



Silicon Dioxide Nanoparticles Enhance Morphophysiological Performance and Biochemical of Soybean (*Glycine Max* L.) cv. Gepak Kuning Under PEG-Induced Drought Stress

¹Satrio Lintang Pambudi, ²Berlian Rosilia Shodiqin, ²Almas Nurazita Suwarno, ³Mohammad Ubaidillah, ⁴Tri Agus Siswoyo

1. Study Program of Magister of Agronomy, Faculty of Agriculture, University of Jember, Jember 68121, Indonesia
2. Graduate School of Biotechnology, University of Jember, Jember 68121, Indonesia
3. Graduate School of Biotechnology, University of Jember, Jember 68121, Indonesia, Graduate School of Biotechnology, University of Jember, Jember 68121, Indonesia and Department of Agronomy, Faculty of Agriculture, University of Jember, Jember 68121, Indonesia
4. Graduate School of Biotechnology, University of Jember, Jember 68121, Indonesia, Departement of Agronomy, Faculty of Agriculture, University of Jember, Jember 68121, Indonesia and The Center of Excellence on Crop Industrial Biotechnology (PUI-PT BioTIn), University of Jember, Jember 68121, Indonesia

Abstract: The application of silicon dioxide (SiO₂) nanoparticles has emerged as a promising approach to enhance plant tolerance to drought stress by improving morphophysiological and biochemistry performance. This study aimed to evaluate the effects of 100 ppm SiO₂ nanoparticles on the morphophysiological responses of the soybean cultivar Gepak Kuning under normal conditions and polythlene glycol (PEG) 6000-induced drought stress. The evaluated parameters included plant height, number of leaves, stem diameter, root length, number of nodules, percentage of active nodules, photosynthetic pigment content, relative water content, electrolyte leakage, protein content, proline content, proline to protein ratio, total flavonoid content, total phenolic content, and plant biomass. The results showed that the application of SiO₂ nanoparticles significantly improved morphological characteristics, including plant height, number of leaves, stem diameter, root length, number of nodules, and the percentage of active nodules. The treatment also enhanced photosynthetic pigment content, including chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll, as well as protein content, relative water content, and plant biomass. In addition, SiO₂ nanoparticles reduced oxidative stress by decreasing the chlorophyll a/b ratio, carotenoid to total chlorophyll ratio, hydrogen peroxide (H₂O₂), malondialdehyde (MDA), proline content, proline to protein ratio, total flavonoid content, total phenolic content, and fresh and dry biomass losses under drought stress. These findings indicate that the application of SiO₂ nanoparticles enhances soybean tolerance to drought stress by improving morphophysiological performance, maintaining plant water status, regulating stress-related metabolite accumulation, and promoting biomass production.

Keywords: Drought stress, PEG, *Glycine max* L., Silicon Dioxide Nanoparticles, Morphophysiology

INTRODUCTION

Drought stress is one of the major abiotic factors threatening global agricultural productivity, causing substantial reductions in crop growth and yield [1]. In soybean (*Glycine max* L.), drought stress is projected to reduce grain yield by up to 60%, depending on the

severity and duration of water deficit [2] Water deficiency limits water and nutrient uptake, disrupts metabolic processes, alters root architecture, impairs morphophysiological performance, and disturbs cellular osmotic balance, ultimately resulting in reduced plant growth and productivity [3] Moreover, drought stress promotes the excessive accumulation of reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2) and malondialdehyde (MDA), which induce oxidative damage to cellular components such as lipids, proteins, and nucleic acids, thereby impairing normal physiological functions [4]

Nanotechnology has emerged as a promising approach to address the challenges of modern agriculture, particularly those associated with abiotic stress [5]. Among the various nanomaterials, SiO_2 nanoparticles have attracted considerable attention because of their ability to improve nutrient use efficiency and enhance plant tolerance to drought stress [6], [7]. The effects of SiO_2 nanoparticles have been attributed to several physiological and biochemical mechanisms, including improved nutrient uptake, maintenance of plant water status, enhanced accumulation of protective metabolites, and increased plant growth and biomass under water-deficit conditions [8].

In soybean production, the application of SiO_2 nanoparticles is expected to enhance drought tolerance by improving plant growth, maintaining morphophysiological performance, preserving plant water status, regulating oxidative stress, promoting the accumulation of stress-related metabolites, and increasing biomass production. Although the beneficial effects of SiO_2 nanoparticles have been reported in several crop species, information regarding their effects on local soybean cultivars under drought stress remains limited. In this study drought stress was simulated using polyethylene glycol (PEG) 6000 [9].

Therefore, this study aimed to evaluate the effectiveness of SiO_2 nanoparticles in improving the drought tolerance of the local soybean cultivar Gepak Kuning under PEG 6000-induced drought stress by assessing morphophysiological characteristics, photosynthetic pigments, plant water status, oxidative stress markers, stress-related metabolites, and biomass production.

MATERIAL AND METHODS

The experiment was conducted in February 2026 at the Greenhouse and Center for Excellence in Science and Technology for Industrial Plant Biotechnology (PUI-PT BioTIn), University of Jember, East-Java, Indonesia (-8.164630° N, 113.716932° E).

SiO₂ Preparation

Commercial SiO_2 nanoparticles were purchased from PT Maxlab Indonesia (Indonesia). The physicochemical properties of the nanoparticles were consistent with the manufacturer's specifications.

A 100 ppm SiO_2 nanoparticle suspension was prepared by dispersing the nanoparticles in sterile distilled water and homogenizing the suspension using a vortex mixer for 2 min to minimize particle aggregation. Fresh suspension was prepared immediately before each application. The SiO_2 nanoparticle suspension was applied to the soil at a final concentration of 100 ppm.

Plant Material and Growth Conditions

Soybean (*Glycine max* L.) seeds of the local cultivar Gepak Kuning were planted in 20 x 20 cm polybags filled with inceptisol soil collected from Banyuwangi, East Java, Indonesia (-8.3276304° N, 114.1217776° S). The plants were watered regularly until 12 days after planting (DAP). Drought stress was induced by applying 150 mL of a 10% polyethylene glycol (PEG) 6000 solution to each polybag every 3 days for a total of four applications, beginning at 12 DAP [9]. Silicon dioxide (SiO₂) nanoparticles were applied as a soil treatment at a concentration of 100 ppm, starting at 14 DAP, with four applications at 4-day intervals. The PEG treatment was initiated before the application of SiO₂ nanoparticles to ensure the establishment of drought stress. Leaf, stem, root, and nodule samples were collected at 28 DAP for morphological, physiological, biochemical, and biomass analyses.

Morpho-physiological Parameters

Morphological parameters, including plant height, number of leaves, stem diameter, root length, number of nodules, and percentage of active nodules, were evaluated at 28 days after planting (DAP). Plant height was measured from the base of the stem to the shoot tip, while the number of leaves was determined by counting fully expanded trifoliate leaves. Stem diameter was measured at the stem base using a digital caliper. Root length was measured from the base of the primary root to the root tip after carefully washing the root system. The total number of nodules was counted for each plant, and the percentage of active nodules was determined by dissecting the nodules and identifying active nodules based on presence of a pink or reddish internal color [10]. Leaf samples were collected at 28 DAP and immediately used for physiological and biochemical analyses.

Pigment Photosynthesis Content

Photosynthetic pigment contents were determined following a method described by Porra [11]. Briefly, 0.3 g of fresh leaf tissue was homogenized with 80% methanol (1:5 w/v), prior centrifuged at 10,000 rpm for 10 min, and the supernatant obtained was collected for pigment analysis. The absorbance of the extract was measured at 665, 652, and 470 nm using a UV-Vis spectrophotometer. Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents were calculated using the following equations:

$$\text{Chlorophyll a (mg/g)} = \frac{[(16,29 \times \text{Abs}_{665}) - (8,54 \times \text{Abs}_{652})]}{1000} \times \text{volume/weight} \quad (1)$$

$$\text{Chlorophyll b (mg/g)} = \frac{[(30,66 \times \text{Abs}_{652}) - (13,58 \times \text{Abs}_{665})]}{1000} \times \text{volume/weight} \quad (2)$$

$$\text{Carotenoid (mg/g)} = \frac{[(1000 \times \text{Abs}_{470}) - (22,12 \times \text{Abs}_{665}) - (2,71 \times \text{Abs}_{652})]}{227} \times \text{volume/weight} \quad (3)$$

$$\text{Total chlorophyll (mg/g)} = \frac{[(22,12 \times \text{Abs}_{665}) + (2,71 \times \text{Abs}_{652})]}{1000} \times \text{volume/weight} \quad (4)$$

Relative Water Content (RWC), Electrolyte Leakage (EL)

The relative water content was determined following method by Rosa et al [12]. Leaf samples (0.3 g) were soaked in 30 mL of distilled water for 24 hours at 4°C in the dark, and

their saturated weights were recorded. The samples were oven dried at 70 °C for 48 hours, and their dry weights were recorded. RWC was calculated using the following equations:

$$\text{RWC (\%)} = \frac{\text{FW}-\text{DW}}{\text{SW}-\text{DW}} \times 100\% \quad (5)$$

- FW = fresh weight
- SW = saturated weight
- DW = dry weight

EL was determined following method described by Rosa et al [12]. Fresh leaf samples (0.3 g) were cut into approximately 5 mm segments and immersed in 10 mL of distilled water in bottle, prior incubated at room temperature for 2 hours, then the initial electrical conductivity (EC1) of the solution was measured using an electrical conductivity (EC) meter. The samples were then autoclaved at 121 °C for 20 min to completely release cellular electrolytes, cooled to room temperature, and the final electrical conductivity (EC2) was measured. EL was calculated using the following equation:

$$\text{EL (\%)} = \frac{\text{EC1}}{\text{EC2}} \times 100\% \quad (6)$$

H₂O₂ and MDA content

H₂O₂ and MDA content was determined following method described by Velikova [13]. Fresh leaf tissue (0.3 g) was homogenized in 0.1% trichloroacetic acid (TCA) (1:5 w/v) prior centrifuged at 12,000 rpm for 15 min at 4 °C. Subsequently for H₂O₂, 250 µL of the supernatant was vortex-mixed with 250 µL 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of potassium iodide (KI) and incubated at 4 °C for 15 min before measuring the absorbance at 390 nm using a UV-Vis spectrophotometer. The H₂O₂ concentration was determined from a standard calibration curve and expressed as µmol g⁻¹ fresh weight (FW). For MDA, 250 µL of the supernatant was mixed with 1 mL of a reaction solution containing 20% TCA and 0.5% thiobarbituric acid (TBA) (1:4 v/v). The absorbance of the reaction mixture was measured at 532 and 600 nm using a UV-Vis spectrophotometer. The MDA concentration was calculated using an extinction coefficient 155 Mm⁻¹ cm⁻¹ and expressed as µmol g⁻¹ FW.

Protein and Proline Content

Protein content was determined using the Bradford assay [14]. Fresh leaf tissue (0.3 g) was homogenized in potassium buffer (pH 7.4) (3:1 w/v), prior centrifuged at 12,000 rpm for 15 min at 4 °C, and the supernatant was incubated a 4 °C for 24 hours. Subsequently, 5 µL of the extract was mixed with 45 µL of distilled water and 950 µL of Bradford reagent. After incubation, the absorbance was measured at 595 nm using a UV-Vis spectrophotometer. Protein concentration was determined from a Bovine Serum Albumin (BSA) standard curve and expressed as mg g⁻¹ FW. Proline content was determined using the method described by Lee et al [15] Briefly, 200 µL of the extract was mixed with 800 µL of the reaction reagent. The reaction mixture was heated at 95 °C for 20 min using a dry block heater, cooled to room temperature, and centrifuged at 10,000 rpm for 1 min at 25 °C. The absorbance of the supernatant was measured at 520 nm using a UV-Vis spectrophotometer. Proline concentration was calculated from standard curve and expressed as µmol g⁻¹ FW

Phenolic and Flavonoid Content

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method described by Taga et al [16]. Briefly, 5 μ L of the extract was vortex-mixed with 45 μ L of analytical-grade methanol, 1 mL of 2% sodium carbonate (Na_2CO_3), and 50 μ L of 50% Folin-Ciocalteu reagent, prior incubated for 30 min at room temperature before measuring the absorbance at 750 nm using a UV-Vis spectrophotometer. Total phenolic content was calculated from a gallic acid standard curve and expressed as mg Gallic Acid Equivalents (GAE) g^{-1} FW.

Total flavonoid content (TFC) was determined according to Pradipta et al [17] using Quercetin as the standard. Briefly, 10 μ L of the extract was mixed with 40 μ L of analytical-grade methanol, 400 μ L of distilled water, and 30 μ L of 10% aluminum chloride (AlCl_3) was added, and the mixture was incubated for an additional 6 min. finally, 200 μ L of 1 N sodium hydroxide (NaOH) and 240 μ L of distilled water were added before measuring the absorbance at 415 nm using a UV-Vis spectrophotometer. Total flavonoid content was calculated from a quercetin standard curve and expressed as mg Quercetin Equivalent (QE) g^{-1} FW.

Statistical Analysis

The study was conducted using a non-factorial completely randomized design consisting of eight treatments with three replicates. The analysis used was ANOVA, followed by the Duncan Multiple Range Test ($p < 0.05$).

RESULT AND DISCUSSION

SiO₂ Nanoparticle Improve Soybean Vegetative Growth Under Drought Stress

The application of SiO₂ nanoparticles significantly affected all vegetative growth parameters of soybean plants (Fig.1). Among the treatments, plants treated with SiO₂ nanoparticles (n-SiO₂) exhibited the greatest plant height (51.6 cm), whereas drought-stressed plants (DS) showed the lowest value (37.06 cm). Similarly, the highest number of leaves was recorded in the n-SiO₂ treatment (5.6 leaves plant⁻¹), while the DS treatment produced the fewest leaves (4.3 leaves plant⁻¹). Stem diameter followed the same trend, reaching a maximum of 2.4 mm in the n-SiO₂ treatment and a minimum of 1.6 mm under drought stress.

A similar response was observed in root growth. Plants treated with n-SiO₂ produced the longest roots (34.5 cm), whereas drought-stressed plants had the shortest roots (22.7 cm). drought stress also markedly reduced nodulation, as reflected by the lowest number of nodules (8.6 nodules plant⁻¹) in the DS treatment. In contrast, the n-SiO₂ treatment produced the highest number of nodules (34.3 nodules plant⁻¹), accompanied by the highest percentage of active nodules (98%). These result indicate that SiO₂ nanoparticles effectively alleviated the adverse effects of drought stress by promoting vegetative growth and maintaining nodulation, thereby supporting nitrogen fixation and overall plant development. Improved growth under SiO₂ nanoparticle treatment may be associated with enhanced plant water status, nutrient uptake, and photosynthetic efficiency, wich collectively contribute to greater drought tolerance [18]

Drought stress induced by 10% PEG 6000 during the vegetative stage significantly reduced the morphological performance of soybean plants. Polyethylene glycol is widely

used to simulate drought stress because it lowers the osmotic potential of the growing medium, thereby limiting water availability to plant roots and mimicking soil water deficit conditions [9], [19]. Reduced water availability inhibits cell division and cell expansion, decreases tissue water potential, and consequently suppresses plant growth and development [20]. These adverse effects are reflected not only in reduced morphological growth but also in altered physiological responses, changes in stress-related metabolite accumulation, and decreased biomass production.

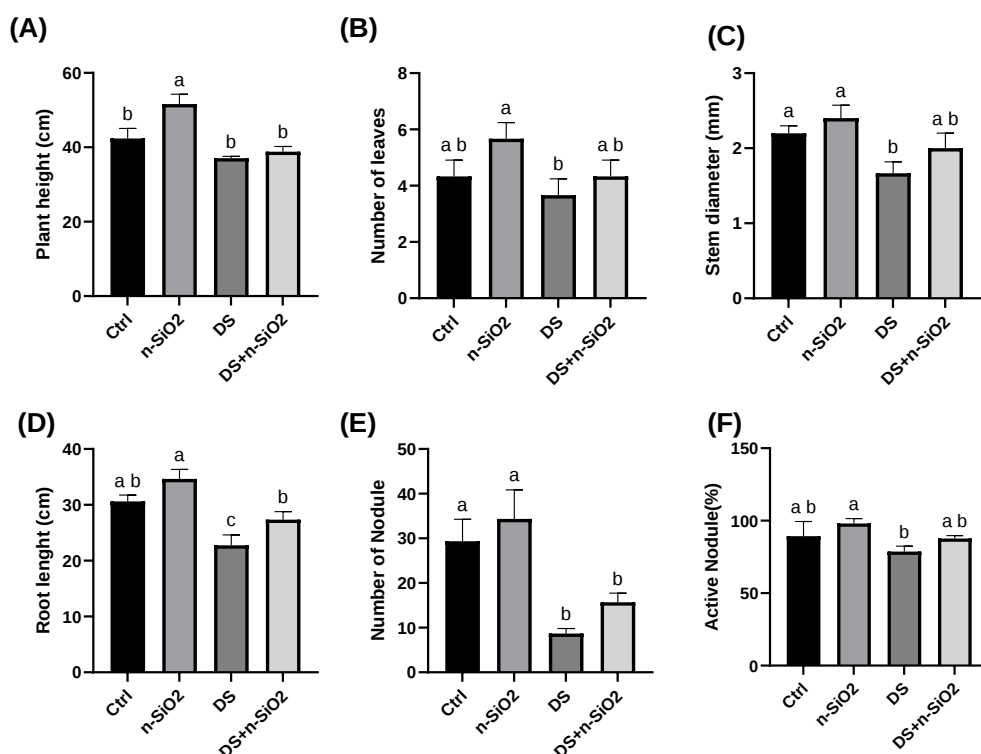


Fig. 1: Plant height, number of leaves, stem diameter, root length, number of nodule, and active nodule for the morphological appearance of soybean plants after treatment with SiO₂ nanoparticle. Different letters at the same level indicate a significant difference ($p < 0.05$)

Response of Photosynthetic Pigments in Soybean after Treatment with SiO₂ Nanoparticle and PEG

Table 1: Effect after treatment with SiO₂ nanoparticle and PEG on photosynthesis pigment of soybean plants.

Treatment	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Carotenoid (mg/g FW)	Chlorophyll Content (mg/g FW)
Ctrl	7.92 ± 0.12b	5.95 ± 0.26b	4.61 ± 0.51b	13.87 ± 0.38b
n-SiO ₂	8.99 ± 0.04a	6.90 ± 0.07a	3.13 ± 0.09c	15.89 ± 0.04a
DS	6.07 ± 0.04c	2.54 ± 0.16d	6.01 ± 0.05a	8.61 ± 0.16c
DS + n-SiO ₂	6.16 ± 0.11c	3.20 ± 0.18c	5.20 ± 0.50ab	9.36 ± 0.08c

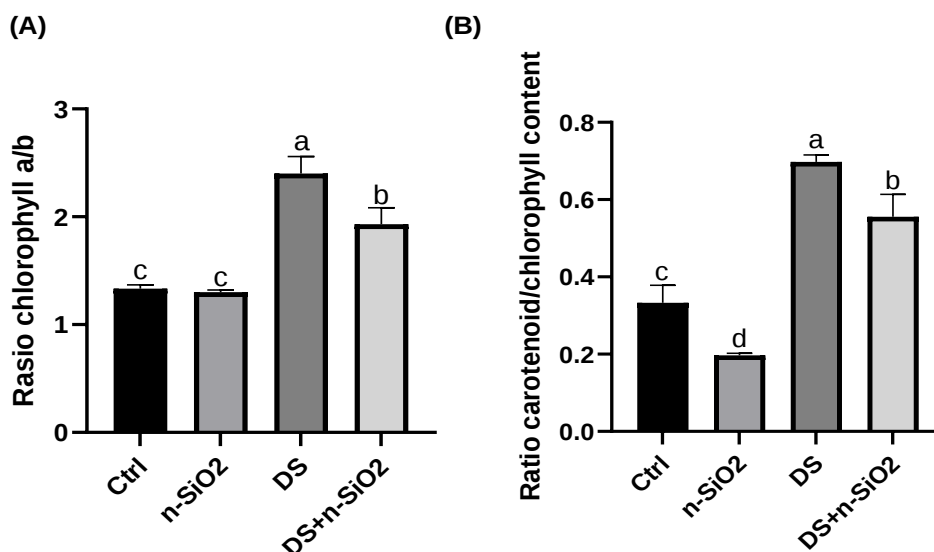


Fig. 2: Ratio chlorophyll a/b and ratio carotenoid/chlorophylls content of soybean plants after treatment SiO₂ nanoparticle. Different letters at the same level indicate a significant difference ($p < 0.05$)

The result of the study that the photosynthetic pigment content was significantly affected by the application of n-SiO₂ and PEG. The n-SiO₂ treatment showed the highest levels of chlorophyll a, chlorophyll b, and total chlorophyll, with average values of 8.99 mg/g FW, 6.90 mg/g FW, and 15.89 mg/g FW, respectively. Meanwhile, for carotenoid content, chlorophyll a/b ratio, and carotenoid/total chlorophyll ratio showed the highest results under the DS treatment with values of 6.01 mg g⁻¹ FW, 2.40, and 0.69, respectively. Conversely, the DS treatment exhibit the lowest chlorophyll a, chlorophyll b, and total chlorophyll contents, at 6.07, 2.54, and 8.61 mg g⁻¹ FW, respectively. The n-SiO₂ treatment, showed the lowest carotenoid content, chlorophyll a/b ratio, and carotenoid/total chlorophyll ratio, with values of 3.13 mg g⁻¹ FW, 1.30, and 0.19, respectively.

Photosynthetic pigments are key indicators of a plant's photosynthetic capacity and the stability of its photosynthetic apparatus [21]. The results of this study demonstrated that drought stress significantly reduced the contents of chlorophyll a, chlorophyll b, and total chlorophyll while increasing carotenoid levels, the chlorophyll a/b ratio, and the carotenoid/total chlorophyll ratio.

Plants develop adaptive mechanisms to mitigate the effect of drought stress by reducing the rate of transpiration and photosynthetic activity, resulting in decreased chlorophyll content, stomatal closure, and a lower photosynthetic rate [22]. Treatment with n-SiO₂ effectively alleviated the adverse effects of drought stress by improving nutrient uptake efficiency, silica deposition in plant tissues, enhancing tissue water balance, and photosynthetic activity [21]. In addition, the application of n-SiO₂ increased chlorophyll contents in soybean plants under drought stress, which is consistent with previous studies [23], [24].

Response of Water Status and Oxidative Stress in Soybean After Treatment with SiO₂ Nanoparticle and PEG

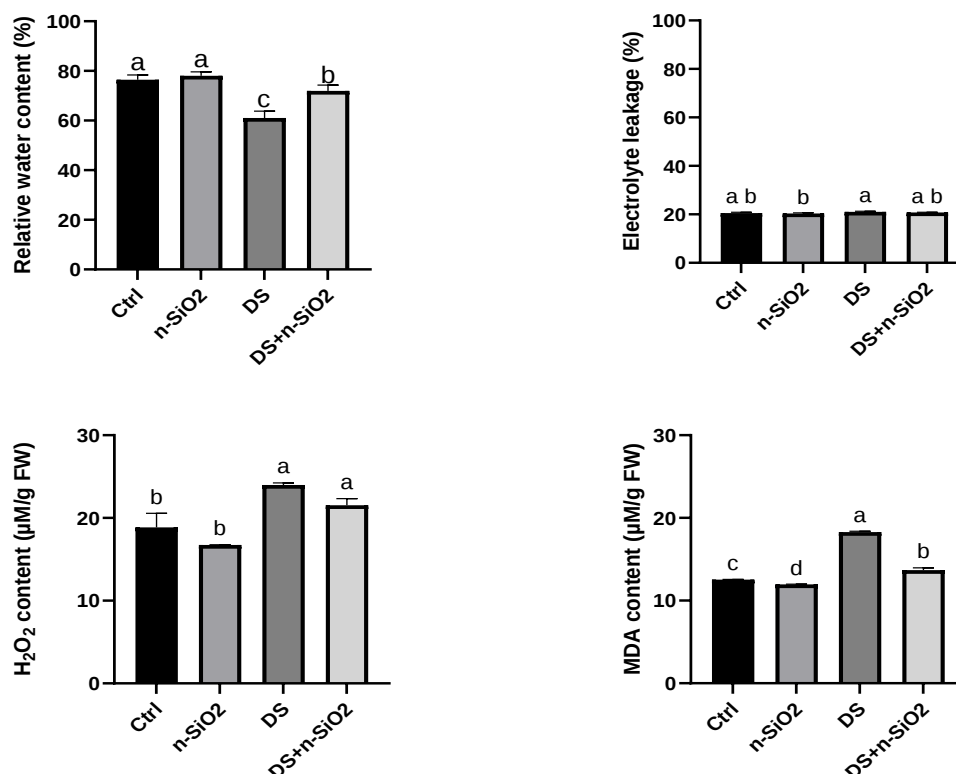


Fig. 4: Relative water content, electrolyte leakage, H₂O₂ content and MDA content of soybean plants after treatment SiO₂ nanoparticle. Different letters at the same level indicate a significant difference ($p < 0.05$)

Treatment with n-SiO₂ had a significant effect on relative water content (RWC), electrolyte leakage (EL), H₂O₂ and MDA levels (Fig. 4). The n-SiO₂ treatment showed the highest RWC, with an average value of 78%, whereas the DS treatment exhibited the lowest RWC of 61%. In contrast, EL showed the opposite trend with the DS treatment produced the highest EL at 21.04%, while the n-SiO₂ treatment recorded the lowest result at 20.37%. H₂O₂ accumulation also increased significantly as a result of drought stress. The highest H₂O₂ content was observed in the DS treatment at 23.98 $\mu\text{M g}^{-1}$ FW. Meanwhile, the n-SiO₂ treatment recorded the lowest result of 16.74 $\mu\text{M g}^{-1}$ FW. A similar trend was also observed in MDA content. The DS treatment showed the highest MDA content at 18.00 $\mu\text{M g}^{-1}$ FW, whereas the n-SiO₂ treatment produced the lowest at 11.95 $\mu\text{M g}^{-1}$ FW.

Plant water status is a key factor determining a plant's ability to survive during drought stress. The decrease in RWC observed in the DS treatment indicated that water deficit impairs root water uptake, leading to a reduction in turgor pressure and disruption of cellular metabolism [18]. Treatment with n-SiO₂ is linked to silicon's ability to enhance root development, thereby improving water-holding capacity. Furthermore, silica deposition can reduce water loss through transpiration and improve water-use efficiency. These mechanisms enabled plants to maintain turgor pressure and physiological activity under drought stress [25]. An increase in RWC was accompanied by a decrease in EL,

indicating that n-SiO₂ maintained cell membrane stability [26] Electrolyte leakage is an important indicator of membrane damage caused by the accumulation of reactive oxygen species (ROS), which leads to lipid peroxidation in the plasma membrane. The lower EL value observed in the n-SiO₂ treatment indicated that greater membrane stability at preventing the loss of ions and cellular metabolites into the surrounding environment [27]

Improved water status contributed to reduced H₂O₂ and MDA accumulation. H₂O₂ is one of the main ROS that increases during drought stress due to an imbalance between ROS formation and detoxification [28] Excessive accumulation of H₂O₂ triggered membrane lipid peroxidation, as indicated by increased MDA levels. Therefore, high levels of H₂O₂ and MDA levels indicated that the DS treatment experienced severe oxidative stress. Conversely, the reduction in H₂O₂ and MDA levels in the n-SiO₂ treatment suggested that silicon is capable of reducing ROS accumulation. Silicon has been reported to enhance the activity of antioxidant enzymes, thereby enabling ROS scavenging before they cause membrane damage [29] In addition, silicon has also been reported to maintain ionic homeostasis and alleviate abiotic stress [30].

Response of Osmotic Adjustment Oxidative Stress and Yield in Soybean After Treatment with SiO₂ Nanoparticle and PEG

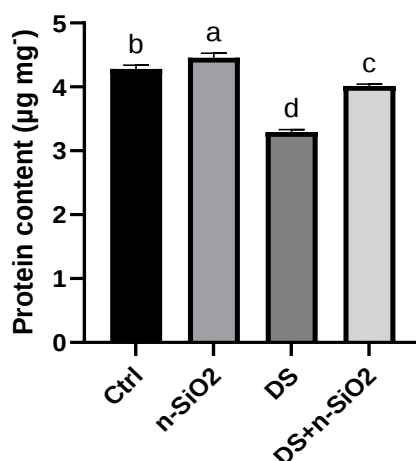


Fig. 5: Protein content soybean plants after treatment with SiO₂ nanoparticle. Different letters at the same level indicate a significant difference ($p < 0.05$)

The n-SiO₂ treatment showed significant differences among treatments. The n-SiO₂ treatment resulted in the highest protein content at 4.45 µg mg⁻¹, representing a 4.7% increase compared with the control. Conversely, the DS treatment produced the lowest protein content of 3.29 µg mg⁻¹, representing 22.4% reduction compared with the control. In contrast, The DS + n-SiO₂ treatment increased protein content to 4.01 µg mg⁻¹. These results indicated that drought stress inhibited protein accumulation. The reduction in protein content in the DS treatment inhibited protein metabolism while accelerating protein degradation due to increased oxidative stress. Protein is a major component of primary metabolism and contributes to the formation of enzymes, structural proteins, transport

proteins, and various regulatory proteins involved in plant growth and physiological responses [31]

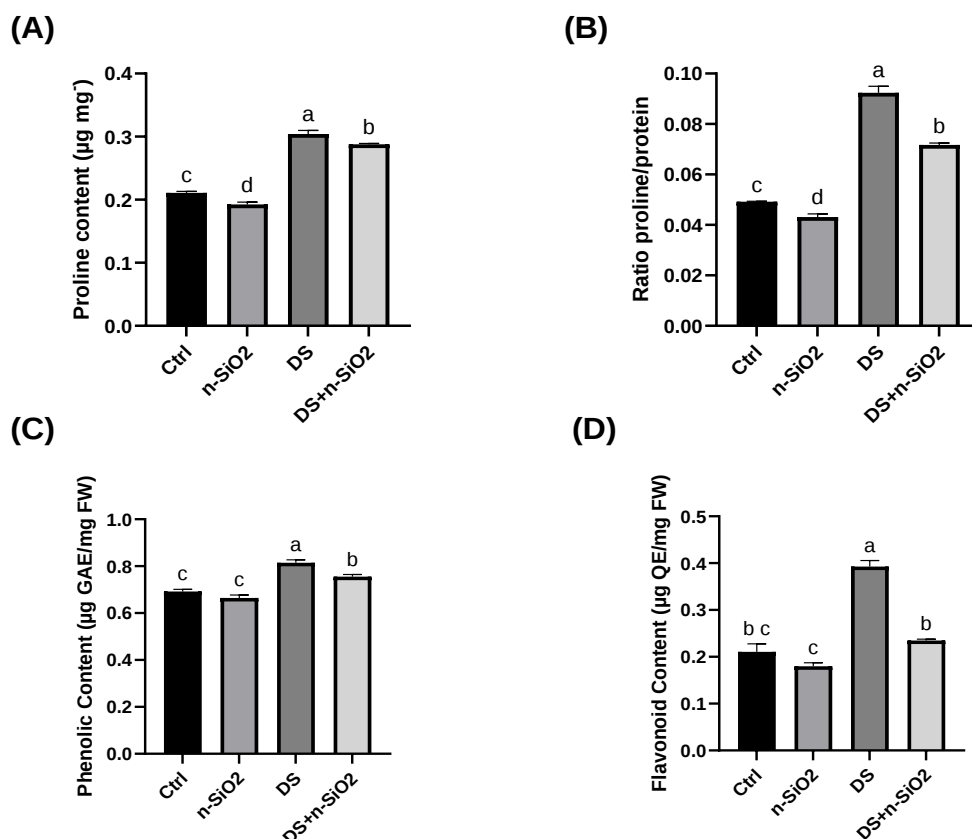


Fig. 6: Metabolite secondary content soybean plants after treatment with SiO₂ nanoparticle. Different letters at the same level indicate a significant difference ($p < 0.05$)

Treatment with n-SiO₂ resulted in significant differences in the proline content, proline-to-protein ratio, flavonoid content, and phenolic content in soybean plants (Fig. 6). The highest proline content was observed in the DS treatment at $0.30 \mu\text{g mg}^{-1}$, while the n-SiO₂ treatment exhibited the lowest result at $0.19 \mu\text{g mg}^{-1}$. The DS + n-SiO₂ treatment reduced proline content to $0.29 \mu\text{g mg}^{-1}$, representing a 3.4% reduction compared to the control. A similar trend was also observed in the proline-to-protein ratio. The highest ratio was found in the DS treatment at 0.09, whereas the n-SiO₂ treatment exhibited the lowest ratio of 0.04. The DS + n-SiO₂ treatment reduced the ratio to 0.07.

The accumulation of secondary metabolites showed a similar trend. The DS treatment resulted in the highest phenolic and flavonoid contents of $0.81 \mu\text{g GAE g}^{-1} \text{FW}$ and $0.39 \mu\text{g QE mg}^{-1} \text{FW}$, respectively. Conversely, the n-SiO₂ treatment reduced phenolic and flavonoid contents to $0.66 \mu\text{g GAE g}^{-1} \text{FW}$ and $0.17 \mu\text{g QE mg}^{-1} \text{FW}$.

The accumulation of proline is one of the primary physiological responses to drought stress. The increase in proline content in the DS treatment indicated that soybean plants experienced severe osmotic stress. Proline acted as a compatible osmolyte to maintain

turgor pressure, protects proteins and cell membranes, and stabilizes enzyme structures during water deficit[15] . In contrast, the lower proline content in the n-SiO₂ treatment indicated that the treatment effectively alleviated drought-induced stress. The reduction in proline content in the DS + n-SiO₂ treatment compared with the DS treatment further supports this finding. This response was also reflected by the proline-to-protein ratio, which represents the balance between protein degradation and osmolyte synthesis during drought stress [32] The higher ratio observed in the DS treatment indicated a metabolic shift from protein synthesis toward stress defense mechanisms. A similar trend was also observed for flavonoid and phenolic contents. Both compounds are secondary metabolites that act as non-enzymatic antioxidants to neutralize ROS under drought stress. The highest flavonoid and phenolic contents observed in the DS treatment indicated that soybean plants activated their antioxidant defense system in response to excessive ROS accumulation. However, the n-SiO₂ treatment decreased the accumulation of both metabolites. These findings indicated that n-SiO₂ shifted the morphophysiological response from a stress defense toward a drought tolerance mechanism [33]

Table 2: Effect after treatment with SiO₂ and PEG on shoot and root biomass of soybean at 28 days

Treatment	Shoot weight (g)	Root weight (g)
Ctrl	8.10 ± 0.22b	4.17 ± 0.26b
n-SiO ₂	11.60 ± 0.83a	4.90 ± 0.16a
DS	6.23 ± 0.12c	2.83 ± 0.12c
DS + n-SiO ₂	7.70 ± 0.16bc	3.97 ± 0.12b

The n-SiO₂ treatment significantly affected the shoot and root biomass of soybean plants at 28 days after planting. The n-SiO₂ treatment produced the highest shoot and root biomass at 11.60 g and 4.90 g, respectively, whereas the DS treatment exhibited the lowest biomass at 6.23 g and 2.83 g, respectively. Plant biomass is a key indicator of photosynthetic performance and metabolic efficiency during the vegetative growth stage. The reduction in shoot and root biomass observed under the DS treatment indicated that drought stress limited plant growth by reducing water uptake and disrupting the stability of the photosynthetic apparatus. These conditions inhibited cell division and enlargement in plants, resulting in lower biomass accumulation. In contrast, the n-SiO₂ treatment significantly increased shoot and root biomass under both normal and drought stress conditions. Overall, these findings demonstrated that silicon improved biomass accumulation primarily by maintaining the integration of morphophysiological responses, reduced oxidative damage, and optimized primary metabolism, thereby allowing greater carbon allocation toward vegetative growth and biomass accumulation [34]

CONCLUSION

The application of n-SiO₂ effectively improved the morphophysiological performance of soybean plants under both normal and PEG 6000-induced drought stress conditions. SiO₂ nanoparticles positively affected the physiological responses of soybean plants by increasing RWC and reducing EL, thereby reducing oxidative damage, as indicated by H₂O₂

accumulation and MDA levels. In addition, n-SiO₂ maintained photosynthetic pigment levels and protein content while suppressing proline accumulation and stress-induced secondary metabolites. Overall, n-SiO₂ treatment promoted vegetative growth and biomass accumulation under drought stress conditions.

Conflict of interest

Authors declare no competing financial interests or personal relationships that could have influenced the work reported in this paper.

Acknowledgments

The authors gratefully acknowledge the University of Jember and the Ministry of Research, Technology and Higher Education of the Republic of Indonesia for their support of this research.

REFERENCES

- [1] A. Kumar, P. Dhansu, and A. Mann, 'Salinity and Drought Tolerance in Plants: Physiological Perspectives', *Salinity and Drought Tolerance in Plants: Physiological Perspectives*, no. October, pp. 1-602, 2023, doi: 10.1007/978-981-99-4669-3.
- [2] I. S. Petrushin, I. A. Vasilev, and Y. A. Markova, 'Drought Tolerance of Legumes: Physiology and the Role of the Microbiome', *Curr. Issues Mol. Biol.*, vol. 45, no. 8, pp. 6311-6324, 2023, doi: 10.3390/cimb45080398.
- [3] O. Basal and A. Szabó, 'Physiology, yield and quality of soybean as affected by drought stress', *Asian Journal of Agriculture and Biology*, vol. 8, no. 3, pp. 247-252, 2020, doi: 10.35495/AJAB.2019.11.505.
- [4] H. Arya, M. B. Singh, and P. L. Bhalla, 'Towards Developing Drought-smart Soybeans', *Front. Plant Sci.*, vol. 12, no. October, 2021, doi: 10.3389/fpls.2021.750664.
- [5] K. Gautam, H. Singh, and A. K. Sinha, 'Nanotechnology in Plant Nanobionics: Mechanisms, Applications, and Future Perspectives', *Adv. Biol.*, vol. 9, no. 4, 2025, doi: 10.1002/adbi.202400589.
- [6] N. Thakur and A. N. Yadav, 'Nanotechnology in Agriculture: A Review on Precision Farming and Sustainable Crop Production', *Bionanoscience*, vol. 15, no. 2, 2025, doi: 10.1007/s12668-025-01861-2.
- [7] S. Roghie, G. Shiade, R. De Mello, P. Amin, F. Rogério, and F. Carvalho, 'Silicon at the Frontline: Enhancing Plant Multistress Tolerance', pp. 3089-3111, 2026.
- [8] S. Esmaili, V. Tavallali, and B. Amiri, 'Nano-Silicon Complexes Enhance Growth, Yield, Water Relations and Mineral Composition in *Tanacetum parthenium* under Water Deficit Stress', *Silicon*, vol. 13, no. 8, pp. 2493-2508, 2021, doi: 10.1007/s12633-020-00605-z.
- [9] B. Sanusi, A. Z. Inyass, S. Zubaidatu, T. E. Barbra, H. Saleh, and M. L. Umar, 'Effect of drought stress on some morphological, physiological and biochemical parameters in soybean (TGX-1835-10E) variety', *Discover Plants*, vol. 2, no. 1, 2025, doi: 10.1007/s44372-025-00221-4.

- [10] Z. I. Mufida, 'Morpho-physiological and Production Responses of Peanut (*Arachis hypogaea* L.) to Biostimulant Protein Hydrolysate Under Drought Stress', vol. 13, no. 04, pp. 113-122, 2025, doi: 10.14738/aivp.1304.19159.Mufida.
- [11] R. J. Porra, 'Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy', vol. 975, pp. 384-394, 1989.
- [12] V. do Rosário Rosa *et al.*, 'Drought stress during the reproductive stage of two soybean lines', *Pesqui. Agropecu. Bras.*, vol. 55, pp. 1-11, 2020, doi: 10.1590/S1678-3921.PAB2020.V55.01736.
- [13] Velikova, 'oxidative stress and some antioxidant systems in acid rain-treated bean plants', *Physical Review*, vol. 176, no. 5, pp. 1709-1714, 1999, doi: 10.1103/PhysRev.176.1709.
- [14] Bradford, 'a rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye binding', *Curr. Opin. Struct. Biol.*, vol. 30, pp. 1-6, 1976, doi: 10.1016/j.sbi.2014.10.005.
- [15] M. R. Lee, C. S. Kim, T. Park, Y. S. Choi, and K. H. Lee, 'Optimization of the ninhydrin reaction and development of a multiwell plate-based high-throughput proline detection assay', *Anal. Biochem.*, vol. 556, no. June, pp. 57-62, 2018, doi: 10.1016/j.ab.2018.06.022.
- [16] M. S. Taga, E. E. Miller, and D. E. Pratt, 'Chia seeds as a source of natural lipid antioxidants', *J. Am. Oil Chem. Soc.*, vol. 61, no. 5, pp. 928-931, 1984, doi: 10.1007/BF02542169.
- [17] S. Pradipta, T. R. I. A. Siswoyo, and M. Ubaidillah, 'Nutraceuticals and bioactive properties of local Java pigmented rice', no. January, 2023, doi: 10.13057/biodiv/d240166.
- [18] S. A. Abdulla and K. A. Rahman, 'Combination Effects of Rhizobium sp. and Nano-Fe on Growth, Nodulation, and Nutrient Uptake of Chickpea (*Cicer arietinum* L.)', *Diyala Agricultural Sciences Journal*, vol. 15, no. 2, pp. 25-33, 2023, doi: 10.52951/dasj.23150203.
- [19] M. Bukan, S. Kereša, I. Pejić, A. Sudarić, A. Lovrić, and H. Šarčević, 'Variability of Root and Shoot Traits under PEG-Induced Drought Stress at an Early Vegetative Growth Stage of Soybean', *Agronomy*, vol. 14, no. 6, pp. 1-13, 2024, doi: 10.3390/agronomy14061188.
- [20] M. Sintaha, C. K. Man, W. S. Yung, S. Duan, M. W. Li, and H. M. Lam, 'Drought Stress Priming Improved the Drought Tolerance of Soybean', *Plants*, vol. 11, no. 21, 2022, doi: 10.3390/plants11212954.
- [21] C. Wang *et al.*, 'Drought and rewatering effects on soybean photosynthesis, physiology and yield', *PeerJ*, vol. 13, pp. 1-22, 2025, doi: 10.7717/peerj.19658.
- [22] P. Negi *et al.*, 'Transcriptional reprogramming and enhanced photosynthesis drive inducible salt tolerance in sugarcane mutant line M4209', *J. Exp. Bot.*, vol. 71, no. 19, pp. 6159-6173, 2020, doi: 10.1093/jxb/eraa339.
- [23] J. Xu, L. Guo, and L. Liu, 'Exogenous silicon alleviates drought stress in maize by improving growth, photosynthetic and antioxidant metabolism', *Environ. Exp. Bot.*, vol. 201, no. June, p. 104974, 2022, doi: 10.1016/j.envexpbot.2022.104974.
- [24] K. S. Siddiqi, A. Husen, N. Zahra, and A. Moheman, 'Harnessing silicon nanoparticles and various forms of silicon for enhanced plant growth performance under salinity stress: application and mechanism', *Discover Nano*, vol. 20, no. 1, 2025, doi: 10.1186/s11671-025-04270-2.
- [25] M. Chen *et al.*, 'Integrated physiological, transcriptomic, and metabolomic analyses of drought stress alleviation in *Ehretia macrophylla* Wall. seedlings by SiO₂ NPs (silica

- nanoparticles)', *Front. Plant Sci.*, vol. 15, no. February, pp. 1-13, 2024, doi: 10.3389/fpls.2024.1260140.
- [26] Y. M. Rashad, H. H. A. El-Sharkawy, B. E. A. Belal, E. S. Abdel Razik, and D. A. Galilah, 'Silica Nanoparticles as a Probable Anti-Oomycete Compound Against Downy Mildew, and Yield and Quality Enhancer in Grapevines: Field Evaluation, Molecular, Physiological, Ultrastructural, and Toxicity Investigations', *Front. Plant Sci.*, vol. 12, no. October, 2021, doi: 10.3389/fpls.2021.763365.
- [27] S. Namjoyan, A. Sorooshzadeh, A. Rajabi, and M. Aghaalikhani, 'Nano-silicon protects sugar beet plants against water deficit stress by improving the antioxidant systems and compatible solutes', *Acta Physiol. Plant.*, vol. 42, no. 10, 2020, doi: 10.1007/s11738-020-03137-6.
- [28] J. Wang, H. Wu, Y. Wang, W. Ye, X. Kong, and Z. Yin, 'Small particles, big effects: How nanoparticles can enhance plant growth in favorable and harsh conditions', *J. Integr. Plant Biol.*, vol. 66, no. 7, pp. 1274-1294, 2024, doi: 10.1111/jipb.13652.
- [29] M. Chen *et al.*, 'Integrated physiological, transcriptomic, and metabolomic analyses of drought stress alleviation in *Ehretia macrophylla* Wall. seedlings by SiO₂ NPs (silica nanoparticles)', *Front. Plant Sci.*, vol. 15, no. February, pp. 1-13, 2024, doi: 10.3389/fpls.2024.1260140.
- [30] E. S. Okeke, E. J. Nweze, T. C. Ezike, C. O. Nwuche, T. P. C. Ezeorba, and C. E. I. Nwankwo, 'Silicon-based nanoparticles for mitigating the effect of potentially toxic elements and plant stress in agroecosystems: A sustainable pathway towards food security', *Science of the Total Environment*, vol. 898, no. July, p. 165446, 2023, doi: 10.1016/j.scitotenv.2023.165446.
- [31] V. D. Rajput *et al.*, 'Effects of silicon and silicon-based nanoparticles on rhizosphere microbiome, plant stress and growth', *Biology (Basel)*, vol. 10, no. 8, pp. 1-19, 2021, doi: 10.3390/biology10080791.
- [32] W. Weisany, J. Razmi, A. H. Eshaghabadi, and D. Pashang, 'Silicon Nanoparticles (SiNP): A Novel and Sustainable Strategy for Mitigating Environmental Stresses in Plants', *J. Soil Sci. Plant Nutr.*, vol. 24, no. 2, pp. 2167-2191, 2024, doi: 10.1007/s42729-024-01790-1.
- [33] S. Daler *et al.*, 'Silicon Nanoparticles (SiO₂ NPs) Boost Drought Tolerance in Grapevines by Enhancing Some Morphological, Physiological, and Biochemical Traits', *Plant Mol. Biol. Report.*, no. 0123456789, 2024, doi: 10.1007/s11105-024-01520-y.
- [34] J. Wei *et al.*, 'Silicon Nano-Fertilizer-Enhanced Soybean Resilience and Yield Under Drought Stress', *Plants*, vol. 14, no. 5, 2025, doi: 10.3390/plants14050751.