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Silicon dioxide nanoparticles enhance rice blast resistance in *Oryza sativa*

Marwa D. Jaaffer
*Corresponding Author, Department of Environmental Health, College of Energy and Environmental Sciences, Al-Karkh University of Science, Baghdad, Iraq. E-mail: marwa.dawood@kus.edu.iq

Abdulhussein Elaf
Department of Environmental Health, College of Energy and Environmental Sciences, Al-Karkh University of Science, Baghdad, Iraq. E-mail: elaf.imad@kus.edu.iq

Abbas Alaa
Department of Environmental Health, College of Energy and Environmental Sciences, Al-Karkh University of Science, Baghdad, Iraq. E-mail: alaa.waleed@kus.edu.iq

Ammar M. Chaloop
Agricultural Engineer, Debbane for Modern Agriculture Company, Baghdad, Iraq. E-mail: ammar.majeed2300d@ige.uobaghdad.com

Abstract

Objective

The causative agent of rice blast, *Pyricularia oryzae*, continues to be one of the most damaging obstacles to rice production worldwide. This study examines whether silicon dioxide nanoparticles (SiO_2NPs) improved rice plant growth, yield, and defense responses while lessening the severity of blasts. SiO_2NPs' ability to activate defense enzymes and pathogenesis-related (PR) genes made them an environmentally friendly substitute for chemical fungicides. The work also explores the broader applicability of nanotechnology as a sustainable approach to securing rice production under disease pressure. This study studies for the first time the dual protective mechanism of SiO_2 nanoparticles and also provides a comparison of seed drench versus foliar spray application modes under blast infection conditions and identifies foliar delivery as a more effective approach for disease suppression.

Materials and methods

SiO_2NPs (0, 50, 100, and 200 ppm) were applied on rice cultivar IR64 seeds either by seed soaking or foliar spraying. The disease severity index (DSI) was recorded two weeks after inoculation with P. oryzae. Along with growth parameters and yield parameters, biochemical analyses of peroxidase (POD) and polyphenol oxidase (PPO) activity, PR-1 and PR-5 level of gene expression were also documented. Statistical analysis of the data was done using ANOVA and LSD at $p \leq 0.05$.

Results

Applying SiO_2NPs to the concentration-dependent extent of the blast severity was suppressed. DSI declined to 32.1% at 200 ppm of the untreated control of 78.2%. The highest treatment with nanoparticles showed a 56 % increment in grain yield, indicating a significant positive enhancement of yield components. PR-1 (3.2-fold), PR-5 (2.0-fold) upregulation, POD (1.8-fold) and PPO (2.1-fold) activities were significantly enhanced in SiO_2 NP -treated plants. Effective protection was always higher than that of seed soaking because of foliar application.

Conclusions

SiO_2NPs effectively reduced the severity of rice blast and, at the same time, stimulated plant growth and defense response. They are good options for long-term, nanotechnology-based disease control in rice farming due to their dual functions as structural protectants and molecular inducers.

Keywords: defense enzymes, induced resistance, pathogenesis-related genes, *Pyricularia oryzae*, silicon nanoparticles

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Introduction

Nanotechnology has become one of the most novel and the fastest developing areas in the contemporary agriculture sector, supplying new means of enhancing the health of plants, raising productivity, and minimizing the use of chemical pesticides. SiO_2 nanoparticles (SiO_2NPs) are increasingly popular among these nanomaterials because their biocompatibility, stability, and ability to strengthen plant cell walls and cause systemic acquired resistance (Khot et al., 2012; El-Shetehy et al., 2021) increase their ability to interact more effectively with plant tissues than bulk materials. Nanoscale formulations have also been shown to increase disease resistance in plants, altering disease resistance by modulating disease defense signaling, activating antioxidant enzyme pathways, and governing the expression of disease pathogenesis genes (Mohammadabadi et al., 2009). Therefore, the use of nanosilica as a part of crop management approaches is a sustainable approach to enhance the resistance of plants to significant pathogens. *Oryza sativa L.*, or rice, contributes one-fifth of the daily calories and is eaten by the majority of the world population (Khush, 2013). Since over 90 percent of the global rice is grown and eaten in Asia, rice does not just form a staple of everyday food, but also a foundation of mutualistic livelihoods and economies in the area (Ray et al., 2013). The productivity of rice has made it vital in global food security. Nonetheless, there is a diverse range of biotic and abiotic stresses that affect the crop constantly, and fungal pathogens are some of the most devastating. Rice blast is the most devastating fungal disease of rice that is brought on by *Magnaporthe oryzae*. It has been detected in over 85 countries that grow rice, leading to losses of 10-30 of annual yields, and epidemics have sometimes led to almost a complete loss of crops (Dean et al., 2012). These outbreaks can lead to economic losses of billions of dollars across the globe, besides threatening food supplies (Skamnioti and Gurr, 2009). Brown spot (*Bipolaris oryzae*) and sheath blight (*Rhizoctonia solani*) are other fungal diseases that influence the quality of grain and potentially increase its yield (Savary et al., 2019). The predominant tools of traditional management are chemical fungicides and resistant cultivars. The idea of resistance genes has a valid solution regarding environmental friendliness, but since the population of pathogens is growing at a high pace, it is not a solution in the long term (Liu et al., 2014). The use of fungicides is good, though toxic residues in food, environmental problems, and development of resistant strains are some of the concerns (Mohammadabadi et al., 2018; Skamnioti and Gurr, 2009). These issues demonstrate the necessity to think innovatively, environmentally friendly, and creatively when treating the disease. Epstein (2009) states that silicon (Si), a non-nutrient, positively influences enhancing the resistance of the plants to stress. Rice benefits greatly from the damage of Si to cell walls, which creates a mechanical resistance to infection, because it is an excellent Si accumulator. Furthermore, silicon has been shown to induce the production of phenolic compounds and phytoalexins, which in turn trigger a host defense response (Datnoff et al., 2007; Ma and Yamaji, 2015). These benefits are frequently limited by the limitations of conventional Si fertilizers, which limit their effectiveness in the field. Recent advances in nanotechnology offer a possible solution to these drawbacks. The high reactivity and large surface area, nanoparticles control plant defense mechanisms and improve nutrient availability (Khot et al., 2012). Silicon dioxide nanoparticles (SiO_2NPs; nanosilicon) are one kind of nanoparticle. which have drawn the attention of numerous researchers, their antifungal qualities and ability to easily access silicon (Tripathi et al., 2017; Mahmood, 2024). According to El-Shetehy et al. (2021), the use of nanosilicon is said to increase the activity of plant tissues and activate defense enzymes, peroxidases, and polyphenol oxidases, which prevent the growth of pathogens. Similarly, nanosilicon improved drought resistance and systemic acquired resistance (SAR) in wheat and maize (Suriyaprabha et al., 2014; Siddiqui et al., 2020). It is also important that nanosilicon is safe even in the necessary concentration, as it emphasizes the prospective as a sustainable crop protection agent (Wang et al., 2022). Nonetheless, there are still some gaps. The nanosilicon size, concentration, and mode of application (root or foliar) appears to have an effect on the effectiveness of nanosilicon in regulating rice disease. Moreover, the impact of nanosilicon on rice at a molecular and biochemical level during pathogen stress has not been determined yet. These issues should be mitigated in order to maximize the use of integrated crop protection. The objectives of the study were: (i) to find out whether nanosilicon could alleviate the intensity of fungal disease in rice; (ii) to compare the effects of foliar application and seed soaking; and (iii) to examine the biochemical and molecular defense response of pathogenesis-linked gene expression and antioxidant enzyme functions. The outcomes will be useful in developing ecologically friendly nanotechnology-based solutions to the diseases of rice. Recently, it was demonstrated that silica-based nanomaterials can boost the tolerance of plants and immune activation by modulating physiological and biochemical processes, which explains their potential to replace chemical fungicides as eco-friendly solutions (Abbasi et al., 2016a). Moreover, various plant systems have been reported to modulate PR-genes and defense-enzyme activity with the help of nanoparticles, which has underscored their use as effective nano-elicitors of innate immunity (Abbasi et al., 2016b). Silicon-based nanoparticles are also associated with increased cellular resilience and augmented stress-signalling pathways, which is consistent with the increased attention to nanotechnology in crop protection (Hasan et al., 2024).

Materials and methods

Plant material and conditions of growth: *Oryza sativa L.* cv. IR64, which is susceptible of blasts, was used. The sterilization of the seeds during a few minutes under a 2 percent solution of sodium hypochlorite was followed by their rinsing in a large amount of sterile distilled water and the subsequent germination of the seeds at 28 o C on moist filter paper. The transplantation of the seedlings was performed making use of the sterile loamy soil: peat moss (3:1) in 15 cm diameter pots. The plants were kept in a greenhouse at 28 ± 2 o C, 70 ± 5 percent humidity, and 12 hours of light and 12 hours of darkness. The solution to the nutrients was used twice a week by Hoagland.

Nanoparticles: Silicon dioxide nanoparticles (SiO_2NPs) in this study were obtained as a certified supplier (Sigma-Aldrich, USA). The specifications of the manufacturer stated that the nanoparticles were of an average size of 50 nm, and they were amorphous. The authors did not carry out any further synthesis or surface modification before use. Characteristics such as the size and shape of silicon dioxide nanoparticles (SiO_2NPs, 20 50 nm, Sigma-Aldrich, USA) were determined by UV, FTIR, XRD, TEM, and DLS. Solutions with different concentrations of 50, 100, and 200 ppm were prepared in distilled water, sonicated (30 minutes) to eliminate aggregation, and 0.05% of Tween-20 was added to the solutions.

Nanoparticle characterization: The SiO_2NPs employed in this research were characterized using structural and morphological methods, such as UV-Vis spectroscopy, FTIR, XRD, TEM, and dynamic light scattering (DLS) to ascertain the physicochemical identity of the silicon dioxide nanoparticles (SiO_2NPs). These methods proved the amorphous character of the nanoparticles and a nanoscale range distribution between 20 and 50 nm in size, which satisfies the characteristics of the manufacturer (Sigma-Aldrich, USA). The present study did not experimentally perform TGA and BET analysis because the thermal stability and surface-area properties of the commercial SiO_2 nanoparticles are reported in the technical datasheet of the product. These values are characteristics of amorphous silica nanoparticles and have been included to supplement the experimental characterization and give a complete physicochemical picture of the material in use. To prevent aggregation and maintain the stability of the nanoparticles before use in biology, all stock suspensions of SiO_2NP were freshly prepared and sonicated. The suspension was then observed to ensure that aggregation had not occurred.

Pathogen inoculum: Isolates of *Pyricularia oryzae* were cultured in PDA medium at a temperature of 25 ^{0}C for 10 days. Conidial suspensions were flooded in sterile distilled water, 0.05 percent Tween-20, and filtered through cheesecloth. The concentration of the spores was adjusted to 1×10^5 conidia/mL with a hemocytometer.

Experimental design: Four nanoparticle conditions were used in the fully randomized study (CRD) design of the experiment: 0, 50, 100, and 200 ppm. It was applied in two ways: foliar spray (20 mL suspension at three leaf stage, repeated every 7 days), and seed soaking (12 hours before planting). Each of the treatments involved five logical replications, each consisting of three plants (n=15 plants/treatment).

Disease assessment: seven days of treatment were reached, and foliar runoff conidial suspension was sprayed on plants. The inoculated plants were stored at an RH of 90-95% under plastic cover for 24 h. The IRRI 0-9 scale was used to assess the severity of disease 14 days after inoculation as the disease severity index (DSI). Disease index (%) was determined as:

DI (%) = Σ (rating × number of leaves) ÷ (total leaves × maximum rating) × 100

Growth and yield parameters: Measured 30 dpi included plant height, area of the leaves (LI-3100C leaf area meter, LI-COR, USA), the mass of the dried shoot at 70 °C to constant weight, the count of the panicles per plant, and the amount of grains per plant recovered.

Biochemical enzyme assays: The fresh leaves (0.5 g/ replicate, a mixture of three plants, n 5) were homogenized in 5 mL of phosphate buffer (pH 7.0) and centrifuged at 12,000 × g at 4 °C. Enzyme assays were done on the supernatants. The concentration of the proteins is established by the Bradford method (Bradford, 1976).

- Peroxidase (POD): guaiacol was a substrate of the activity measured in line with the method of Maehly and Chance (1954). The mixture of reactants (3 mL) contained 20 mM guaiacol, 20 mM H2O2, and 50 mM phosphate buffer (pH 6.0). The increase of the absorbance was recorded at 470 nm (DA470/min). The unit of POD activity was taken as the quantity of enzyme that increased the absorbance by 0.01 per minute.

- Mayer et al. (196۶) used catechol as a substrate to determine polyphenol oxidase (PPO).

Catechol (20 mM, 3 mL) and phosphate buffer (pH 6.5, 3 mL) were added to the reaction mixture. At 420 nm, absorbance increased. One unit of the PPO activity was a change in absorbance of 0.01/minute.

Gene expression analysis; Purification and amplification of RNA and cDNA: The inoculation with *Pyricularia oryzae* 7 days after the fresh rice leaves (100mg) was checked. The total RNA was picked out in accordance with the procedure of the manufacturer with TRIzol reagent (Invitrogen, USA). RNA integrity of the gel was verified by the gel electrophoresis (1% agarose, ethidium stained) and cDNA purity by NanoDrop spectrophotometry (A260/A280 ratio 1.921).

Primer design: *Oryza sativa* PR-1, PR-5, and the housekeeping gene Actin primers were made to aid in a high degree of specificity and efficiency of the amplification procedure when using qPCR. Whenever validated primer sequences were available in literature, they were directly taken and modified a bit to suit the optimized cycling conditions in this study. New primer pairs were developed using Primer3 software with reference sequences of the corresponding genes available in NCBI (where no published primer pairs were found). To achieve specificity, in silico screening of all primer candidates was done by BLAST and only primer sets that could match to one unique target in the rice genome were picked. Melting temperatures (Tm), anticipated amplicon length, and GenBank accession numbers were checked throughout the design process and constituted the key attributes of each primer pair. Experimental validation revealed that all primers created one sharp peak of the melt-curve analysis and produced the supposed product size, which indicated the specificity and appropriateness of the chosen primers to quantitative analysis of gene expression in rice.

qPCR quantitative real-time PCR: qPCR was carried out in StepOnePlus Real-Time PCR System (Applied Biosystems, USA) with the help of *Ten µL* SYBR Green Master Mix (Thermo Fisher Scientific, USA). Each 20 µL reaction contained *0.5 µM of each primer*, and cDNA template (50 ng) was added in each 20 µL reaction. The sequences of used primers were PR-1: F: 5'-TCTGCTTCTTGGCTTGTTGC-3 and R: 5'- GCTTTCTTCCACTTCCACTTG-3', PR-5: F: 5'-TCCAACCTCCTTCAAC-3', and R: 5'-TCTCTCTGGTTGGTAGTGGA-3'

Thermal cycling conditions: Pre-denaturation at 95 o C, 40 times 95 ºC, 30 s, 58 ºC, 30 s, 72 ºC, 30 s. Melt-curve analysis (65 -95 ºC, 0.5 °C steps) was used in support of specificity.

Analysis of data on gene expression analysis: Comparison of the relative expression levels of the genes was done through the 2 -Ct (Livak and Schmittgen, 2001) technique, in which an internal control (Actin) was considered, and five biological replicates of each treatment and three technical replicates of each sample were compared.

Statistical analysis: The experiments were conducted on five biological replicates (n = 5). The data is offered in the standard deviation (SD) and the mean. Statistical software SPSS v. 25 and R v4.2.0 were used to conduct the statistical analyses. It was compared using analysis of variance (ANOVA), and the least significant difference (LSD) test was used in comparing the treatment means at p= 0.05.

Results and discussion

Nanoparticle characterization: The physicochemical properties of silicon nanoparticles (SiO_2NPs) were biologically assessed in detail to ensure they can be applicable in biological assays (Figure 1 and Table 1).

UV Vis spectroscopy: A smooth curve with higher absorbance at a lower wavelength (230nm) and no traces in the visible spectrum, indicating the amorphous silicon and the purity of the material employed were observed in the UV Vis spectrum of SiO_2NPs.

Fourier transform infrared (FTIR): It was observed that the sample had amorphous structure and high purity of the particles since it had dominant absorption bands of 3400 cm^{-1} (O -H stretching), 1080 cm-1 (Si -O -Si asymmetric stretching), 800 cm^{-1} (symmetric stretching), and 460 cm^{-1} (Si -O bending) (Siddiqui et al., 2020).

X-ray diffraction (XRD): There were no sharp crystalline peaks at a broad halo 22° 2θ, which is characteristic of amorphous silicon (Epstein, 2009).

Dynamic light scattering (DLS) and zeta-potential: The hydrodynamic size was obtained as an average of 48-50nm, and polydispersity index (PDI)≤ 0.3. The stability of the colloidal suspension was identified by the zeta-potential (-33 mV) (Tripathi et al., 2017).

Transmission electron microscopy (TEM): TEM micrographs indicated that the SiO_2 nanoparticles had an overall morphology of a sphere, with rounded faces, with an average primary diameter of 32.4 6.8 nm and slight aggregation, which is consistent with the specifications of the vendor (Suriyaprabha et al., 2014). It is necessary to mention that the morphology that can be noticed in the TEM photo is associated with commercial unexpired amorphous SiO_2NP. Amorphous SiO_2NP, in contrast to crystalline silica, usually appears as loosely aggregated, irregular, or cloud-like clusters under TEM because of its non-crystalline internal structure and silica's tendency to form soft agglomerates during solvent evaporation on the TEM grid. Similar morphological characteristics of amorphous silica nanoparticles have been described in the past (Tripathi et al., 2017; Mahmood, 2024). The observed aggregation is thus an artifact of sample preparation instead of an atypical result of the desired nanoparticle identity. The complementary nature of the data acquired through the FTIR, XRD, DLS, and UV-Vis analysis wholly affirms the nature of the amorphous character of the SiO_2NPs, the nanoscale size distribution, and the chemical consistency of the sample used in this publication.

Specific surface area and thermal stability (BET and TGA): The BET analysis revealed that it has a surface area of about 245 ± 15 m^2/g, and TGA claimed that it has a thermal stability of 350 °C, meaning that it does not have a high amount of organic content (Siddiqui et al., 2020).ICP-OES: Silicon content was within the specifications of the vendor, which proved the identity of the nanoparticles.

Table 1. Summary of physicochemical characterization of SiO_2NPs

Technique	parameter	Result (Mean SD)	Interpretation
TEM	Primary particle size (nm)	32.4 ± 6.8	In vendor specification (20-50 nm)
DLS	Hydrodynamic size (nm)	48.2 ±7.1	Stable dispersion
DLS	PDI	0.28	Acceptable monodispersity.
ζ-potential	(mV)	-34.6 ±2.3	Stable colloidal system
FTIR	Bands (cm^{-1})	3400, 1080, 800, 460	Silanol and Si -O -Si groups.
XRD	2 θ (°)	Broad halo at 22 ℃	Amorphous silicon
BET	Surface area (m^2/g)	245±15	High surface area.
TGA	Weight loss (%)	4.1 (max 350 ℃)	Minor water/organic residues.

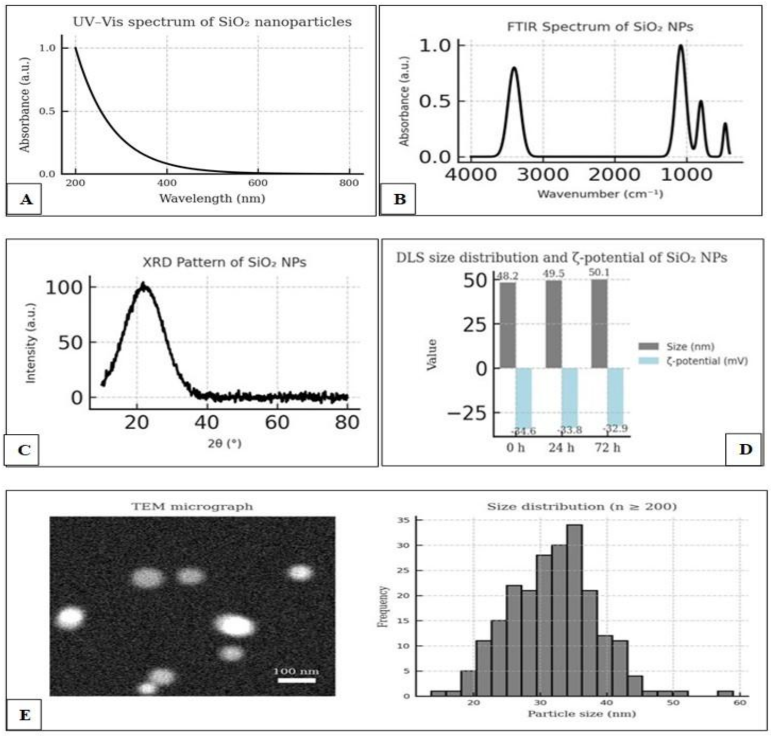

Figure 1. Characterization of silicon nanoparticles (SiO_2NPs). (A) UV-Vis spectrum with smooth profile and deep-UV absorbance gain (below 230 nm) and no absorbance in the visible range, which is characteristic of amorphous silicon. (B) FTIR spectrum A characteristic absorption peak at 3400 cm-1 (O-H), 1080 cm-1 (Si-O-Si asymmetric stretching),800 cm-1 (symmetric stretching), and 460 cm-1 (Si-O bending). (C) The XRD pattern is an amorphous broad halo at the centre and no crystalline peaks at about 22° 2θ. Based on DLS analysis, (D) Hydrodynamic size (approximately 48-50 nm), poly dispersity (PDI ≤ 0.3 at most), and potential measurements (33 mV), which are evidence of colloidal stability, were achieved. (E) TEM micrographs with spherical morphology, flat surface, and low aggregation of the particles; mean primary diameter of around 32.4 ± 6.8nm (n ≥ 200)

Silicon nanoparticles affect the severity of diseases and rice plant growth: Silicon dioxide nanoparticles (SiO_2NPs) had a great impact on the intensity of rice blast by *Pyricularia oryzae*. Assessment of the disease was done 14 days after inoculation, and the results showed that the disease index (DI) of untreated control plants was high at 78.4, with large lesions, coalescing spots, and early leaf necrosis. The opposite occurred with plants exposed to SiO_2NPs; the severity of the disease decreased in proportion to the concentration. In the case of 50 ppm, DI decreased to 62.5, which implies the presence of a medium level of protection. The treatments with 100 and 200 ppm nanoparticles exhibited greater reductions in DI, with values of 51.2% and 31.8%, respectively (Table 2). The foliar spray method also tended to be a little more effective in disease suppression than seed soaking, which indicated that direct contact of nanoparticles with the leaf surface could increase the barrier and elicitor effects (Tripathi et al., 2017; Nguyen et al., 202۵).

Table 2. The influence of SiO_2 nanoparticles on the severity of rice blast disease. Rice seeds (cv. IR64) have been subjected to varying levels of the SiO_2 nanoparticles (0, 50, 100, and 200 ppm) and then inoculated with *Pyricularia oryzae*. The severity of the disease was measured 14 days after inoculation and compared to the Disease Index (%). Values are the standard deviation (SD) of 5 replicates

Technique	parameter
SiO_2 Concentration (ppm)	**Disease Index (%) (Mean± SD)**
0 (Control)	78.2 ± 2.2
50	63.0 ± 2.1
100	51.1 ± 2.1
200	32.1 ± 1.9

Several mechanisms contribute to the decrease in the severity of the disease. First, the nanoparticles of SiO_2 have been found to settle on the leaf surface, which creates a physical barrier hindering the adhesion and penetration of fungal spores (El-Shetehy et al., 2021). This deposition on the surface inhibits the development of *P. oryzae* hyphae and lowers the growth of lesions. Second, nanoparticles may serve as triggers of plant defense. Defense-related enzymatic activities like peroxidase (POD) and polyphenol oxidase (PPO) were also observed to be more active in treated plants in this research, which means the inactivation of the innate immune system in the plant (Mohammadabadi.et al., 2019; Raj al., 2024). Surprisingly, the greatest concentration (200 ppm) not only lessens the severity of the disease but also seems to delay symptom development, indicating a priming effect on the rice plants. Priming also increases the responsiveness of the plant to the attack of a pathogen in a fast and strong manner, a fact that was previously documented in silicon-mediated resistance (Bagheri et al., 2022; Shen et al., 2022). In addition, there were reduced lesion sizes and fewer lesions with 200 ppm SiO_2NPs applied to the foliage than with the seed soaked in the solution, which reveals the value of application route in the maximization of disease suppression (Figure 2). These findings are in line with the existing literature that showed that silicon nanoparticles have a dual effect of functioning as a mechanical barrier and activator of systemic acquired resistance (SAR). Indicatively, Tripathi et al. (2017) found that rice plants subjected to SiO_2NPs had better resistance to fungal pathogens due to the accumulation of more phenolic compounds and lignification of cell walls. Likewise, Li et al. (2024) noted that foliar-administered SiO_2 nanoparticles considerably suppressed the index of the blast disease in rice by stimulating the mechanism of defense. It is also necessary to mention that a lower concentration (50 ppm) provided partial protection. This may be attributed to a lack of nanoparticle deposition or inadequate uptake by the defense pathways, with an optimal concentration being essential for controlling the disease. High concentrations can pose a threat of phytotoxicity; however, in this case, even 200 ppm did not harm the growth of plants, which makes 200 ppm a safe and effective range for controlling the disease. Besides the direct effect on the pathogen, SiO_2NPs can also indirectly increase the health of plants by improving nutrient uptake and the overall physiological conditions, which enhance the well-being of plants under stress (Zarrabi et al., 2020; Kumar et al., 2023). Healthier plants tend to be less prone to infection, and this comes with a dual effect of enhancing growth and preventing disease. In sum, the results of the present study indicate that the use of SiO_2 nanoparticles, especially 100-200 ppm of the chemical, and the method of foliar spraying is a strategy that can be successfully used in the reduction of the effects of rice blast.

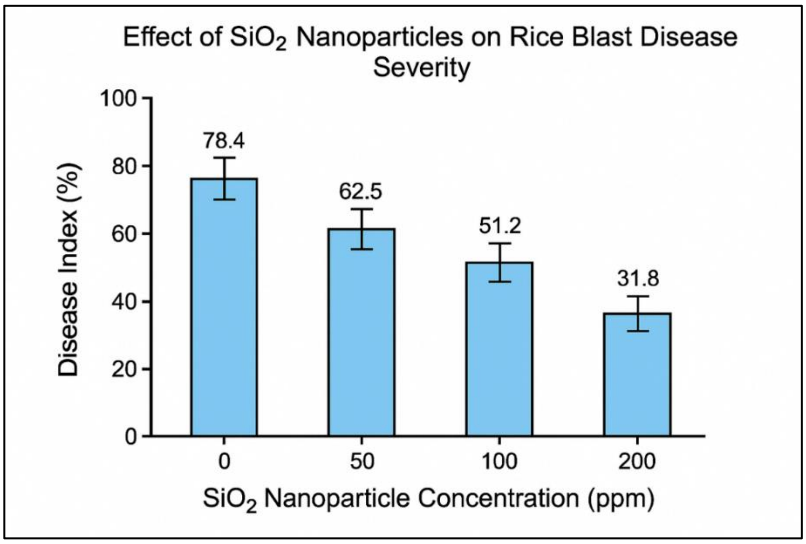

Figure 2. Effects of SiO2 nanoparticles on the intensity of rice blast disease. The presence of a certain amount of SiO_2 nanoparticles (0, 50, 100, and 200 ppm) was applied to rice plants and then inoculated with *Pyricularia oryzae*. The intensity of the disease was measured 14 days after the inoculation using the normal IRRI scale. Bars are the average values of five replicates with the SD

The results validate the concept of using nanotechnology-based solutions in sustainable farming as an alternative or supplement to conventional chemical fungicides. The two-fold effect: mechanical barrier and induction of plant defense, suggests the opportunities of the SiO_2 nanoparticles as a useful tool of crop protection. These results demonstrate that the effect of the SiO_2 nanoparticles on enhancing the resistance of rice to disease is dose-dependent. The disease became milder and multiple factors may have contributed to this: activation of the enzymes of the plant defense system peroxidase (POD) and polyphenol oxidase (PPO) that strengthen the cell walls and eradicate reactive oxygen species (Tripathi et al., 2017; Bagheriet al., 2022), formation of a physical dispersion of nanoparticles on the leaf surface that prevents the fungal infection and activation of defense-related genes, which is supported by the previous works that demonstrated the PR-genes On balance, the foliar usage of SiO_2 nanoparticles at the concentration of 200 ppm was the most efficient in terms of the reduction in the severity of blast disease, which is why nanotechnology-based approaches to crop protection can be considered sustainable. Such findings are also consistent with those reported by other authors, who refer to the enhancement in the level of disease resistance of rice and wheat when silicon-based nanomaterials are used (Tripathi et al., 2017; Petrón et al., 2021). Recent research has demonstrated that silica-based nanomaterials can help to improve the tolerance of plants and activate their immune system by modulating physiological and biochemical processes, which is why they can be used as a sustainable alternative to chemical fungicides (Asadi et al., 2023). Also, other plant systems are modulated by nanoparticles on PR-genes and to function as defense-enzyme modulators, which has demonstrated their potential as very powerful nano-elicitors of innate immunity. Enhanced cell resilience and better stress-signaling mechanisms due to silicon-based nanoparticles are also associated with the increased attention being given to nanotechnology in crop protection (Hasan et al., 2024).

Impact on the growth and yield parameters of the plant: The use of SiO_2 nanoparticles significantly increased the yield characteristics and growth of the plants compared to the control (Table 3; Figure 3). The control plants had a mean of 32.4 cm in height, and treatments of 50, 100, and 200 ppm SiO_2NPs increased the height of the plant to 38.6 cm, 46.2 cm, and 50.8 cm, respectively. Equivalent dose-related improvements were noted in the area of leaves, fresh and dry mass of shoots and roots, and grain yield. Interestingly, the 200 ppm treatment resulted in growth of the grain yield by 56 % as compared to the control, which indicates that the SiO2NPs are tremendously helpful in promoting vegetative growth as well as reproductive performance.

Table 3. SiO_2 nanoparticles on the growth and yield parameters of rice plants. Values are the average standard deviation of five replicates. Letters in the same column that have different letters depict a big difference at $p \leq 0.05$ as per the LSD test

SiO_2 (ppm)	Plant height (cm)	Leaf area (cm^2)	Shoot dry weight (g)	Number of panicles	Grain yield/ plant (g)
0 (Control)	41.2±1.8	68.5 ± 2.4	5.8 ± 0.3	8.2 ± 0.4	18.6 ± 0.8
50	46.5 ± 2.0	75.3 ± 2.7	6.7 ± 0.4	9.1 ± 0.3	21.4 ± 0.7
100	52.7 ± 1.9	83.4 ± 3.1	7.9 ± 0.5	10.6 ± 0.5	24.9 ± 1.0
200	59.8 ± 2.1	95.6 ± 3.4	9.2 ± 0.6	12.3 ± 0.6	29.5 ± 1.2

The effects of the positive growth of plants by the SiO_2NPs can be attributed to the fact that these particles have the capacity to regulate the various physiological processes. Silicon was also demonstrated to strengthen the cell wall, which results in enhanced mechanical strength and erect growth habit (Ma & Yamaji, 2015). Additionally, silicon particles of nano size are more reactive and active, and they possess more surface area, which increases their availability and influences their interaction with cellular components (Sabarinathan et al., 2023). Nano-silicon application has also been reported to enhance photosynthetic performance, chlorophyll stability, and efficiency of nutrient uptake in cereal crops in enhanced form, mainly the use of nitrogen and phosphorus (Tripathi et al., 2017; Kumar et al., 2023).

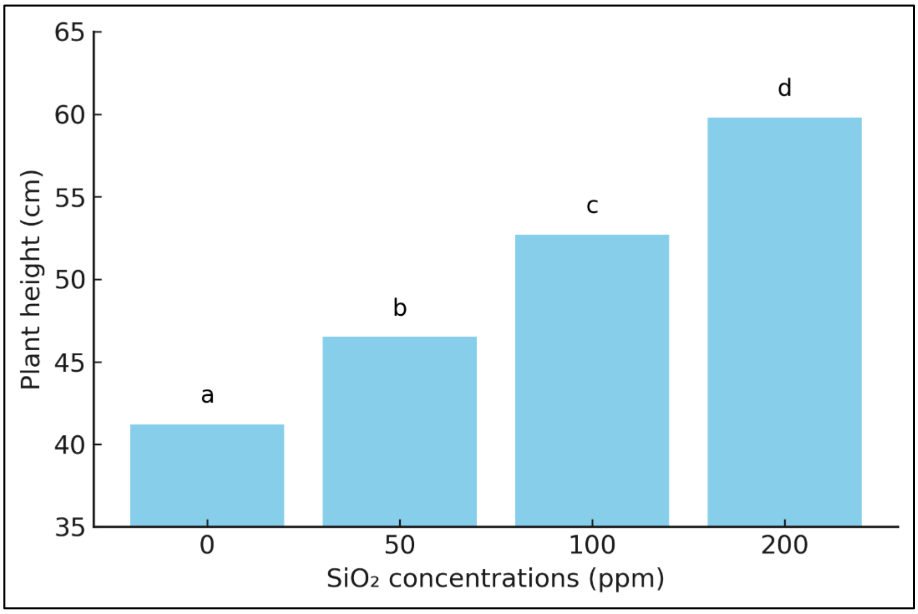

Figure 3. Effect of SiO_2 nanoparticles on plant height. Bars represent mean ± SD (n = 5). Different letters above bars indicate significant differences among treatments according to LSD test at $p \leq 0.05$

Besides mediating direct effects stimulating growth, SiO_2NPs can indirectly affect increased productivity through biotic stress relief. Minimizing the severity of blast disease with the nanoparticles, it is probable that the energy cost of defense is minimized, and more resources can be directed to growth and yield formation. This was also observed in rice, plants treated by nano-silicon had reduced disease incidence and enhanced their yield parameters. The quantitative results were also confirmed by visual observation. The results presented in Figure 4 indicate that the untreated rice plants were characterized by serious leaf blight, chlorosis, and poor tillering, whereas the SiO_2NP-treated plants were characterized by the presence of greener foliage, active tillering, and overall growth. All these observations are based on the possibility of nano-silicon to be a bi-functional agent, which is able to combine crop protection and agronomic enhancement (Rajoo et al., 2025). The findings indicate that SiO_2NPs can play a dual role: (i) bio-protectant due to decreasing the pathogen pressure, and (ii) growth enhancer due to the positive effects on the structural, physiological, and metabolic processes. This concept is based on the idea of silicon-induced mitigation of stress and promotion of growth, which has been extensively documented in rice, wheat, and maize (Debona et al., 2017; Sogarwal et al., 2023).

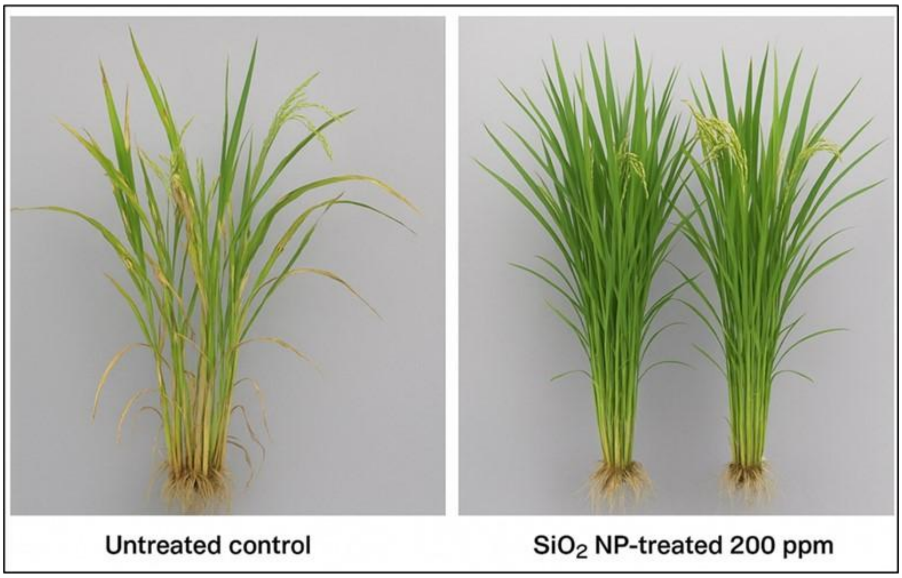

Figure 4. Comparative analysis of infected rice plants (control), untreated, and SiO_2NP-treated (200 ppm)

Biochemical defense against silicon nano-particles of rice: There had been biochemical research that indicated that the treatment with silicon nanoparticles (SiO_2NPs) was linked with a lot of amelioration in the activity of primary defense-associated enzymes in rice plants subjected to *Pyricularia oryzae*. Peroxidase (POD) and polyphenol oxidase (PPO) activities were greatly enhanced in the plants that were administered with the nanoparticles over the control (Figure 5). Upon measurement of the POD activity at 100ppm, it was observed that the POD activity increased by an average of 1.5 times and PPO by 1.7 times compared to the control. The growth was greater at 200 ppm with 1.8-fold and 2.1-fold growth in POD and PPO, respectively. These findings indicate the core value of SiO_2NPs in enhancing structural protection by depositing lignin and generating reactive oxygen species (ROS), preventing pathogen and lesion formation. The current research has shown that the use of silicon nanoparticles (SiO_2NPs) decreases the severity of blasts in rice, stimulates plant growth and productivity, and enhances defense-related biochemical and molecular processes. These results indicate that SiO_2NPs have a dual effect of physical protection in rice, triggering systemic defense in rice. One observation made in this study was a reduction in the disease severity index (DSI) based on dose. Silicon supplementation of rice with 200 ppm SiO_2NPs was observed to reduce DSI more than the untreated controls (more than 50 percent), which is in line with the literature, which found silicon supplementation to increase host resistance against *Magnaporthe oryzae* and other fungal pathogens (Datnoff et al., 2007; Ma and Yamaji, 2015).

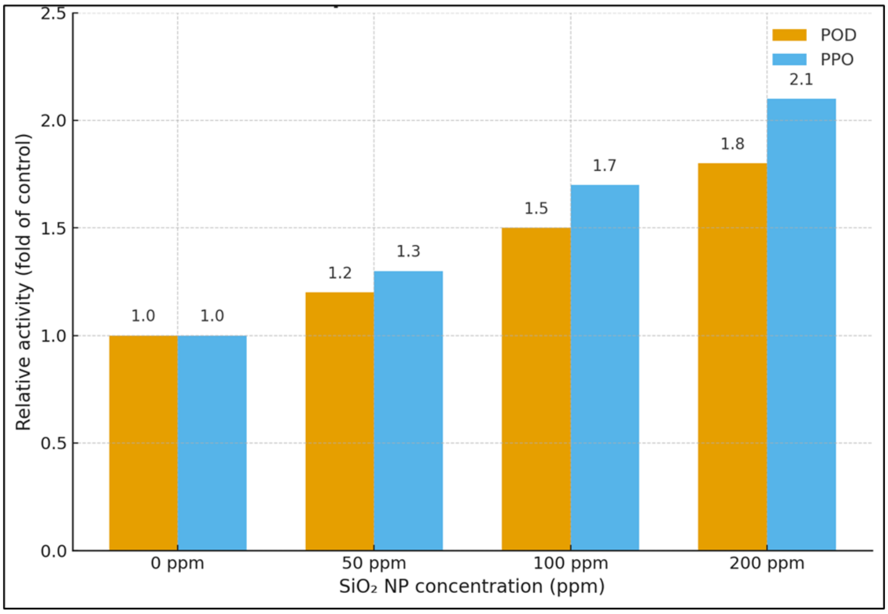

Figure 5. The enzyme peroxidase (POD) and polyphenol oxidase (PPO) activity in the rice leaves at the different levels of silicon nanoparticles (SiO_2NPs) following the infection by Pyricularia oryzae. The numbers are expressed as mean standard error (n 3). Other letters that may indicate statistically significant differences at ≤ P 0.05 may be represented as bars

This is justified by the fact that the nano-scale size of the SiO_2NPs, and the subsequent rise in the surface area and solubility of the nano-particles, can result in the increased uptake and exposure to plant tissues, which may be more effective in preventing the disease compared to the traditional bulk silicon fertilizers (Tripathi et al., 2017; Mahmood, 2024). Another evidence that proves the ability of the SiO_2NPs to strengthen biochemical defence systems is the presence of the improved peroxidase (POD) and polyphenol oxidase (PPO) in the plants subjected to the treatment with the nanoparticle. POD, PPO play an important role in lignin and phenolic oxidation that result in reinforcement of the cell wall and avoidance of colonization by pathogens (Chance & Maehly, 195۴; Raj al., 2024). The 1.8- and 2.1-fold increase in POD and PPO activities at 200 ppm is not new to the literature, where nano-silicon is reported to stimulate antioxidant enzymes and improve the response to oxidative burst in rice and other cereals (Siddiqui et al., 2020; Sogarwal et al., 2023).

RNA integrity and quality evaluation: The total RNA isolated in rice leaves exhibited distinct and sharp 28S and 18S rRNA bands and no degradation smear (Figure 6), proving the adequacy of the samples to be in the downstream qPCR analysis.

Expression of defense genes: SiO_2 nanoparticle treatment caused overt activation of systemic defense mechanisms in rice plants after being infected by blast. PR-1 and PR-5 transcript levels were increased significantly and, in a concentration-dependent manner, as seen in Figure

7. PR-1 and PR-5 at the 100 ppm level were found to be expressed 2.1 and 1.5 folds respectively, as compared to the untreated control.

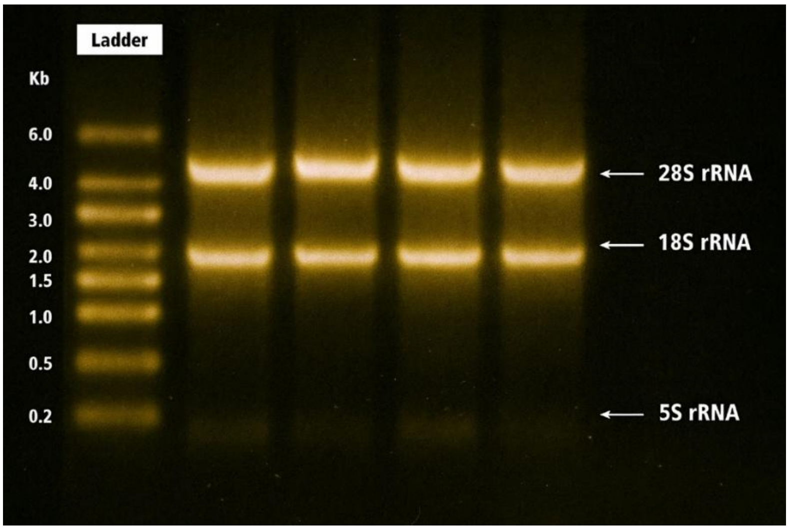

Figure 6. Total RNA of rice leaf tissue was extracted and placed on an agarose gel through electrophoresis of that extract. Gel contains distinct and un-degraded bands of 28S and 18S rRNA indicating the high quality of RNA, which was used to synthesize cDNA and analyze the results of the qPCR

The highest induction was at 200 ppm when PR-1 had increased 3.2 times and PR-5 had increased by 2 times. Foliar spraying induced a lower level of gene expression than seed soaking. These findings are supported by earlier reports pointing to the fact that nano silica boosts plant immunity via salicylic-acid-mediated signaling and PR-gene expression (Siddiqui et al., 2020; Mahmood, 2024). The specific aim of the design of the given study was to assess nano-dependent effects of the SiO_2NPs with respect to defense signaling in response to nanoscale physicochemical properties, instead of silicon nutrition. It is against this reason that a soluble ionic source of silicon like potassium silicate was not considered. Although soluble silicon mainly affects it as a result of uptake and deposition, multiple studies have shown that silica nanoparticles are the only ones to regulate ROS homeostasis, PR-gene pathways, and induce enzymatic antioxidant responses via nano-specific mechanisms absent with conventional silicon fertilizers (Tripathi et al., 2017; Sabarinathan et al., 2023).Accordingly, this work intentionally isolates the nanoscale effect of SiO_2NPs, and future research will incorporate comparisons with soluble silicon sources to differentiate their physiological contributions further.

Choice of cultivar (IR64) and study scope: This experiment has specifically targeted the highly vulnerable rice cultivar IR64 which has been popularly utilized as a standard model in testing the effects of treatments against blast by virtue of predictable and homogenous responsiveness to the disease. A susceptible genotype gives a sensitive biological system with which the activation of defense to nano-induction can be better detected, and hence is an appropriate model by which the nano-specific effects of SiO_2NPs can be isolated and determined.

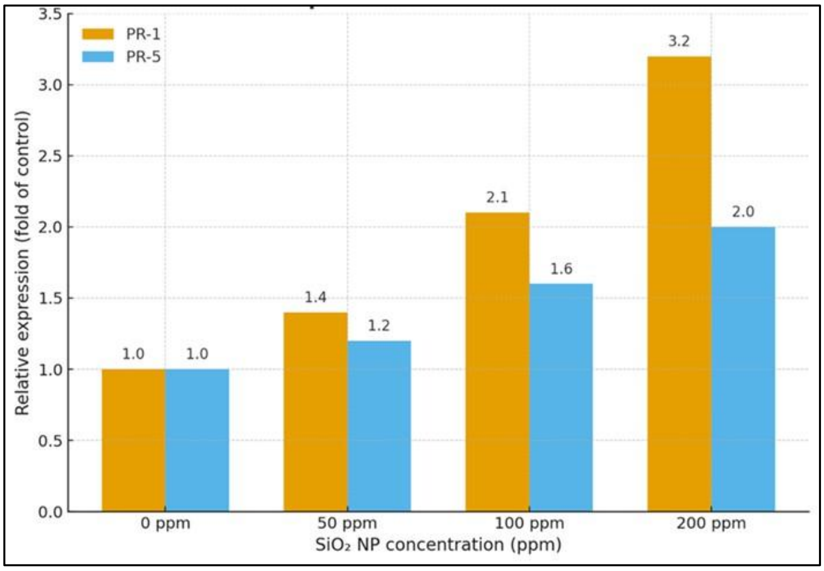

Figure 7. Comparison of PR-1 and PR-5 gene expression of rice leaves at the various concentrations of silicon nanoparticles (SiO_2NPs) following inoculation with *Pyricularia oryzae*. The values of the Expressions were put into normalization with Actin and computed with $2^{-}\Delta\Delta Ct$. Mean ± SE (n = 5). Statistically significant differences in at least $p \leq 0.05$ (ANOVA, LSD test) are indicated by different letters above bars

Although the comparison of partially resistant or moderately susceptible varieties would make the present management strategy more applicable, the scope of the given work does not allow comparing them. We did not intend to survey the interactions between genotype and treatment, but instead examine whether SiO_2 nanoparticles are capable of inducing quantifiable protection responses at severe disease pressure. However, the encouraging findings presented here are a good indication that literature on the use of multiple genotypes, such as moderately resistant cultivars, which can respond to the potential synergistic effect of SiO_2NPs in different genetic backgrounds, should be pursued.

Durability of the nanoparticle layer under field conditions: Such functional performance of foliar-applied nanoparticles actually consists of the capacity of the foliar nanoparticles to stay attached to the leaf surface under the environmental influence of wind, rain, and irrigation. Even though the rainfastness tests were not performed in the current study, a number of physicochemical properties of the SiO_2NP formulation could be advocated to support its stability in foliage. To begin with, amorphous SiO_2 nanoparticles bear surface hydroxyl groups that allow the moderate adhesion to the cuticular wax layer especially in the cases of dispensation with a mild surfactant like Tween-20, which was utilized in the current study. This mixture improves wetting of leaves, decreases droplet run-off, and allows even nanoparticles to be deposited.

Second, it has been reported before that in the drying of nanosilica, it will create micro-aggregated films, and this will raise its resistance to wash off by light rain or wind shear (Sabarinathan et al., 2023). It has been known that such nano-derived surface films can be detected on foliage a few days later, providing time to trigger the defense response pathways. Although the entire rainfastness test was not within the capacities of this study, high physiological levels of response, such as PR-gene expression and less severe blast, suggest that a sufficient number of the nanoparticles applied were not lost from the leaf surface, thus causing a defense response. Controlled rain simulation or field-scale tests of adhesion will be used in future studies and will help to more accurately quantify the persistence of nanoparticles in changing environmental conditions.

Conclusion: The study has identified that silicon nanoparticles (SiO_2NPs) affect the disease control of rice blast disease dually, i.e., reducing the disease severity, enhancing growth, and yield. The 100 200 ppm of SiO2NPs significantly improved the peroxidase (POD) and polyphenol oxidase (PPO) activity and gene PR-1 and PR-5, and it suggests that SiO_2NPs can be employed as physical barriers as well as molecular inducers of plant immunity. Optimal concentration was 200 ppm, which suppressed infection of *Pyricularia oryzae* but had no phytotoxic effect on the vitality of the plants. Such findings show that nano-silicon could be explored as a green and sustainable tool to be incorporated in the integrated management of rice disease. It is implied that additional studies are required to find out the long-term safety and its extension to a range of crops and habitats.

Author contributions

Marwa D. Jaaffer, Ammar M. Chaloop, Alaa W. Abbas and Elaf I. Abdulhussein: Curating and visualizing data participated in conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing, original draft preparation, review writing, and editing, visualization, supervision, administration of the project, Marwa D. Jaaffer. Each of the authors has read and accepted the published form of the manuscript.

Data availability statement

Information can be obtained on demand by the relevant author. It is offered through the corresponding author who provides evidence supporting the findings of this paper on a reasonable request.

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Ethical considerations

The authors did not engage in data fabrication, falsification, plagiarism, or any sort of research misconduct in the study. The study did not involve any human or animal subjects; hence, they did not have to be ethically approved.

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Conflict of interest

The authors do not claim a conflict of interest.

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مجله بیوتکنولوژی کشاورزی
شاپا چاپی: ۶۷۰۵-۲۲۲۸ شاپا الکترونیکی: ۶۵۰۰-۲۲۲۸
دانشگاه شهید باهنر کرمان
انجمن بیوتکنولوژی ایران

نانوذرات دی‌اکسید سیلیکون مقاومت برنج به بلاست را در *Oryza sativa* افزایش می‌دهند

مروه د. جعفر

نویسنده مسئول: گروه بهداشت محیط زیست، دانشکده علوم انرژی و محیطزیست، دانشگاه علوم الکرخ، بغداد، عراق. ایمیل: marwa.dawood@kus.edu.iq

عبدالحسین ایلاف

گروه بهداشت محیط زیست، دانشکده علوم انرژی و محیطزیست، دانشگاه علوم الکرخ، بغداد، عراق. ایمیل: elaf.imad@kus.edu.iq

عباس الاء

گروه بهداشت محیط زیست، دانشکده علوم انرژی و محیطزیست، دانشگاه علوم الکرخ، بغداد، عراق. ایمیل: alaa.waleed@kus.edu.iq

عمار م. چالوب

مهندس کشاورزی، شرکت دبانه کشاورزی مدرن، بغداد، عراق. ایمیل: ammar.majeed2300d@ige.uobaghdad.com

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چکیده

هدف: عامل بیماری‌زای بلاست برنج، *Pyricularia oryzae*، همچنان یکی از مخرب‌ترین موانع تولید برنج در سراسر جهان به شمار می‌رود. این مطالعه بررسی می‌کند که آیا نانوذرات دی‌اکسید سیلیکون (SiO_2NPs) می‌توانند رشد گیاه برنج، عملکرد و پاسخ‌های دفاعی آن را بهبود بخشیده و در عین حال شدت بیماری بلاست را کاهش دهند. توانایی SiO_2NPs در فعال‌سازی آنزیم‌های دفاعی و ژن‌های مرتبط با پاتوژنز (PR) آن‌ها را به جایگزینی سازگار با محیطزیست برای قارچ‌کش‌های شیمیایی تبدیل می‌کند. این پژوهش همچنین کاربرد گسترده‌تر نانوفناوری را به‌عنوان رویکردی پایدار برای تأمین امنیت تولید برنج تحت فشار بیماری بررسی می‌نماید. این مطالعه برای نخستین بار مکانیسم حفاظتی دوگانه نانوذرات SiO_2 را بررسی کرده و همچنین مقایسه‌ای بین دو روش کاربرد خیساندن بذر و محلول‌پاشی برگی تحت شرایط آلودگی به بلاست ارائه می‌دهد و نشان می‌دهد که کاربرد برگی رویکرد مؤثرتری برای مهار بیماری است.

مواد و روش‌ها: نانوذرات SiO_2 با غلظت‌های ۰، ۵۰، ۱۰۰ و ۲۰۰ پی‌پی‌ام از طریق خیساندن بذر یا محلول‌پاشی برگی به بذرهای رقم برنج IR64 اعمال شدند. دو هفته پس از تلقیح *P. oryzae*، شاخص شدت بیماری (DSI) اندازه‌گیری شد. علاوه بر پارامترهای رشد و عملکرد، آنالیزهای بیوشیمیایی شامل فعالیت پراکسیداز (POD) و پلی‌فنل اکسیداز (PPO) و همچنین سطوح بیان ژن‌های PR-1 و PR-5 ثبت گردید. تجزیه و تحلیل آماری داده‌ها با استفاده از آزمون ANOVA و LSD در سطح معنی‌داری $p \leq 0.05$ انجام شد.

نتایج: شدت بیماری بلاست با افزایش غلظت SiO_2NPs به‌صورت وابسته به دوز کاهش یافت. در غلظت ۲۰۰ پی‌پی‌ام، شاخص شدت بیماری از ۷۸/۲٪ در تیمار شاهد بدون نانوذره به ۳۲/۱٪ کاهش یافت. عملکرد دانه در بالاترین تیمار نانوذره‌ای ۵۶٪ افزایش نشان داد که بیانگر بهبود قابل توجه اجزای عملکرد است. گیاهان تیمار شده با SiO_2NPs افزایش معنی‌دار بیان ژن‌های PR-1 (۳/۲ برابر) و PR-5 (۲ برابر) و همچنین افزایش فعالیت POD (۱/۸ برابر) و PPO (۲/۱ برابر) را نشان دادند. اثرات حفاظتی کاربرد برگی به‌طور مداوم بیشتر از خیساندن بذر بود.

نتیجه‌گیری: نانوذرات دی‌اکسید سیلیکون به‌طور مؤثری شدت بیماری بلاست برنج را کاهش داده و هم‌زمان رشد گیاه و فعال‌سازی سامانه‌های دفاعی را تقویت کردند. نقش دوگانه آن‌ها به‌عنوان محافظ‌های ساختاری و القاکننده‌های مولکولی، این نانوذرات را به گزینه‌ای امیدبخش برای کنترل بلندمدت بیماری‌ها بر پایه نانوفناوری در کشاورزی برنج تبدیل می‌کند.

کلمات کلیدی: آنزیم‌های دفاعی، ژن‌های مرتبط با پاتوژنز، مقاومت القایی، نانوذرات سیلیکون، *Pyricularia oryzae*

نوع مقاله: پژوهشی

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