APPLICATION OF NATURAL BIOSTIMULANTS IN CACTUS *IN VITRO* CULTURES: EVALUATING THE EFFECTIVENESS OF CHITOSAN AND NANOSILICON

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Abstract

This study investigates the impact of chitosan (CH) and nanosilicon (nSi) on the micropropagation of two cactus species. Stem explants were cultured on Murashige and Skoog medium supplemented with chitosan at 20 - 60 mg L^{-1} *or silicon nanoparticles at 100 - 500 mg* L^{-1}*. After 35 days of* in vitro *culture, morphological parameters were evaluated. Plantlets were acclimatised* ex vitro *to assess survival rates and root development. Chitosan significantly promoted shoot multiplication and root formation in both species, with optimal effects at moderate concentrations (40 mg* L^{-1} *for Echinopsis chamaecereus and 20 mg* L^{-1} *for* Opuntia microdasys*). In contrast, lower concentrations of nSi (100 or 200 mg* L^{-1}*) stimulated rhizogenesis in E. chamaecereus but had variable effects on* O. microdasys*. At 500 mg* L^{-1}*, nSi increased shoot biomass but reduced root growth. The survival and rooting rates during* ex vitro *acclimatisation were treatment- and concentration-dependent. These findings highlight the potential of CH and nSi as eco-friendly biostimulants in cactus micropropagation systems. Optimising their concentrations could enhance shoot and root development while reducing reliance on synthetic growth regulators.*

Keywords: *Echinopsis chamaecereus, Opuntia microdasys, micropropagation, chitosan, nanosilicon*

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1. Introduction

The *Cactaceae* family comprises approximately 130 genera and over 2,000 species with diverse morphological characteristics. These plants are predominantly found in arid and semi-arid regions across the Americas, the Mediterranean, Africa, Asia, Europe, and Australia [1, 2]. Cacti are highly adaptable to harsh environmental conditions, serving as a natural barrier against soil erosion and playing a role in land reclamation. Cacti cultivation has increased rapidly in recent decades. Traditional propagation methods, including seed germination and vegetative propagation, are often insufficient due to limited flowering, low seed viability, and slow growth rates [3]. Cacti are characterised by the content of many valuable chemical compounds with healing and nutritional effects used in natural medicine. North American Indians have long used the properties of cacti to treat various ailments and disorders. Among the most widespread and economically significant cacti are species from the genus *Opuntia*, commonly called prickly pears, which are widely cultivated for their edible fruits, medicinal properties, and ornamental value. Prickly pear extracts are available in powder or tablet form and are used as a dietary supplement. Prickly pear is a rich source of flavonoids, which are known for their health-promoting properties [1]. The simplest and most common method of propagating cacti in nature is propagation by seeds or vegetatively by suckers. However, the significant limitations of flowering, and therefore the insufficient production of seeds and their low germination capacity, are that they do not meet the needs of protection and mass propagation of this species [2]. Cacti are among the most endangered taxonomic groups. In addition, some types of cacti are at risk of extinction due to illegal trade and unsustainable harvesting or the degradation of their natural habitat due to human activity [4, 5]. A technique that allows for the mass reproduction of endangered cactus species in a short time is *in vitro* culture. Current research on micropropagation of cacti focuses mainly on determining the effect of growth regulators (auxins and/or cytokinins) on the multiplication and rooting of plants. Cactus micropropagation methods have been developed for over 60 years [3]. Numerous micropropagation techniques have been applied, such as regeneration through somatic embryogenesis, regeneration through direct or indirect organogenesis, and *in vitro* grafting. Micropropagation of cacti has been extensively studied, particularly regarding the effects of synthetic plant growth regulators such as auxins and cytokinins [6]. However, limited research exists on applying natural biostimulants such as chitosan (CH) and nanosilicon (nSi) in cactus tissue culture. Chitosan, a chitin derivative with biostimulatory and antimicrobial properties, has been reported to enhance shoot and root development in various plant species [7, 8]. Faizan *et al.* [9] demonstrated that foliar application of chitosan nanoparticles in *Solanum lycopersicum* enhanced shoot growth, particularly by increasing shoot length, while simultaneously reducing reactive oxygen species accumulation, as indicated by decreased H_2O_2 and MDA levels. Similarly, in maize (*Zea mays* L.), chitosan seed treatments (priming/coating) have been reported to accelerate early root initiation, increase shoot and root length and seedling dry mass, and attenuate oxidative damage by reducing malondialdehyde (MDA) while enhancing peroxidase and catalase activities [10]. These findings underscore chitosan’s potential role in modulating hormonal and enzymatic pathways associated with plant development and stress mitigation. In some experiments, it also increased the ability of plants to accumulate selected metabolites [11]. However, chitosan's exact mode of action in plant systems remains only partially understood. According to Lopez-Moya *et al.* [12], chitosan stimulates the accumulation of auxins, particularly indole-3-acetic acid (IAA), in the root tip, causing distinct morphological changes in this region.

Silicon (Si) is another promising biostimulant, known for improving plant resistance to biotic and abiotic stressors [13, 14]. Although not classified as an essential element, Si strengthens cell walls, enhances stress tolerance, and promotes growth when supplied in nanoparticle form [15, 16]. However, its effects can vary depending on concentration and plant species, necessitating careful optimisation [17, 18]. Occurring mainly in soil solutions as silicic acid (H_4SiO_4) at concentrations of about 0.1 - 0.6 mM, this mineral is easily absorbed, leading to its significant accumulation in plants. It accumulates mainly in cell walls, strengthening their structure, which improves tissue integrity and reduces susceptibility to mechanical damage and infections. One way in which silicon can be used in plants is in the form of nanoparticles. The use of silicon nanoparticles is considered an effective alternative to commercially used silicon solutions [15]. Nanosilicon was used to increase the efficiency of germination, growth and flowering of plants (*Arachis hypogaea* L.) by Prasad *et al.* [19]. Moreover, it was observed that after the use of silicon oxide nanoparticles, the thickness of the cell wall increased, which positively affected the penetration of fungi, bacteria and nematodes, increasing resistance to diseases caused by them. The positive effects of nSi in alleviating plant stress and promoting plant growth have attracted wide attention. However, it seems that the application of nSi may be beneficial but also ineffective [18] or even cause harm [17] to the plant defence system, depending on the dose and duration of application or the system studied. Hence, it is essential to select the appropriate concentration and form of nSi to achieve the intended effect.

We hypothesised that the application of chitosan and nanosilicon at specific concentrations would significantly enhance the efficiency of *in vitro* multiplication and rooting of two cactus species, compared to the control, by modulating physiological and biochemical responses. This study aims to evaluate the effects of chitosan and nanosilicon on the *in vitro* propagation, morphological development, and *ex vitro* acclimatisation success of *E. chamaecereus* and *O. microdasys*.

2. Materials and Methods

2.1. Plant Material

Shoot explants were obtained from established *in vitro* cultures of *Echinopsis chamaecereus* and *Opuntia microdasys*. Stem segments (17 - 20 mm long) were excised and used for experiments.

2.2. Medium and Culture Conditions

Shoot explants were initiated on Murashige and Skoog [20] medium under a laminar flow hood. The medium was supplemented with chitosan (CH) of molecular weight 3.33 kDa in the concentration of 20 - 60 mg L^{-1} or MS with the addition of nanosilicon (nanopowder < 100 nm particle size, Sigma-Aldrich), in concentrations of 100 - 500 mg L^{-1}. A control medium without biostimulants was also included. Chitosan samples were obtained by continuously adding hydrogen peroxide (0.8 - 6.4 mM/g of polysaccharide) to a 2.5% chitosan solution with pH 3.5 - 4.0 at 80°C. After degradation, all samples, as chloride salts, had similar polydispersity and a high degree of deacetylation (> 95%). The molar mass of each sample was determined using the high-performance liquid chromatography/gel permeation chromatography (HPLC/GPC) method (HPLC SmartLine system with an isocratic pump 1000 equipped with an RI Detector 2300; Knauer, Germany) [21].

Chitosan and nanosilicon concentration ranges were chosen based on preliminary experiments and literature data indicating their optimal and suboptimal physiological thresholds in *in vitro* culture of various plant species, including cacti. The applied doses aimed to verify these biostimulants' stimulatory and potentially inhibitory effects, allowing for species-specific optimisation.

Each culture medium was enriched with 3% (w/v) sucrose (Chempur, Poland), 0.8% (w/v) agar (Biocorp, Poland), and 100 mg L^{-1} myo-inositol (Duchefa Biochemie B.V., The Netherlands). The mixtures were heated, and 30 ml portions were dispensed into 450 ml flasks. Sterilisation was carried out at 121°C (0.1 MPa), with the duration adjusted to the medium volume in each vessel. Cultures were maintained in a growth chamber under a 16-hour photoperiod at 24 ± 2°C (a photosynthetic photon flux density (PPFD) of 40 μmol m^{-2} s^{-1}). Each experimental variant comprised 32 shoots (8 flasks, each containing 4 explants). After 35 days, the explants were removed and rinsed with deionised water. Measurements included shoot and root length, the number of newly formed shoots and fresh mass.

Explants of the *E. chamaecereus* and *O. microdasys* with a well-developed root system from all experimental combinations were removed from the culture vessels and thoroughly washed with deionised water to remove any remaining substrate. For acclimatisation, rooted plantlets were transferred to plastic pots (10 cm diameter) with a universal soil substrate (pH 5.5) and maintained at 22 ± 2°C with 90% relative humidity for six weeks. The survival rate and root development were assessed at the end of the acclimatisation period.

2.3. Statistical Analysis

Experimental data were analysed using Statistica 13.0 software (StatSoft, distributed in Poland by StatSoft Poland, Kraków). Normality and homogeneity of variance were tested, followed by ANOVA and Tukey's post hoc test ($p < 0.05$).

3. Results and Discussion

Plant development can be stimulated at various growth stages by applying plant growth regulators or biostimulators. The positive effect of chitosan on the growth and development of many plant species has been described in the literature many times [22–24]. However, there are no reports on the influence of this biostimulant on the growth of *E. chamaecereus* and *O. microdasys* plants in *in vitro* cultures.

The mean values of morphological traits of *E. chamaecereus* and *O. microdasys* are presented in Tables 1 and 2. Chitosan proved to be more effective in stimulating the multiplication of *E. chamaecereus* shoots compared to nSi (Figure 1). The explants from the MS+40 mg L^{-1} CH medium were 14% shorter than the control but developed 6% more shoots and 13% more roots, with roots being 8% longer compared to the control (Table 1). Furthermore, the fresh weight of plants from this medium was 45% higher than that of the control. Plants from the MS medium supplemented with nSi were lower on average by 30 - 47% compared to the control. Additionally, it was observed that the number of shoots and fresh mass decreased with the increase in nSi concentration. The addition of 100 mg L^{-1} nSi stimulated the number of new shoots (137% of the control) and inhibited the rhizogenesis process (Table 1).

In the case of *O. microdasys*, it was observed that chitosan stimulated the growth of shoots and roots (Figure 2). The explants were higher than the control by 23 - 32% (Table 2). Moreover, with the increase in the concentration of chitosan in the medium, the number of new shoots, the number and length of roots and the fresh weight decreased, which indicates the need for optimal selection of the dose. The addition of nSi to the medium inhibited the number of new shoots and the length of roots (Table 2). It was observed that with the increase in the concentration of silicon nanoparticles in the medium, the height of shoots increased (107 - 130% of the control) and the fresh weight (93 - 142% of the control).

Table 1. Effect of different medium composition on morphological traits of *E. chamaecereus* plants under *in vitro* conditions after 35 days of culture (n = 32 shoots per treatment).

Medium	Plant length [mm]	No. of new explants	No. of roots	Roots length [mm]	Fresh mass [mg]
MS – control	18.25 ± 5.2^{a}	1.9 ± 1.6^{a}	2.9 ± 1.2^{a}	19.9 ± 11.7^{a}	178.2 ± 51.3^{a}
MS+20 mg L^{-1} CH	9.63 ± 4.6^{b}	2.9 ± 1.9^{a}	2.1 ± 1.6^{a}	5.3 ± 3.8^{b}	139.6 ± 45.0^{ab}
MS+40 mg L^{-1} CH	11.38 ± 6.5^{ab}	2.6 ± 1.6^{a}	1.6 ± 1.6^{a}	4.1 ± 4.0^{b}	89.9 ± 29.3^{b}
MS+60 mg L^{-1} CH	12.75 ± 5.7^{ab}	1.8 ± 1.4^{a}	2.9 ± 1.6^{a}	7.0 ± 3.7^{b}	82.5 ± 28.6^{b}
MS+100 mg L^{-1} nSi	17.57 ± 6.2^{a}	1.7 ± 1.2^{a}	2.9 ± 1.6^{a}	15.1 ± 9.2^{a}	166.5 ± 66.9^{a}
MS+200 mg L^{-1} nSi	15.75 ± 4.8^{a}	2.0 ± 1.6^{a}	3.3 ± 1.8^{a}	21.5 ± 11.8^{a}	258.2 ± 95.8^{a}
MS+500 mg L^{-1} nSi	19.57 ± 5.3^{a}	1.6 ± 1.2^{a}	3.3 ± 2.2^{a}	11.7 ± 7.5^{ab}	173.3 ± 58.0^{a}

Note. Means (± SD) followed by the same letter do not differ significantly at $p < 0.05$ according to Tukey multiple ranges.

Table 2. Effect of different medium composition on morphological traits of *O. microdasys* plants under *in vitro* conditions after 35 days of culture (n = 32 shoots per treatment).

Medium	Plant length [mm]	No. of new explants	No. of roots	Roots length [mm]	Fresh mass [mg]
MS – control	11.0 ± 3.8^{a}	1.7 ± 0.7^{a}	0.9 ± 0.4^{a}	24.7 ± 16.9^{ab}	218.2 ± 152.6^{a}
MS+20 mg L^{-1} CH	14.5 ± 5.8^{a}	1.8 ± 0.7^{a}	1.9 ± 1.0^{a}	52.0 ± 50.5^{a}	298.5 ± 215.0^{a}
MS+40 mg L^{-1} CH	13.5 ± 6.4^{a}	1.4 ± 0.5^{a}	1.8 ± 1.1^{a}	47.6 ± 40.0^{a}	268.3 ± 223.7^{a}
MS+60 mg L^{-1} CH	14.0 ± 5.1^{a}	1.1 ± 0.3^{a}	0.8 ± 0.4^{a}	11.8 ± 11.0^{b}	246.4 ± 160.4^{a}
MS+100 mg L^{-1} nSi	11.8 ± 3.2^{a}	1.3 ± 0.5^{a}	1.0 ± 0.4^{a}	5.4 ± 4.6^{b}	149.3 ± 53.1^{ab}
MS+200 mg L^{-1} nSi	14.0 ± 5.0^{a}	1.6 ± 0.5^{a}	1.1 ± 0.7^{a}	22.6 ± 19.4^{ab}	273.6 ± 158.8^{a}
MS+500 mg L^{-1} nSi	14.3 ± 4.9^{a}	1.6 ± 0.7^{a}	1.3 ± 0.5^{a}	25.4 ± 14.9^{ab}	310.3 ± 152.9^{a}

Note. Means (± SD) followed by the same letter do not differ significantly at $p < 0.05$ according to Tukey multiple ranges.

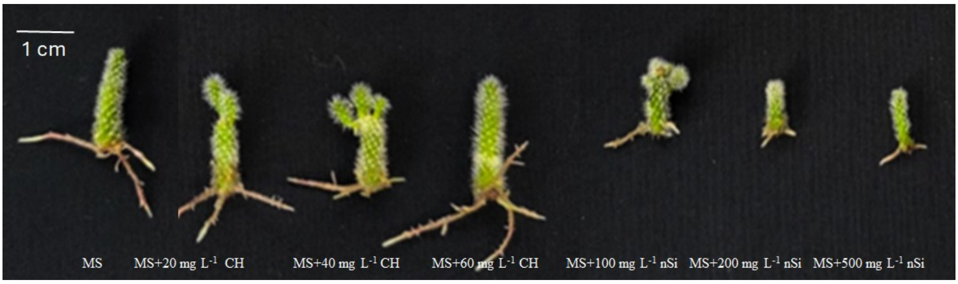


Figure 1. *In vitro* cultures of *E. chamaecereus* after 35 days of incubation.

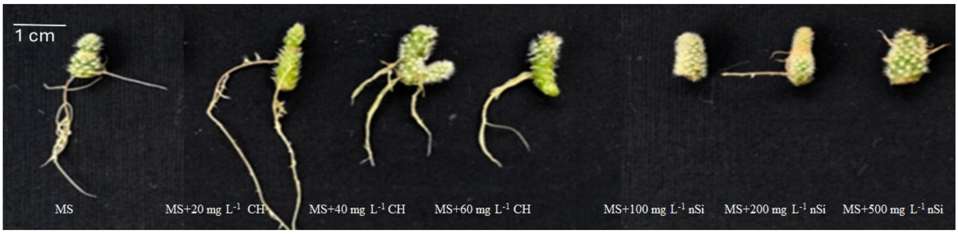
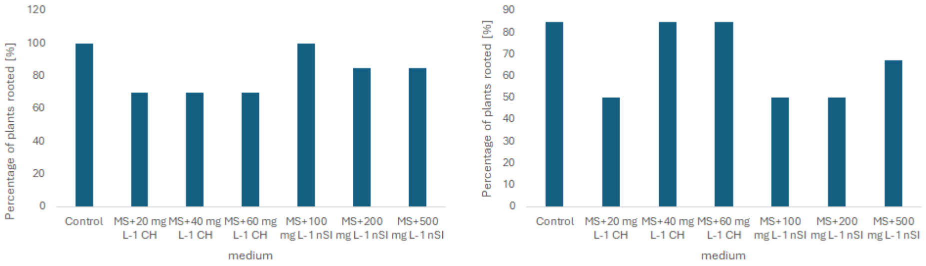


Figure 2. *In vitro* cultures of *O. microdasys* after 35 days of incubation

It has been shown that chitosan significantly stimulates the proliferation of many plant species and has a beneficial effect on the root system. According to Nge *et al.* [25], chitosan can stimulate the induction of differentiation of orchid plant tissues. In a study conducted by Krupa-Małkiewicz and Fornal [26], 15 mg L^{-1} of chitosan was shown to serve as a plant growth stimulator for *Petunia×atkinsiana* D. don micropropagation. On the other hand, Obsuwan *et al.* [27] used chitosan to promote the morphological characteristics of *Rhynchostylis giganteaprotocorms in vitro* cultures. Similar results were obtained by Kruczek *et al.* [7], observing a significant increase in the number of roots in micropropagated goji plants (*Lycium chinense*). According to Lopez-Moya *et al.* [12], the mechanism of action of chitosan is closely related to, among others, the induction of auxin accumulation in the root zone. Avestan *et al.* [28] investigated the influence of nanosilicon dioxide (SiO_2) and chitosan on the growth and proliferation of apple (*Malus domestica* Borkh. 'Gala') explants under osmotic stress. The use of 50 or 100 mg L^{-1} SiO_2 or 40 mg L^{-1} chitosan increased the growth of apple explants under stress conditions. This research suggests that the use of SiO_2 or chitosan may improve plant growth and tolerance to stress. According to Mani *et al.* [29], silicon nanoparticles are characterised by unique physiological properties and are effective carriers enabling the transport of components involved in the synthesis of proteins and nucleotides in plant tissue culture. In addition, they stimulate the secretion of naturally occurring growth regulators, antioxidants, and enzymes, as well as the production and accumulation of lignin, chitin, and phenolic compounds. Using silicon nanoparticles in *in vitro* cultures affects morphophysiological features such as increased biomass, higher photosynthetic efficiency, accelerated seed germination and flowering induction in various crop species [16].

The micropropagation process's success depends on the plants' ability to adapt to *ex vitro* conditions. The present study determined the percentage of rooted plants after 6 weeks of adaptation to *ex vitro* conditions (Figure 3).



(a) **(b)**

Figure 3. Percentage of plants rooted after 6 weeks of adaptation to *ex vitro* conditions: (a) *E. chamaecereus*, (b) *O. microdasys*.

Our study confirmed that adding 100 mg L^{-1} nSi stimulated the rhizogenesis of *E. chamaecereus* explants in 100% (Figure 3a). Moreover, the plants were more robust and better rooted, thus reducing labour requirements. It was further observed that the percentage of acclimatised plants decreased to 85% with increasing nSi concentration and was lower than that of the control. In addition, plants developed 14 to 25% more roots than the control. However, they were 10 to 40% shorter than the control (Table 3). The addition of chitosan, independent of its concentration, did not influence the rhizogenesis of *E. chamaecereus*. The percentage of rooted explants was at the same level, 70%. Additionally, they were shorter than the control by 9 to 28%. The opposite relationship was observed in *O. microdasys* (Figure 3b). Plants from the medium containing chitosan in higher concentrations (40 mg L^{-1} and 60 mg L^{-1}) were rooted in 85% corresponding to the control level. On the other hand, the number of roots and their length were higher than the control by 15 - 80% and 60 - 66%, respectively (Table 4). On the other hand, the lower the nSi concentration in the MS medium (100 mg L^{-1} and 200 mg L^{-1}), the fewer plants were rooted (50%). *O. microdasys* explants from the MS medium+500 mg L^{-1} were rooted in 67%. It was also observed that the higher the nSi concentration in the medium, the lower the length of roots and their number (Table 4).

Table 3. Effect of different medium composition on the number and root length of *E. chamaecereus* plants under *ex vitro* conditions after 6 weeks of adaptation.

Medium	**No. of roots**	**Roots length [mm]**
MS – control	2.9 ± 1.0^{a}	24.4 ± 9.9^{a}
MS+20 mg L^{-1} CH	2.9 ± 1.5^{a}	15.2 ± 5.1^{b}
MS+40 mg L^{-1} CH	3.3 ± 1.0^{a}	22.2 ± 9.2^{a}
MS+60 mg L^{-1} CH	3.7 ± 1.7^{a}	20.43 ± 10.2^{a}
MS+100 mg L^{-1} nSi	2.9 ± 1.1^{a}	17.6 ± 4.4^{ab}
MS+200 mg L^{-1} nSi	2.6 ± 1.0^{a}	21.9 ± 9.2^{a}
MS+500 mg L^{-1} nSi	1.9 ± 0.9^{a}	14.7 ± 3.3^{b}

Note. Means (± SD) followed by the same letter do not differ significantly at $p < 0.05$ according to Tukey multiple ranges.

Table 4. Effect of different medium composition on the number and root length of *O. microdasys* plants under *ex vitro* conditions after 6 weeks of adaptation.

Medium	**No. of roots**	**Roots length [mm]**
MS – control	1.2 ± 0.4^{a}	32.4 ± 13.0^{ab}
MS+20 mg L^{-1} CH	2.0 ± 0.9^{a}	52.0 ± 18.9^{a}
MS+40 mg L^{-1} CH	2.2 ± 1.3^{a}	52.2 ± 13.1^{a}
MS+60 mg L^{-1} CH	1.4 ± 1.0^{a}	53.8 ± 14.4^{a}
MS+100 mg L^{-1} nSi	2.5 ± 1.5^{a}	16.7 ± 10.0^{b}
MS+200 mg L^{-1} nSi	1.7 ± 1.0^{a}	25.0 ± 15.0^{b}
MS+500 mg L^{-1} nSi	1.6 ± 1.1^{a}	27.5 ± 11.1^{b}

Note. Means (± SD) followed by the same letter do not differ significantly at $p < 0.05$ according to Tukey multiple ranges.

Auxins are commonly added to the media to induce rhizogenesis. IBA (indole-3-butyric acid), IAA (3-indoleacetic acid), or NAA (1-naphthaleneacetic acid) are widely used to induce rhizogenesis in *Opuntia* species. In the case of *O. ficus-indica*, *O. robusta*, *O. amyclaea* and *Opuntia* spp., 100% rooting was recorded in the presence of low or medium amounts of IBA, IAA or NAA (0.5 - 2 mg L^{-1}) [2, 30, 31]. However, in the case of *O. ellisiana*, rhizogenesis could only be induced in the presence of high amounts of auxin (5 mg L^{-1} IBA) [32]. The plants rooted well, developed well in the soil, and adapted to greenhouse conditions. In the case of cacti, Sawsan *et al.* [33] obtained 100% rooted plants of *Cereus peruvianus* L. with the longest roots on full, half or quarter strength MS medium with or without 1 g L^{-1} activated carbon (AC). Lema-Rumińska and Kulus [3] described that rooting can sometimes occur spontaneously without the participation of PGR, which results from high levels of endogenous auxins. In addition, according to García-Rubio and Malda-Barrera [34], to increase the chances of survival of seedlings *ex vitro*, they can be additionally inoculated with mycorrhiza. The results obtained in this study may find practical applications in the large-scale commercial production of cactus seedlings. Using chitosan and nanosilicon reduces the need for synthetic growth regulators, promoting more eco-friendly and sustainable cultivation methods. Moreover, the developed micropropagation method could be important in protecting endangered cactus species by enabling their efficient reintroduction into natural habitats.

4. Conclusions

This study demonstrates that chitosan and nanosilicon are effective biostimulants in cactus micropropagation. Their impact is concentration- and species-dependent, necessitating careful optimisation. For *E. chamaecereus*, nanosilicon at 100 mg L^{-1} significantly enhanced rooting and improved survival in *ex vitro* conditions, while chitosan (40 mg L^{-1}) effectively stimulated shoot proliferation and biomass accumulation. In contrast, *O. microdasys* showed better overall shoot and root development with chitosan, although the addition of nanosilicon also influenced shoot growth and fresh mass. These outcomes underscore the importance of carefully selecting and optimising concentration levels for each biostimulant. The findings support the use of these natural compounds as sustainable alternatives to synthetic growth regulators, contributing to eco-friendly and large-scale cactus production.

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6. References

[1] Shedbalkar UU, Adki VS, Jadhav JP, Bapat VA; (2010) *Opuntia* and other cacti: applications and biotechnological insights. Trop Plant Biol 3(3), 136–150. **DOI:** 10.1007/s12042-010-9055-0

[2] Bouzroud S, El Maaiden E, Sobeh M, Devkota KP, Boukcim H, Kouisni L, El Kharrassi Y; (2022) Micropropagation of *Opuntia* and other cacti species through axillary shoot proliferation: A comprehensive review. Front Plant Sci 13, 926653. **DOI:** 10.3389/fpls.2022.926653

[3] Lema-Rumińska J, Kulus D; (2014) Micropropagation of cacti – a review. Haseltonia 19, 46–63. **DOI:** 10.2985/026.019.0107

[4] Ortega-Baes P, Sühring S, Sajama J, Sotola E, Alonso-Pedano M, Bravo S, Godínez-Alvarez H; (2010) Diversity and conservation in the cactus family. In: Ramawat KG (ed), Desert plants: Biology and Biotechnology. Springer, Berlin, Heidelberg, 157–173. **DOI:** 10.1007/978-3-642-02550-1_8

[5] Goettsch B, Hilton-Taylor C, Cruz-Piñón G, Duffy JP, Frances A, Hernández HM, Inger R, Pollock C, Schipper J, Superina M, Taylor NP; (2015) High proportion of cactus species threatened with extinction. Nat Plants 1(10), 15142. **DOI:** 10.1038/nplants.2015.142

[6] Coelho N, Romano A; (2022) Impact of chitosan on plant tissue culture: recent applications. Plant Cell Tissue Organ Cult 148, 1–13. **DOI:** 10.1007/s11240-021-02156-6

[7] Kruczek A, Krupa-Małkiewicz M, Ochmian I; (2021) Micropropagation, rooting, and acclimatization of two cultivars of goji (*Lycium chinense*). Not Bot Horti Agrobo 49(2), 12271. **DOI:** 10.15835/nbha49212271

[8] Krupa-Małkiewicz M, Jurkiewicz A, Ochmian I, Figiel-Kroczyńska M; (2022) Effect of chitosan and meta-topolin in micropropagation of *Vaccinium corymbosum*. Prog Chem Appl Chitin Deriv 27, 154–161. **DOI:** 10.15259/PCACD.27.011

[9] Faizan M, Rajput VD, Al-Khuraif AA, Arshad M, Minkina T, Sushkova S, Yu F; (2021) Effect of Foliar Fertigation of Chitosan Nanoparticles on Cadmium Accumulation and Toxicity in *Solanum lycopersicum*. Biology, 10(7), 666. **DOI:** 10.3390/biology10070666

[10] Badawy, MEI, Rabea, EI (2011) A Biopolymer Chitosan and Its Derivatives as Promising Antimicrobial Agents against Plant Pathogens and Their Applications in Crop Protection. Int J Carbohydr Chem, 2011(1), 460381. **DOI:** 10.1155/2011/460381

[11] Ahmed KBM, Khan MMA, Siddiqui H, Jahan A; (2020) Chitosan and its oligosaccharides, a promising option for sustainable crop production—a review. Carbohydr Polym 227, 115331. **DOI:** 10.1016/j.carbpol.2019.115331

[12] Lopez-Moya F, Escudero N, Zavala-Gonzalez EA, Esteve-Bruna D, Blázquez MA, Alabadí D, Lopez-Llorca LV; (2017) Induction of auxin biosynthesis and WOX5 repression mediate changes in root development in *Arabidopsis* exposed to chitosan. Sci Rep 7(1), 16813. **DOI:** 10.1038/s41598-017-16874-5

[13] Luyckx M, Hausman JF, Lutts S, Guerriero G; (2017) Silicon and plants: current knowledge and technological perspectives. Front Plant Sci 8, 411. **DOI:** 10.3389/fpls.2017.00411

[14] Boldt JK, Locke JC, Altland JE; (2018) Silicon accumulation and distribution in petunia and sunflower grown in a rice hull-amended substrate. HortSci 53(5), 698–703. **DOI:** 10.21273/HORTSCI12325-17

[15] Avestan S, Ghasemnezhad M, Esfahani M, Byrt CS; (2019) Application of nano-silicon dioxide improves salt stress tolerance in strawberry plants. Agronomy 9(5), 246. **DOI:** 10.3390/agronomy9050246

[16] Krupa-Małkiewicz M, Calomme M; (2021) Actisil application affects growth, flowering, and biochemical parameters in petunia *in vitro* and greenhouse. Plant Cell Tissue Org Cult 146(3), 449–459. **DOI:** 10.1007/s11240-021-02078-3

[17] Rastogi A, Tripathi DK, Yadav S, Chauhan DK, Živčák M, Ghorbanpour M, El-Sheery NI, Brestic M; (2019) Application of silicon nanoparticles in agriculture. 3 Biotech 9, 90. **DOI:** 10.1007/s13205-019-1626-7

[18] Hajizadeh HS, Asadi M, Zahedi SM, Hamzehpour N, Rasouli F, Helvacı M, Alas T; (2021) Silicon dioxide-nanoparticle nutrition mitigates salinity in gerbera by modulating ion accumulation and antioxidants. Folia Hortic 33, 91–105. **DOI:** 10.2478/fhort-2021-0007

[19] Prasad TNVKV, Sudhakar P, Sreenivasulu Y, Latha P, Munaswamy V, Reddy KR, Sreeprasad TS, Sajanlal PR, Pradeep T; (2012) Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. J Plant Nutr 35, 905–927. **DOI:** 10.1080/01904167.2012.663443

[20] Murashige T, Skoog F; (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15(3), 473–479. **DOI:** 10.1111/j.1399-3054.1962.tb08052.x

[21] Bartkowiak A; (2001) Binary polyelectrolyte microcapsules based on natural polysaccharides. Prace Nauk Politech Szczec 566. Wydawnictwo Politechniki Szczecińskiej, Szczecin.

[22] Sopalun K, Thammasiri K, Ishikawa K; (2010) Effects of chitosan as the growth stimulator for *Grammatophyllum speciosum in vitro* culture. Int J Biol Life Agric Sci 4(11), 828–830.

[23] Veraplakorn V, Kudan S; (2021) Chitosan elicitor stimulation of *in vitro* growth and *ex vitro* acclimatization of *Lantana camara* L. Agr Nat Resour 55, 431–439. **DOI:** 10.34044/j.anres.2021.55.3.13

[24] Ochmian I, Lachowicz S, Krupa-Małkiewicz M; (2022) The effect of different molecular weights of chitosan on the yield, quality, and health-promoting properties of strawberries. Prog Chem Appl Chitin Deriv 27, 194–203. **DOI:** 10.15259/PCACD.27.015

[25] Nge KL, Nwe N, Chandrkrachang S, Stevens WF; (2006) Chitosan as a growth stimulator in orchid tissue culture. Plant Sci 170(6), 1185–1190. **DOI:** 10.1016/j.plantsci.2006.02.006

[26] Krupa-Małkiewicz M, Fornal N; (2018) Application of chitosan *in vitro* to minimize adverse effects of salinity in *Petunia* × *atkinsana* D. Don. J Ecol Eng 19(1), 143–149. **DOI:** 10.12911/22998993/79410

[27] Obsuwan K, Yoodee S, Uthairatanakij A; (2010) Application of chitosan on *in vitro* growth of *Rhynchostylis gigantea* protocorms and seedlings. Acta Hortic 878, 283–288. **DOI:** 10.17660/ActaHortic.2010.878.35

[28] Avestan S, Naseri L, Barker AV; (2017) Evaluation of nanosilicon dioxide and chitosan on tissue culture of apple under agar-induced osmotic stress. J Plant Nutr 40(20), 2797–2807. **DOI:** 10.1080/01904167.2017.1382526

[29] Mani M, Faisal M, Alatar AA, Shekhawat MS; (2024) *In vitro* propagation and assessment of the impact of nano silicon on growth and structural developments in *Neomarica longifolia* (Link & Otto) Sprague (Yellow Iris). S Afr J Bot 171, 147–155. **DOI:** 10.1016/j.sajb.2024.05.046

[30] García-Saucedo PA, Valdez-Morales M, Valverde ME, Cruz-Hernández A, Paredes-López O; (2005) Plant regeneration of three *Opuntia genotypes* used as human food. Plant Cell Tissue Org Cult 80(2), 215–219. **DOI:** 10.1007/s11240-004-9158-0

[31] Zoghlami N, Bouamama B, Khammassi M, Ghorbel A; (2012) Genetic stability of long-term micropropagated *Opuntia ficus-indica* (L.) Mill. plantlets as assessed by

molecular tools: perspectives for *in vitro* conservation. Ind Crops Prod 36(1), 59–64. **DOI:** 10.1016/j.indcrop.2011.08.006

[32] Juárez MC, Passera CB; (2002) In vitro propagation of Opuntia ellisiana Griff. and acclimatization to field conditions. Biocell 26, 319–324. **DOI:** 10.32604/biocell.2002.26.319

[33] Sawsan SS, Abou-Dahab TA, Youssef EMA; (2005*) In vitro* propagation of cactus (*Cereus peruvianus* L.). Arab J Biotechnol 8(1), 169–176.

[34] García-Rubio OR, Malda-Barrera G; (2009) Conservación *in situ* y *ex situ* de *Mammillaria mathildae*, cactácea endémica en peligro de extinción de la ciudad de Querétaro. Ciencia Edición especial Premio Alejandrina a la Investigación en Ciencias Exactas y Ambientales 2, 3–16.