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# Impact of silicon oxide nanoparticles on drought resistance and growth of *in vitro* cultured banana (*Musa spp*.) plants

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## Abstract

Banana (*Musa* spp.) fruits are a globally important fruit crop, widely cultivated in tropical and subtropical regions. However, its growth, development and productivity are significantly impacted by abiotic stresses such as drought, heat, salinity and nutrient deficiencies. As a shallow-rooted plant with high transpiration rates, bananas are particularly susceptible to water deficits, which disrupt its physiological, biochemical and molecular processes. Drought stress leads to reduced photosynthesis, impaired water relations, oxidative damage and hormonal imbalances, ultimately affecting yield and fruit quality. Drought stress poses a significant threat to the production of banana, especially *in vitro* cultures, where water availability is critical. This study investigates the role of silicon oxide ($SiO_2$) nanoparticles (NPs) in enhancing drought tolerance and promoting growth in *in vitro*-cultured banana plants subjected to water deficit conditions induced by polyethylene glycol (PEG) at different concentrations, viz., 1 mg, 2 mg, 3 mg and 4 mg. Polyethylene glycol , a high-molecular-weight, non-penetrating osmotic agent, widely used to simulate drought stress by creating water-deficient conditions without causing direct toxicity to plant tissues. Polyethylene glycol-treated banana plantlets were incubated with varying concentrations of $SiO_2$ NPs, viz., 100 mg/L, 200 mg/L, 300 mg/L and 400 mg/L and their cumulative effect on morpho-physiological parameters was assessed. Results showed that $SiO_2$ NPs application improved drought tolerance by enhancing water-use efficiency, maintaining relative water content (RWC) and increasing chlorophyll levels. Findings suggest that $SiO_2$ NPs play a pivotal role in mitigating drought stress by modulating morpho-physiological attributes in banana plantlets, making them a promising strategy for improving the resilience of *in vitro*-cultured bananas in water-limited environments.

**Keywords:** drought stress; *in vitro* micropropagation; *Musa acuminata*; nanoparticles; polyethylene glycol; silicon dioxide

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## Introduction

Banana (*Musa spp.*) is a most vital and staple food for about one billion people all over the world according to Food and Agriculture Organization of the United Nations (FAO) (1). It has become one of the major fruit crops in tropical countries due to its high-income potential for the local farmers (2, 3). Since they are seedless, the plant must be propagated vegetatively or can be produced by *in vitro* micropropagation (4). In the modern era, *in vitro* micropropagation is the preferable technique in modern breeding because it allows the production of many virus-free plants in a relatively short time and in a small space (5, 6). Bananas and plantains constitute a major dietary staple and an important export commodity in numerous countries with a global average banana production rising from 115 million tons in 2017–19 at an approximately value of 50 billion USD (FAOSTAT 2019) to 135 million tons in 2022–23 with an estimated USD 64 billion and 141 million tonnes in 2023, marking a 4.2 % increase from the previous year (7). Though India ranks first in banana production, it contributes about 26 % (26.5 million metric tonnes) to the world's total banana pool and covers approximately 776000 ha of agricultural land. The average national productivity is 34.2 Mt/ha. Banana also has the highest production of all fruit in India (33 %) (8). India is a major exporter of bananas, playing a significant role in its agricultural economy. During 2022–23, India exported bananas worth 176 million USD, which increased to approximately 300 million USD in 2023–24. The primary export destinations include the UAE, Saudi Arabia, Iran, Kuwait and Bahrain. Banana production accounts for about 37 % of the total fruit production in India and supports the livelihoods of millions of people

(9). Banana (*Musa spp.*) is a most vital food as well as a cash crop in many tropical and subtropical regions. Despite its importance, banana cultivation faces several challenges, such as water scarcity, pest and disease pressures and the need for improved agronomic practices (10, 11).

Silicon dioxide ($SiO_2$) nanoparticles (NPs), also known as silica NPs, are tiny particles of $SiO_2$ with sizes typically ranging from 1–100 nm. Silicon dioxide exhibits unique physicochemical properties such as a high surface area-to-volume ratio, excellent mechanical stability and strong adsorptive capabilities. In plant science, $SiO_2$ NPs are used to improve plant growth and stress tolerance (12). They can penetrate plant tissues more easily due to their nanoscale size, providing plants with silicon, an element known to enhance resilience to biotic and abiotic stresses, including drought (13). Silicon dioxide NPs provide multiple protective benefits against polyethylene glycol (PEG)-induced water stress. They improve water uptake and retention, enhance osmotic adjustment, boost antioxidant defences, maintain photosynthesis, promote root growth and regulate hormonal balance (14). By mitigating the adverse effects of PEG-induced drought stress, $SiO_2$ NPs help plants maintain growth and productivity in water-limited conditions (15). The present study aims to investigate the potential of $SiO_2$ NPs in mitigating drought stress and enhancing growth performance in *in vitro*-cultured banana (*Musa spp.*) plantlets. Specifically, the study seeks to (i) simulate water deficit conditions using varying concentrations of PEG, (ii) evaluate the effects of different concentrations of $SiO_2$ NPs on plantlet growth under induced drought stress and (iii) assess the associated changes in key morpho-physiological parameters, including water-use efficiency, relative water content (RWC) and chlorophyll content. Through this approach, the study intends to elucidate the role of $SiO_2$ NPs in improving drought tolerance and supporting the resilience of banana plantlets under water-limited conditions.

## Materials and Methods

### Plant materials

Three-month-old suckers of two cultivars of *Musa spp.* (Grand Naine and Karibale Monthan) were obtained from the Horticulture Research Centre, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut. The *in vitro* micropropagation experiments were conducted at the Tissue Culture Laboratory at the College of Biotechnology.

### Explant preparation

The suckers were thoroughly washed with tap water to remove any debris. The leaf sheaths and roots were carefully removed and the basal portion of the corm was cut into pieces measuring 12 × 12 × 15 mm. The explants were then rinsed under running tap water for 30 min, followed by soaking in a clean solution with continuous shaking for another 30 min. To remove any residual detergent, the explants were washed with distilled water. Subsequently, they were treated with a fungicide solution (Bavistin) for 30 min and rinsed again with distilled water. Finally, the explants were transferred to a laminar airflow chamber for further sterilisation (16).

### Preparation of culture media

Inside the laminar airflow chamber, the explants were treated with 70 % ethanol for 2 min, followed by washing with sterile water. They were then treated with 0.1 % $HgCl_2$ for 5 min and rinsed three times with sterile water, each rinse lasting 5 min. The explants were then trimmed to a size of 8 × 8 × 10 mm and inoculated onto Murashige and Skoog (MS) medium supplemented with 6 g/L agar, 30 g/L sucrose, 5 mg/L BAP and 1.0 mg/L IAA under sterile conditions (17). The cultures were incubated in the dark at 27 ± 2 °C for 21 days. To prevent growth inhibition caused by phenolic compounds released into the medium, the culture medium was replaced three times after the initial 21-day incubation period.

### Incubation with polyethylene glycol

To determine the lethal concentration of polyethylene glycol for each cultivar, PEG-6000 was added to solid basal medium at different concentrations, viz., 1, 2, 3 and 4 mg/L. The produced shoots were cultured for 4 weeks on these media and the percentage of survival was observed. After 4 weeks, shoot or root growth and development were determined. The stressed shoots were subsequently transferred to the medium supplemented with $SiO_2$ NPs (0, 100 ppm, 200 ppm, 300 ppm and 400 ppm). After six weeks of inoculation of explants, morphological and physiological parameters were determined.

### Preparation of silicon dioxide nanoparticle suspensions

The size of the $SiO_2$ NPs was characterised using transmission electron microscopy (TEM) analysis. The TEM images for the nanoparticles were shown in Fig. 1. The product was spherical and crystalline in structure, as shown in the image.

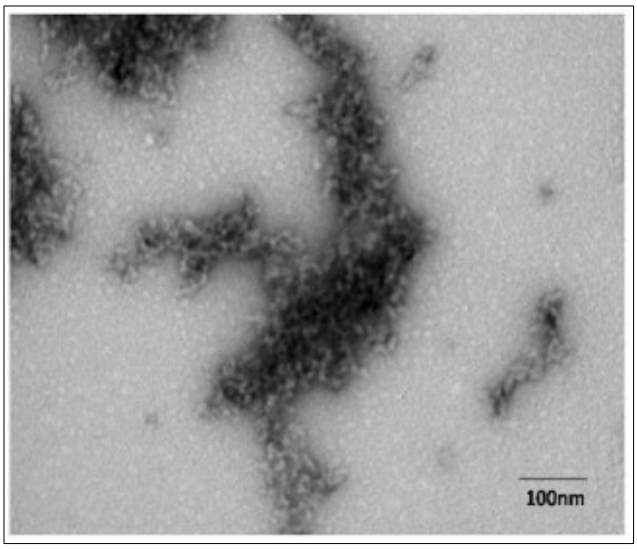



**Fig. 1.** Transmission electron microscopy (TEM) image of silicondioxide ($SiO_2$) nanoparticle.

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The $SiO_2$ NPs were suspended directly in distilled water and dispersed using ultrasonic vibration (100 W, 40 kHz) for 30 min to ensure uniform distribution. To minimise particle aggregation and maintain suspension stability, small magnetic stir beads were added and the suspensions were continuously stirred prior to application (18). Subsequently, different concentrations of $SiO_2$ NP suspensions (100, 200, 300 and 400 ppm) were prepared and used for the experiment.

### Statistical analysis

All experiments were repeated twice and conducted using a completely randomised design in a factorial arrangement with at least 4 replicates. All data were averaged and statistically analysed using online package for statistical analysis (OPSTAT) and statistical package for the social sciences (SPSS). The experiment was conducted using a completely randomised design (CRD) with 3 replications, each consisting of three plants per treatment combination. Statistical analyses were carried out using International Business Machines (IBM) SPSS Statistics (Version 19). Prior to analysis, the data were tested for normality, homogeneity of variance and sphericity using Mauchly's test. A two-way ANOVA under a fixed-factor model with a full factorial arrangement was employed to evaluate the effects of treatments and their interactions. Mean comparisons among treatment groups were performed using Duncan's multiple range test (DMRT) at a 5 % significance level ($p \leq 0.05$). Percentage data were subjected to arcsine transformation prior to ANOVA to stabilise variance.

## Results and Discussion

### Effect of polyethylene glycol treatment on morphological characters

The results indicate significant differences in various growth parameters, such as the number of shoots per explant, shoot length, fresh weight, dry weight, number of leaves, chlorophyll content, RWC, proline content, root number per shoot and root length in two banana genotypes, Grand Naine and Karibale Monthan, under varying concentrations of PEG. Maximum morphological performance was recorded in the control plantlets for both cultivars. For Grand Naine, T0 control plantlets showed the highest values with 9.0 ± 1.15 shoots per explant, a shoot length of 5.0 ± 0.57 cm, fresh weight of 2.68 ± 1.12 g, dry weight of 0.24 ± 0.12 g and 11.0 ± 0.33 leaves per shoot on MS medium without PEG. Similarly, T1 (1 % PEG) also yielded high morphological values, although slightly reduced from the control, with 8.6 ± 0.88 shoots, a shoot length of 3.6 ± 0.16 cm, fresh weight of 2.59 ± 0.33 g, dry weight of 0.20 ± 0.03 g and 10.2 ± 1.12 leaves per shoot. The findings show a general decline in all morphological parameters as PEG concentration increased, with the lowest values observed at 4 % PEG ($T_4$), where Grand Naine plantlets had 3.2 ± 0.52 shoots, a shoot length of 2.0 ± 0.32 cm, fresh weight of 2.22 ± 0.33 g, dry weight of 0.09 ± 0.54 g and 6.24 ± 0.33 leaves per shoot. For Karibale Monthan, similar observations were recorded, with the control group exhibiting the highest values, including 11.6 ± 0.88 shoots, a shoot length of 4.6 ± 0.55 cm, fresh weight of 2.84 ± 0.66 g, dry weight of 0.38 ± 1.12 g and 14.9 ± 0.08 leaves per shoot. The 1 % PEG concentration also showed high growth parameters for Karibale Monthan but lower values than the control as mentioned in Tables 1 and 2. At 4 % PEG, Karibale Monthan plantlets demonstrated minimum values, with 4.2 ± 0.52 shoots, a shoot length of 3.2 ± 0.1 cm, fresh weight of 2.10 ± 0.04 g, dry weight of 0.12 ± 0.01 g and 8.40 ± 0.03 leaves per shoot. The study shows that increasing PEG-induced drought stress significantly reduces growth and physiological performance in banana plantlets. This decline is mainly due to reduced water availability, which lowers cell turgor, restricts cell division and elongation and impairs nutrient uptake. Decreased chlorophyll content and RWC indicate disruption of the photosynthetic system and poor water status. Although mild stress (1 % PEG) had limited impact due to initial adaptive responses, higher concentrations (2–4 %) caused severe inhibition as metabolic activities and enzymatic functions were disturbed. Proline accumulation under stress reflects a protective response, but it is insufficient at higher stress levels. Overall, these effects explain the reduced growth and highlight the sensitivity of banana to drought conditions (19). Although Karibale Monthan performed better under control conditions, both genotypes exhibited similar sensitivity to drought stress. Overall, the results highlight the detrimental effects of water deficit and the need for effective strategies, such as silicon-based interventions, to enhance drought tolerance in banana (Fig. 2).

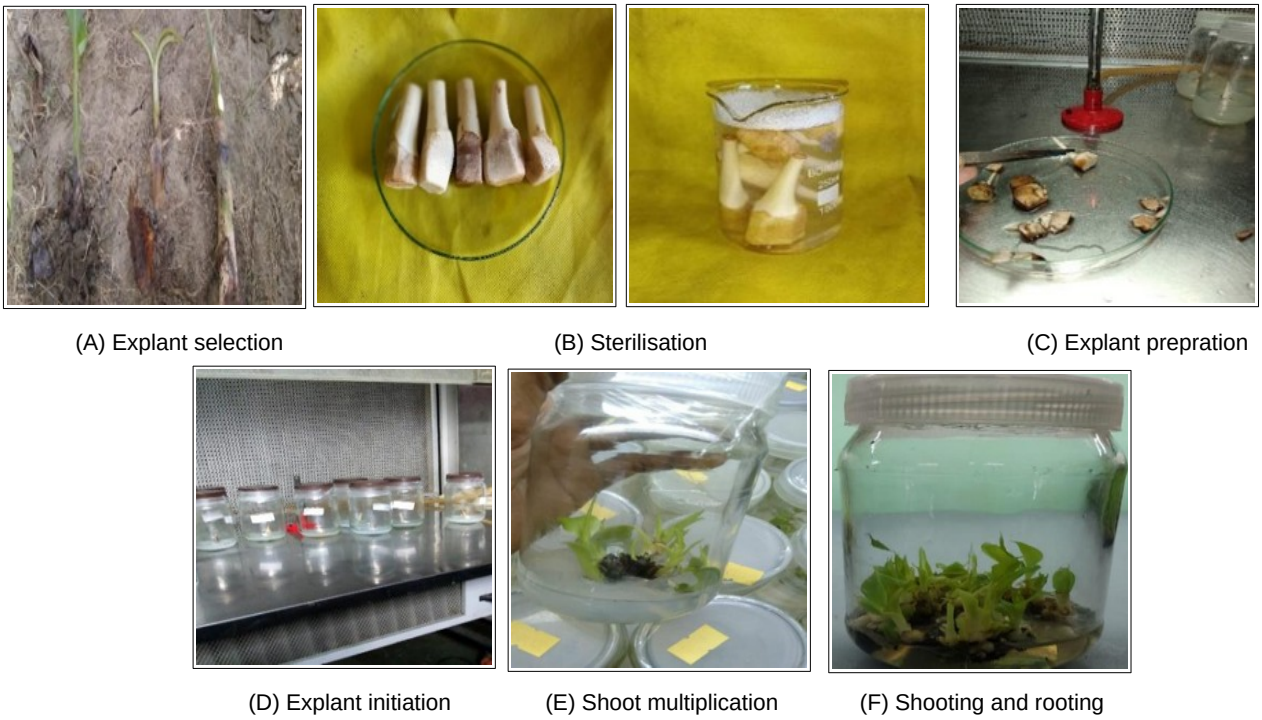
**Table 1.** Effect of various polyethylene glycol (PEG) concentrations on plant growth of banana genotype (Grand Naina)

| Treatment | No. of shoots | shoot length (cm) | Fresh wt (g) | Dry wt (g) | No. of leaves |
|---|---|---|---|---|---|
| MS + PEG 0 % | 9.0 ± 1.15$^{a}$ | 5.0 ± 0.57$^{a}$ | 2.68 ± 1.12$^{a}$ | 0.24 ± 0.12$^{a}$ | 11.0 ± 0.33$^{a}$ |
| MS + PEG 1 % | 8.6 ± 0.88 $^{ab}$ | 3.6 ± 0.16$^{b}$ | 2.59 ± 0.33$^{b}$ | 0.20 ± 0.03$^{a}$ | 10.2 ± 1.12$^{a}$ |
| MS + PEG 2 % | 6.3 ± 0.33 $^{b}$ | 2.9 ± 0.03$^{bc}$ | 2.54 ± 0.57$^{bc}$ | 0.13 ± 0.05$^{b}$ | 10.0 ± 0.88$^{b}$ |
| MS + PEG 3 % | 4.0 ± 0.57$^{b}$ | 2.2 ± 0.23$^{c}$ | 2.42 ± 0.14$^{c}$ | 0.10 ± 1.00$^{bc}$ | 7.20 ± 0.03$^{b}$ |
| MS + PEG 4 % | 3.2 ± 0.52$^{c}$ | 2.0 ± 0.32$^{c}$ | 2.22 ± 0.33$^{c}$ | 0.09 ± 0.54$^{c}$ | 6.24 ± 0.33$^{c}$ |

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**Table 2.** Effect of various polyethylene glycol (PEG) concentrations on plant growth of banana genotype (Karibale Monthan)

| Treatment | No. of shoots | shoot length(cm) | Fresh wt (g) | Dry wt (g) | No. of leaves |
|---|---|---|---|---|---|
| MS+ PEG 0 % | 11.6 ± 0.88$^{a}$ | 4.6 ±.0.55$^{a}$ | 2.84 ± 0.66$^{a}$ | 0.38 ± 1.12$^{a}$ | 14.9 ± 0.08$^{a}$ |
| MS+ PEG 1 % | 8.0 ± 0.57$^{b}$ | 4.4 ± 0.55$^{ab}$ | 2.72 ± 0.03$^{ab}$ | 0.22 ± 0.88$^{b}$ | 13.2 ± 0.12$^{b}$ |
| MS+ PEG 2 % | 6.0 ± 0.03$^{b}$ | 3.8 ± 0.06$^{b}$ | 2.64 ± 0.57$^{b}$ | 0.20 ± 0.03$^{b}$ | 11.3 ± 0.33$^{b}$ |
| MS+ PEG 3 % | 5.6 ± 0.33$^{c}$ | 3.6 ± 0.20$^{b}$ | 2.48 ± 0.12$^{c}$ | 0.17 ± 0.01$^{bc}$ | 10.8 ± 0.88$^{c}$ |
| MS+ PEG 4 % | 4.2 ± 0.52$^{c}$ | 3.2 ± 0.1b$^{c}$ | 2.10 ± 0.04$^{bc}$ | 0.12 ± 0.01$^{c}$ | 8.40 ± 0.03$^{c}$ |



(A) Explant selection (B) Sterilisation (C) Explant prepration

(D) Explant initiation (E) Shoot multiplication (F) Shooting and rooting

**Fig. 2.** *In vitro* micropropagation of banana (*Musa* spp.).

### Effect of polyethylene glycol treatment on proline content, chlorophyll content and relative water content

Additionally, significant genotype-specific responses were noted in proline, chlorophyll content and RWC. While proline content increased under all drought conditions for both cultivars, no statistically significant changes were observed in chlorophyll content and RWC for the Grand Naine cultivar at various drought levels compared to the control. In Karibale Monthan, however, statistically significant reductions were observed in chlorophyll content and RWC as PEG concentration increased. Proline content showed a gradual increase with rising PEG levels, reaching 3.78 ± 0.01 µmoles/g in Grand Naine and 5.83 ± 0.21 µmoles/g in Karibale Monthan at 4 % PEG. It was observed from the results that chlorophyll and RWC decreased as the concentration of PEG increased (Table 3). It was noticed that control cultures had the highest values for chlorophyll content i.e., 7.15 ± 0.03 for Grand Naine and 7.84 ± 0.02 for Karibale Monthan and RWC of 69.93 ± 1.67 % for Grand Naine and 74.93 ± 2.35 % for Karibale Monthan. At 4 % PEG, these values decreased to 5.34 ± 0.88 and 49.86 ± 1.36 for Grand Naine and 5.10 ± 0.03 and 53.10 ± 2.40 for Karibale Monthan respectively. Similar trends were observed in *in vitro* banana cultures subjected to PEG and sorbitol treatments (19). Their study demonstrated that osmotic stress caused significant reductions in shoot length, root length and RWC, while proline content increased with higher concentrations of stress inducers. These biochemical and physiological alterations were considered important indicators of drought tolerance mechanisms.

The formation and length of roots followed a similar pattern when cultured on rooting medium with varying PEG concentrations in both the banana cultivars. According to the data presented in Table 4, the highest root count and maximum root length were recorded in the control group without PEG supplementation, while the lowest number was observed at 4 % (w/v) PEG. This pattern suggests that increased PEG concentrations reduce root formation and growth in both banana genotypes.

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**Table 3.** Effect of different polyethylene glycol (PEG) doses applied to banana varieties on different physiological parameters

| | Grand Naine | | | Karibale Monthan | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Proline content** | **Chlorophyll** | **RWC (%)** | **Proline content** | **Chlorophyll content (%)** | **RWC (%) (µmoles/g)** |
| MS + PEG 0 % | 2.06 ± 0.26$^{a}$ | 7.15 ± 0.03$^{a}$ | 69.93 ± 1.67$^{a}$ | 2.38 ± 0.29$^{a}$ | 7.84 ± 0.02$^{a}$ | 74.93 ± 2.35$^{a}$ |
| MS + PEG 1 % | 2.30 ± 0.32$^{ab}$ | 6.56 ± 0.02$^{b}$ | 66.13 ± 1.68$^{b}$ | 4.33 ± 0.25b$^{c}$ | 7.58 ± 0.08$^{b}$ | 69.80 ± 1.92$^{ab}$ |
| MS + PEG 2 % | 2.51 ± 0.02$^{b}$ | 6.12 ± 0.04$^{ab}$ | 62.43 ± 2.32$^{b}$ | 4.78 ± 0.09$^{b}$ | 7.52 ± 0.04$^{ab}$ | 64.96 ± 2.69$^{b}$ |
| MS + PEG 3 % | 2.98 ± 0.03$^{bc}$ | 5.58 ± 0.12$^{bc}$ | 59.53 ± 2.41$^{c}$ | 4.93 ± 0.03$^{bc}$ | 6.12 ± 0.01$^{b}$ | 62.96 ± 3.64$^{bc}$ |
| MS + PEG 4 % | 3.78 ± 0.01$^{c}$ | 5.34 ± 0.88$^{c}$ | 49.86 ± 1.36$^{bc}$ | 5.83 ± 0.21$^{c}$ | 5.10 ± 0.03$^{c}$ | 53.10 ± 2.40$^{c}$ |

**Table 4.** Effect of different polyethylene glycol (PEG) treatment on root formation in two banana genotypes

| | Grand Naine | | Karibale Monthan | |
|---|---|---|---|---|
| **Treatment** | **No. of roots** | **Average root length(cm)** | **No. of roots** | **Average root length(cm)** |
| MS + PEG 0 % | 10.0 ± 0.57$^{a}$ | 8.6 ± 0.76$^{a}$ | 12.0 ± 1.15$^{a}$ | 8.3 ± 0.56$^{a}$ |
| MS + PEG 1 % | 9.60 ± 0.33$^{b}$ | 5.8 ± 0.29$^{ab}$ | 10.3 ± 1.45 $^{b}$ | 8.1 ± 0.66$^{ab}$ |
| MS + PEG 2 % | 9.33 ± 0.66$^{ab}$ | 5.4 ± 0.28$^{b}$ | 10.0 ± 0.57 $^{ab}$ | 7.7 ± 0.62$^{b}$ |
| MS + PEG 3 % | 8.00 ± 0.33$^{b}$ | 4.5 ± 0.54$^{bc}$ | 9.66 ± 0.33$^{bc}$ | 7.4 ± 0.61$^{bc}$ |
| MS + PEG 4 % | 7.26 ± 1.14$^{c}$ | 3.8 ± 0.36$^{c}$ | 9.00 ± 0.32 $^{c}$ | 7.1 ± 0.46$^{c}$ |

## Effect of silicon dioxide nanoparticle treatment on morphological characters

The results demonstrate that 1 % PEG yielded the highest number of shoots, shoot length, root number and root length, while increased PEG concentrations progressively reduced these traits. Notably, 3 % PEG was determined to produce moderate drought stress effects, making it the concentration of choice for further experiments. Subsequently, *in vitro* banana plantlets stressed at 3 % PEG were transferred to a shoot multiplication medium supplemented with various concentrations (100 ppm, 200 ppm, 300 ppm and 400 ppm) of $SiO_2$ NPs to analyse their cumulative effect. After 4 weeks of inoculation, we observed the change in treated plants. By the data, we can evaluate that the maximum number of shoots and shoot length were achieved in both the genotypes at $T_1$ (3 % PEG + 100 ppm $SiO_2$ NP) (20). In banana explants of Grand Naine genotype, the maximum number of shoots was (10.3 ± 0.33) and maximum shoot length was (5.66 ± 0.17), maximum fresh weight was observed (2.46 ± 0.08) and maximum dry weight was (0.26 ± 0.04) when compared with previous data of PEG-induced drought alone. At $T_1$ (3 % PEG + 100 ppm $SiO_2$-NP) combination followed by $T_2$ (3 % PEG + 200 ppm) at which the number of shoots/explant (9.00 ± 0.57), shoot length (5.13 ± 0.17), fresh weight was (2.43 ± 0.08), dry weight was (0.19 ± 0.01). Minimum recordings were observed in the T4 (3 % PEG + 400 ppm $SiO_2$) at which the number of shoots were obtained (7.00 ± 0.57), shoot length was (3.26 ± 0.14), fresh weight was (2.12 ± 0.02) and dry weight was (0.15 ± 0.01) (Table 5). Similarly, in the case of Karibale Monthan banana, in the treatment combination of $T_1$ (3 % PEG + 100 ppm $SiO_2$-NP), the maximum number of shoots was (11.6 ± 0.33), maximum shoot length was found (5.56 ± 0.14), fresh weight was (2.69 ± 0.09) and dry weight (0.25 ± 0.02) followed by $T_2$ (3 % PEG + 200 ppm $SiO_2$ NP), at which the number of shoots was (11.0 ± 0.57), shoot length was (4.56 ± 0.21), fresh weight was (2.53 ± 0.06) and dry weight (0.21 ± 0.01). The minimum values were observed in the $T_4$ (3 % PEG + 400 ppm $SiO_2$), at which the number of shoots was (8.33 ± 0.33), shoot length was (3.56 ± 0.12), fresh weight was (2.24 ± 0.03) and dry weight was (0.17 ± 0.02). However, it was investigated that the treatment with $T_2$ (3 % PEG + 200 ppm $SiO_2$-NP) had a greater number of leaves in both the genotypes, i.e., in Grand Naine genotype it was higher, i.e., (11.3 ± 0.88) and in Karibale Monthan it was (10.3 ± 0.88) as compared to the control (untreated plants) and lowest in $T_4$ (4 % PEG + 100 ppm $SiO_2$-NP).

**Table 5.** Effect of silicon dioxide nanoparticle ($SiO_2$ NPs) on morphological characters of *in vitro* banana plantlets

| **Banana variety** | **Treatments** | **Number of roots** | **Root length (cm)** |
|---|---|---|---|
| Grand Naine | T0 (Control) | 14.6 ± 0.66$^{a}$ | 11.0 ± 1.00$^{a}$ |
| | T1 (3 % PEG + 100 ppm $SiO_2$) | 12.3 ± 1.76$^{ab}$ | 9.00 ± 0.57$^{ab}$ |
| | T2 (3 % PEG + 200 ppm $SiO_2$) | 10.3 ± 1.45$^{b}$ | 7.00 ± 0.57$^{b}$ |
| | T3 (3 % PEG + 300 ppm $SiO_2$) | 9.33 ± 0.33$^{bc}$ | 8.33 ± 0.88$^{bc}$ |
| | T4 (3 % PEG + 400 ppm $SiO_2$) | 7.66 ± 0.88$^{c}$ | 6.33 ± 0.33$^{c}$ |
| Karibale Monthan | $T_0$ (Control) | 16.3 ± 0.33$^{a}$ | 12.0 ± 1.15$^{a}$ |

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| | | |
|---|---|---|
| T1 (3 % PEG + 100 ppm $SiO_2$) | 15.0 ± 1.15$^{a}$ | 10.3 ± 0.88$^{b}$ |
| T2 (3 % PEG + 200 ppm $SiO_2$) | 12.3 ± 1.20$^{ab}$ | 7.00 ± 0.57$^{bc}$ |
| T3 (3 % PEG + 300 ppm SiO2) | 10.3 ± 0.33$^{b}$ | 8.33 ± 0.88$^{c}$ |
| T4 (3 % PEG + 400 ppm SiO2) | 9.00 ± 0.57$^{c}$ | 6.66 ± 0.33$^{c}$ |

PEG: polyethylene glycol.

### Effect of silicon dioxide nanoparticle treatment on physiological characters

In the present investigation, data suggested that application of the treatment (3 % PEG + 100 ppm $SiO_2$-NP) in Grand Naine banana plants recorded the highest RWC (71.2 ± 1.66) and highest chlorophyll content (6.93 ± 0.17) and the lowest RWC level was (52.3 ± 1.73) and minimum chlorophyll content (4.50 ± 0.28) respectively in $T_4$ (3 % PEG + 400 ppm $SiO_2$-NP) as shown in Table 6 (Fig. 3). On the other hand, similar findings were recorded in the Karibale Monthan banana genotype, where the highest RWC (74.0 ± 1.00) and chlorophyll content (7.20 ± 0.11) were shown in $T_1$ (3 % PEG + 100 ppm $SiO_2$-NP) and minimum RWC was found (59.0 ± 2.62) and lowest chlorophyll content was recorded (5.26 ± 0.14) with the treatment of $T_4$ (3 % PEG + 400 ppm $SiO_2$-NP) (Fig. 4). Similar response was previously observed, who reported that silicon increased mesophyll thickness, stomatal density and chlorophyll a content in *in vitro* 'Dwarf Cavendish' banana plants cultured in bioreactors. The content of proline was affected by the application of $SiO_2$ NPs concentration and drought regimes. As shown in the previous study, the level of proline was increased as the drought stress increased, induced by PEG at concentration-dependent manner. In the present experiment, when the stressed shoots were shifted to the treatment of $SiO_2$-NP, the highest content of proline was obtained in the treatment combination (3 % PEG + 400 ppm $SiO_2$ NP). A remarkable increase in proline level was observed in both the genotypes of banana. In contrast, the lowest content of proline was observed in the treatment combination of $T_1$ (3 % PEG + 100 ppm $SiO_2$-NP). Similar findings were reported by Lamiaa M. Mahmoud et al., (2020) in banana cv. 'Grand Nain', where $SiO_2$ nanoparticles enhanced shoot growth, chlorophyll content, photosynthetic performance, potassium uptake and $K^+/Na^+$ ratio under salinity and water deficit stress (21).

In both the genotypes of banana, it was shown that the highest proline accumulation was recorded in $T_4$ (3 % PEG + 400 ppm $SiO_2$-NP). In Grand Naine, it was found highest (2.98 ± 0.13) when compared to PEG treatments alone and (4.76 ± 0.31) in Karibale Monthan compared with PEG-treated cultures. A similar trend was observed for root parameters, where the highest root number and root length were recorded in the $T_1$ treatment supplemented with 100 ppm $SiO_2$ NPs in both varieties, compared to PEG-treated plantlets (Table 7). However, increasing the concentration of NPs beyond this level adversely affected root formation (22). The results indicate that 100 ppm and 200 ppm $SiO_2$ NP treatments significantly enhance both shoot and root growth in *in vitro*-cultured banana plantlets under PEG-induced drought stress conditions (23).



$T_0$ (Control) $T_1$(3 % PEG + 100 ppm$SiO_2$) $T_2$ (3 % PEG + 200 ppm$SiO_2$) $T_3$(3 % PEG+300 ppm$SiO_2$) $T_4$(3 % PEG+400 ppm $SiO_2$)

**Fig. 3.** Effect of silicon dioxide nanoparticle (SiO2 NPs) on shoot multiplication of *in vitro* cultured Grand Naine banana plantlets.



$T_0$ (Control) $T_1$ (3 % PEG+100 ppm$SiO_2$) $T_2$ (3 % PEG+200 ppm$SiO_2$) $T_3$ (3 %PEG+300 ppm $SiO_2$) $T_4$(3 % PEG+400 ppm $SiO_2$)

**Fig. 4.** Effect of silicon dioxide nanoparticle ($SiO_2$ NPs) on shoot multiplication of *in vitro* cultured Karibale Monthan banana plantlet.

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**Table 6.** Effect of treatment of silicon dioxide nanoparticle ($SiO_2$ NPs) on relative water content (RWC), chlorophyll and proline content

| Banana variety | Treatments | Chlorophyll content | Proline content (µmoles/g) | RWC (%) |
|---|---|---|---|---|
| Grand Naine | T0 (Control) | 7.00 ± 0.28$^{a}$ | 1.63 ± 0.03$^{a}$ | 73.0 ± 0.57$^{a}$ |
| | $T_1$ (3 % PEG + 100 ppm $SiO_2$) | 6.93 ± 0.17$^{ab}$ | 1.77 ± 0.06$^{b}$ | 71.2 ± 1.66$^{a}$ |
| | $T_2$ (3 % PEG + 200 ppm $SiO_2$) | 6.06 ± 0.06$^{b}$ | 1.94 ± 0.18$^{ab}$ | 68.7 ± 0.68$^{b}$ |
| | $T_3$ (3 % PEG + 300 ppm $SiO_2$) | 5.26 ± 0.17$^{bc}$ | 2.29 ± 0.36$^{bc}$ | 62.8 ± 2.17$^{bc}$ |
| | $T_4$ (3 % PEG + 400 ppm $SiO_2$) | 4.50 ± 0.28$^{c}$ | 2.98 ± 0.13$^{c}$ | 52.3 ± 1.73$^{c}$ |
| Karibale Monthan | T0 (Control) | 8.26 ± 0.14$^{a}$ | 2.05 ± 0.19$^{a}$ | 75.0 ± 0.63$^{a}$ |
| | $T_1$ (1 % PEG + 100 ppm $SiO_2$) | 7.20 ± 0.11$^{ab}$ | 2.94 ± 0.06$^{ab}$ | 74.0 ± 1.00$^{ab}$ |
| | $T_2$ (2 % PEG + 200 ppm $SiO_2$) | 6.00 ± 0.28$^{b}$ | 4.03 ± 0.08$^{b}$ | 71.0 ± 0.71$^{b}$ |
| | $T_3$ (3 % PEG + 300 ppm $SiO_2$) | 6.43 ± 0.23$^{bc}$ | 4.12 ± 0.26$^{c}$ | 67.8 ± 1.65$^{bc}$ |
| | $T_4$ (4 % PEG + 400 ppm $SiO_2$) | 5.26 ± 0.14$^{c}$ | 4.76 ± 0.31$^{c}$ | 59.0 ± 2.62$^{c}$ |

**Table 7.** Effect of treatment of silicon dioxide nanoparticle ($SiO_2$ NPs) on root formation in *in vitro* banana

| Banana variety | Treatments | Number of shoots | Shoot length (cm) | Number of leaves | Fresh weight (g) | Dry weight (g) |
|---|---|---|---|---|---|---|
| Grand Naine | $T_0$ (Control) | 11.6 ± 0.66$^{a}$ | 6.03 ± 0.14$^{a}$ | 13.0 ± 0.57$^{a}$ | 2.67 ± 0.04$^{a}$ | 0.28 ± 0.02$^{a}$ |
| | $T_1$ (3 % PEG + 100 ppm $SiO_2$) | 10.3 ± 0.33$^{ab}$ | 5.66 ± 0.17$^{b}$ | 9.00 ± 0.57$^{b}$ | 2.46 ± 0.08$^{ab}$ | 0.26 ± 0.04$^{ab}$ |
| | $T_2$ (3 % PEG + 200 ppm $SiO_2$) | 9.00 ± 0.57$^{b}$ | 5.13 ± 0.17$^{b}$ | 11.3 ± 0.88$^{ab}$ | 2.43 ± 0.08$^{b}$ | 0.19 ± 0.01$^{b}$ |
| | $T_3$ (3 % PEG + 300 ppm $SiO_2$) | 8.00 ± 0.57$^{bc}$ | 4.26 ± 0.26$^{bc}$ | 8.00 ± 1.15$^{b}$ | 2.32 ± 0.02$^{bc}$ | 0.15 ± 0.01$^{bc}$ |
| | $T_4$ (3 % PEG + 400 ppm $SiO_2$) | 7.00 ± 0.57$^{c}$ | 3.26 ± 0.14$^{c}$ | 7.66 ± 0.66$^{c}$ | 2.12 ± 0.02$^{c}$ | 0.15 ± 0.01$^{c}$ |
| Karibale Monthan | T0 (Control) | 14.6 ± 1.76$^{a}$ | 6.50 ± 0.17$^{a}$ | 12.0 ± 1.15$^{a}$ | 2.73 ± 0.04$^{a}$ | 0.48 ± 0.01$^{a}$ |
| | $T_1$ (3 % PEG + 100 ppm $SiO_2$) | 11.6 ± 0.33$^{b}$ | 5.56 ± 0.14$^{b}$ | 9.66 ± 0.33$^{b}$ | 2.69 ± 0.09$^{ab}$ | 0.25 ± 0.02$^{ab}$ |
| | $T_2$ (3 % PEG + 200 ppm $SiO_2$) | 11.0 ± 0.57$^{b}$ | 4.56 ± 0.21$^{b}$ | 10.3 ± 0.88$^{ab}$ | 2.53 ± 0.06$^{b}$ | 0.21 ± 0.01$^{b}$ |
| | $T_3$ (3 % PEG + 300 ppm $SiO_2$) | 9.66 ± 0.33$^{bc}$ | 3.83 ± 0.12$^{c}$ | 8.33 ± 0.88$^{bc}$ | 2.35 ± 0.13$^{b}$ | 0.18 ± 0.01$^{c}$ |
| | $T_4$ (3 % PEG + 400 ppm $SiO_2$) | 8.33 ± 0.33$^{c}$ | 3.56 ± 0.12$^{c}$ | 7.33 ± 0.33$^{c}$ | 2.24 ± 0.03$^{c}$ | 0.17 ± 0.02$^{c}$ |

## Conclusion

This study highlights the potential of silicon oxide nanoparticles ($SiO_2$ NPs) as an effective strategy to enhance drought tolerance and improve growth performance in *in vitro*-cultured banana (*Musa* spp.) under polyethylene glycol (PEG)-induced osmotic stress. Polyethylene glycol is widely used to simulate drought conditions by reducing water availability without causing direct toxicity, thereby providing a reliable model for studying plant responses to water deficit. The application of $SiO_2$-NPs under PEG-induced stress significantly improved both morphological and physiological attributes of banana plantlets. Treated plants showed enhanced root and shoot development, increased chlorophyll content, improved relative water status and better water-use efficiency compared to PEG-stressed controls. These improvements suggest that $SiO_2$ NPs help maintain cellular hydration, stabilise photosynthetic activity and support overall metabolic functions under stress conditions. The beneficial effects of $SiO_2$ NPs may be attributed to their role in strengthening cell structure, enhancing antioxidant defence systems and regulating physiological processes associated with stress adaptation. By mitigating osmotic imbalance and reducing stress-induced damage, $SiO_2$ NPs act as a protective agent that supports sustained plant growth even under adverse conditions. These findings indicate that $SiO_2$ NPs can effectively alleviate drought-induced constraints in banana plantlets, making them a promising tool for improving crop resilience in water-limited environments. This approach holds particular significance for banana cultivation in arid and semi-arid regions, where drought stress is a major limiting factor for productivity. However, further studies are required to optimise NP concentrations, understand their long-term physiological and molecular effects and evaluate their environmental safety under field conditions. Overall, $SiO_2$ NPs represent a promising, eco-compatible approach for enhancing drought tolerance and supporting sustainable banana production and may also have wider applications in improving stress resilience in other economically important crops.

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## Authors' contributions

JS and RSS drafted, manuscript and designed the methodology. JS carried out the experiments and analyse the data. JS and AY performed the statistical analysis. RR, VB, AR and SM assisted in compiling the data. All authors have read and approved the final manuscript.

## Compliance with ethical standards

### Conflict of interest

Authors do not have any conflict of interests to declare.

### Ethical issues

None

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