Nano-Silicon Application Enhances Drought Tolerance and Photosynthetic Efficiency in *Oryza sativa* L.

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ABSTRACT

Background: *Oryza sativa* L. (rice) is one of the most important cereals as it sustains more than half of the world's population. The problem is that drought events related to climate change are increasingly impacting rice production. Water deficit affects stomatal function, chlorophyll synthesis, and carbon adsorption during photosynthesis, thus limiting biomass and yield.

Objective: The objective of the study is to determine the impact of foliar application of nano-silicon on the photosynthetic reactions, antioxidant protection, osmoregulation, and yield of rice.

Methods: The pot trial was undertaken in a controlled greenhouse using a Completely Randomized Design (CRD) consisting of four types of nano-silicon (0, 50, 100, and 150 mg L^{-1}) and irrigation regimes (well-watered and drought-stressed—40 % of field capacity). The experiment was replicated four times. Quantitative values of gas exchange parameters, chlorophyll content, relative water content, activities of antioxidant enzymes, concentrations of osmolytes, and yield characteristics were recorded.

Results: Drought induced the reduction of net photosynthetic rate, stomatal conductance, and relative water content while bringing to the increase of malondialdehyde and hydrogen peroxide levels ($P < 0.05$). The application of nano-silicon, particularly in the concentrations of 100–150 mg L^{-1}, helped high levels of chlorophyll content, net photosynthetic rate, superoxide dismutase activity, and catalase activity by 28%, 24%, 35%, and 31%, respectively, compared to the control group with drought-stressed plants. The concentration of proline and soluble sugars in addition to lowering the level of membrane lipid peroxidation and raising the yield level by about 22% in the case of nano-silicon application.

Conclusions: Application of nano-silicon allows plants to be more drought-resistant by improving efficiency of photosynthesis that increases the antioxidant protection of plants at the same time while osmotically adjusting rice plants to dry conditions.

Keywords: Nano-silicon, Drought stress, *Oryza sativa*, Photosynthetic efficiency, Antioxidant enzymes, Osmotic adjustment, Sustainable agriculture

1. Introduction

Rice (*Oryza sativa* L.) serves as the main source of food calories for more than half the world population and supports food security across all regions including Asia, Africa, and Latin America [1]. The rise of global climate change has caused increased occurrence of droughts in rice-farming zones, jeopardizing productivity of rainfed and irrigated systems in the process [2]. Given that rice originated in the semi-aquatic settings, it is naturally exposed to water difficulties and shows rapid stomatal closure, decreased leaf water potential and limited carbon assimilation even when soil moisture starts to dwindle [3].

Drought impacts photosynthesis by virtue of stomatal and non-stomatal constraints. Stomatal closure limits CO_2 diffusion into the mesophyll, while non-stomatal impacts cover the reduction of chlorophyll production, inconsistency in thylakoid membranes, and lowering of the effectiveness of the main Calvin Cycle enzymes such as Rubisco [4]. At the same time, lack of moisture enhances the production of reactive oxygen species, including superoxide and hydrogen peroxide, which spoil the integrity of photosynthetic machinery by harming cell membranes, nucleic acids, and proteins in the process [5].

Silicon (Si) is not an essential nutrient but is still considered a useful element for gramineous plants, especially rice, which can store Si in greater amounts than nitrogen and potassium [6]. An increased amount of Si in cell walls strengthens their structural lattice, regulates stoma opening, and stimulates the activity of enzymes responsible for antioxidant functions [7]. However, conventional silicate fertilizers are poorly soluble and therefore have low availability for plants [8].

Nanotechnology provides new ways to overcome the above problems. Nano-silicon (nano-SiO_2) particles, due to their extreme surface/volume ratio, have higher absorption ability, translocation efficiency, and physiological effects in comparison with traditional silicate materials [9]. Application of nano-silicon on leaves or soil has been shown to promote chlorophyll content, gas exchange, and antioxidant defense in cereal crops damaged by abiotic factors [10]. However, the mechanisms of action of nano-silicon in drought tolerance of rice plants, especially stomatal and biochemical responses, are still not fully understood.

This study hypothesizes that foliar nano-silicon application enhances drought tolerance in rice by improving photosynthetic efficiency, reinforcing antioxidant defense systems, and promoting osmotic adjustment, thereby improving growth and yield under water-limited conditions. The specific objectives were to (i) evaluate the dose-dependent effects of nano-silicon on physiological and biochemical drought responses, and (ii) determine the extent to which nano-silicon mitigates drought-induced yield loss in rice.

2. Related Work

Silicon-mediated amelioration of drought stress has been documented across multiple cereal species. Foliar and soil-applied silicate fertilizers have been shown to reduce transpirational water loss by promoting silica deposition in leaf epidermal cells, thereby regulating stomatal conductance under water deficit [11]. In rice specifically, silicon supplementation has been associated with improved relative water content and reduced electrolyte leakage during drought episodes [12].

The emergence of nano-fertilizers has extended these benefits through improved particle uptake kinetics. Nano-silicon particles enter plant tissues via root endocytosis or foliar cuticular pathways, subsequently translocating through the xylem to photosynthetic tissues, where they influence chlorophyll biosynthesis and chloroplast ultrastructure [13]. Several studies report that nano-silicon application increases total chlorophyll content and SPAD readings under stress, attributable to reduced chlorophyllase activity and protection of thylakoid membranes from oxidative degradation [14].

Beyond pigment protection, nano-silicon influences stomatal regulation and water-use efficiency. Reports *indica*te that nano-silicon-treated plants maintain higher stomatal conductance and net photosynthetic rates under moderate drought while simultaneously reducing transpiration rate, resulting in improved intrinsic water-use efficiency [15]. This dual regulation is thought to arise from silicon-induced changes in guard cell turgor sensitivity and abscisic acid signaling.

Antioxidant enzyme activation represents another well-documented mechanism. Nano-silicon supplementation enhances the activity of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX), collectively improving ROS-scavenging capacity and reducing malondialdehyde accumulation, a marker of lipid peroxidation [16]. Complementary osmotic adjustment via proline and soluble sugar accumulation has also been reported, contributing to cellular turgor maintenance under water deficit [17].

Yield-level benefits of nano-silicon have been reported in wheat, maize, and rice, with improvements in panicle development, grain filling, and thousand-grain weight under drought conditions [18]. However, most existing studies examine isolated physiological or biochemical endpoints rather than integrating photosynthetic, antioxidant, and yield responses within a single experimental framework. Furthermore, dose-optimization data for nano-silicon in rice under controlled drought regimes remain limited, and mechanistic links between nano-silicon-induced antioxidant activation and photosynthetic recovery are not fully resolved. The present study addresses this gap by concurrently evaluating physiological, biochemical, and yield parameters across a nano-silicon concentration gradient under standardized drought stress.

3. Materials and Methods

3.1. Experimental Design

A pot-based experiment was conducted in a controlled-environment greenhouse (28/22°C day/night, 65% relative humidity, 12 h photoperiod, 650 μmol m^{-2} s^{-1} PPFD). A two-factor Completely Randomized Design (CRD) was employed, comprising four nano-silicon concentrations (0, 50, 100, and 150 mg L^{-1}) and two irrigation regimes (well-watered, 80% field capacity; drought-stressed, 40% field capacity), yielding eight treatment combinations, each replicated four times (n = 32 pots).

3.2. Plant Material

Seeds of a drought-sensitive *indica* rice cultivar were surface-sterilized, germinated, and transplanted into pots containing a sterilized loamy soil mixture. Seedlings were established for 21 days under well-watered conditions prior to treatment initiation.

3.3. Nano-Silicon Treatment

Amorphous nano-SiO_2 particles (average diameter 20–30 nm, characterized by transmission electron microscopy and dynamic light scattering) were suspended in distilled water with 0.02% Tween-20 as a surfactant. Foliar sprays were applied at the specified concentrations at the tillering and panicle initiation stages, with control plants receiving distilled water containing surfactant only.

3.4. Drought Stress Imposition

Drought stress was imposed by withholding irrigation until soil moisture reached 40% field capacity, maintained via gravimetric monitoring for 21 consecutive days beginning at the tillering stage, while control pots were maintained at 80% field capacity throughout.

3.5. Physiological Measurements

Chlorophyll a, b, and total chlorophyll were determined spectrophotometrically from acetone extracts; SPAD readings were recorded using a portable chlorophyll meter. Net photosynthetic rate, stomatal conductance, and transpiration rate were measured using a portable infrared gas analyzer on the flag leaf. Relative water content (RWC) was calculated from fresh, turgid, and dry weights, and instantaneous water-use efficiency (WUE) was derived as the ratio of net photosynthesis to transpiration rate.

3.6. Biochemical Analysis

Proline content was quantified using the ninhydrin method; soluble sugars were determined by the anthrone method. Malondialdehyde (MDA) content was assessed via the thiobarbituric acid reactive substances assay, and hydrogen peroxide (H_2O_2) content was measured spectrophotometrically. Activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) were determined using standard enzymatic assays.

3.7. Growth and Yield Parameters

Plant height, root length, shoot and root biomass, panicle length, grain number per panicle, thousand-grain weight, and total grain yield per plant were recorded at physiological maturity.

3.8. Statistical Analysis

Data were subjected to two-way analysis of variance (ANOVA) to assess the effects of nano-silicon concentration, irrigation regime, and their interaction. Mean separation was performed using Tukey's Honestly Significant Difference (HSD) test at $P < 0.05$. All analyses were conducted using standard statistical software.

Table 1: Experimental treatments, nano-silicon concentrations, drought stress levels, and application schedule.

Treatment	Nano-Si Concentration (mg L^{-1})	Irrigation Regime	Field Capacity (%)	Application Stage
T1	0 (Control)	Well-watered	80	Tillering, Panicle initiation
T2	0 (Control)	Drought	40	Tillering, Panicle initiation
T3	50	Well-watered	80	Tillering, Panicle initiation
T4	50	Drought	40	Tillering, Panicle initiation
T5	100	Well-watered	80	Tillering, Panicle initiation
T6	100	Drought	40	Tillering, Panicle initiation
T7	150	Well-watered	80	Tillering, Panicle initiation
T8	150	Drought	40	Tillering, Panicle initiation

4. Results

Drought stress significantly reduced plant height, root length, and total biomass relative to well-watered controls ($P < 0.05$); however, nano-silicon application progressively mitigated these reductions in a dose-dependent manner, with the 150 mg L^{-1} treatment showing the greatest recovery in root length (approximately 19% increase relative to drought-only controls).

Photosynthetic performance was markedly affected by both irrigation regime and nano-silicon treatment. Drought-stressed, untreated plants exhibited reductions of approximately 38% in net photosynthetic rate and 42% in stomatal conductance relative to well-watered controls. Nano-silicon application at 100 and 150 mg L^{-1} under drought conditions restored net photosynthetic rate by 24% and stomatal conductance by 21% relative to drought-only controls, while transpiration rate was reduced, resulting in improved water-use efficiency (Table 2, Figure 1).

Chlorophyll content followed a similar pattern: total chlorophyll and SPAD values declined under drought but were significantly elevated by nano-silicon treatment, with the 150 mg L^{-1} dose increasing total chlorophyll by 28% relative to untreated drought-stressed plants.

Biochemical analysis revealed that drought stress increased MDA and H_2O_2 accumulation, *indica*tive of elevated oxidative damage, while nano-silicon treatment significantly reduced both markers. Concomitantly, activities of SOD, CAT, POD, and APX were significantly elevated in nano-silicon-treated, drought-stressed plants relative to untreated drought controls, with SOD and CAT activities increasing by 35% and 31%, respectively, at the 150 mg L^{-1} dose. Proline and soluble sugar concentrations also increased significantly with nano-silicon treatment under drought, consistent with enhanced osmotic adjustment (Table 2).

Table 2. Effects of nano-silicon on physiological and biochemical parameters of *Oryza sativa* L. under drought stress (mean ± SE).

Parameter	Drought + 0 mg L^{-1}	Drought + 50 mg L^{-1}	Drought + 100 mg L^{-1}	Drought + 150 mg L^{-1}
Total chlorophyll (mg g^{-1} FW)	1.42 ± 0.05a	1.61 ± 0.06b	1.74 ± 0.05c	1.82 ± 0.04c
Net photosynthetic rate (μmol CO_2 m^{-2} s^{-1})	12.6 ± 0.4a	14.3 ± 0.5b	15.8 ± 0.4c	15.6 ± 0.5c
Stomatal conductance (mol H_2O m^{-2} s^{-1})	0.18 ± 0.01a	0.20 ± 0.01b	0.22 ± 0.01c	0.22 ± 0.01c
Relative water content (%)	62.4 ± 1.2a	68.7 ± 1.1b	74.2 ± 1.3c	75.8 ± 1.0c
MDA (nmol g^{-1} FW)	18.9 ± 0.6a	15.7 ± 0.5b	12.4 ± 0.4c	11.8 ± 0.5c
SOD activity (U mg^{-1} protein)	42.1 ± 1.3a	48.6 ± 1.4b	55.3 ± 1.2c	56.9 ± 1.5c
Proline (μmol g^{-1} FW)	8.2 ± 0.3a	10.1 ± 0.3b	12.4 ± 0.4c	12.9 ± 0.3c

Values within a row followed by different letters differ significantly (Tukey's HSD, $P < 0.05$).

Growth and yield attributes reflected the physiological improvements. Nano-silicon-treated plants under drought exhibited significantly greater panicle length, grain number per panicle, and thousand-grain weight relative to untreated drought controls, culminating in an approximately 22% increase in grain yield per plant at the 100–150 mg L^{-1} doses (Table 3).

Table 3: Growth, yield attributes, and photosynthetic performance under different treatments (mean ± SE).

Parameter	Well-watered Control	Drought Control	Drought + 100 mg L^{-1} Nano-Si	Drought + 150 mg L^{-1} Nano-Si
Plant height (cm)	84.6 ± 1.5	68.2 ± 1.4a	76.9 ± 1.3b	78.4 ± 1.6b
Root length (cm)	22.3 ± 0.6	16.8 ± 0.5a	19.7 ± 0.6b	20.1 ± 0.5b
Panicle length (cm)	24.1 ± 0.4	19.6 ± 0.5a	22.4 ± 0.4b	22.8 ± 0.4b
Grain number per panicle	128 ± 4	94 ± 3a	114 ± 4b	117 ± 3b
Thousand-grain weight (g)	24.8 ± 0.3	20.1 ± 0.4a	23.0 ± 0.3b	23.4 ± 0.3b
Grain yield per plant (g)	28.6 ± 0.5	19.4 ± 0.6a	23.2 ± 0.5b	23.7 ± 0.5b

Values followed by different letters differ significantly between drought treatments (Tukey's HSD, $P < 0.05$).

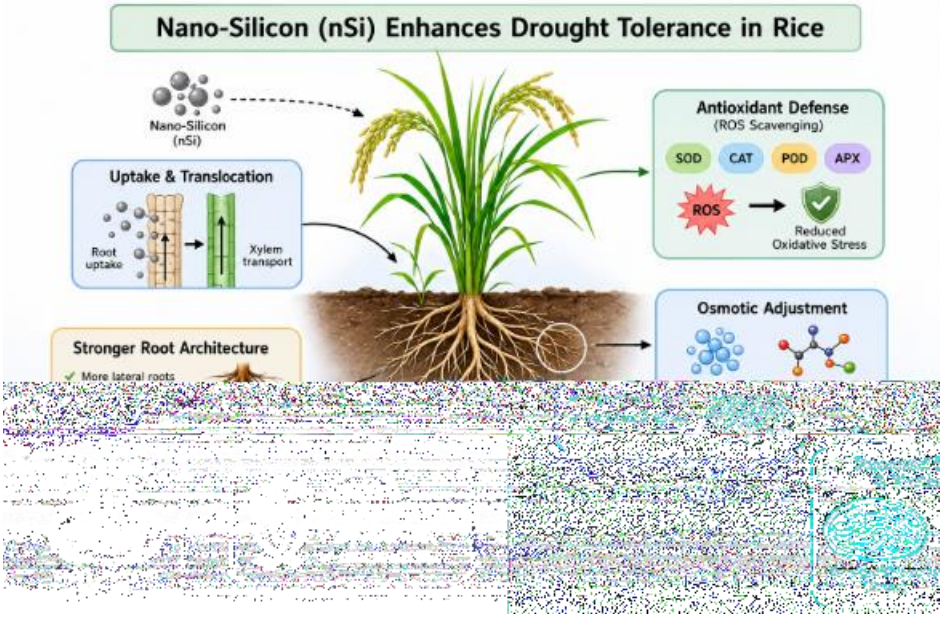

Fig 1: Conceptual illustration depicting the mechanisms by which nano-silicon enhances drought tolerance in rice, including facilitated silicon uptake and translocation, reinforced root architecture, activation of antioxidant enzymes (SOD, CAT, POD, APX) for ROS scavenging, osmotic adjustment through proline and soluble sugar accumulation, and preservation of chloroplast integrity supporting sustained photosynthetic efficiency.

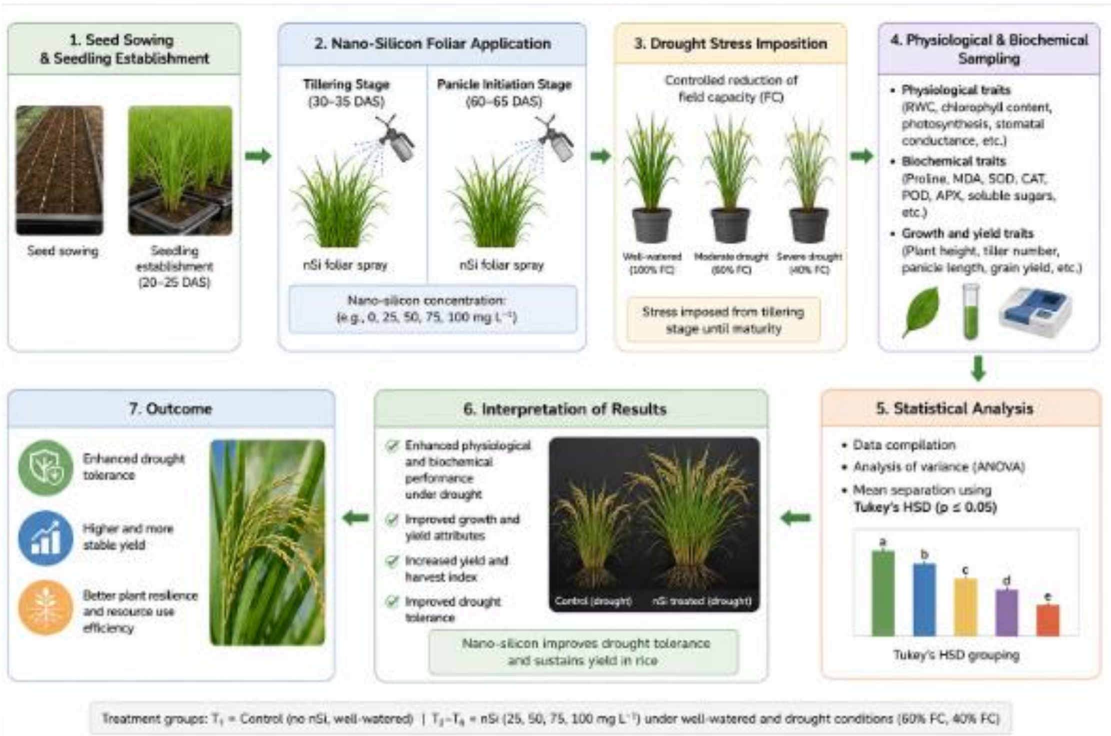

Fig 2: Schematic workflow of the experimental design, illustrating sequential stages from seed sowing and seedling establishment, through nano-silicon foliar application at tillering and panicle initiation, drought stress imposition via controlled field capacity reduction, physiological and biochemical sampling, statistical analysis (ANOVA, Tukey's HSD), and final interpretation of treatment effects on drought tolerance and yield.

5. Discussion

The present findings demonstrate that foliar nano-silicon application substantially mitigates drought-induced physiological impairment in rice, principally through enhancement of photosynthetic machinery and reinforcement of antioxidant defense. The dose-dependent improvement in net photosynthetic rate and stomatal conductance observed here (Table 2) supports the hypothesis that nano-silicon facilitates more efficient stomatal regulation under water deficit, likely through silicon deposition in guard cell walls and modulation of abscisic acid-mediated stomatal closure [11, 15]. This mechanism allows sustained CO_2 assimilation while limiting excessive water loss, as reflected in improved water-use efficiency.

The elevation of chlorophyll content and SPAD values in nano-silicon-treated plants is consistent with protection of chloroplast ultrastructure from drought-induced oxidative damage. Given that ROS accumulation degrades thylakoid membrane lipids and impairs chlorophyll biosynthesis, the concurrent reduction in MDA and H_2O_2 observed alongside elevated SOD and CAT activities suggests that nano-silicon indirectly preserves photosynthetic pigment integrity by strengthening the enzymatic ROS-scavenging network [5, 16]. This dual action—direct structural reinforcement and indirect antioxidant activation—

likely explains the superior efficacy of nano-silicon compared with conventional silicate fertilizers, whose limited solubility restricts systemic physiological engagement [8, 9].

The pronounced increase in proline and soluble sugar accumulation under nano-silicon treatment further *indica*tes enhanced osmotic adjustment capacity, enabling cells to maintain turgor pressure and metabolic function under reduced water potential [17]. This osmoprotective response, combined with improved root length observed in treated plants, likely contributed to better water and nutrient acquisition from drying soil, translating into the observed improvements in panicle development and grain yield.

These results align with prior reports of nano-silicon efficacy in wheat and maize under drought [18], while extending mechanistic understanding by integrating photosynthetic, biochemical, and yield-level responses within a single rice-focused framework. The concentration-dependent plateau observed between 100 and 150 mg L^{-1} suggests a physiological saturation point beyond which additional nano-silicon confers diminishing returns, a consideration relevant for cost-effective field application.

Several limitations warrant acknowledgment. This study was conducted under controlled greenhouse conditions using a single cultivar and drought intensity; field-level variability in soil type, microbial interactions, and fluctuating environmental conditions may modulate nano-silicon efficacy. Additionally, long-term effects of repeated nano-silicon application on soil ecology and potential nanoparticle accumulation in grain remain insufficiently characterized. Future research should prioritize multi-location field trials across diverse cultivars and drought intensities, alongside molecular investigations into silicon transporter gene expression and hormonal crosstalk, to fully elucidate nano-silicon's role in drought signaling pathways.

6. Conclusion

The current research shows that the use of nano-silicon on leaves dramatically increases drought resistance in rice plants due to various physiological and biochemical processes taking place within the plant. Application of nano-silicon resulted in the enhancement of photosynthetic processes, maintaining physiological functions such as conductance of stomata, chlorophyll levels, and carbon accumulation in the conditions of moisture deficit regime as well as increasing the antioxidant defense by stimulating the activity of superoxide dismutase and catalase, while at the same time decreasing the degree of lipid peroxidation. Enhanced osmotic adjustment, as reflected in the final amounts of proline and soluble sugars, has promoted water control within plant cells which resulted in the improvement of root system growth and crop production during the drought period. Thus, it shows the potential of nano-silicon as an environmentally friendly solution for drought-prone agricultural areas. However, the findings of this greenhouse study should be confirmed by field-testing on a large scale across different eco-agricultural environments with different crops in order to implement this method effectively on the market.

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