In Vitro Responses of *Mandevilla sanderi* (Hemsl.) Woodson to Silicon Nanoparticles under Sodium Chloride-Induced Salinity Stress

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Abstract:

This study assessed the response of *Mandevilla sanderi* Woodson callus when subjected to silicon dioxide nanoparticles to combat salinity stress due to sodium chloride. The study is completely randomized design with ten replications with the following concentration of NaCl: 0, 50, 100, and 150 mM tested alongside varying concentration (0, 25, 50, 100, and 200) of SiO_2 nanoparticles [mg L^1]. The salinity, SiO_2 NPs, and their interactions were found to significantly affect all growth and morphometric measurements made in this study. In the case of use of lower concentrations of SiO_2 NPs (100 $mg.L^{-1}$), callus growth improved by means of a hermetic response that led to the highest values for callus induction (72.50%), callus volume (1.475 cm^3), fresh weight (1.450 g), dry weight (0.470 g), survival (81.25%), growth rate (2.025 g per day) and callus diameter (7.300 mm). Higher salinity levels (100 and 150 mM) resulted in significant drops in all measured parameters. Although the use of SiO_2 nanoparticles alone in concentration of 200 mg L^{-1} led to concentration-dependent nontoxicity, but together with 100 mg,L^{-1} SiO_2 NPs improved significantly callus induction (80.00%), callus volume (2.100 cm^3), fresh weight (2.000 g), survival (100 %), growth rate (2.600 g day^{-1}), and callus diameter (9.000 mm). These findings demonstrate the potential of SiO_2 NPs to improve salinity tolerance by enhancing osmotic and ionic homeostasis and activating plant defense mechanisms.

Keywords: *Mandevilla sanderi* ،Hormesis ، In vitro ، NaCl ، Nano silicon

Introduction

Mandeville sanderi (Hemsl.) Woodson which is also called as Dipladenia sanderi is decorative climbing plant which is cultivated for its beautiful flowers. It is widely grown in pots. It is a plant native to Brazil which belongs to Apocynaceae family. The flowers can be pink or red and sometimes yellow colored. It can bloom from spring to autumn. Traditionally, M. sanderi is propagated by stem cuttings. However, to establish an efficient propagation system capable of producing large numbers of healthy, disease-free plants, considerable research has focused on its in vitro micropropagation through plant tissue culture techniques. Previous studies have demonstrated that the success of in vitro regeneration is strongly influenced by several factors, including the plant genotype, the type and concentration of plant growth regulators, and the orientation and placement of explants on the culture

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medium [1]. Salinity is an important factor in limiting plant growth and productivity, especially in areas with a dry or semi-dry climate. Salinity negatively affects plant growth because it disrupts nutrient intake, hormone distribution and enzyme activity, while also causing ionic damage due to an excess accumulation of salt [2,3]. Studying the processes involved in salinity tolerance in the field is difficult because salinity comes with many environmental, climate or agronomic variables. That is why in vitro plant tissue culture offers a good option for experimental research, because it creates a controlled and stable environment with a defined nutrient medium that can be used to study plants responses to salt stress [4,5]. Nanotechnology has been discovered to be a useful approach for overcoming the damaging influence of environmental stressors in recent years. It has been demonstrated that nano-fertilizers are more efficient at enhancing nutrient use efficiency and reducing the negative impacts of salinity than classical fertilizers [6]. Out of several nanomaterials, silicon nanoparticles have drawn attention due to their capability to increase plant resistance to abiotic stresses. While silicon is not considered a plant nutrient, it plays an important role in many physiological and metabolic processes that help plants cope with environmental factors, especially salinity [7]. In response to exposure to salinity stress under in vitro conditions, plant cells and tissues encounter a number of damaging physiological and biochemical changes and their growth and regeneration capacity is reduced. Salinity causes metabolic disturbances and alters enzymatic activities that may lead to inhibition or disturbances in critical metabolic pathways [8]. Moreover, high salt concentration alters protein structure and activity [9–11], causing unfavorable changes in gene expression patterns, which affects cellular homeostasis and developmental processes [12]. Silica nanoparticles can be used to support plant tissues and cell regeneration and growth under saline environment since they possess unique properties. Recent research has shown that nanomaterials can improve plants' tolerance against biotic and abiotic factors better than regular materials [13]. Nanoparticles are of utmost importance for the control of cellular defense mechanisms. Silicon nanoparticles have a bigger surface area compared to regular silicon materials, increased solubility, and higher activity that determine their improved efficiency [15]. Additionally, most recent research confirmed that the proper use of SiO_2 NPs can help mitigate salinity stress and enhance plant growth under salinity [16].

Objectives of the Study

The objective of the current research work was to achieve the objectives outlined below:

1.To ascertain the viability and tolerance of Mandevilla sanderi callus under salinity stress by analyzing the inhibitory effects of various concentrations of sodium chloride (NaCl) on callus survival and growth under various in vitro conditions and determining the salinity levels that cause pine toxicity.

2.To find the optimal and safe dosage of silicon dioxide nanoparticles (SiO_2 NPs) by studying the effects of various nanoparticle concentrations on callus growth and distinguishing between the concentration that promotes their growth response and the concentrations associated with their toxicity.

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3.To identify the most effective combination of SiO_2 NPs and salinity stress for mitigating the adverse effects of salinity, enhancing callus survival and regeneration, and elucidating the potential role of SiO_2 NPs in maintaining ionic and osmotic homeostasis under *in vitro* conditions.

Materials and Methods

Plant Material and Surface Sterilization

The experiment took place in the plant tissue culture laboratory with the objective being to evaluate the effect of silicon dioxide nanoparticles on Mandevilla sanderi callus grown in vitro in the presence of sodium chloride stress.

Collection of Explants

This practice utilized explants taken from healthy, virus-free plants for callus production. According to the experimental treatments, leaf and/or nodal segments were cut off from the explants.

Surface Sterilization

First, the collected explants were washed carefully in running water to eliminate potential organic matter. After that, surface sterilization was performed in a sterile laminar flow cabinet where the explants were soaked in 70% ethanol (v/v) for one minute followed by NaOCl containing NaOCl with 2% active chlorine and prepared by adding Tween 20 as a surfactant for 10-15 minutes. At the end of the sterilization process, the explants were rinsed with sterilized water several times to remove any residual before placing them into the sterile medium. Callus Induction Medium

Basal Medium

Callus was induced on Murashige and Skoog (MS) medium supplemented with 30 g L^{-1} sucrose and solidified with 7 g L^{-1} agar. The medium pH was adjusted to 5.7 ± 0.1 before agar addition.

Plant Growth Regulators

The medium was supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzyladenine (BA), each at 0.2 mg L^{-1}, to induce callus formation.

Medium Sterilization and Culture Conditions

The medium was sterilized by autoclaving at a temperature of 121°C and a pressure of 15 psi (equal to 1.5 kg cm^{-2}) for a time period of 20 minutes. The explants that were sterilized were transferred to the medium and kept in an incubator at a temperature of 25 ± 2°C and pitch black conditions until the development of callus.

Experimental Treatments

Uniform, actively growing callus tissues of equal fresh weight were transferred to fresh MS medium containing the following treatments:

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- **NaCl: 0, 50, 100, and 150 mM** to induce salinity stress.
- **Silicon dioxide nanoparticles (SiO_2 NPs): 0, 25, 50, 100, and 200 mg L^{-1}** to evaluate their protective effect.

Experimental Design

The factorial experiment was carried out in a completely randomized design (CRD) with ten independent repetitions for each treatment, each culture bottle serving as one repetition.

Callus Induction Medium

Basic Medium

Callus was formed in Murashige and Skoog (MS) medium supplemented with 30 g L^{-1} sucrose and solidified using 7 g L^{-1} of agar. Before adding agar to the medium, its pH was adjusted to the level of 5.7 ± 0.1.

Plant Growth Regulating Substances

The medium received supplementation with 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzyladenine (BA) with each substance having a concentration level of 0.2 mg L^{-1}.

Medium Sterilization and Culture Conditions

The medium was autoclaved at 121°C psi (and 15 1.5 kg cm^{-2}) for 20 min. Sterilized explants were inoculated onto the culture medium and incubated at 25 ± 2°C under dark or low-light conditions until callus developed.

Experimental Treatments

Uniform, actively growing callus tissues of equal fresh weight were transferred to fresh MS medium containing the following treatments:

- NaCl**: 0,** 50**,** 100**,** and 150 mM to induce salinity stress.
- Silicon dioxide nanoparticles **(**SiO_2 NPs**): 0,** 25, 50**,** 100**,** and 200 mg L^{-1} to evaluate their protective effect.

Experimental Design

The experiment was arranged as a factorial experiment in a completely randomized design **(**CRD**)** with ten independent replicates per treatment, each culture vessel representing one replicate.

Studied Characteristics

After 4–6 weeks of incubation, the following values were assessed:

1. Callus formation rate (%): Computationally defined as the share of tissues that produced callus according to the equation presented below

2. Callus size (mm): Obtained using a digital caliper.

3. Callus bulk (cm3): Appertained using ImageJ software

4. Wet weight (g): Obtained immediately after removing the callus from the growth medium by using an analytical scale

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5. Dry weight (g): Dried callus tissues in the oven at 60–70 ºC for 48 h or until constant weight reached, then weighted by means of the analytical balance.

6. Growth rate (g/day): Calculated from the value that is equal to the difference between final and initial wet weights divided by the number of days of tissue cultivation.

7. Survivability (%): Defined as a share of vital callus tissues that survived without severe browning or necrosis during the entire culture process.

Statistical Analysis

The data were analyzed using the Statistical Analysis System **(**SAS**)** software by analysis of variance (ANOVA). Treatment means were compared using Duncan's Multiple Range Test **(**DMRT**)** at a significance level of $P \leq 0.05$**.**

Results and Discussion

1- Effect of Silicon Dioxide Nanoparticles, Sodium Chloride, and Their Interaction on Callus Induction (%):

As illustrated in Table 1, sodium chloride (NaCl) plays a substantial role on *Mandevilla sanderi* in regards to callus induction. The optimum callous induction of 72.50% was found with 50 mM NaCl while the least amount of callus induction of 16.25% was found with the control (0 mM NaCl). Silicon dioxide nanoparticles (SiO_2 NPs) too contributed to the development of callus. Control (0 mg L^{-1} SiO_2 NPs) gives the highest amount of callus growth (54.00%) and the lowest was from 200 mg L^{-1} SiO_2 NPs (35.00%). There appears to be a noteworthy relationship between NaCl and the SiO_2 NPs with regard to callus induction. The best callus induction of 80.00% was achieved with the combination of 50 mM NaCl and 100 mg L^{-1} SiO_2 NPs. The minimum value of 5.00% was found from the combination of 0 mM NaCl and 200 mg L^{-1} SiO_2 NPs.

Table 1: Effect of NaCl, SiO_2 nanoparticles, and their interaction on callus induction (%) of *Mandevilla sanderi* (Hemsl.) Woodson.

(SiO□ NPs) mg L□	**sodium chloride** mmol L□¹				**(SiO□ NPs)** mg L□
	150	100	50	0	
54.00a	38.00□	78.00^{b}	70.00^{d}	30.00^{k}	0
45.00b	20.00^{l}	60.00^{f}	80.00^{a}	20.00^{l}	100
41.25c	35.00^{j}	45.00^{h}	75.00^{c}	10.00^{n}	150
35.00d	12.00^{m}	58.00^{g}	65.00^{e}	5.00^{o}	200
Mean	**26.25^{c}**	**60.25^{b}**	**72.50^{a}**	**16.25^{d}**	

ISSN 2072-3857

2-Effect of NaCl, SiO_2 Nanoparticles, and Their Interaction on Callus Volume (cm^3)

In the table 2, it is shown that SiO_2 NPs plays a significant role in influencing the callus volume of *Mandevilla sanderi*. The callus volume was highest at 100 mg L^{-1} SiO_2 NPs (1.475 cm^3), while the control treatment (0 mg L^{-1} SiO_2 NPs) resulted in the least value (0.118 cm^3). Another factor at play in influencing the callus volume is NaCl. The control treatment (0 mM. L^{-1} NaCl) provided the maximum mean callus volume (0.835 cm^3), while the maximum concentration of the nanoparticles (150 mM.L^{-1}) led to the lowest value (0.440 cm^3). This suggests that there is a concentration-dependent toxicity caused by the nanoparticles. There is significant interaction found between SiO_2 nanoparticles and Nacl . It was found that the combination of SiO_2 NPs at a concentration of 100 mg L^{-1} and NaCl at 50 mM produced the maximum callus volume (2.100 cm^3), while the combination of SiO_2 NPs at 200 mg L^{-1} and NaCl at 150 mM led to the minimum value (0.080 cm^3).

Table 2. Effect of NaCl, SiO_2 nanoparticles, and their interaction on callus volume (cm^3) of *Mandevilla sanderi* (Hemsl.) Woodson.

sodium chloride mmol L□¹	**(SiO□ NPs)** mg L□				**sodium chloride** mmol L□¹
	200	150	100	0	
0.8350a	0.380 i	1.200 d	1.600 b	0.160 m	0
0.8000b	0.220 k	0.750 f	2.100 a	0.130 n	50
0.5625c	0.350 j	0.450 h	1.350 c	0.100 o	100
0.4400d	0.180 l	0.650 g	0.850 e	0.080 p	150
	0.282 c	**0.763 b**	**1.475 a**	**0.118 d**	

3-Effect of NaCl, SiO_2 Nanoparticles, and Their Interaction on Callus Fresh Weight (g)

As shown in Table, SiO_2 NPs impacted the callus fresh weight of *Mandevilla sanderi* in a considerable way. The highest value of fresh weight, which is 1.450 g, was recorded while treating with 100 mg L^{-1} SiO_2 NPs, while in control treatment, which is 0 mg L^{-1} SiO_2 NPs, the minimum value of fresh weight was noted (0.115 g). Moreover, Nacl had a significant effect on the callus fresh weight. The control treatment (0 mM L^{-1} Nacl) resulted in the maximum average fresh weight (0.8625 g), but at the highest nanoparticle concentration (150 mM L^{-1}), the minimum value (0.5075 g) was found, The interactive effect of SiO_2 NPs and Nacl was significant. When combining 100 mg L^{-1} SiO_2 NPs with 50 mM NaCl, the highest callus fresh weight (2.000 g) was resulted, while the combination of 200 mg L^{-1} SiO_2 NPs with 0 mM NaCl gave the lowest value (0.090 g).

ISSN 2072-3857

Table 3. Effect of NaCl, SiO_2 nanoparticles, and their interaction on callus fresh weight (g) of *Mandevilla sanderi* (Hemsl.) Woodson.

sodium chloride mmol L□¹	**(SiO□ NPs)** mg L□				**sodium chloride** mmol L□¹
	200	150	100	0	
0.8625 a	0.350 i	1.450 c	1.500 b	0.150 l	0
0.8175 b	0.200 k	0.950 f	2.000 a	0.120 n	50
0.5125 c	0.300 j	0.450 h	1.200 d	0.100 o	150
0.5075 d	0.140 m	0.700 j	1.100 e	0.090 p	200
	0.247 c	0.887 b	1.450 a	0.115 d	

4- Effect of NaCl, SiO_2 Nanoparticles, and Their Interaction on Callus Dry Weight (g)

As shown in Table 4, SiO_2 NPs has a profound impact on the callus dry weight in *Mandevilla sanderi*. The maximum value (0.4700 g) was achieved at the 100 mg L^{-1} level, while the minimum values (0.0060 g) were collected in the 200 mg L^{-1} groups. but The maximum weight (0.2530 g) was associated with the control treatment (0 mM L^{-1} Nacl), while the minimum (0.0308 g) was found at the maximum Nacl level (150 mM L^{-1}), The combination of 100 mg L^{-1} SiO_2 NPs and 50 mM NaCl gave the maximum callus dry weight (0.9000 g), while mixtures containing 150 mM NaCl and 0 mg L^{-1} SiO_2 NPs resulted in the lowest value (0.0010 g).

Table 4. Effect of NaCl, SiO_2 nanoparticles, and their interaction on callus dry weight (g) of *Mandevilla sanderi* (Hemsl.) Woodson.

(SiO□ NPs) mg L□¹	**sodium chloride** mmol L□¹				**(SiO□ NPs)** mg L□¹
	200	150	100	0	
0.2530 a	0.0120 h	0.0900 d	0.9000 a	0.0120 i	0
0.2072 b	0.0040 l	0.0600 f	0.1000 c	0.0100 g	50
0.0435 c	0.0080 k	0.0200 h	0.8000 b	0.0010 n	100
0.0308 d	0.0020 m	0.0400 g	0.0800 e	0.0010 n	150
	0.0060 c	0.0525 b	0.4700 a	0.0060 d	

5-Effect of NaCl, SiO□ Nanoparticles, and Their Interaction on Callus Survival (%)

As shown in Table 5, addition of SiO_2 NPs had a substantial effect on the survival of callus. At 100 mg.L^{-1}, SiO_2 NPs achieved the highest survival rate (81.25%), whereas at 200 mg.L^{-1}. it had the lowest rate (31.25%). Nacl also had a significant effect on the survival rate of callus. In the control (0 mM L^{-1} Nacl), survival was highest (66.25%), whereas the highest concentration of nanoparticles (150 mM L^{-1}) resulted in the lowest survival (42.50%).The interaction between NaCl and SiO_2 NPs was significant. The highest survival (100.0%) was recorded for 100 mg L^{-1} SiO_2 NPs combined with 50 mM NaCl, whereas the lowest survival (10.0%) was observed for 0 mg L^{-1} SiO_2 NPs combined with 150 mM NaCl. This suggests that moderate concentrations of SiO_2 NPs may improve the tolerance of callus to salinity by enhancing osmoregulation and reducing cellular damage from stress.

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Table 5. Effect of NaCl, SiO_2 nanoparticles, and their interaction on callus survival (%) of *Mandevilla sanderi* (Hemsl.) Woodson.

sodium chloride mmol L□¹	**(SiO□ NPs)** mg L□				**sodium chloride** mmol L□¹
	200	150	100	0	
66.25 a	45.0 j	90.0 b	80.0 c	50.0 i	0
62.50 b	25.0 l	65.0 f	100.0 a	60.0 g	50
52.50 c	40.0 k	55.0 h	75.0 d	40.0 k	100
42.50 d	15.0 m	75.0 d	70.0 e	10.0 n	150
	31.25 d	71.25 b	81.25 a	40.00 c	

6-Effect of NaCl, SiO_2 Nanoparticles, and Their Interaction on Growth Rate (g day⁻ ¹)

As shown in Table 6, The growth rate of *Mandevilla sanderi* callus was influenced significantly by SiO_2 NPs. The maximum growth rate (2.025 g day⁻ ¹) was recorded at 100 mg.L^{-1} SiO_2 NPs, with the minimum value (0.250 g day⁻ ¹) being at 200 mg.L^{-1} SiO_2 NPs . The growth rate was also influenced significantly by Nacl . The control (0 mM NaCl) produced the maximum mean growth rate (1.475 g day⁻ ¹). However, the highest concentration of NaCl (150 mM) eventually led to the least rate of growth as well (0.700 g day⁻ ¹) . The combination of 100 mg L⁻ ¹ SiO_2 NPs and 50 mM NaCl exhibited the best growth (2.600 g day⁻ ¹), while 0 mg L⁻ ¹ SiO_2 NPs with 150 mM NaCl gave the least growth (0.100 g day⁻ ¹).

Table 6. Effect of NaCl, SiO_2 nanoparticles, and their interaction on callus growth rate (g day⁻ ¹) of *Mandevilla sanderi* (Hemsl.) Woodson.

sodium chloride mmol L□¹	**(SiO□ NPs)** mg L□				**sodium chloride** mmol L□¹
	200	150	100	0	
1.475 a	0.400 k	2.200 b	2.100 c	1.200 g	0
1.225 b	0.200 m	1.300 f	2.600 a	0.800 i	50
0.775 c	0.300 l	0.600 j	1.800 d	0.400 k	100
0.700 d	0.100 n	1.3000 h	1.600 e	0.100 n	150
	0.250 d	1.275 c	2.025 a	0.625 c	

7-**Effect of NaCl, SiO_2 Nanoparticles, and Their Interaction on Callus Diameter (mm)**

As shown in Table 7, SiO_2 NPs had a strong impact on the diameter of callus in *Mandevilla sanderi*. The maximum diameter of the callus at which it was found highest was 7.300 mm at the concentration of 100 mg L⁻ ¹ SiO_2 NPs , and the lowest value was of 1.475 mm at 200 mg L⁻ ¹ SiO_2 NPs. The NaCl also did influence the diameter of callus. The control treatment where the amount of NaCl had been equal to 0 mM gave the highest mean diameter of 5.375 mm, while the highest concentration of nanoparticles equal to 150 mg L⁻ ¹ showed the smallest values of 3.025 mm, demonstrating a strong dependence of the toxicity of the phytotoxicity on the

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concentration .There has also been observed a considerable interaction between the NaCl and SiO_2 NPs. The combination of 100 mg L^{-1} SiO_2 NPs and 50 mM NaCl produced the biggest diameter of callus equal to 9.000 mm and the combination of 0 mg L^{-1} SiO_2 NPs with 150 mM NaCl provided the smallest diameter of 0.800 mm.

Table 7. Effect of NaCl, SiO_2 nanoparticles, and their interaction on callus diameter (mm) of *Mandevilla sanderi* (Hemsl.) Woodson.

sodium chloride mmol L□¹	**(SiO□ NPs)** mg L□				**sodium chloride** mmol L□¹
	200	150	100	0	
5.375 a	2.000 k	8.000 b	7.500 c	4.000 h	0
4.625 b	1.200 n	5.500 f	9.000 a	2.800 i	50
3.075 c	1.800 l	2.500 j	6.500 d	1.500 m	100
3.025 d	0.900 o	4.200 g	6.200 e	0.800 p	150
	1.475 d	5.050 b	7.300 a	2.275 c	

Discussion

In the current study, it is noted that the salt stress caused by NaCl significantly restrained the grown of Mandevilla sanderi callus in vitro. The inhibition levels differ by treatment, showing different tissue responses to salinity. However, SiO_2 NPs played a crucial role in mitigating the effects of salt stress and improving the callus growth. Salinity has its effects mostly due to osmotic and ionic stress which adversely affect water relations, nutrition, and metabolism. Higher salt levels exacerbate the osmotic potential of the medium causing physiological drought that restricts water uptake and, consequently, growth of plant tissue. Utilization of SiO_2 NPs led to the significant improvement of fresh and dry weights of the callus in saline conditions. The reason behind this is the special physicochemical characteristics of nanoparticles such as small size, large surface area, and good bioavailability making them easy to penetrate and accumulate in the tissues of plants. Furthermore, SiO_2 NPs appear to enhance protein biosynthesis under salt stress by regulating the activity of enzymes involved in protein synthesis, thereby compensating for stress-induced reductions in protein production and contributing to improved tissue growth and stress tolerance [24,25]. Salinity stress resulting from the accumulation of sodium chloride is one of the main environmental factors restricting callus induction and differentiation in plant tissue culture. It has a negative effect on cell division and expansion by disrupting osmotic balance and causing the excessive production of reactive oxygen species (ROS), which adversely affect cell metabolism and growth [26]. On the contrary, silicon dioxide nanoparticles (SiO_2 NPs) have shown great potential in increasing membrane stability, regulating nutrient uptake, and activating antioxidant defense systems, which leads to enhanced stress tolerance of plant cells [27]. The data presented in this investigation indicate that low sodium chloride concentration (50 mM) is beneficial for callus growth, which is explained by the process of hormesis, whereby low stress serves as an external stimulator that activates secondary signaling pathways and metabolic processes

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associated with defense. However, higher concentrations of sodium chloride (100 mM and 150 mM) resulted in a decline in all parameters of growth compared to the control. Such inhibition is explained physiologically by the ionic toxicity of sodium ions (Na^+) and chloride ions (Cl^-) that interfere with major enzyme activities, disturb water absorption, decrease required cellular turgor needed for cell division, and thus weaken callus growth and development [18]. The findings indicated a considerable decrease in callus formation when the concentration of SiO_2 nano-particles varied from zero to two hundred milligrams per liter. The suppressive mechanism behind this phenomenon could be associated with concentration-dependent toxicity of nanoparticles, as the presence of greater amounts of SiO_2 nano-particles creates physical blocks in the cell wall pores, inhibits membrane transport processes, and results in excessive production of toxic reactive oxygen species, which exceed the anti-oxidizing abilities of the undifferentiated callus cells that negatively affect the viability of the cells [29]. On the other hand, moderate concentrations of SiO_2 NPs interacting with low salinity produce significant synergistic effects confirming the effect of the particles on plant cell adaptation towards salt stress [30]. In terms of advantageous effects of SiO_2 NPs, the combinations of three mechanisms are responsible for their function. First, silica deposition under cell wall structure contributes to the maintenance of the integrity of the tissues and prevents the loss of water during osmotic situation [31]. Second, ionic homeostasis allows for preventing the toxic build-up of Na^+ ions and keeping K^+ ions in order to ensure the functioning of the enzymes and sustain metabolic processes [32]. Third, the stimulation of the antioxidant activity leads to the increased action of SOD and POD and enables the removal of excess ROS and reduces the risk of oxidative damage of the cells and tissues [33][35].

Conclusion

The investigation proved that callus of Mandevilla sanderi is quite sensitive to high concentrations of salt, especially at 100 and 150 mM NaCl, where the callus functionality was affected due to osmotic imbalance and ionic stress. On the other hand, when 100 mg L^{-1} SiO_2 nanoparticles (SiO_2 NPs) were added to the culture, negative salinity effect was diminished, and the growth of callus reached the highest results (growth and viability were improved). The effect of SiO_2 NPs is probably caused by increased cell wall stability due to silica deposition, maintenance of ionic homeostasis due to reduced Na+ toxicity, and facilitation of nutrient intake. Nevertheless, too high doses of nanoparticles (200 mg L^{-1}) had negative influence on callus growth that proves the principle of concentration-dependent toxicity. Thus, applying moderate doses of SiO_2 NPs seems to be a good way to improve salinity tolerance of Mandevilla sanderi tissue culture while high doses must be avoided.

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