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Impact of plant-derived melanin on *in vitro* rooting efficiency and biochemical characteristics of grapevines

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Submission Date: August 19th, 2025; **Acceptance Date:** October 8th, 2025; **Publication Date:** October 15th, 2025

Please cite this article as: Melyan G., Mikaelyan A., Dangyan K., Galstyan M., Mkrtchyan A., Asatryan N., Martirosyan Y., Aghajanyan A. Impact of plant-derived melanin on in vitro rooting efficiency and biochemical characteristics of grapevines. *Bioactive Compounds in Health and* Disease 2025; 8(10): 386 – 397. DOI: https://doi.org/10.31989/bchd.v8i10.1750

ABSTRACT

Background: Grapevine (*Vitis vinifera* L.) is a globally important crop, and effective *in vitro* propagation is essential for producing healthy, uniform plants. However, optimizing rooting and growth remains a challenge. Plant-derived melanin, known for its antioxidant and auxin-like properties, has shown potential in promoting plant development.

Objectives: This study aimed to assess the impact of plant-derived melanin on *in vitro* rooting efficiency and key biochemical characteristics—such as chlorophyll content, total soluble sugars, and vitamin C levels—in two grapevine cultivars (*'Deghin Yerevani'* and *'Parvana'*). The study also explored the potential of melanin to enhance the nutritional profile of grapevine leaves, which may contribute to the health-promoting properties of grape-derived products.

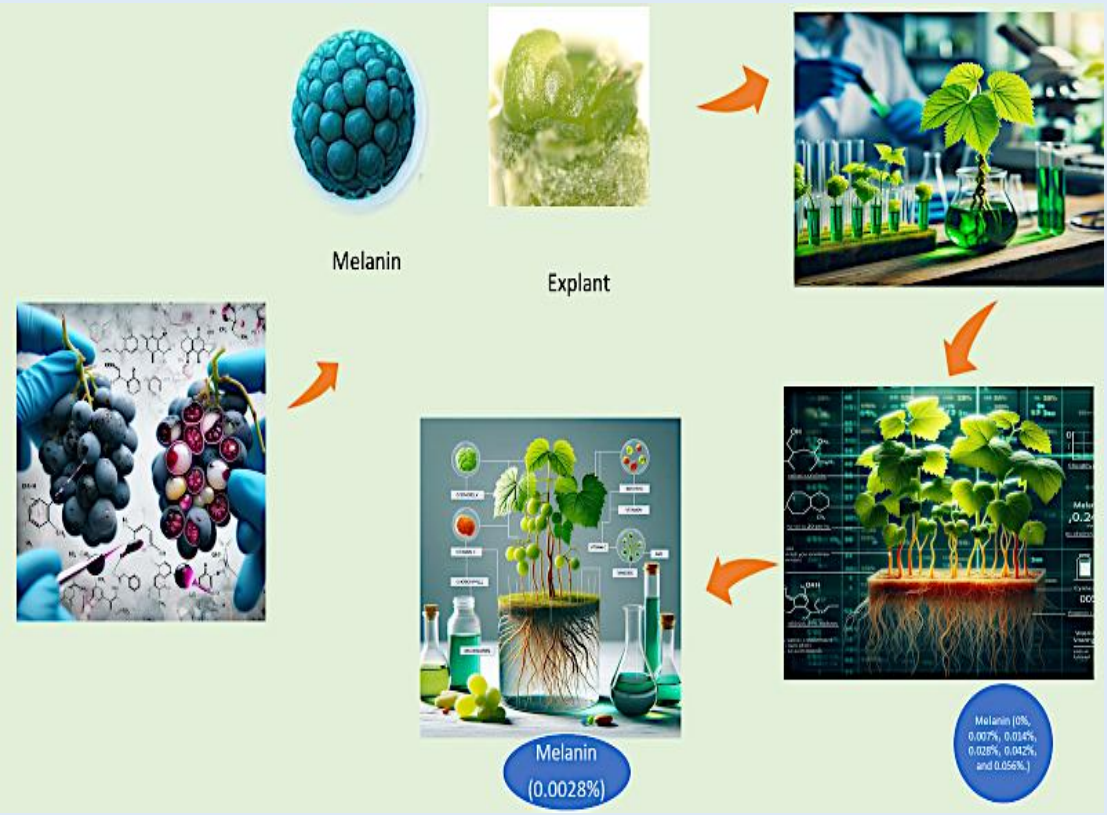
Materials and Methods: Virus-free grapevine microcuttings were cultured on Murashige and Skoog (MS) medium supplemented with melanin at concentrations of 0.007%, 0.014%, 0.028%, 0.042%, and 0.056%. The control group received no melanin. After 30 days, rooting parameters (root number and root length) were assessed. The regenerated plants were then transferred to an aeroponic system for further growth. Biochemical analyses included measurements

of chlorophyll, total soluble sugars, and vitamin C content in the leaves. All experiments were conducted in triplicate, and data were expressed as mean ± standard deviation (SD). Statistical comparisons were made using Student's *t*-test ($P < 0.05$), and a two-way analysis of variance (ANOVA) without replication was performed to assess the effects of cultivar and melanin concentration on rooting efficiency.

Results: Melanin enhanced rooting and improved the biochemical profile of grapevine microcuttings in a dose-dependent manner. The 0.028% melanin concentration yielded the highest rooting success and significantly increased chlorophyll, sugar, and vitamin C levels. Higher concentrations (0.042% and 0.056%) did not result in statistically significant improvements ($p > 0.05$).

Conclusion: Plant-derived melanin at an optimal concentration of 0.028% significantly improved rooting efficiency and enhanced essential biochemical traits, including chlorophyll, sugar, and vitamin C content in grapevine microcuttings. These improvements suggest that melanin can serve as a sustainable, natural alternative to synthetic growth regulators in micropropagation systems. Furthermore, the enrichment of bioactive compounds in grapevine leaves indicates a possible link between melanin application and the nutritional quality of grape-derived foods, offering promising implications for both sustainable agriculture and human health.

Keywords: melanin, *in vitro*, virus-free, grapevine, rooting, biochemical composition



Graphical Abstract: Boosting Rooting and Biochemical Composition in Grapevines with Melanin

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INTRODUCTION

Grapevine (*Vitis vinifera* L.) propagation is essential for the commercial production of both wine and table grapes. Conventional methods such as cuttings and grafting are widely practiced but are often slow and associated with a high risk of disease transmission. In contrast, *in vitro* culture techniques offer a rapid, disease-free alternative for generating high-quality planting material and conserving valuable grapevine germplasm [1–4]. These modern biotechnological approaches are increasingly vital for sustainable viticulture and enhancing vineyard productivity.

Beyond their agricultural and economic value, grapes are also well known for their nutritional and health-promoting properties. They are rich in vitamins, essential minerals, and a wide range of bioactive compounds—particularly polyphenols and flavonoids—that contribute to antioxidant activity and reduce inflammation [5–6]. Notably, the byproducts of grape processing, such as pomace, leaves, and seeds, are increasingly used in the development of functional foods due to their high content of bioactive phytochemicals [7–8].

Natural bioactive compounds—whether nutritive or non-nutritive—function as primary and secondary metabolites and play crucial roles in maintaining health and preventing disease. Their use in plant biotechnology and functional food production is growing, owing to their therapeutic potential, biocompatibility, and structural diversity [9–14].

In plant tissue culture, synthetic auxins such as indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA) are commonly used to stimulate root formation [15–18]. However, recent research has identified plant-derived melanin as a promising natural biostimulant. Owing to its antioxidant properties and auxin-like physiological activity, melanin has the potential to promote root development, enhance overall plant growth, and increase resilience to environmental stress. As a naturally occurring pigment found across various biological kingdoms, melanin plays a protective role by shielding cells from ultraviolet radiation, oxidative damage, and other stressors [19–24]. Although the use of melanin in plant systems remains underexplored, interest in its potential to improve abiotic stress tolerance and plant performance is growing rapidly.

Melanin is also recognized as a biodegradable, multifunctional biomaterial with diverse applications in agriculture, biotechnology, and medicine [25–27]. In this study, melanin was extracted from grapevine waste—a byproduct of winemaking—using a simple, energy-efficient caustic soda method. This waste material, often discarded or composted, is rich in phenolic compounds and melanin, offering an accessible and sustainable source of natural plant biostimulants.

Authors [28–30] have described the application of water-soluble melanin as a biostimulant, most often derived from Bacillus thuringiensis strains. Nevertheless, when cultivated in complex nutrient media, this microorganism produces both melanin and insecticidal toxins. Isolating melanin from such fermentation broths requires a lengthy, multistep, and labor-intensive procedure. In addition, the possible presence of insecticidal toxins in the culture medium raises concerns about their transfer and accumulation in the edible tissues of treated plants.

By contrast, melanin derived from grape processing waste can be obtained through a much simpler process involving only three to four technological steps. This plant-based melanin is comparable to microbial melanin [31] in terms of physicochemical properties and biological activity, but it is free from toxic compounds. These advantages underscore the sustainability, safety, and practical relevance of developing biostimulants from plant-derived raw materials.

Previous studies have demonstrated that melanin can stimulate plant growth, promote root development, and enhance tolerance to abiotic stresses, positioning it as a viable natural alternative to synthetic agrochemicals [28–32]. The present study aims to evaluate the potential of grape-derived melanin as a sustainable, plant-based substitute for synthetic auxins in grapevine

micropropagation. In addition to supporting eco-friendly and efficient propagation practices, this approach may also enhance the biochemical quality of grapevine leaves—such as their vitamin C and chlorophyll content—which are increasingly recognized for their health-promoting properties. By enriching grape-derived products with beneficial bioactive compounds, melanin-based biostimulants may contribute not only to plant health and agricultural sustainability but also to the development of functional foods with potential benefits for human health.

MATERIALS AND METHODS

Study Location: The study was conducted from 2022 to 2024 at the Tissue Culture Laboratory of the Scientific Center of Agrobiotechnology.

Plant Material: The research focused on two grapevine cultivars (*Vitis vinifera* L.): *'Deghin Yerevani'* and *Parvana'*.

- *'Deghin Yerevani'*: An indigenous Armenian seedless grape variety, widely cultivated in Armenia's grape-growing regions.
- *'Parvana'*: A medium-ripening seedless variety, developed from a cross between 'Katta *Kurgan*' and '*Kishmish Khishrau*', predominantly grown in the Armavir region.

Shoot apical meristems were obtained from the National Grape Field Collection (40.157419° N, 44.291986° E). Virus-free microcuttings were cultured *in vitro* on Murashige and Skoog (MS) medium supplemented with plant-derived melanin at concentrations of 0% (control), 0.007%, 0.014%, 0.028%, 0.042%, and 0.056%.

Cultures were maintained under controlled conditions: 25 ± 1 °C, 70% relative humidity, and a 16-hour photoperiod. The experiment included 15 microcuttings per treatment, with four independent repetitions to ensure reproducibility. Rooting efficiency was evaluated after 30 days of cultivation by measuring the number of roots per shoot in both cultivars.

Aeroponic System Setup: Rooted plantlets were transferred to a closed aeroponic cultivation system for acclimatization and further development. In this system, plant roots were suspended in air and periodically misted with a nutrient solution via high-pressure nozzles. The system operated under controlled environmental conditions (25 ± 1 °C, 70% relative humidity, 16-hour photoperiod). Plants remained in the aeroponic system for 30 days. This method ensured optimal root aeration and nutrient uptake, supporting enhanced shoot elongation, root branching, and overall plant vigor.

Leaves from aeroponically grown plants were harvested for biochemical analyses, including chlorophyll content, total soluble sugars, and ascorbic acid levels.

Melanin Extraction: Water-soluble melanin was extracted from black grape pomace, a winemaking byproduct, using a modified alkaline extraction method:

1. Pomace was thoroughly washed, air-dried, and treated with 0.4 M NaOH at 60 °C for 2.5 hours (solid-to-liquid ratio: 1:14).
2. The extract was centrifuged at 5000 × g for 30 minutes to separate the melanin-rich supernatant from the insoluble residue.
3. The pH of the supernatant was adjusted to stabilize the melanin for subsequent plant treatments and analyses.

Characterization of the Melanin Extract: The chemical composition of the obtained melanin extract was as follows: melanin content, 18–21 g/L; mineral ions, 5.8–6.4 g/L; pH, 8.0–8.2; polyphenols, 21–23 g/L; flavonoids, 0.9–1.7 g/L.

Biochemical Analyses

Chlorophyll Content: Chlorophyll content in fresh leaf samples was measured spectrophotometrically using the method proposed by Lichtenthaler (1987) [33]. Leaf tissue (0.1 g) was homogenized in 80% acetone and centrifuged to obtain a clear extract. The absorbance of

the supernatant was measured at 663 nm and 645 nm to determine the concentrations of chlorophyll a, chlorophyll b, and total chlorophyll. Calculations were based on Lichtenthaler's extinction coefficients and expressed as milligrams per gram of fresh weight (mg/g FW).

Ascorbic Acid (Vitamin C) Content: Ascorbic acid content was determined via iodine titration, following the AOAC official method (2000) [34]. A known volume of plant extract was titrated with standardized iodine solution until a persistent light pink color indicated the endpoint. Results were reported in milligrams per 100 grams of fresh weight (mg/100 g FW).

Total Soluble Sugar Content: Total soluble sugars were quantified using the modified phenol-sulfuric acid method described by Melgarejo et al. (2000) [35]. Fresh tissue samples were homogenized in distilled water, and the extract was treated with phenol and sulfuric acid, producing a yellow-orange complex. Absorbance was measured at 490 nm. A glucose standard curve was used to calculate sugar concentrations, expressed as milligrams of glucose equivalent per gram of fresh weight (mg/g FW).

Statistical Analysis: All experiments were performed in triplicate. Results are presented as the mean ± standard deviation (SD). To compare data between treatment groups, Student's t-test was used, with differences considered statistically significant at $P < 0.05$.

Additionally, a two-way analysis of variance (ANOVA) without replication was conducted in Microsoft Excel to evaluate the influence of two factors—grapevine cultivar (*'Deghin Yerevani'* and *'Parvana'*) and melanin concentration—on rooting efficiency. The ANOVA results included the sources of variation, calculated F-values, and corresponding P-values. Statistical significance was determined at the 5% probability level ($P < 0.05$).

RESULTS

The effects of varying melanin concentrations on rooting percentage, root number per shoot, and root length were assessed in two grapevine cultivars, *'Deghin Yerevani'* and *'Parvana'*. Figures 1 and 2 show the rooting percentages for each variety across the different melanin concentrations. Table 1 summarizes the impact of these treatments on root development, highlighting significant differences among the tested concentrations.

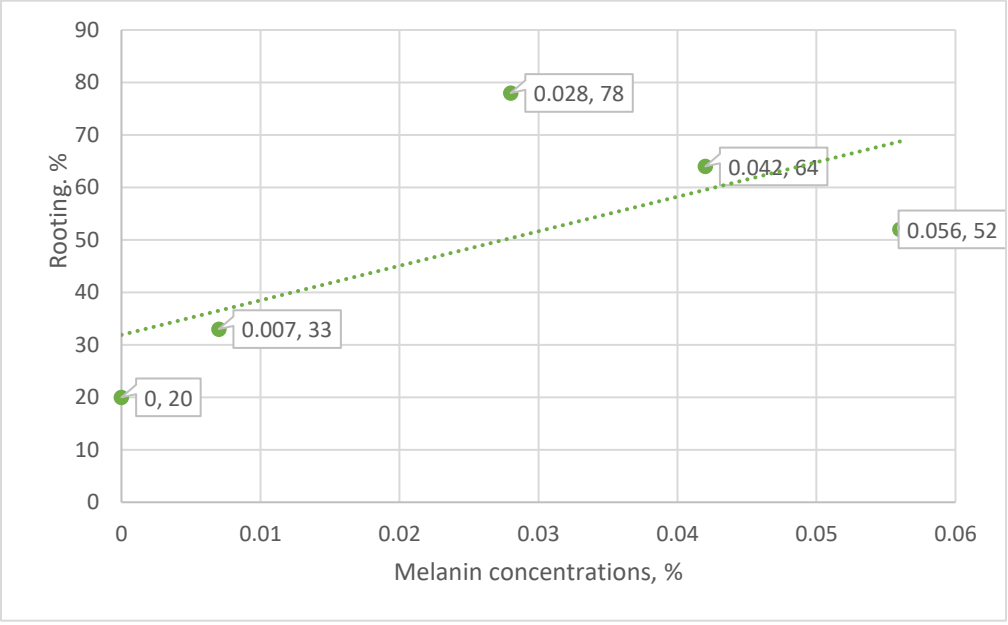

Figure 1. Effect of melanin concentrations on the rooting percentage of grapevine microshoots cv. *'Deghin Yerevani'*.

As shown in Figure 1, the highest rooting percentage for *'Deghin Yerevani'* (78%) was observed at a melanin concentration of 0.028%. Higher concentrations (0.042% and 0.056%) resulted in a noticeable decline in rooting efficiency, indicating a dose-dependent inhibitory effect at elevated melanin levels. Similarly, Figure 2 illustrates that *'Parvana'* achieved its peak rooting percentage (72%) at 0.028% melanin. Consistent with the results for *'Deghin Yerevani'*, increasing the concentration to 0.042% and 0.056% led to a significant reduction in rooting efficiency, indicating that the optimal concentration for root induction in this cultivar is also around 0.028%.

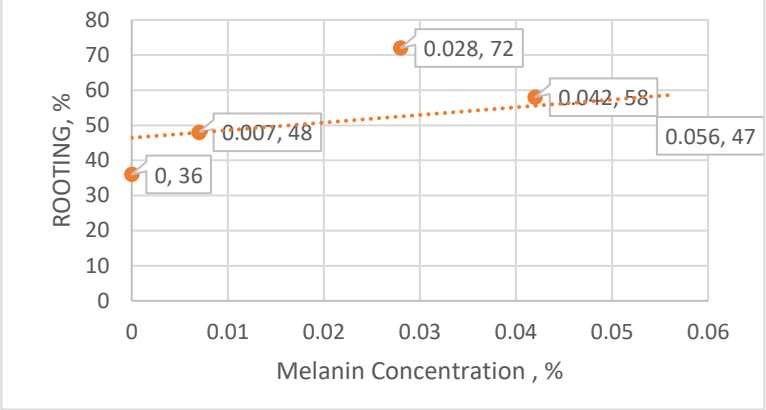

Figure 2. Effect of melanin concentrations on the rooting percentage of grapevine microshoots cv. '*Parvana*'.

A two-way ANOVA was conducted to assess the effects of grapevine cultivar and melanin concentration on rooting percentage. The analysis revealed:

- No significant difference between the two grapevine cultivars ($F = 0.291$, $P = 0.618$).
- A significant effect of melanin concentration on rooting percentage ($F = 9.779$, $P = 0.024$), with the highest rooting percentages recorded at 0.028% and 0.042%.

The influence of melanin on root development—measured by average root number per shoot and root length—was assessed in both grapevine cultivars (Table 1). Significant improvements were observed at lower melanin concentrations, particularly at 0.028%, with declines noted at higher concentrations.

Table 1. Influence of Melanin Concentrations on Root Development in *'DeghinYerevani'* and *'Parvana'*

Variety	Melanin (%)	Roots per Shoot (Mean ± SD)	Root Length (cm, Mean ± SD)
'Deghin Yerevani'	0	1.1 ± 0.1	3.5 ± 0.1
	0.007	2.7 ± 0.1	4.0 ± 0.2
	0.028	5.6 ± 0.2	6.3 ± 0.3
	0.042	4.2 ± 0.1	4.4 ± 0.2
	0.056	3.3 ± 0.2	3.8 ± 0.1
'Parvana'	0	1.3 ± 0.2	3.2 ± 0.2
	0.007	2.4 ± 0.1	4.0 ± 0.2
	0.028	3.7 ± 0.3	5.2 ± 0.1
	0.042	3.0 ± 0.1	4.6 ± 0.1
	0.056	2.2 ± 0.2	2.8 ± 0.2

In the *'Deghin Yerevani'* cultivar, the number of roots per shoot reached a maximum at 0.028% melanin concentration (5.6 ± 0.2), significantly higher than at 0% (1.1 ± 0.1). However, further increases in melanin concentration led to a gradual decline in root number, with values of 4.2 ± 0.1 at 0.042% and 3.3 ± 0.2 at 0.056%. A similar pattern was observed in the *'Parvana'* cultivar, where root number peaked at 0.028% (3.7 ± 0.3), then decreased to 3.0 ± 0.1 at 0.042% and 2.2 ± 0.2 at 0.056%. Root length in *'Deghin Yerevani'* also showed a notable increase, rising from 3.5 ± 0.1 cm at 0% to 6.3 ± 0.3 cm at 0.028%. Higher melanin concentrations resulted in shorter roots, with lengths of 4.4 ± 0.2 cm at 0.042% and 3.8 ± 0.1 cm at 0.056%. The *'Parvana'* cultivar exhibited a similar trend, with the longest roots observed at 0.028% (5.2 ± 0.1 cm), followed by a decline at higher concentrations.

Two-way ANOVA results demonstrated that both variety and melanin concentration significantly influenced root development parameters. For the number of roots per shoot, a significant effect of variety was observed ($F(1,13) = 31.56$, $P = 0.0021$), indicating that *'Deghin Yerevani'* generally produced more roots than *'Parvana'* across treatments. Additionally, melanin concentration significantly affected root number ($F(4,13) = 5.40$, $P = 0.0289$), with peak values at 0.028% in both cultivars, followed by a decline at higher concentrations. Regarding root length, the effect of variety was marginally significant ($F(1,13) = 3.73$, $P = 0.0775$), suggesting a tendency for longer roots in *'Deghin Yerevani'* compared to *'Parvana'.* Melanin concentration had a significant impact on root length ($F(4,13) = 3.08$, $P = 0.0384$), with the greatest root elongation observed at 0.028% in both cultivars, followed by reductions at higher concentrations.

These results indicate that while both grapevine cultivars responded positively to lower melanin concentrations, *'Deghin Yerevani'* exhibited a stronger rooting response in terms of both root number and length compared to *'Parvana'*.

Chlorophyll Content in Grapevine Microcuttings under Melanin Treatments: The effects of melanin on chlorophyll accumulation were assessed in microcuttings of two *Vitis vinifera* cultivars, *'Deghin Yerevani'* and *'Parvana'.* Measurements included chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll (Chl a + b), with results summarized in Table 2.

Table 2. Chlorophyll content in grapevine microplants under different melanin treatments.

Variety	Treatment	Melanin Concentration (%)	Chl a (mg/g FW) (Mean ± SD)	Chl b (mg/g FW) (Mean ± SD)	Total Chlorophyll (mg/g FW) (Mean ± SD)
'Deghin Yerevani'	Control	-	1.19 ± 0.04	0.40 ± 0.02	1.59 ± 0.06
	Melanin	0.007	1.36 ± 0.04	0.52 ± 0.03	1.88 ± 0.07
		0.028	1.53 ± 0.04	0.58 ± 0.03	2.11 ± 0.11
		0.042	1.42 ± 0.07	0.50 ± 0.04	1.92 ± 0.11
		0.056	1.39 ± 0.05	0.55 ± 0.04	1.94 ± 0.09
'Parvana'	Control	-	1.18 ± 0.03	0.42 ± 0.02	1.60 ± 0.05
	Melanin	0.007	1.28 ± 0.05	0.46 ± 0.03	1.74 ± 0.08
		0.028	1.48 ± 0.06	0.56 ± 0.03	2.04 ± 0.09
		0.042	1.40 ± 0.06	0.51 ± 0.04	1.91 ± 0.10
		0.056	1.20 ± 0.07	0.41 ± 0.04	1.61 ± 0.11

In *'Deghin Yerevani'*, the control group recorded moderate chlorophyll levels, with Chl a at 1.19 ± 0.04 mg/g FW, Chl b at 0.40 ± 0.02 mg/g FW, and total chlorophyll at 1.59 ± 0.06 mg/g FW**.** Melanin treatments increased chlorophyll content across all concentrations, with the highest values observed at 0.028% melanin **(**Chl

a: 1.53 ± 0.04 mg/g FW, Chl b: 0.58 ± 0.03 mg/g FW, total chlorophyll: 2.11 ± 0.11 mg/g FW**).** A slight decline occurred at 0.042% and 0.056%**,** though levels remained higher than in the control group.

In *'Parvana'*, a similar trend was observed. Chlorophyll content increased with melanin treatment, peaking at 0.028% melanin **(**Chl a: 1.48 ± 0.06 mg/g FW, Chl b: 0.56 ± 0.03 mg/g FW, total chlorophyll: 2.04 ± 0.09 mg/g FW**).** Higher concentrations (0.042% and 0.056%) led to a reduction in chlorophyll content, but values remained above the control level.

Additionally, melanin's effects on sugar and vitamin C (ascorbic acid) content were assessed in grapevine leaves. Results are summarized in Table 3.

Table 3. Biochemical analysis of grapevine leaves under different melanin treatments.

Melanin Concentration (%)	Variety	Sugar Content (mg/g FW, Mean ± SD)	Vitamin C Content (mg/100 g FW, Mean ± SD)
0 (Control)	*'Deghin Yerevani'*	5.10 ± 0.20	13.50 ± 0.50
0 (Control)	*'Parvana'*	4.80 ± 0.18	12.80 ± 0.45
0.007	*'Deghin Yerevani'*	5.30 ± 0.22	14.00 ± 0.55
0.007	*'Parvana'*	5.00 ± 0.20	13.20 ± 0.50
0.028	*'Deghin Yerevani'*	5.80 ± 0.25	15.20 ± 0.60
0.028	*'Parvana'*	5.50 ± 0.23	14.50 ± 0.58
0.042	*'Deghin Yerevani'*	5.60 ± 0.24	14.80 ± 0.57
0.042	*'Parvana'*	5.30 ± 0.22	14.00 ± 0.53
0.056	*'Deghin Yerevani'*	5.50 ± 0.23	14.50 ± 0.58
0.056	*'Parvana'*	5.10 ± 0.20	13.80 ± 0.55

The highest sugar and vitamin C levels in both varieties were recorded at 0.028% melanin. In *'Deghin Yerevani',* sugar content reached 5.80 ± 0.25 mg/g FW, while vitamin C content increased to 15.20 ± 0.60 mg/100 g FW. Similarly, '*Parvana*' showed peak values at this concentration, with 5.50 ± 0.23 mg/g FW sugar and 14.50 ± 0.58 mg/100 g FW vitamin C. At higher concentrations (0.042% and 0.056%), slight declines were observed, though values remained above control levels, indicating a concentration-dependent response. These results indicate that while melanin enhances biochemical traits at optimal levels, excessive amounts may induce metabolic saturation or trigger regulatory mechanisms limiting further increases.

DISCUSSION

The application of melanin at varying concentrations significantly influenced rooting parameters in both *'Deghin Yerevani'* and '*Parvana*'. Among the tested treatments, 0.028% melanin was the most effective, increasing rooting percentages to 78% in *'Deghin Yerevani'* and 72% in *'Parvana'.* These results highlight melanin's potential as a natural enhancer of root initiation in grapevine micropropagation.

Although direct studies on melanin's role in grapevine rooting are limited, its effects appear comparable to those of plant hormones such as auxins and cytokinins, which are known to regulate oxidative stress and stimulate cell differentiation during root development. Previous research has shown that melanin exhibits auxin-like activity in various plant species. For example, in potatoes, melanin treatment significantly enhanced rhizogenesis and in vitro growth, promoting earlier root emergence, increased root biomass, and improved biochemical traits—such as elevated dry matter, sugar, starch, and ascorbic acid levels, especially in the *'Nevsky'* variety [32].

The enhanced rooting observed in grapevines may stem from melanin's antioxidant properties and its interaction with hormone signaling pathways. Supporting this, earlier studies have shown that melanin can

enhance stress tolerance and plant development [36]. Additionally, melanin-based nanoparticles of plant origin have demonstrated antioxidant and antibacterial activity [37, 38], which could further contribute to the improved rooting efficiency and plant vigor observed in this study.

Melanin treatment also positively affected root number and root length. At 0.028% melanin, *'Deghin Yerevani'* produced an average of 5.55 ± 0.2 roots per shoot with a root length of 6.3 ± 0.3 cm, while *'Parvana'* produced 3.7 ± 0.3 roots with a root length of 5.21 ± 0.1 cm. These results indicate that melanin not only initiates rooting but also supports root elongation and proliferation—consistent with its auxin-like effects.

However, higher melanin concentrations (0.042% and 0.056%) led to reduced rooting efficiency, possibly due to hormone imbalances or saturation effects. Genotypic differences were also evident, as *'Deghin Yerevani'* responded more favorably than *'Parvana',* suggesting cultivar-specific sensitivity to melanin. These results emphasize the need to optimize melanin concentration—particularly around 0.028%—for maximal rooting success, with *'Deghin Yerevani'* emerging as a highly responsive cultivar.

In addition to rooting improvements, melanin treatment increased chlorophyll content in grapevine leaves. At 0.028% melanin, total chlorophyll content reached 2.11 ± 0.11 mg/g FW *in 'Deghin Yerevani'* and 2.04 ± 0.09 mg/g FW in *'Parvana'*. These findings are consistent with reports that exogenous melanin or melatonin application can enhance chlorophyll biosynthesis and antioxidant capacity in plants [39].

However, higher melanin concentrations (0.042% and 0.056%) caused slight reductions in chlorophyll levels, suggesting a dose-dependent response. Excessive melanin may interfere with pigment biosynthesis pathways, potentially affecting photosynthetic efficiency [22]. This highlights the importance of fine-tuning melanin application to balance its beneficial effects with potential physiological disruptions.

The observed stimulation of rooting, root elongation, and improved biochemical traits *in vitro* could result from several synergistic mechanisms. Melanin may have acted as an antioxidant, reducing ROS damage and supporting cell division and elongation. Its auxin-like activity likely promoted root initiation, while its chelating capacity could have optimized micronutrient availability and redox balance, enhancing chlorophyll synthesis and metabolism. Together, these effects likely contributed to the significant increases in rooting and leaf biochemical parameters observed at 0.028% melanin.

Plant-derived melanin shares features with several natural biostimulants while retaining unique advantages. Like humic substances, it provides nutrient chelation and auxin-like effects, and, similar to seaweed extracts, it supports oxidative stress protection without supplying external hormones. Its strong antioxidant capacity resembles microbial melanins, but its plant origin and simple extraction from grape pomace minimize concerns about microbial toxins. This combination of antioxidant and chelating actions makes melanin particularly suitable for controlled root stimulation and oxidative stress protection *in vitro* [40]**.**

The observed inverted-U response indicates that melanin promotes rooting and biochemical activity at optimal concentrations (0.028%), but higher levels may induce inhibitory effects, possibly due to micronutrient chelation, osmotic stress, or hormonal feedback mechanisms. This highlights the importance of fine-tuning melanin concentrations for *in vitro* applications.

In addition to melanin, the alkaline extraction procedure used for grape pomace may have co-extracted other bioactive compounds, including flavonoids and polyphenols. These compounds are known for their antioxidant and growth-modulating properties and could have contributed synergistically to the enhanced rooting, root elongation, and chlorophyll content observed. While the relative contribution of these co-extracted compounds was not quantified in this study, their

presence may have reinforced melanin's effects, and further chemical analysis is warranted to clarify their role.

Beyond its role in micropropagation, plant-derived melanin may also have potential in functional food development. Its strong antioxidant activity and ability to modulate plant metabolism point to opportunities for enhancing the nutritional quality of grapevine-derived products by increasing health-promoting compounds such as chlorophylls, polyphenols, and vitamins. Incorporating melanin or melanin-rich extracts into tissue culture protocols could therefore support biofortification strategies aimed at producing grape leaves or fruits with improved antioxidant capacity [31,41].

CONCLUSION

This study demonstrated that a 0.028% concentration of plant-derived melanin was most effective in enhancing rooting efficiency and chlorophyll content in the grapevine cultivars *'Deghin Yerevani'* and *'Parvana'*. At this concentration, both root number and length increased significantly, with *'Deghin Yerevani'* showing the strongest physiological response. Higher melanin concentrations (0.042% and 0.056%) led to reduced performance, indicating a threshold beyond which melanin's benefits decline. These findings highlight melanin's potential as a sustainable biostimulant in viticulture and its possible contribution to functional food systems through the production of grapevine products enriched in bioactive compounds. By utilizing grape pomace, a winemaking byproduct, this approach supports circular bioeconomy principles, offering dual benefits for agriculture—through improved plant growth—and for human health, by potentially enhancing the nutritional value of grape-derived products.

Future Directions: To build on these findings, future studies should:

- Explore melanin's interactions with key plant hormones such as auxins and cytokinins to
- clarify its role in root and shoot development.
- Investigate its influence on a broader range of biochemical markers, including phenolics**,** flavonoids, and antioxidant enzyme activities, which are relevant to human health.
- Assess the impact of environmental factors (light, temperature, humidity) on melanin efficacy.
- Evaluate melanin's role in improving fruit quality, shelf-life, and resistance to biotic and abiotic stressors—traits that influence the health benefits and safety of grape-derived products**.**

These efforts will further elucidate melanin's biological functions and promote its application in sustainable and health-oriented viticulture**.**

Abbreviations: ANAU: Armenian National Agrarian University, SPC: Scientific and Production Center, NAS RA: National Academy of Sciences of the Republic of Armenia, ANOVA: Analysis of Variance, FW: Fresh Weight, AOAC: Association of Official Analytical Chemists, Chl a: Chlorophyll a, Chl b: Chlorophyll b, MS: Murashige and Skoog, SD: Standard Deviation.

Conflicts of Interest: The authors have no conflict of interest to declare

Author Contributions: GM, AM, KD, AA, and YuM drafted the experimental design. GM, KD, AA, and YuM performed the experiments. AM, AA, NA, MG, and GM assisted with data collection, data analysis, and the initial manuscript draft. KT contributed to data interpretation and manuscript revision. All authors read and approved the final version of the manuscript.

Acknowledgement: We gratefully acknowledge the financial support from the State Committee of Science of Armenia for the conducted research.

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