microstructural features. The dynamic light scattering (DLS) was used to determine the hydrodynamic particle size distribution and surface charge (zeta potential) to assess colloidal stability.

Germination experiment

A laboratory study was conducted at the Department of Agronomy, Vellore Institute of Technology, Vellore, to evaluate the impact of seed priming with synthesised nanocomposites on the germination and growth performance of the rice variety 'TRY 5'. Seeds were surface-sterilised with 2.5 % sodium hypochlorite solution for 5 min and subsequently washed thoroughly with deionised water to remove residual chlorine. Different concentrations of the g-C₃N₄/SiO₂ nanocomposite (5, 10, 15, 20 and 25 ppm) were prepared along with an absolute control (CT; no treatment) and a positive control (PT; NaCl), resulting in a total of 7 treatments arranged in a completely randomised design (CRD). For seed treatment, the required quantity of g-C₃N₄/SiO₂ nanocomposite was weighed and added to deionised water. The ultrasonication was performed for 45 min to disperse the nanocomposite. After that, the required volume was made up by utilising deionised water. Then the sterilised seeds were soaked in the prepared solution for 24 hr under darkness for the respective treatments. The germination test was demonstrated using the between-paper method with 7 treatments and 3 replications. Except for the absolute control, all treatments were maintained at 80 mM NaCl to induce moderate salinity, as in a previous study, which represented low and high levels (40). For each treatment, 15 seeds were used for testing seed germination parameters. Seed germination was conducted under controlled conditions in a germination chamber maintained at 80 % relative humidity and 25 °C throughout the experimental period. According to the International Seed Testing Association (ISTA) protocol for rice seed germination, the first observation was taken on the 5th day and the final observation on the 14th day.

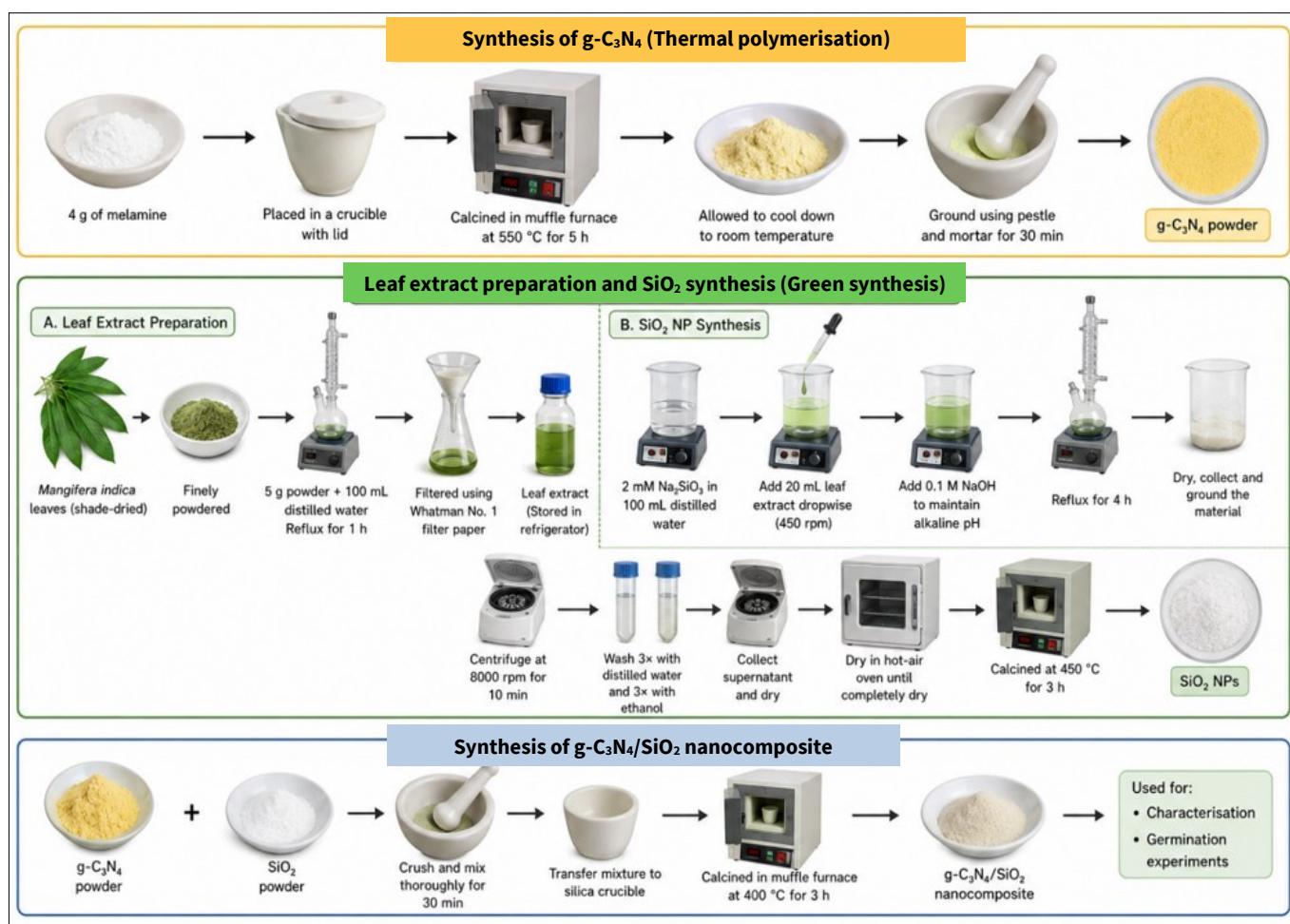


Fig. 1. Depicts the synthesis procedure for the g-C₃N₄/SiO₂ nanocomposite.

The analysis of variance for the growth attributes of rice seeds treated with six different concentrations of nano g-C₃N₄/SiO₂ composite revealed statistically significant differences in germination percentage, mean germination time, germination rate index, coefficient of velocity of germination, mean seedling length, root length, shoot length and vigour index indicating that the application of g-C₃N₄/SiO₂ had a significant effect on these parameters at $p < 0.05$.

Germination percentage

The germination percentage (GP) was calculated as the ratio of the number of seeds that germinated to the total number of seeds sown, expressed as a percentage, followed by previous researchers (41).

Germination percentage (%) =

$$\frac{\text{Number of germinated seed}}{\text{Total number of seed}} \times 100 \quad \text{Eq. (1)}$$

Mean germination time

Mean germination time (MGT) was determined using the equation proposed by previous researchers (42).

$$\text{Mean germination time} = \sum(n \times d)/N \quad \text{Eq. (2)}$$

In this equation, n represents the number of seeds that germinated during a specific time interval (day), d denotes the number of days elapsed from the beginning of the experiment and N refers to the total number of seeds germinated by the end of the experiment.

Coefficient of velocity of germination

The coefficient of velocity of germination (CVG) was calculated using the formula described by previous researchers (43).

$$\text{Coefficient of velocity of germination} = \frac{\sum(N)}{NT} \times 100 \quad \text{Eq. (3)}$$

In this formula, N indicates the number of seeds that germinated on a given day, while T represents the number of days after sowing corresponding to that germination count.

Germination rate index

The germination rate index (GRI) was calculated using the formula as described previously (44).

$$\text{Germination rate index} = \frac{G_1}{1} + \frac{G_2}{2} + \frac{G_3}{3} + \dots + \frac{G_n}{n} \quad \text{Eq. (4)}$$

In this method, G₁, G₂, ..., G_n denote the germination percentage ($\times 100$) recorded on the first, second and subsequent days up to the final (ninth) day, while 1, 2, ..., n represent the corresponding days of the first, second and final germination counts, respectively.

Vigour index

The seedling vigour index (VI) was calculated as the product of germination percentage and total seedling length, following the method described by in previous reports (45). Accordingly, the seedling VI was expressed as:

Seedling vigour index

$$= \text{Germination (\%)} \times \text{Total seedling length (cm)} \quad \text{Eq. (5)}$$

Results and Discussion

Characterisation of nanocomposite

X-ray diffraction analysis

The XRD technique was used to analyse the crystalline structure and phase composition of the synthesised materials. The diffracted patterns of g-C₃N₄, SiO₂ and the g-C₃N₄/SiO₂ nanocomposite are shown in Fig. 2. Pristine g-C₃N₄ exhibited a prominent diffraction peak at 27.5°, corresponding to the (002) plane, which is associated with the interlayer stacking of the conjugated aromatic system of graphitic carbon nitride (46). On the other hand, the SiO₂ displayed a broad diffraction centred at 23°, indicating a predominantly amorphous nature. The XRD pattern of g-C₃N₄/SiO₂ nanocomposite contained characteristic diffraction peaks of both g-C₃N₄ and SiO₂. A slight broadening and reduction in peak intensity compared with pristine g-C₃N₄ suggest interfacial interaction between the two components, indicating the successful formation of g-C₃N₄/SiO₂ (47).

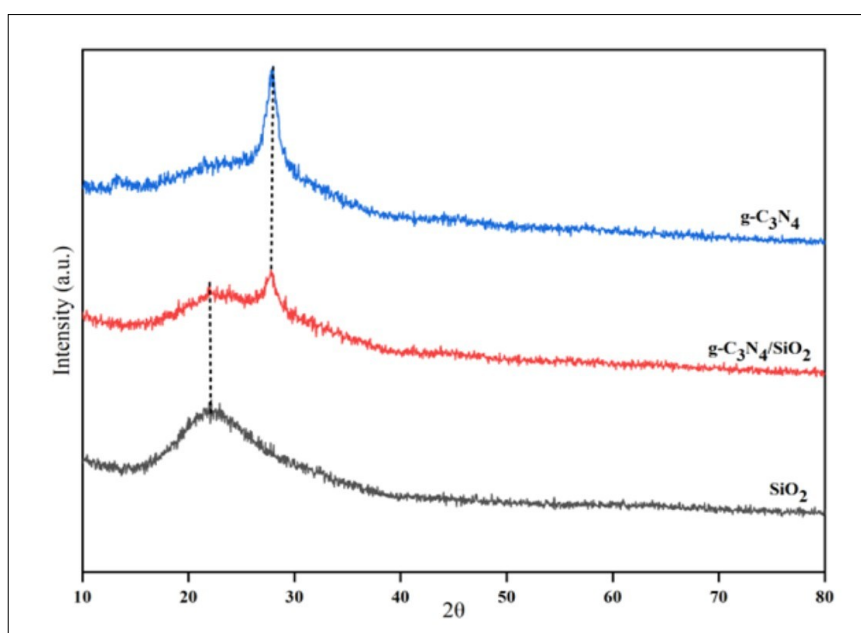


Fig. 2. X-ray diffraction (XRD) patterns of SiO₂, g-C₃N₄/SiO₂ and g-C₃N₄.

Fourier-transform infrared spectroscopy analysis

The FTIR spectra of g-C₃N₄, SiO₂ and the g-C₃N₄/SiO₂ nanocomposite were analysed to determine the presence of functional groups and confirm the successful synthesis of the composite material (Fig. 3). The g-C₃N₄ spectrum exhibited a broad absorption band at 3000–3400 cm⁻¹ (48), attributed to N-H stretching vibrations and adsorbed moisture. The distinct bands seen between 1200–1650 cm⁻¹ were assigned to C-N and C=N stretching vibrations of the heptazine framework, while the peak at ~810 cm⁻¹ was the breathing mode of the triazine ring (49). For SiO₂, the strong absorption in the region at ~1080 cm⁻¹ was attributed to the asymmetric Si-O-Si stretching vibrations. The symmetric stretching and bending vibrations of the Si-O-Si were assigned to the peaks of ~460 cm⁻¹ and ~800 cm⁻¹, respectively (50). The nanocomposite exhibits characteristic bands of both g-C₃N₄ and SiO₂, which were retained. However, a slight shift and overlap of the C-N/C=N and Si-O-Si bands were observed (51). These spectral changes indicate interfacial interaction between g-C₃N₄ and SiO₂, characterised by successful composite formation and the presence of surface functional groups.

Ultraviolet-visible analysis

The UV-Vis absorption spectra of g-C₃N₄, SiO₂ and the g-C₃N₄/SiO₂ nanocomposite are shown in Fig. 4. Pristine g-C₃N₄ exhibited strong absorption in the 300–420 nm region with a maximum absorption peak near 380 nm, attributed to the π - π^* electronic transition within the conjugated C-N heterocyclic structure (52). The SiO₂ showed weak adsorption due to its wide band gap (53). The nanocomposite displayed intermediate absorption behaviour, indicating successful incorporation of SiO₂ into the g-C₃N₄ matrix. The slight reduction in absorption intensity relative to pristine g-C₃N₄ may be attributed to improved dispersion and to the influence of silica on the composite structure (51). In general, the absorption properties support the formation of the nanocomposite.

Scanning electron microscopy analysis

The SEM was employed to examine the surface morphology of g-C₃N₄, SiO₂ and the g-C₃N₄/SiO₂ nanocomposite (Fig. 5). The SEM image of g-C₃N₄ (Fig. 5A) revealed aggregated granular particles forming dense clusters, which can be attributed to the layered

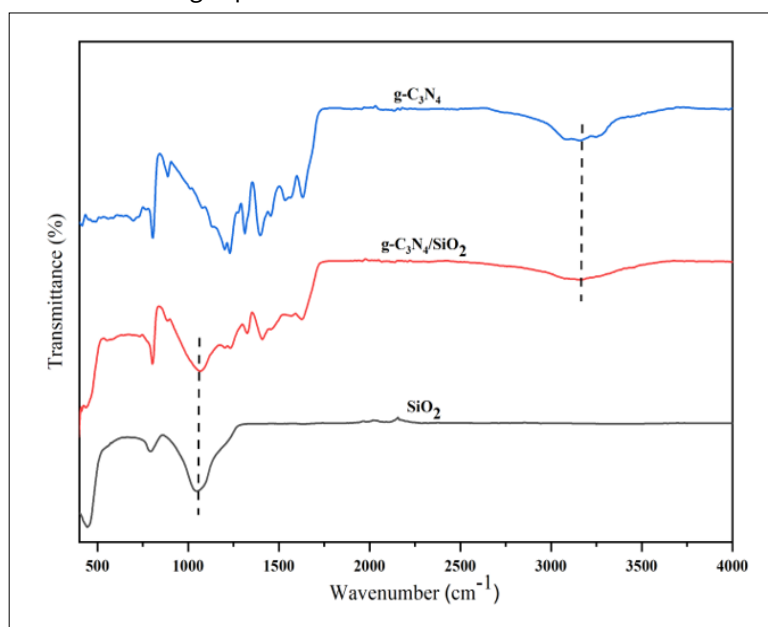


Fig. 3. Fourier-transform infrared (FTIR) spectra of SiO₂, g-C₃N₄/SiO₂ and g-C₃N₄.

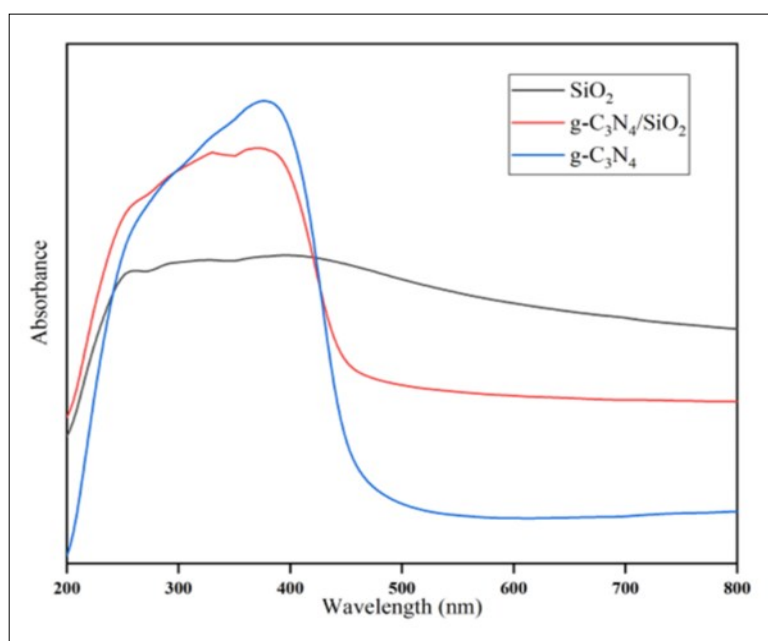


Fig. 4. Ultraviolet-visible (UV-Vis) spectra of SiO₂, g-C₃N₄/SiO₂ and g-C₃N₄.

structure and stacking of $g\text{-C}_3\text{N}_4$. In contrast, green-synthesised SiO_2 nanoparticles (Fig. 5B) exhibited a porous, sponge-like morphology consisting of interconnected nanoscale particles with irregular surfaces and visible voids. The $g\text{-C}_3\text{N}_4/\text{SiO}_2$ nanocomposite (Fig. 5C) displayed a comparatively compact and homogeneous morphology with reduced aggregation. The observed morphological differences between the pristine materials and the nanocomposite suggest the successful incorporation of SiO_2 with the $g\text{-C}_3\text{N}_4$ matrix.

Field-emission scanning electron microscopy analysis

The FESEM coupled with EDS was further employed to investigate the elemental composition and distribution of the nanocomposite (Fig. 6A–F). The FESEM image of the elemental overlap, Fig. 6A, showed a compact nanostructured morphology. The EDS spectrum Fig. 6B confirmed the presence of the major constituent elements, namely carbon (C), nitrogen (N), oxygen (O) and silicon (Si), corresponding to $g\text{-C}_3\text{N}_4$ and SiO_2 . Furthermore, elemental mapping images demonstrate the uniform distribution of C, N, O and Si throughout the nanocomposite structure. The elemental homogeneous dispersion indicates effective incorporation of $g\text{-C}_3\text{N}_4$ and SiO_2 within the matrix, which ascertains the successful formation of the $g\text{-C}_3\text{N}_4/\text{SiO}_2$ nanocomposite.

Dynamic light scattering and zeta potential analysis

Dynamic light scattering analysis showed that the nanocomposite possessed an average hydrodynamic diameter of 416.71 ± 36.28 nm with a polydispersity index (PDI) of 0.296 ± 0.050 , indicating a moderately uniform particle size distribution. Furthermore, the zeta potential ranged from -22.29 to $+8.74$ mV with a mean value of

-11.02 mV. The negative surface charge and moderate zeta potential suggest moderate colloidal stability of the nanocomposite suspension.

Effect of $g\text{-C}_3\text{N}_4/\text{SiO}_2$ nanocomposite on seed germination and growth

The germination and seedling growth parameters of rice were significantly influenced by seed priming with different concentrations of $g\text{-C}_3\text{N}_4/\text{SiO}_2$ nanocomposite under salinity stress conditions (80 mM NaCl). Different treatments (CT, PT and 5, 10, 15, 20 and 25 ppm) affected seed germination parameters, including germination percentage (GP), germination rate index (GRI), mean germination time (MGT) and coefficient of velocity of germination (CVG), as shown in Fig. 7 (A–D). Salinity is a major concern in agriculture, inducing osmotic stress, ionic toxicity and oxidative damage, thereby impairing nutrient uptake and metabolic activities essential for seed germination. In this study, the nanoprimering with $g\text{-C}_3\text{N}_4/\text{SiO}_2$ effectively mitigated salt stress and improved germination performance compared with the saline control (PT).

In Fig 7A, GP varied significantly among the nanocomposite seed treatments. The highest germination percentage was obtained with treatment T_6 (20 ppm $g\text{-C}_3\text{N}_4/\text{SiO}_2$ nanocomposite) with 93.3 %. The PT with salinity stress exhibited 86.7 % germination. The lowest GP was 77.8 % for T_5 (15 ppm). The superior GP observed at 20 ppm indicates that the nanocomposite created a favourable environment for the seed germination under saline stress. Nanoprimering is known to improve seed hydration and

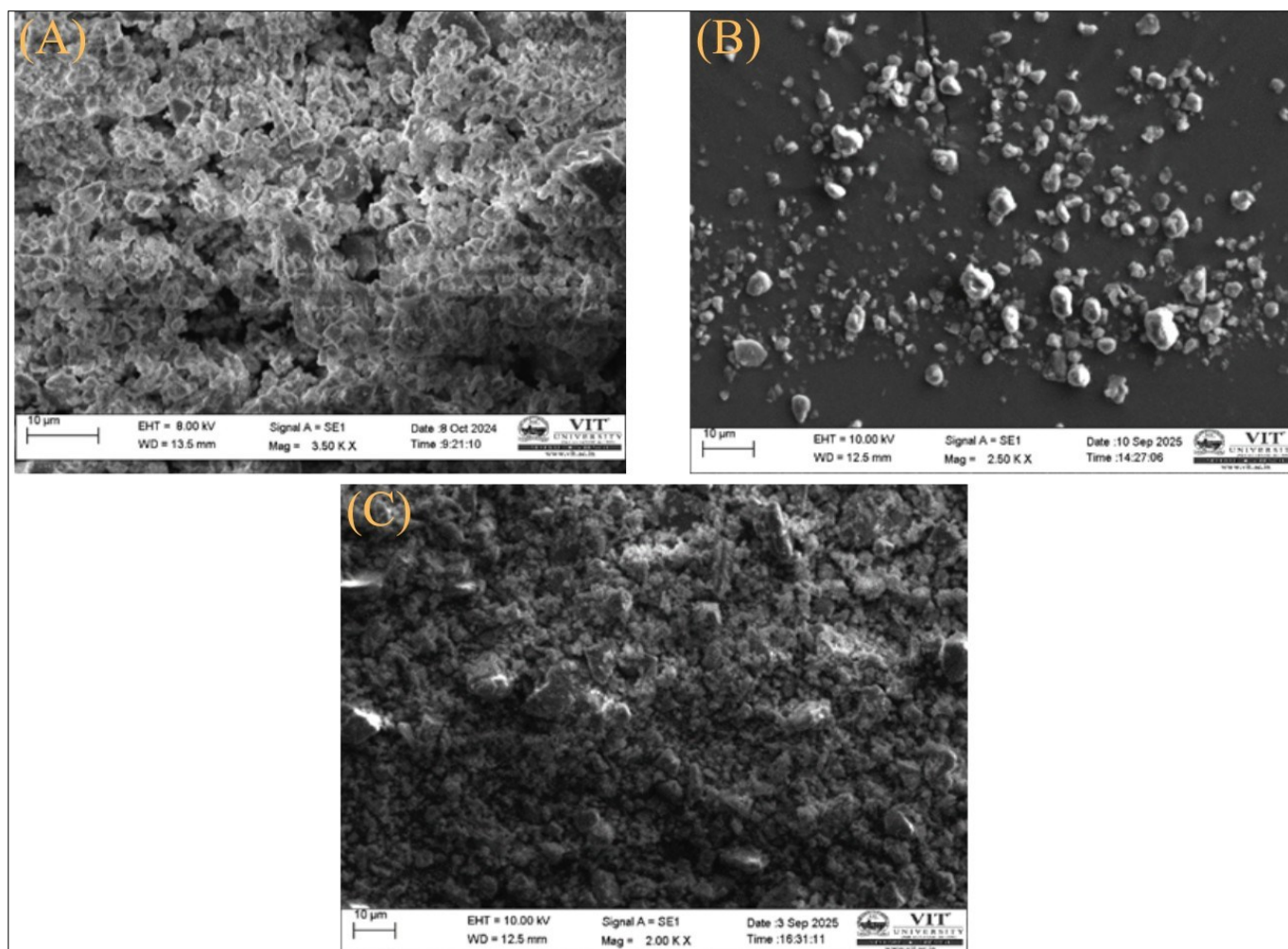


Fig. 5. Scanning electron microscopy (SEM) images of (A) SiO_2 ; (B) $g\text{-C}_3\text{N}_4$; (C) $g\text{-C}_3\text{N}_4/\text{SiO}_2$.

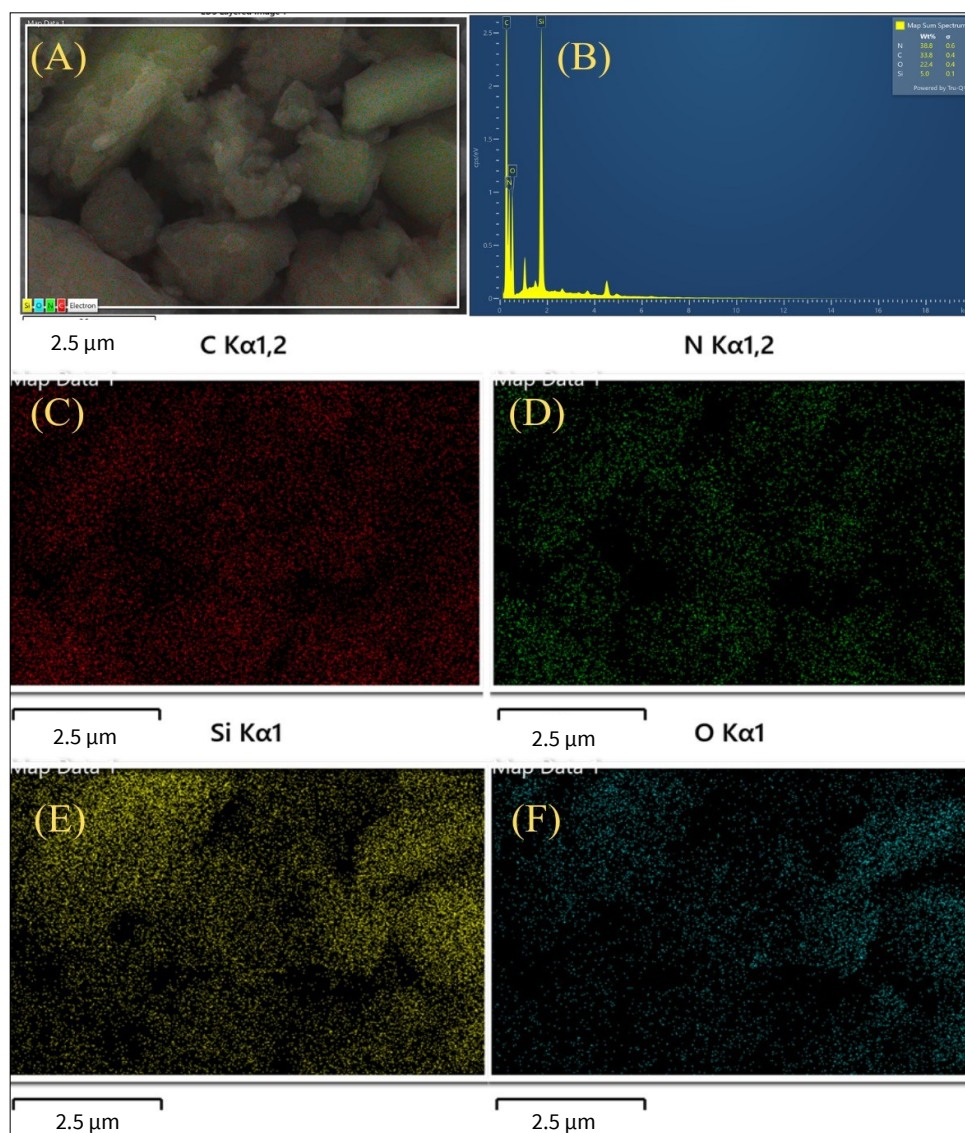


Fig. 6. Field-emission scanning electron microscopy (FESEM), energy-dispersive X-ray spectroscopy (EDS) and elemental mapping analyses of the g-C₃N₄/SiO₂ nanocomposite. (A) FESEM image showing the elemental overlap morphology; (B) EDS spectrum confirming the elemental composition; (C–F) Elemental mapping of C, N, Si and O showing their uniform distribution.

initiate early metabolic activity by enhancing seed coat permeability. The high specific surface area and abundant surface-active sites of the g-C₃N₄/SiO₂ nanocomposite facilitate better interaction with seed tissues, resulting in enhanced water absorption and enzyme activation (54). These processes stimulate embryo growth and promote successful radicle emergence even under stress conditions.

A similar trend was noted for the GRI (Fig. 7B). The maximum GRI value of 2.60 was recorded in T₆, while the lowest value of 1.89 was observed in T₅. A higher GRI indicates uniform and rapid seedling growth under unfavourable conditions. The enhanced GRI may be attributed to the nanocomposite's ability to promote water imbibition and physiological processes within the seed. Furthermore, g-C₃N₄ has been reported to mitigate heavy-metal toxicity and oxidative stress by controlling reactive oxygen species (ROS) generation (55), thereby safeguarding germination tissues against oxidative injury.

Mean germination time exhibited an inverse correlation with overall germination performance (Fig. 7C). Seeds priming treatment T₆ (20 ppm g-C₃N₄/SiO₂ nanocomposite) recorded the shortest MGT (5.50 days), indicating better performance, whereas T₄ (10 ppm) showed the longest duration (6.3 days). The application of

Si nanoparticle improves MGT in tomato seeds (56). These results validate the dose-optimised increase in germination performance, as reflected in reduced mean germination time and enhanced seedling establishment.

Similarly, the CVG also differed significantly among the treatments (Fig. 7D). The highest CVG value (18.21) was recorded in T₆ (20 ppm g-C₃N₄/SiO₂ nanocomposite), whereas T₄ (10 ppm) recorded the lowest value of 15.86. The significant increase in CVG indicates faster and uniform germination among the seeds in the treatment. The increase in germination velocity may be attributed to enhanced water imbibition in the seeds due to the hydrophilic nature of the Si nanoparticles (56, 57). Enhanced CVG contribute to improved initial crop stand, thereby facilitating better growth and development.

Different treatments (CT, PT and 5, 10, 15, 20 and 25 ppm) influenced seedling growth parameters, including vigour index (VI), root length (RL), shoot length (SL) and mean seedling length (MSL), as illustrated in Fig. 8 (A–D). Seedling vigour index was significantly enhanced by g-C₃N₄/SiO₂ treatment. Notably, the highest vigour index was recorded at the highest value of 3224 in T₆ (20 ppm g-C₃N₄/SiO₂ nanocomposite), indicating the best seedling vigour, whereas T₅ (15 ppm) recorded the lowest value of 2119. A higher

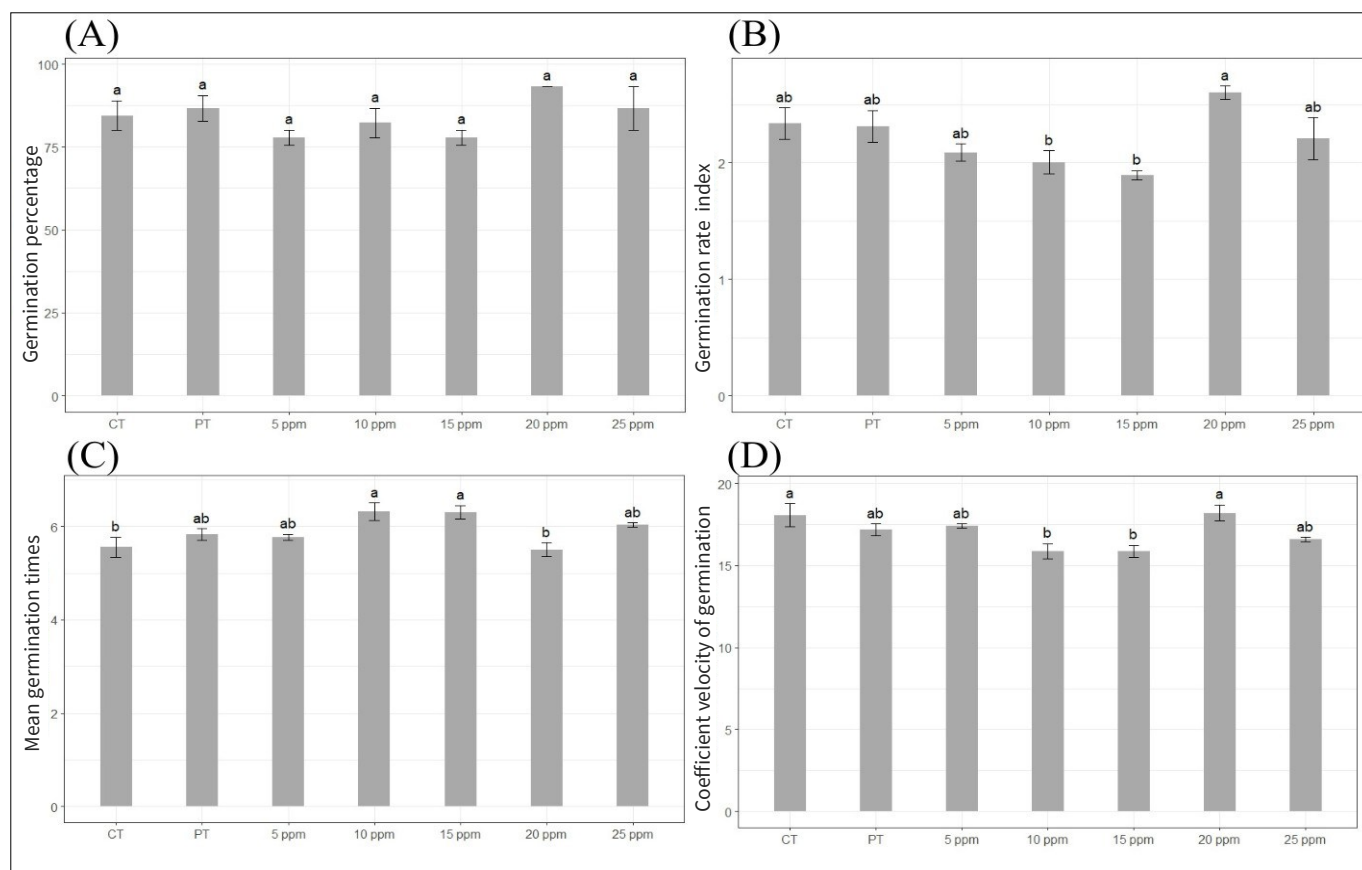


Fig. 7. Effects of different treatments (CT, PT and 5, 10, 15, 20 and 25 ppm) on seed germination parameters: (A) germination percentage; (B) germination rate index; (C) mean germination time; (D) coefficient of velocity of germination. Bars represent mean $\pm$ standard error (3 replicates) and different letters indicate significant differences among treatments ($p < 0.05$).

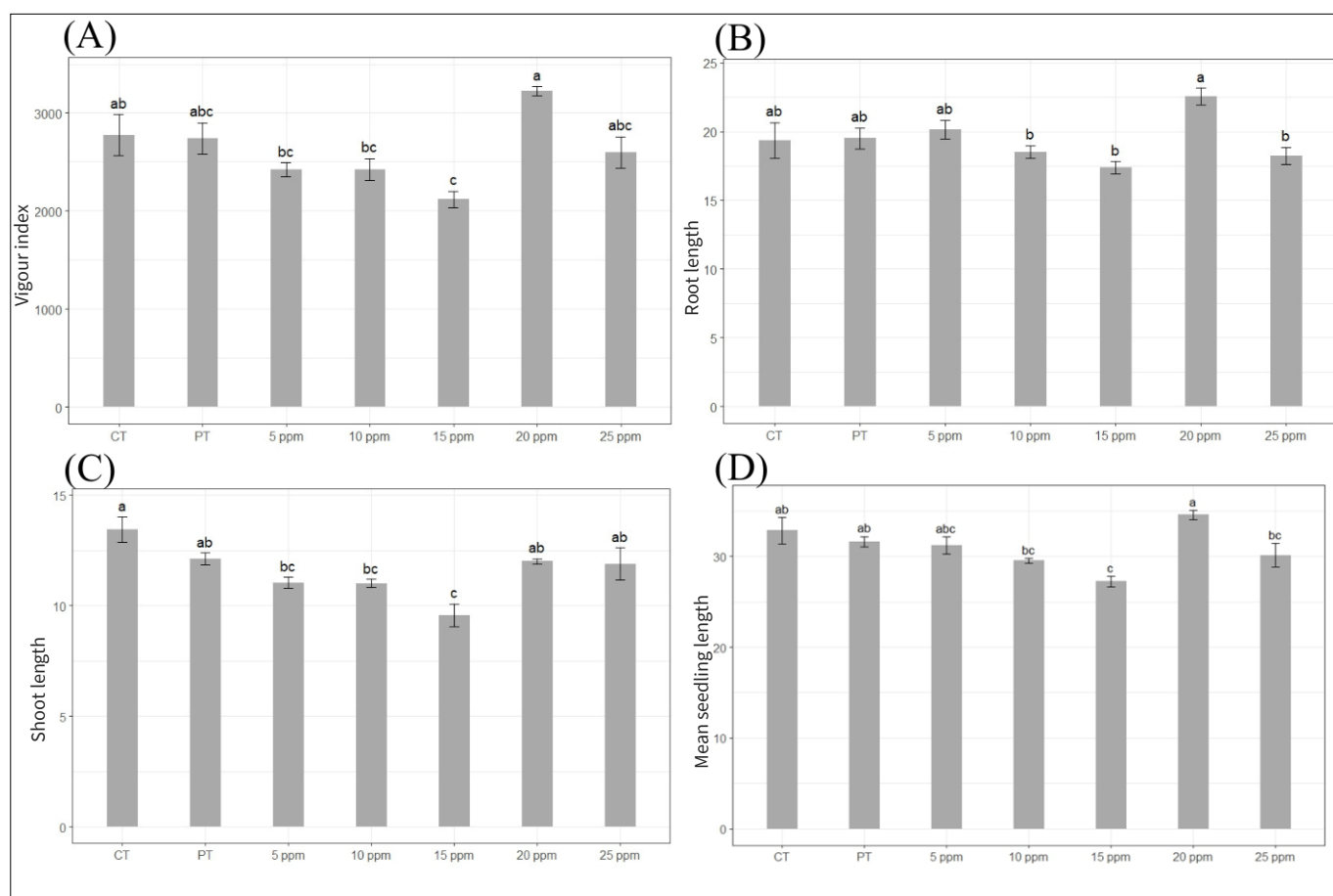


Fig. 8. Effects of different treatments (CT, PT and 5, 10, 15, 20 and 25 ppm) on seedling growth parameters: (A) vigour index; (B) root length; (C) shoot length; (D) mean seedling length. Bars represent mean $\pm$ standard error (3 replicates) and different letters indicate significant differences among treatments ($p < 0.05$).

vigour index indicates improved seedling establishment potential and enhanced seedling tolerance to environmental stress. The rise in vigour index may result from superior germination performance, improved nutrient uptake and reduced oxidative damage.

Root length varied among treatments, with concentration-responsive gradient outcomes. The maximum RL (22.6 cm) was observed in T₆, followed by T₃ (20.2 cm), while the absolute control (T₁) recorded the lowest value. Enhanced root growth is advantageous under stressful circumstances, improving nutrient and water uptake. Similarly, a study reported that rice seeds treated with Si nanoparticles showed increased nutrient uptake, including Zn, Cu and Mg (57). The larger root growth in the nanoprimered treatments ensures improved stress adaptation and seedling establishment.

Shoot length displayed varied results as compared with the other parameters. Notably, T₁ (absolute control) recorded the highest SL (13.46 cm). Although the absolute control recorded the maximum value, the 20 ppm nanocomposite treatment produced shoot growth while sustaining superior germination and root development. Consequently, the highest MSL, 34.56 cm, was observed in T₆, whereas T₅ recorded the lowest value, 27.24 cm. The improved seedling growth may be associated with enhanced water uptake, increased photosynthetic efficiency and better regulation of physiological processes, which subsequently enhanced seedling development (58).

Pearson correlation analysis showed that germination and seedling growth parameters were significantly related and germination parameters were interdependent of growth parameters under salinity stress (Fig. 9). The germination percentage had a strong positive correlation with GRI ($r = 0.89$) and vigour index (VI; $r = 0.84$), which means that high percentage germination correlated well with high germination rate and high vigour with seedling. Similarly, GRI exhibited the strongest positive correlation

with VI ($r = 0.91$), demonstrating the importance of rapid germination in promoting vigorous seedling establishment.

Root length was highly correlated with MSL ($r = 0.89$), indicating a large contribution of root development to the whole seedling length. The positive correlation of RL and MSL with VI further substantiated the use of these traits to assess seedling performance under stress. In contrast, MGT showed negative correlations with most growth parameters, such as GRI ($r = -0.73$), MSL ($r = -0.62$) and VI ($r = -0.58$), showing that delayed germination had a negative impact on the development of seedlings. The CVG exhibited significant positive correlations with GRI, MSL and VI and was perfectly negatively correlated with MGT ($r = -1.00$). Conclusively, the correlation analysis indicated that these improvements in germination speed and efficiency were positively correlated with the growth and vigour of seedlings, thereby confirming the positive impact of g-C₃N₄/SiO₂ nanoprimering on rice establishment under saline conditions.

Interestingly, the nanocomposite effects are concentration dependent in rice seeds. While lower and moderate concentrations were found to have beneficial effects on germination and growth, the 15 ppm treatment has comparatively poor performance. These variations indicate that nanoparticle-mediated responses are influenced by concentration, uptake and interaction with seed tissues. However, similar nonlinear responses have been observed in several nanoprimering studies, where optimal concentrations stimulate growth. But suboptimal concentrations may not produce the same physiological and morphological effects. We highlight that several findings have already emphasised the significance of Si nanoparticles and very few studies have been conducted on g-C₃N₄. This work provides the first evidence that g-C₃N₄/SiO₂ nanocomposites can be harnessed in promoting plant growth through nanoprimering.

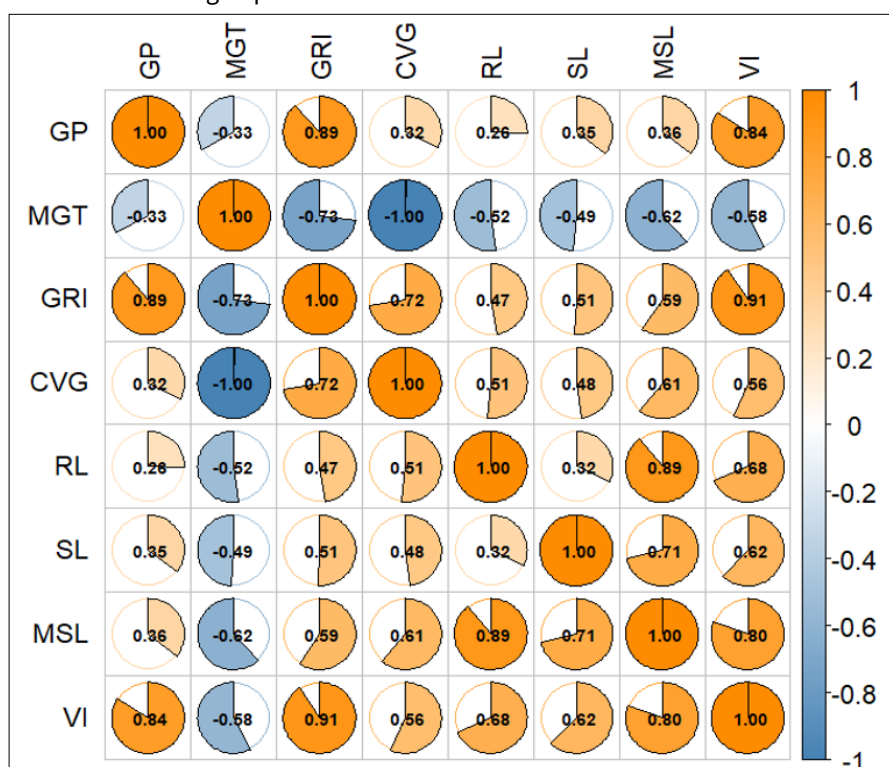


Fig. 9. Correlation plot showing the relationship among the germination parameters.

GP: germination percentage; MGT: mean germination time; GRI: germination rate index; CVG: coefficient of velocity of germination; RL: root length; SL: shoot length; MSL: mean seedling length; VI: vigour index.

Conclusion

In conclusion, the current results demonstrate that seed priming using graphitic carbon nitride/silicon dioxide (g-C₃N₄/SiO₂) nanocomposite is a promising strategy for mitigating the negative impacts of salinity stress on rice germination and seedling establishment. The tested dose of 20 ppm proved to be the optimal dose, significantly improving germination percentage (GP), velocity, vigour index (VI) and mean seedling length (MSL). The synergistic effect between g-C₃N₄ and SiO₂ likely enhanced nutrient utilisation, osmotic adjustment and stress management, thus contributing to better seedling establishment under saline conditions. These findings suggest using the metal-free g-C₃N₄/SiO₂ nanocomposite as a sustainable nanoprimer agent for enhancing rice productivity in salt-affected areas. However, further investigations are anticipated under greenhouse and field conditions to validate their efficiency in real-time environments. Nanocomposites are gaining more visibility and acceptance throughout the world as promising tools for sustainable agriculture, but the commercial exploitation of their high use and regulatory compliance is still evolving. The studies on environmental safety, ensuring biosafety on nontargeted organisms, are to be validated. Even at low concentration, the metal-free composition and favourable biological performance at low concentration represent the need for large-scale industrial production for climate-resilient agriculture.

Acknowledgements

This research was funded by Vellore Institute of Technology (VIT), Vellore, Tamil Nadu, India. The authors thank Vellore Institute of Technology (VIT) for providing the facilities and technical support required to carry out this research. They gratefully acknowledge the VIT SEED Grant (RGEMS) (Sanction Order No. SG20230059) awarded by Vellore Institute of Technology (VIT) in support of the seed germination study.

Authors' contributions

RJ conducted the experiment, recorded observations, analysed the data and drafted the manuscript. NK contributed to conceptualisation, work planning, funding acquisition, methodology, supervision and coordination. VJ performed the experimental and laboratory analyses and assisted with the review and editing of the manuscript. TPK contributed to work planning, coordination, summarising and revising the manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT (OpenAI) for language correction, grammar improvement and enhancement of manuscript readability. After using this tool, the authors carefully reviewed and edited the content as needed and take full responsibility for the content of the publication.

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