OPTIMISATION OF ROOTING AND GROWTH OF GRAPEVINE CUTTINGS THROUGH CHITOSAN APPLICATION AND SUBSTRATE SELECTION

Marcelina Krupa-Małkiewicz[1,a], Ireneusz Ochmian[2,b,*]

[1] – Department of Plant Genetics, Breeding and Biotechnology, West Pomeranian University of Technology in Szczecin, Słowackiego 17 Str., 71-434 Szczecin, Poland
[a] – ORCID: 0000-0002-4333-9122
[2] – Department of Horticulture, West Pomeranian University of Technology in Szczecin, Słowackiego 17 Str., 71-434 Szczecin, Poland
[b] – ORCID: 0000-0002-3606-1927
**corresponding author: iochmian@zut.edu.pl*

Abstract

This study evaluated the effects of chitosan concentration and substrate composition on rooting efficiency, growth performance, and leaf colour parameters in hardwood cuttings of Vitis vinifera L. cv. 'Solaris'. Cuttings were soaked in 0.2% or 0.4% chitosan solutions and planted in five substrate types: peat, sand, perlite, pea-sand (50:50), and peat-perlite (50:50). The highest rooting percentage (92%) and most favourable biometric parameters were observed in cuttings treated with 0.2% chitosan and grown in the peat-perlite mixture. The tallest shoots (77 cm) were recorded with 0.4% chitosan, while the longest roots (52 cm) developed in the sand-perlite substrate. Although chitosan did not significantly affect shoot diameter, it enhanced root system development and overall plant quality, particularly in substrates with high air and water permeability. In addition, leaf colour analysis revealed that chitosan application in the peat-perlite substrate significantly reduced L and a* values, indicating a deeper green foliage colour and enhanced chlorophyll accumulation. In contrast, sandy substrates showed minimal pigment response. These results confirm that chitosan acts as an effective biostimulant when applied in synergy with suitable substrates, and leaf colour metrics may serve as reliable indicators of physiological status during grapevine propagation.*

Keywords: *biostimulation, natural polysaccharides, plant regeneration, shoot growth, root induction, stress physiology*

Received: 07.03.2025
Accepted: 28.06.2025

1. Introduction

The common grapevine (*Vitis vinifera* L.) is among the most economically important fruit crops globally, cultivated extensively in temperate and subtropical regions [1]. Vegetative propagation through hardwood cuttings (canes) is a widely adopted method, allowing for the preservation of varietal traits and the rapid, large-scale production of planting material [2, 3]. Commercial viticulture favours this technique because of its simplicity, high success rate, and ability to maintain genetic fidelity.

Sustainable nursery practices increasingly require the use of environmentally responsible technologies that minimise the application of synthetic growth regulators and fungicides traditionally employed to stimulate rhizogenesis. Consequently, natural biostimulants are gaining prominence, though their efficacy remains subject to empirical validation [4, 5].

Chitosan, a natural biopolymer derived from the deacetylation of chitin, has attracted considerable interest as an eco-friendly growth stimulator, especially in relation to rooting processes [6]. Research has demonstrated that the application of chitosan via soaking or irrigation promotes callus formation, adventitious root initiation, and overall plant development. For instance, soaking cuttings in aqueous chitosan solutions (0.05 - 0.2%) enhanced rooting in *Freesia*, *Gladiolus*, and *in vitro*-propagated winegrape plants [7–9]. Moreover, chitosan irrigation has been shown to improve root development under abiotic stress conditions, likely due to its chelating capacity and biostimulant activity [10]. It is also known to stimulate biochemical pathways, including the synthesis of phytoalexins and activation of oxidative enzymes, thereby enhancing plant resilience and indirectly supporting rhizogenesis [11].

Rooting success in grapevine cuttings is influenced by both endogenous factors, such as tissue maturity and carbohydrate reserves, and exogenous factors, including substrate type, temperature, humidity, light conditions, and the application of growth stimulants [3, 12]. Of these, substrate selection is particularly critical, as it directly affects rooting efficiency and subsequent plant quality [13, 14]. An ideal rooting substrate provides adequate aeration and water retention, a neutral to slightly acidic pH, and should be free from pathogens [15]. Frequently used substrates include perlite, sand, peat, coconut fibre, compost, and organic manures [16, 17].

Haile [15] reported that a mixture of agricultural soil and sand (75:25, v/v) achieved the highest rooting rate (85.96%) and longest root systems, although shoots were comparatively weaker in substrates with high sand content. Similarly, Muhammad *et al.* [16] found that amending clay-based media with coconut peat and bagasse improved rooting percentage, leaf number, and shoot elongation. Tsipouridis *et al.* [18] demonstrated that a peat-perlite mixture (50:50) was superior to single-component substrates in enhancing root development. Ferrer *et al.* [19] confirmed that sand-soil mixtures facilitated better overall seedling growth, although excessive sand content negatively impacted root elongation.

In light of these findings, the present study aimed to assess the combined effects of substrate composition and chitosan application on the rooting efficiency and vegetative growth of hardwood cuttings of *Vitis vinifera* L. cv. 'Solaris'. We hypothesised that the use of low concentrations of chitosan (0.2 - 0.4%) in conjunction with aerated organic-mineral substrates, such as peat-perlite mixtures, would significantly improve rooting percentage, root system development, and overall shoot growth compared to untreated control cuttings or those cultivated in single-component substrates. This hypothesis rests on the premise that chitosan functions as a biostimulant by enhancing physiological responses and nutrient uptake, particularly when applied in growing media that offer optimal moisture and air balance.

2. Materials and Methods

2.1. Characteristics of the Research Area and Plant Material

The experiment was conducted in growth chambers at the Department of Horticulture and the Department of Plant Genetics, Breeding, and Biotechnology, West Pomeranian University of Technology in Szczecin. The study was carried out under controlled conditions during the early spring season.

The plant material comprised dormant, one-year-old hardwood cuttings of grapevine (*Vitis vinifera* L. cv. 'Solaris'), collected in February from healthy, productive vines. Shoots with a diameter of 7 - 10 mm and a length of 20 - 25 cm, containing two buds, were selected. Each cutting was trimmed with a straight top cut (2 cm above the upper bud) and a slanted basal cut (0.5 cm below the lower bud). For each treatment combination, 50 cuttings were prepared, yielding a total of 900 experimental units across all chitosan and substrate variants.

Cuttings were soaked for 24 hours at room temperature (22°C) in aqueous chitosan solutions at concentrations of 0.2% and 0.4%. The following substrate variants were used:

- low peat (pH 5.5 - 6.0),
- fine-grained quartz sand,
- horticultural perlite,
- peat-sand mixture (1:1, v/v),
- peat-perlite mixture (1:1, v/v).

All substrates were sterilised before use and supplemented with Osmocote Exact Standard 5 - 6 (5 g per litre of substrate). Each cutting was planted individually in a 1-litre polyethylene pot (20 cm in height) filled with the respective substrate.

The experiment was conducted under standardised environmental conditions:

- root zone temperature: 20 - 22°C,
- bud zone temperature: ~10°C,
- total darkness for the first three weeks to inhibit bud break and promote rhizogenesis.

Following the initial rooting phase, the cuttings were transferred to a lighted growing chamber and cultivated for an additional eight weeks on flood benches. During this period, chitosan was applied via irrigation (150 mL per pot) four times at fortnightly intervals.

2.2. Chitosan Preparation and Physicochemical Characterisation

Chitosan samples of varying molar masses were prepared by controlled radical degradation of high molecular weight chitosan (MW > 1200 kDa; Sigma-Aldrich). The process involved the gradual addition of hydrogen peroxide (0.8 - 6.4 mmol g^{-1} of polysaccharide) to a 2.5% chitosan solution (pH 3.5 - 4.0) at 80°C. The resulting chloride salt derivatives displayed similar polydispersity indices and a high degree of deacetylation (> 95%).

The molar masses of the degraded chitosan samples were determined using high-performance liquid chromatography/gel permeation chromatography (HPLC/GPC) with a SmartLine system (Knauer, Germany) consisting of an isocratic pump (Model 1000) and a refractive index detector (RI Detector 2300).

To prepare the experimental solution, 12,000 Da (12k) chitosan was dissolved in 300 g of distilled water and titrated to pH 7.7 with 1 M NaOH. The final volume was adjusted to 1,000 g by adding 500 g of 0.2 M acetic acid solution.

2.3. Measurements

The assessment of biometric parameters was carried out after the cultivation was completed. Biometric traits were assessed at the end of the cultivation period. Measurements were taken for all cuttings in each treatment group. The following parameters were evaluated:

- rooting percentage (%): the proportion of cuttings with a visible, well-developed root system, expressed as a percentage of the total number of cuttings per treatment,
- plant height (cm): measured from the substrate surface to the apex of the tallest shoot,
- shoot diameter (mm): measured with a digital calliper, 5 cm above the upper bud,
- root system length (cm): the length of the longest root, measured from its point of origin on the cutting to the tip.

Leaf colour was measured using a portable Konica Minolta CM-700d colourimeter. Readings were taken on three fully expanded leaves per cutting for each treatment combination (substrate × chitosan concentration). Measurements were recorded using the CIE L*a* colour space:

- L*, lightness (0 = black; 100 = white),
- a*, green-red axis (negative values = green; positive values = red).

The instrument was calibrated before each series using a standard white reference plate, and a dark chamber reading was taken to minimise background noise.

2.4. Statistical Analysis

Data were statistically analysed using Statistica software version 12.5 (StatSoft Polska, Kraków, Poland). One-way analysis of variance (ANOVA) was employed to evaluate treatment effects. Differences between means were determined using Tukey's least significant difference (LSD) test at a significance level of $p < 0.05$.

3. Results and Discussion

3.1. Percentage of Rooted Cuttings

The results demonstrated a significant influence of both substrate type and chitosan concentration on the rooting percentage of hardwood cuttings of *Vitis vinifera* L. cv. 'Solaris' (Figure 1). The highest rooting rate was recorded after applying 0.2% chitosan (79%), closely followed by 0.4% (77%). Both values were significantly higher than the control (69%), confirming the biostimulatory effect of chitosan on grapevine rooting. However, the difference between the two chitosan concentrations was not statistically significant, indicating that increasing the concentration above 0.2% did not provide additional benefits.

Substrate composition also had a pronounced effect. The highest rooting percentage was obtained in the peat-perlite mixture (87%), confirming the effectiveness of combining organic and mineral components with favourable air-water properties. These findings align with Jaleta and Sulaiman [3], who reported improved grapevine propagation when using substrates that integrate peat, perlite, or sand-materials known for their moisture retention and aeration capacity. High rooting rates were also achieved in the peat-sand mixture (77%) and in pure perlite (76%), further highlighting the beneficial role of peat due to its high organic matter content and water-holding capacity. By contrast, sand (64%) and the sand-perlite mixture (67%) yielded the lowest percentages of rooted cuttings, suggesting their limited suitability for grapevine propagation.

The highest overall rooting efficiency (92%) was recorded in cuttings treated with 0.2% chitosan and grown in the peat-perlite mixture, confirming that highly aerated organic-mineral substrates (50:50 v/v) offer optimal conditions for root initiation [3]. Conversely, the lowest efficiency (52%) was observed in untreated cuttings planted in the sand-perlite mixture.

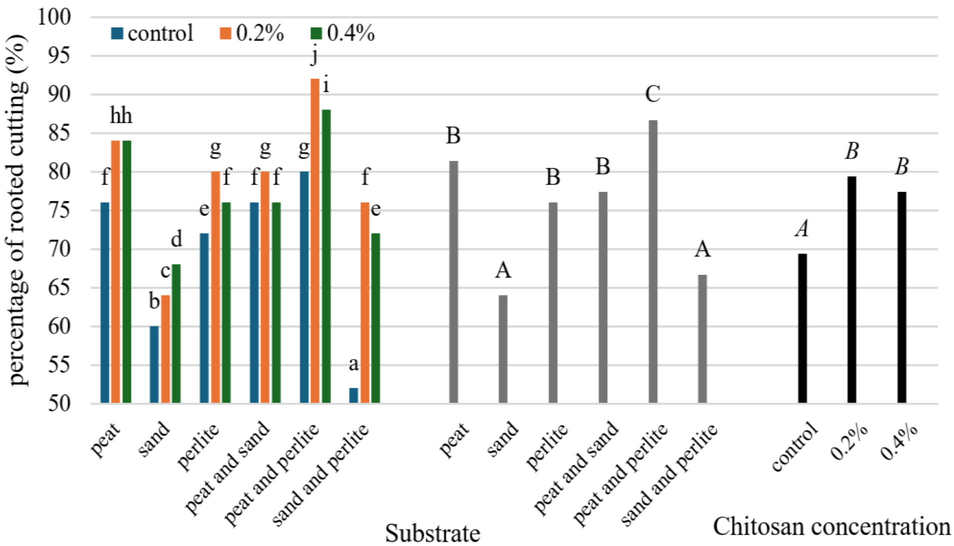

Figure 1. Effect of substrate type and chitosan concentration on the percentage of rooted hardwood grapevine cuttings (*Vitis vinifera* L. cv. 'Solaris') (*Note.* Mean values marked with the same lowercase letter do not differ significantly according to Tukey's test at $p \leq 0.05$, considering the interaction between chitosan concentration and substrate type (NIR = 4). CAPITAL LETTERS indicate significant differences between substrate types (main effect), while *ITALIC CAPITAL LETTERS* denote significant differences between chitosan concentrations (main effect).

These findings are consistent with those reported by Tsipouridis *et al.* [18] and Kawecki and Kozłowski [20], who emphasised the importance of substrate selection, identifying the best results in organic-mineral mixtures. Similarly, Dvin *et al.* [21] noted that the addition of fibres to sandy substrates improved both rooting efficiency and root system development. Although Ferrer *et al.* [19] reported high rooting percentages in pure sand, the resulting root systems were weak, limiting their value in commercial propagation. In contrast, soil-sand mixtures promoted more balanced growth and better cutting quality.

Taken together, these results and supporting literature underscore the critical role of substrate selection in the propagation of grapevine cuttings. Moreover, they confirm the potential of chitosan as an effective biostimulant, particularly when applied in synergy with substrates that support water retention and root aeration. This combination enhances rooting performance and improves nursery efficiency and plant quality.

3.2. Plant Height

The results demonstrated that both substrate composition and chitosan concentration influenced the height of *Vitis vinifera* L. cv. 'Solaris' hardwood cuttings (Table 1). Among the factors analysed, substrate type exerted the strongest effect, whereas the influence of chitosan concentration was less pronounced. The tallest plants were observed in

Table 1. Effect of substrate type and chitosan concentration on the quality of grapevine hardwood cuttings (*Vitis vinifera* L. cv. 'Solaris').

Chitosan concentration (B)	Substrate (A)						
	peat	sand	perlite	peat and sand	peat and perlite	sand and perlite	mean
	plant height [cm]						
control	57 ± 2.2^{de}	39 ± 2.9^{a}	47 ± 3.0^{bc}	52 ± 2.7^{cd}	68 ± 2.5^{g}	63 ± 2.0^{ef}	54^{A}
0.2%	55 ± 2.4^{d}	42 ± 2.0^{ab}	45 ± 2.5^{b}	50 ± 2.6^{c}	65 ± 3.2^{f}	66 ± 1.9^{fg}	54^{A}
0.4%	51 ± 1.9^{c}	45 ± 2.3^{b}	51 ± 2.4^{c}	54 ± 3.1^{d}	77 ± 3.4^{h}	60 ± 1.7^{e}	56^{A}
mean	54^{BC}	42^{A}	48^{AB}	52^{AB}	70^{D}	63^{CD}	
	shoot diameter [mm]						
control	7.5 ± 0.6^{cd}	6.3 ± 0.7^{ab}	6.6 ± 0.8^{b}	7.7 ± 0.6^{d}	10.5 ± 0.7^{f}	5.8 ± 0.4^{a}	7.4^{A}
0.2%	7.3 ± 0.5^{c}	6.5 ± 1.0^{b}	7.0 ± 0.2^{bc}	8.2 ± 0.5^{de}	11.3 ± 0.9^{g}	6.9 ± 0.5^{bc}	7.9^{A}
0.4%	7.6 ± 0.9^{d}	6.0 ± 0.8^{a}	7.1 ± 0.5^{c}	8.5 ± 0.5^{e}	10.9 ± 0.6^{fg}	7.5 ± 0.4^{cd}	7.9^{A}
mean	7.5^{BC}	6.3^{A}	6.9^{AB}	8.1^{C}	10.9^{D}	6.7^{AB}	
	root system length [cm]						
control	27 ± 4.3^{a}	41 ± 4.5^{cd}	43 ± 4.0^{de}	35 ± 2.4^{bc}	39 ± 3.1^{c}	47 ± 4.8^{fg}	39^{A}
0.2%	34 ± 3.7^{b}	49 ± 3.6^{g}	46 ± 3.3^{f}	34 ± 3.2^{b}	42 ± 3.3^{d}	52 ± 4.3^{h}	43^{A}
0.4%	35 ± 4.8^{bc}	45 ± 5.4^{ef}	49 ± 4.8^{g}	39 ± 2.9^{c}	44 ± 3.0^{e}	50 ± 4.5^{gh}	44^{A}
mean	32^{A}	45^{BC}	46^{C}	36^{AB}	42^{BC}	50^{C}	

Note. Mean values denoted by the same letter do not differ statistically significantly at $p < 0.05$ according to t-Tukey test. CAPITAL LETTERS indicate statistically significant differences between the main effects, while lowercase letters denote significant differences for the interactions between chitosan the peat-perlite mixture (mean height of 70 cm), which significantly outperformed all other substrate variants. High values were also recorded in the sand-perlite mixture (63 cm), while the shortest plants were found in the sand substrate (42 cm).

Regarding chitosan treatment, the greatest average plant height (56 cm) was obtained after applying 0.4% chitosan. However, the differences between chitosan concentrations (0.2% and 0.4%) and the control were not statistically significant, suggesting only a limited effect of chitosan on shoot elongation under the given conditions.

These findings are consistent with those reported by Jaleta and Sulaiman [3], who emphasised the pivotal role of substrate selection in promoting successful rooting and shoot growth in grapevine cuttings. They noted that the most favourable outcomes were achieved using mixtures of organic and mineral components, particularly peat, perlite, and sand, due to their capacity to maintain optimal air-water relations. Substrates enriched with organic matter are known to enhance moisture retention and root aeration, thereby stimulating root development and, indirectly, shoot growth.

The strong performance of the peat-perlite substrate observed in the present study further supports this view, highlighting the synergistic benefits of combining water-holding materials (peat) with highly porous ones (perlite). Although the sand-perlite mixture also resulted in relatively tall plants, the observed height was lower, likely due to reduced organic content and more limited nutrient availability.

Notably, the shortest plants were recorded in the sand-only substrate, corroborating previous reports. Jaleta and Sulaiman [3] pointed out that pure sand exhibits poor moisture retention, which constrains root development and diminishes shoot growth. Similar results were reported by Ferrer *et al.* [19], who noted that although high rooting percentages can be achieved in sand, the resulting plants often display weak shoot development, thereby reducing their suitability for commercial propagation.

3.3. Shoot Length and Diameter

The present study demonstrated that both substrate composition and chitosan concentration influenced the shoot diameter of hardwood cuttings of *Vitis vinifera* L. cv. 'Solaris'. The greatest mean shoot diameter (10.9 mm) was recorded in cuttings grown in the peat-perlite mixture, which was significantly higher than in any other substrate. The smallest diameters were observed in sand (6.3 mm) and the sand-perlite mixture (6.7 mm), indicating suboptimal conditions for shoot development in these media.

Regarding chitosan concentration, the highest mean shoot diameter (7.9 mm) was obtained following treatment with either 0.4% or 0.6% chitosan; however, the differences relative to the control (7.4 mm) were not statistically significant. This suggests that chitosan had only a limited direct effect on shoot thickness. Notably, the maximum individual shoot diameter (11.3 mm) was observed in cuttings treated with 0.6% chitosan and grown in the peat-perlite substrate, whereas the smallest value (5.8 mm) was recorded in control plants cultivated in the sand-perlite mixture.

These results are in agreement with findings from previous studies, which emphasise the central role of substrate composition in determining shoot morphology. Amir *et al.* [22] reported the highest shoot diameters in a sand-garden soil mixture (1:1), highlighting the advantages of combining mineral and organic materials to enhance nutrient and water availability. Similarly, the superior performance of the peat-perlite mixture in the present study is likely attributable to the synergistic effect of peat's moisture and nutrient retention capacity with the excellent aeration provided by perlite.

Conversely, the limited growth observed in pure sand reflects its poor water and nutrient retention capacity and negligible organic matter content. These characteristics restrict nutrient availability and root development, thereby constraining shoot growth.

Although chitosan application did not significantly affect shoot diameter across treatments, its combination with the optimal substrate (peat-perlite) resulted in the best growth parameters. This confirms the utility of chitosan as a biostimulant in nursery production, particularly when paired with substrates offering optimal physicochemical properties.

Notably, the same substrate (peat-perlite) also produced the tallest plants, reinforcing its suitability for grapevine propagation. In contrast, the lowest values for both shoot length and diameter were recorded in the sand substrate, underscoring its unsuitability for vigorous plant development. This suggests that environmental conditions that promote shoot elongation also favour radial growth, indicating the simultaneous enhancement of cell expansion and biomass accumulation.

The observed trends are consistent with previous studies on chitosan's biostimulatory effects. Luan *et al.* [23] demonstrated that chitosan application enhanced root length and root number in *Ficus* species. At the same time, Salachna and Bartkowiak [24] reported increased shoot length and leaf production in freesia following bulb soaking in chitosan. Żurawik [7] further confirmed that chitosan stimulates both shoot elongation and stem thickening, with benefits observed under both soaking and irrigation regimes. Similarly, Kruczek *et al.* [25] reported that the application of 20 ppm chitosan in substrate media significantly improved rooting efficiency and shoot development in goji plants (*Lycium chinense*), while Krupa-Małkiewicz and Ochmian [9] successfully employed 10 ppm chitosan for *in vitro* rooting of grapevine.

The enhanced shoot length and diameter recorded in this study suggest that chitosan contributes to improved nutrient and water utilisation and activates physiological mechanisms conducive to vegetative growth. Therefore, its integration with appropriately selected substrates represents a promising strategy for optimising the propagation of grapevine cuttings.

3.4. Root Length

The findings of the study revealed that both substrate type and chitosan concentration significantly influenced the root system length of hardwood grapevine cuttings of *Vitis vinifera* L. cv. 'Solaris'. The longest roots were observed in cuttings cultivated in the sand-perlite mixture (mean 50 cm), highlighting the favourable air-water properties of this substrate, which promote extensive root development. Comparable values were recorded in pure perlite (46 cm) and sand (45 cm), while the shortest roots occurred in the peat substrate (32 cm). Regarding chitosan concentration, the greatest mean root length (44 cm) was recorded following treatment with 0.4% chitosan; however, the differences compared to the control (39 cm) were not statistically significant.

The literature confirms that chitosan improves the morphological traits of grapevine cuttings, particularly shoot and root development. Górnik *et al.* [26] demonstrated that soaking cuttings in aqueous chitosan solutions (0.5 - 2%) for 24 hours increased shoot length, shoot number, and the number of internodes and adventitious roots, with 1% chitosan proving most effective. These effects were observed even under water deficit conditions, suggesting chitosan enhances stress tolerance. Additionally, treated plants exhibited increased leaf chlorophyll content, indicating improved photosynthetic capacity and overall plant condition.

Our findings reinforce the role of chitosan as a biostimulant not only for pigment synthesis but also for early-stage vegetative development under suboptimal conditions. Similar results were reported by Luan *et al.* [23], who noted improved root elongation in *Ficus microcarpa*, and by Salachna and Bartkowiak [24], who observed enhanced shoot growth and leaf production in freesia treated with chitosan. These effects suggest that chitosan influences physiological processes underpinning the development of vegetative organs.

Kukla and Żurawik [27] discovered that 0.4% chitosan significantly boosted root quantity and biomass in *Eucomis comosa* leaf cuttings. Soaking was more effective than foliar application or watering. They also found that chitosan with a lower molecular weight (7,000 Da) was more efficient than that with a higher molecular weight. Badizadegan *et al.* [28] found that treating *Zamioculcas zamiifolia* leaflet cuttings with 250 mg L^{-1} chitosan promoted roots, while greater concentrations resulted in tissue darkening, highlighting the importance of dose optimisation.

In more advanced applications, Korpayev *et al.* [29] reported enhanced rooting efficiency in *Malling Merton 106* apple rootstocks using chitosan-encapsulated auxins, achieving up to 91.7% rooting rates. Kara *et al.* [30] also demonstrated that encapsulated nano-silver and IBA increased shoot and root biomass in *Vitis vinifera* cv. 41B, supporting the synergistic effect of nanomaterials in plant propagation. Although chitosan was not tested in that study, its biostimulant role aligns with the trends observed.

El Amerany *et al.* [31] confirmed that both foliar and substrate-applied chitosan increased root length, dry biomass, and chlorophyll content in tomato seedlings, illustrating the systemic activity of chitosan across species and application methods. The results of the present study corroborate these findings, particularly in the context of mineral substrates with high permeability, such as perlite and sand-perlite mixtures, which provided the best rooting conditions when combined with chitosan.

The importance of substrate composition was also highlighted in studies by Schwab *et al.* [32] and Zamanidis *et al.* [33], who demonstrated that mixtures of organic and mineral materials (e.g., peat and perlite) improved moisture retention and aeration in the rhizosphere, enhancing both shoot and root development. Although the sand-perlite substrate was most effective in promoting root length in our study, it should be noted that peat-based mixtures supported more robust aerial growth, suggesting a trade-off between vegetative and root development depending on substrate composition.

Taken together, the results of this study support the hypothesis that chitosan, when applied in conjunction with appropriately selected substrates, can improve rooting performance in grapevine propagation. Although 0.4% chitosan yielded the longest roots, differences compared to the control group were not statistically significant, indicating that the choice of substrate may have a more pronounced effect than chitosan concentration alone.

Previous studies in *Vitis vinifera* [7–9] and other species [23–25] consistently show that chitosan stimulates root organogenesis, particularly when used at low concentrations or in nanoformulated forms. Nonetheless, responses to chitosan are often species- or cultivar-dependent and may vary with molecular weight, application method (soaking, irrigation, or foliar spraying), and environmental conditions. Further research is needed to elucidate these interactions and determine optimal nursery propagation protocols.

3.5. Leaf Colour Parameters

A significant interaction between chitosan concentration and substrate type was observed for both leaf colour parameters, lightness (L*) and greenness (a*), indicating that the

Table 2. Effect of chitosan concentration and substrate type on the leaf colour parameters of grapevine hardwood cuttings (*Vitis vinifera* L. cv. 'Solaris').

Chitosan concentration (B)	Substrate (A)						
	peat	**sand**	**perlite**	**peat and sand**	**peat and perlite**	**sand and perlite**	**mean**
				lightness L*			
control	44.4 ± 1.7^{cd}	48.5 ± 1.3^{g}	46.5 ± 1.9^{ef}	45.2 ± 1.3^{cde}	43.6 ± 1.8^{bc}	45.4 ± 1.5^{de}	45.6^{A}
0.2%	41.9 ± 2.1^{ab}	48.9 ± 1.7^{g}	45.7 ± 2.0^{de}	44.7 ± 1.5^{cd}	42.0 ± 2.1^{ab}	45.2 ± 1.4^{cde}	44.7^{A}
0.4%	43.5 ± 1.9^{bc}	48.1 ± 1.5^{fg}	45.1 ± 1.7^{cde}	44.3 ± 1.5^{cd}	41.0 ± 1.4^{a}	44.3 ± 1.2^{cd}	44.4^{A}
mean	43.3^{AB}	48.5^{C}	45.8^{BC}	44.7^{AB}	42.2^{A}	45.0^{AB}	
				green a*			
control	–34.1 ± 2.3^{de}	–28.4 ± 1.6^{c}	–32.5 ± 2.0^{d}	–36.9 ± 2.4$^{e\text{-}h}$	–39.3 ± 2.1^{hi}	–34.9 ± 2.0^{def}	–34.3^{A}
0.2%	–36.9 ± 1.7$^{e\text{-}h}$	–25.1 ± 1.1^{b}	–34.3 ± 2.4^{de}	–38.2 ± 1.8^{gh}	–41.9 ± 1.9^{ij}	–37.2 ± 2.7^{fgh}	–35.6^{A}
0.4%	–38.9 ± 2.1^{gh}	–22.0 ± 1.5^{a}	–36.4 ± 1.6^{efg}	–39.5 ± 2.0^{hi}	–44.1 ± 2.5^{j}	–39.4 ± 2.3^{hi}	–36.7^{A}
mean	–36.6^{B}	–25.1^{A}	–34.4^{B}	–38.2^{BC}	–41.7^{C}	–37.2^{B}	

Note. Mean values denoted by the same letter do not differ statistically significantly at $p < 0.05$ according to t-Tukey test. CAPITAL LETTERS indicate statistically significant differences between the main effects, while lowercase letters denote significant differences for the interactions between chitosan pigment response to chitosan was conditional upon the physicochemical properties of the substrate (Table 2).

The most substantial interaction effect was recorded in the peat-perlite substrate, which independently provided the most favourable rooting and growth conditions. In this medium, treatment with 0.4% chitosan led to the most pronounced darkening of the leaves (L* decreased from 43.6 to 41.0) and intensification of green pigmentation (a* decreased from –39.3 to –44.1). These pigment shifts were closely associated with superior vegetative performance, including the tallest shoots (77 cm), thickest stems (11.3 mm), and longest roots (52 cm), as presented in Table 1. Under such conditions, chitosan likely supported key physiological processes, including chlorophyll biosynthesis, which correlated with improved plant growth parameters.

In contrast, grapevine cuttings grown in sand, a substrate with negligible cation exchange capacity and poor water retention, showed no improvement in either L* or a* following the chitosan application. The leaves remained pale (L* ≈ 48) and relatively low in chlorophyll (a* ≈ –22 to –28), which corresponded with inferior biometric traits (e.g., shoot height ≈ 42 cm, rooting percentage of 64%). Intermediate substrates such as perlite, peat-sand, and sand-perlite exhibited moderate pigment enhancement following chitosan application, with reductions in L* of approximately 1 unit and more negative a* values by 2 - 3 units. These changes paralleled moderate improvements in shoot height and root development (Table 1), suggesting partial physiological responsiveness.

Comparable conclusions were drawn in *in vitro* studies on chitosan. Krupa-Małkiewicz and Ochmian [9] did not report distinct changes in grapevine leaf colour; however, the leaves remained intensely green, and L* differences did not exceed 2 units [34]. Leaves of goji and strawberry exhibited similar a* values, confirming the naturally intense green pigmentation typical of these species. Foliar application of chitosan under water stress conditions improved photosynthetic parameters, enhanced chlorophyll content (SPAD index), and increased sugar and anthocyanin levels [35]. This supports its ability to stimulate both primary and secondary metabolism, directly influencing leaf pigmentation. Additionally, the activation of antioxidant enzymes such as SOD and CAT, along with the reduction of H_2O_2 and MDA, highlights chitosan's protective properties under oxidative stress [36]. At the molecular level, chitosan has been shown to induce the expression of genes involved in anthocyanin and phenolic biosynthesis, including PAL, CHS, and UFGT, as demonstrated in the 'Tinto Cão' grapevine cultivar [37]. This may partly explain the enhanced green pigmentation observed in the present study.

In research on pigment stabilisation in bamboo and wine, chitosan was shown to protect chlorophyll and anthocyanins by forming stable complexes with metals (e.g., Cu^{2+}) and phenolic compounds [38, 39]. These physicochemical properties may also play a role in the preservation of leaf colour. Chitosan acts on multiple levels. It improves plant physiology, protects pigments, and activates defence mechanisms. Its effectiveness, however, is environmentally conditional and should therefore be carefully considered when applied in horticultural and viticultural practice.

4. Conclusions

Chitosan concentration statistically significantly affected rooting efficiency and root system development in hardwood cuttings of *Vitis vinifera* L. cv. 'Solaris'. The most favourable outcomes were obtained with a 0.2% solution, which enhanced both the percentage of rooted cuttings and root length. Higher concentrations (0.4% and 0.6%) did not yield further benefits, suggesting that increasing the dosage beyond this level is not economically justified.

Substrate type played a crucial role in determining rooting success and subsequent plant development. The peat-perlite mixture (50:50) was the most effective, providing optimal water retention and aeration. In contrast, single-component substrates such as sand and peat produced the weakest results, underscoring their limited suitability for vegetative propagation.

A significant interaction between chitosan concentration and substrate composition was observed, with the highest rooting percentage (92%) and best overall plant performance recorded in cuttings treated with 0.2% chitosan and grown in the peat-perlite substrate. This confirms that the efficacy of biostimulants such as chitosan is strongly influenced by the physicochemical properties of the growth medium.

In addition to its effects on rooting and shoot development, chitosan application significantly impacted leaf pigmentation. The most intense darkening (lower L*) and greening (more negative a* values) were recorded in the pea-perlite substrate following 0.4% chitosan treatment. These changes correlated with improved chlorophyll biosynthesis and better physiological condition and were consistent with other enhanced growth parameters. In contrast, minimal pigment changes in sandy substrates reflected lower physiological activity and poorer plant performance.

Leaf colour parameters, particularly L* and a*, emerged as reliable, non-invasive indicators of physiological condition, photosynthetic efficiency, and rooting success, and may serve as valuable diagnostic tools in nursery management.

The findings of this study support the use of natural biostimulants such as chitosan, particularly in combination with carefully selected substrate mixtures, to enhance the efficiency of grapevine propagation and improve the quality of nursery material destined for commercial cultivation.

5. References

[1] Tiefenbacher JP, Townsend C; (2020) The Semiofoodscape of Wine: The Changing Global Landscape of Wine Culture and the Language of Making, Selling, and Drinking Wine. In: Brunn SD, Kehrein R (eds), Handbook of the Changing World Language Map. Springer International Publishing, Cham, 4103–4145. **DOI:** 10.1007/978-3-030-02438-3_213

[2] Rao K; (2004) Studies on the propagation of grape rootstocks through hardwood and softwood cuttings. MSc Thesis. Rajendranagar, Hyderabad, 1–97.

[3] Jaleta A, Sulaiman M; (2019) A Review on the Effect of Rooting Media on Rooting and Growth of Cutting Propagated Grape (*Vitis vinifera* L). WJASS 3(4). **DOI:** 10.33552/WJASS.2019.03.000567

[4] Pacholczak A, Szydło W, Jacygrad E, Federowicz M; (2012) Effect of auxins and the biostimulator AlgaminoPlant on rhizogenesis in stem cuttings of two dogwood cultivars (*Cornus alba* ‘Aurea’ and ‘Elegantissima’). Acta Sci Pol Hortorum Cultus 11(2), 93–103.

[5] Świerczyński S, Stachowiak A, Golcz-Polaszewska M; (2017) The influence of treatments applied to common ninebark (*Physocarpus opulifolius* (L.) Maxim.) ‘Diabolo’ cuttings on their rooting and growth. Nauka Przyr Technol 11(3). **DOI:** 10.17306/J.NPT.00184

[6] Krupa-Małkiewicz M, Ochmian I; (2024) Comparison of the effectiveness of chitosan and auxin on *in vitro* and *ex vitro* rooting of *Vaccinium corymbosum* L. Prog Chem Appl Chitin Deriv 29, 156–165. **DOI:** 10.15259/PCACD.29.011

[7] Żurawik P; (2013) Wpływ suszu krewetkowego i chitozanu oraz metod uprawy na wzrost, rozwój, wartość dekoracyjną i plon bulw potomnych frezji (*Freesia* Eckl. ex Klatt). Wydawnictwo Uczelniane Zachodniopomorskiego Uniwersytetu Technologicznego, Szczecin; 1–128. ISBN 978-83-7663-155-4.

[8] Ramos-García M, Ortega-Centeno S, Hernández-Lauzardo AN, Alia-Tejacal I, Bosquez-Molina E, Bautista-Baños S; (2009) Response of Gladiolus (*Gladiolus* spp.) Plants after Exposure Corms to Chitosan and Hot Water Treatments. Sci Hortic 121(4), 480–484. **DOI:** 10.1016/j.scienta.2009.03.002

[9] Krupa-Małkiewicz M, Ochmian I; (2023) Chitosan and meta-topolin supplementation of the multiplication media of *Vitis vinifera* L. Prog Chem Appl Chitin Deriv 28, 46–55. **DOI:** 10.15259/PCACD.28.005

[10] Sheikha SAA, Al-Malki FM; (2015) Chitosan influence on the amino acids and proline content in the plants under drought stress. J Plant Prod 6(4), 447–455.

[11] 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

[12] Patil VN, Chauhan PS, Shivankar RS, Vilhekar SH, Waghmare VS; (2001) Effect of plant growth regulators on survival and vegetative growth of grapevine cuttings. Agric Sci Digest 21(2), 97–99.

[13] Kumar S, Prakash S, Malik S, Dhyani BP; (2021) Effect of different rooting media treatments on rooting and growth of stem cuttings in pomegranate (*Punica granatum* L.). Pharm Innov J 10(3), 475–479.

[14] Riaz A, Arshad M, Younis A, Raza A, Hameed MA; (2008) Effects of different growing media on growth and flowering of *Zinnia elegans* cv. Blue point. Pak J Bot 40(4), 1579–1585.

[15] Haile A; (2017) Effect of cane length and rooting media on rooting and shoot growth of grape (*Vitis vinifera* L.) stem cuttings at Raya Valley, Northern Ethiopia. MSc Thesis. Hawassa University College of Agriculture, Hawassa.

[16] Muhammad F, Kakar K, Golly MK, Ilyas N, Zib B; (2018) Comparative effect of potting media on sprouting and seedling growth of grape cuttings. Int J Environ Agric Res 4, 2454–1850.

[17] Chaudhari BB, Bhatt D, Chawla SL, Patel MA, Bennurmath P; (2018) Effect of rooting hormone and media on root induction in poinsettia (*Euphorbia pulcherrima* Willd.). J Ornamental Hortic 21(1–2), 7–12. **DOI:** 10.5958/2249-880X.2018.00002.6

[18] Tsipouridis C, Thomidis T, Michailides Z; (2005) Factors influencing the rooting of peach GF677 (peach × almond hybrid) hardwood cuttings in a growth chamber. N Z J Crop Hortic Sci 33(2), 93–98. **DOI:** 10.1080/01140671.2005.9514336

[19] Ferrer M, Guarinoni A, Camussi G; (1991) Effect of the rooting media and date of cuttings extraction on the quantity and quality of rooting in the grape rootstock SO4 (*Vitis riparia* × *Vitis berlandieri*) and determination of a critical phenological stage. Bol Investig Fac Agron Univ Repub 31, 1–11.

[20] Kawecki Z, Kozlowski WM; (1995) Effect of different organic substrates on rooting of one-bud hardwood grapevine cuttings. Acta Acad Agric Tech Olstenensis 60, 99–106.

[21] Dvin SR, Moghadam EG, Kiani M; (2011) Rooting Response of Hardwood Cuttings of MM111 Apple Clonal Rootstock to Indolebutyric Acid and Rooting Media. Asian J Appl Sci 4(4), 453–458. **DOI:** 10.3923/ajaps.2011.453.458

[22] Amir M, Hussain S, Martin S, Latif MA, Basit A, Ahmad N, Khan F, Ullah W, Ullah R, Abbas M; (2023) Different media effect on various cultivars of grapes (*Vitis vinifera*) cuttings. J Xi'an Shiyou Univ Nat Sci Ed 19(10), 468–484.

[23] Luan LQ, Ha VTT, Nagasawa N, Kume T, Yoshii F, Nakanishi TM; (2005) Biological Effect of Irradiated Chitosan on Plants *in Vitro*. Biotechnol Appl Biochem 41(1), 49–57. **DOI:** 10.1042/BA20030219

[24] Salachna P, Bartkowiak A; (2008) Wpływ miejsca uprawy i chitozanu o różnym ciężarze cząsteczkowym na wzrost i plonowanie frezji odmiany 'Lisa'. Cz. I. Cechy morfologiczne i kwitnienie. Zesz Probl Postęp Nauk Rol 525, 367–374.

[25] 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

[26] Górnik K, Grzesik M, Romanowska-Duda B; (2008) The effect of chitosan on rooting of grapevine cuttings and on subsequent plant growth under drought and temperature stress. J Fruit Orn Plant Res 16, 333–343.

[27] Kukla P, Żurawik P; (2022) Physiological status, rooting and bulb yield of leaf cuttings of *Eucomis comosa* (Houtt.) H.R. Wehrh. 'Sparkling Burgundy' as affected by chitosan. Hort Sci 49(3), 154–163. **DOI:** 10.17221/67/2021-HORTSCI

[28] Badizadegan F, Solgi M, Taghizadeh M, Abbasifar A; (2023) Effect of Chitosan on Propagation of Zamiifolia as Tropical Ornamental Indoor Plant by Leaf Cutting. Ornam Hortic 29(2), 278–285. **DOI:** 10.1590/2447-536X.v29i2.2626

[29] Korpayev S, Karakeçili A, Dumanoğlu H, Ibrahim Ahmed Osman S; (2021) Chitosan and Silver Nanoparticles Are Attractive Auxin Carriers: A Comparative Study on the Adventitious Rooting of Microcuttings in Apple Rootstocks. Biotechnol J 16(8), 2100046. **DOI:** 10.1002/biot.202100046

[30] Kara Z, Doğan O, Yazar K; (2023) Promoting Rooting and Vegetative Growth in 41 B Vine Rootstock Cuttings with Encapsulated Nano Silver and IBA Applications. Vis 3. **DOI:** 10.52001/vis.2023.20.57.63

[31] El Amerany F, Meddich A, Wahbi S, Porzel A, Taourirte M, Rhazi M, Hause B; (2020) Foliar Application of Chitosan Increases Tomato Growth and Influences Mycorrhization and Expression of Endochitinase-Encoding Genes. Int J Mol Sci 21(2), 535. **DOI:** 10.3390/ijms21020535

[32] Schwab JD, Williams KA, Griffin JJ; (2021) Asexual propagation by stem cuttings of half-high and low-bush blueberries in soilless substrates. J Environ Hortic 39(2), 47–52.

[33] Zamanidis P, Paschalidis C, Maltabar L, Vasiliadis S; (2013) Effect of the substrates on the production of engrafted vine cuttings in heated greenhouses. Commun Soil Sci Plant Anal 44(16), 2488–2495. **DOI:** 10.1080/00103624.2013.803571

[34] Kusuma HS, Jaya DEC, Illiyanasafa N; (2024) Effect of Chitosan Coating on Basil (*Ocimum sanctum*) Leaves Dried by Microwave-Assisted Drying Method: Analysis of Color, Effective Moisture Diffusivity, and Drying Kinetics. Int J Biol Macromol 273, 133000. **DOI:** 10.1016/j.ijbiomac.2024.133000

[35] Khalil HA, Badr Eldin RM; (2021) Chitosan Improves Morphological and Physiological Attributes of Grapevines Under Deficit Irrigation Conditions. J Hortic Res 29(1), 9–22. **DOI:** 10.2478/johr-2021-0003

[36] Farouk S, Ramadan AA, Showler AT; (2013) Chitosan effects on physiochemical indicators of drought-induced leaf stress in cowpea. Plant Knowledge J 2(4), 135–144.

[37] Singh RK, Martins V, Soares B, Castro I, Falco V; (2020) Chitosan application in vineyards (*Vitis vinifera* L. cv. Tinto Cão) induces accumulation of anthocyanins and other phenolics in berries, mediated by modifications in the transcription of secondary metabolism genes. Int J Mol Sci 21(1), 306. **DOI:** 10.3390/ijms21010306

[38] Enyinwa PC, Ren X, Zou C, Han S, Zhang Y, Wu X, Sun F, Wang H; (2023) Novel Formulated Chitosan Copper Complex as a Green Color Protection Agent of Moso Bamboo and Its Reaction Characteristics on Bamboo Epidermis. Ind Crops Prod 195, 116391. **DOI:** 10.1016/j.indcrop.2023.116391

[39] Artem V, Antoce AO, Geana EI, Ranca A; (2023) Effect of Foliar Treatment with Chitosan on Phenolic Composition of ˈFetească Neagră' Grapes and Wines. Not Bot Horti Agrobo 51(1), 12920. **DOI:** 10.15835/nbha51112920