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The role of plant-derived melanin in enhancing *in vitro* growth and nutrient accumulation in potato varieties

Armen Aghajanyan[1], Aram Mikaelyan[2]*, Hamlet Martirosyan [2], Gayane Melyan [2]

[1] Scientific and Production Center "Armbiotechnology," National Academy of Sciences of the Republic of Armenia, Yerevan, Armenia; [2]Agrobiotechnology Scientific Center, Branch of ANAU (Armenian National Agrarian University) Foundation, Ejmiatsin, Armenia.

***Corresponding Author**: Aram Mikaelyan, PhD, Head of Department, Agrobiotechnology Scientific Center, Branch of ANAU Foundation, 1 Isi le Mulino St., Ejmiatsin 1101, Republic of Armenia.

Submission Date: August 28th, 2024**; Acceptance Date:** September 30th**,** 2024**; Publication Date:** October 8th, 2024

Please cite this article as: Aghajanyan A., Mikaelyan a., Martirosyan H., Melyan G. The role of plant-derived melanin in enhancing in vitro growth and nutrient accumulation in potato varieties. *Bioactive Compounds in Health and Disease* 2024; 7(10): 511-524. DOI: https://doi.org/10.31989/bchd.v7i10.1445

ABSTRACT

Background: Melanins are versatile biopolymers found in a wide range of living organisms, contributing to the diverse colors observed in nature. Beyond their pigmentation role, melanins exhibit a variety of biological activities, including antioxidant, antitoxic, antitumor, photoprotective, antimicrobial, and radioprotective properties. These attributes make melanins valuable in fields such as medicine, pharmaceuticals, and agriculture. In this study, we investigated grape marc, a byproduct of wine production, as a novel source of water-soluble melanin extracted using sodium hydroxide. This approach not only repurposes agricultural waste but also explored melanin's role in optimizing *in vitro* plant cultivation. We evaluated the impact of melanin on the biochemical properties of potato plants, focusing on improvements in growth, rhizogenesis, and the nutritional composition of tubers.

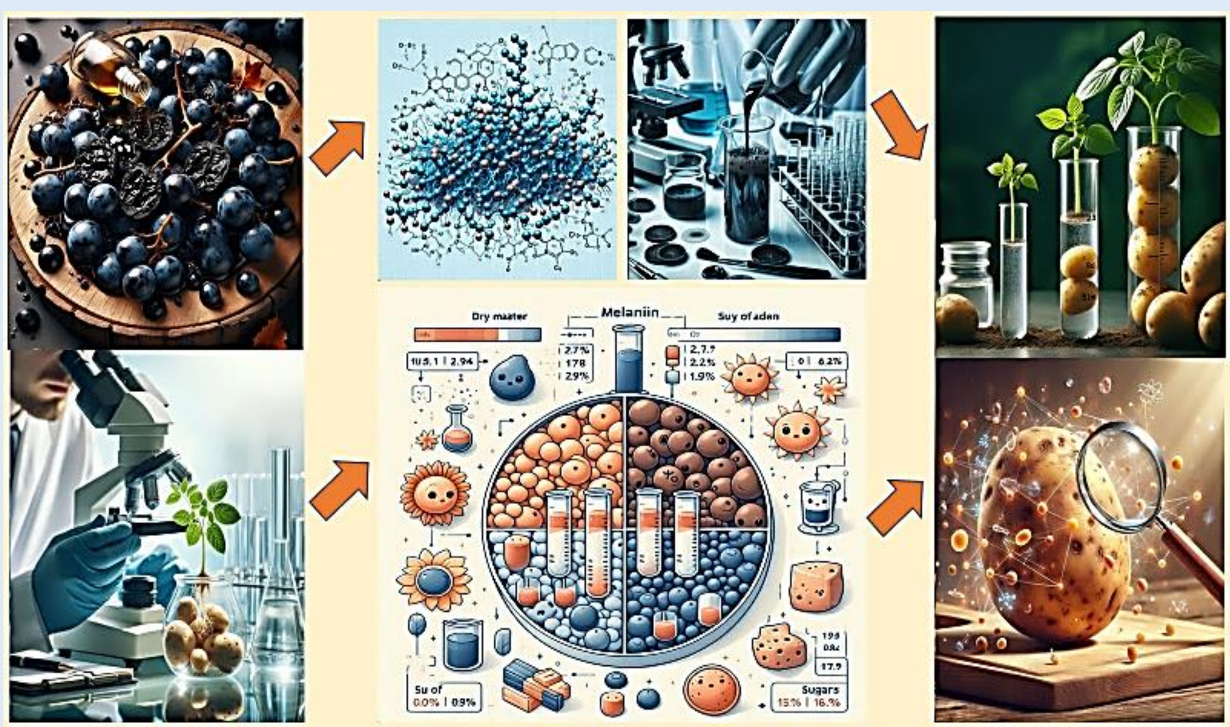
Objective: The aim of this study was to evaluate the effects of plant-derived melanin on the *in vitro* growth characteristics and biochemical composition of two potato varieties.

Methods: This study took place in the tissue culture laboratory at the Scientific Center of Agrobiotechnology from 2021 to 2024. Apical meristems from the *Nevsky* and *Impala* potato varieties were used for *in vitro* regeneration. The research focused on the *in vitro* growth and adaptation of these plants under different melanin concentrations. Key growth parameters, chlorophyll content, and biochemical composition were analyzed to assess the impact of melanin on potato development.

Results: The study found that melanin at a concentration of 0.028% significantly enhanced the *in vitro* growth and rhizogenesis of potato microstems, with optimal results in root number (12.30 per shoot) and shoot length (16.91 cm). Plants also showed earlier and more vigorous root formation, resembling an auxin-like effect. Higher melanin concentrations reduced these benefits. Additionally, plants treated with 0.028% melanin had a high acclimatization success rate (96%) and improved biochemical composition in tubers, with increases in dry matter, sugars, starch, and ascorbic acid, particularly in the *Nevsky* variety.

Conclusion: Melanin significantly stimulated the in vitro growth and development of potato plants. Increasing melanin levels to an optimal threshold accelerated rhizogenesis and promoted vigorous root and shoot growth, evidenced by increased length and thickness. Acting similarly to auxin, melanin not only encouraged root formation but also enhanced shoot development, leading to improved plant adaptation. Additionally, melanin influenced the biochemical composition of potato tubers, including their nutritional content. These findings highlight the crucial role of melanin concentration in optimizing the growth and biochemical properties of potato varieties.

Keywords: in vitro propagation, potato, melanin, plant adaptation, biochemical compound, micropropagation



Graphical abstract: plant-derived melanin, potato, *in vitro* growth, micropropagation, biochemical compound

INTRODUCTION

Melanins, which are abundant in nature, contribute to the color diversity observed in living organisms. These biopolymers, found in microbial, animal, and plant sources, exhibit significant physiological activities, including anti-inflammatory, antitoxic, antitumor, photoprotective, and phytostimulating properties [1-2]. For example, melanin derived from fungal sources has shown potential in radioprotection and UV protection, making it a valuable ingredient in cosmetic formulations [3]. The role of melanins in plant development is particularly intriguing. Recent studies have indicated that melanins can positively influence plant growth and development, underscoring their value in in vitro plant cultivation. Optimizing nutrient media composition is crucial for successful in vitro cultivation, as it significantly affects root and shoot development [4]. Additionally, research has demonstrated that melanin not only enhances plant growth but also imparts resistance to various stresses, suggests its role in improving plant development and resilience [5]. Melanin's multifunctionality and biodegradability make it a promising biomaterial with diverse applications [6-8]. Bioactive compounds, derived from both nutritive and non-nutritive natural sources, serve as primary and secondary metabolites, contributing to health by preventing or managing chronic diseases and their symptoms. In functional foods, bioactive compounds play a crucial role, offering mechanisms for disease management, prevention, and treatment. Their multifunctionality and compatibility underscore their importance in promoting overall well-being [9-14].

Potato (Solanum tuberosum) serves as a fundamental food crop, supplying essential nutrients to millions worldwide. Its nutritional profile includes proteins, carbohydrates, minerals, carotenoids, dietary fiber, vitamins, and minimal fat and sodium. Additionally, potato tubers contain bioactive compounds with potential health benefits [15-16].The goal of this study is to assess the impact of plant-derived melanin on the *in vitro* growth and biochemical properties of two potato varieties.

MATERIAL AND METHODS:

The study was conducted in the tissue culture laboratory at the Scientific Center of Agrobiotechnology from 2021 to 2024.

Water-soluble melanin was obtained from grape peel at the SPC NAS RA through a series of steps [17]. First, the grape pomace was thoroughly washed with water to remove soluble impurities and residues. Next, the washed pomace was filtered to separate the solid sediment, which was then dried for further processing. The dried sediment was treated with an alkaline solution to extract melanin, which dissolved into the solution. Following this, the mixture was centrifuged to separate the insoluble mass from the melanin-rich extract. Finally, the pH of the extract was adjusted to stabilize the melanin and optimize its bioactive properties. The resulting solution contained water-soluble melanin with high bio-inhibitory properties, making it suitable for further applications and analysis.

Apical meristems from potato tuber sprouts served as the explants for plant regeneration. Surface sterilization of the sprout fragments was performed using 1.0% sodium hypochlorite for 15 minutes with a few drops of Tween-20. Research was conducted on two varieties of potatoes, *Nevsky* and *Impala*.

Nevsky Potato Variety: Originating from Russia, the *Nevsky* potato variety is characterized by its tuber weight ranging from 90 to 130 grams. The peel is light beige, while the flesh is white. It has a smooth peel structure, rounded tuber shape, and pink eyes. The starch content is between 10-12%. The variety was approved in 1982 [18].

Impala Potato Variety: The *Impala* potato variety, of Dutch origin, is an early-maturing variety. It produces large tubers, ranging from 80 to 160 grams, with an oval to long shape. The skin color is yellow with a smooth texture, and the flesh is light yellow. It is known for its high yield and good resistance to common scab, and medium to high resistance to potato virus Y. The variety is suitable for table use, renowned for its good taste and cooking quality. It thrives in light, slightly damp soils with moderate nitrogen content [19].

The microcuttings of *in vitro* regenerated potato plants were cultured on MS medium supplemented with varying concentrations of melanin (0%, 0.007%, 0.014%, 0.028%, 0.042%, and 0.056%). The control group was grown on basal MS medium without melanin. The data were collected on the number of roots per shoot and shoot length after 15 days of growth. The cultures were maintained under controlled conditions, with a 16-hour photoperiod, at 25°C, and 70% relative humidity. The number of roots and shoots per explant were recorded, and the lengths of roots and shoots were measured using a digital caliper. The adaptation of *in vitro* plants, influenced by the different melanin concentrations, was conducted by cultivating them in a soil mix composed of a 1:3 ratio of perlite to compost.

Chlorophyll content was determined using the method outlined by Lichtenthaler and Wellburn (1983) [20]. The soluble sugars in potato tubers were measured according to the procedure described by Maness (2010) [21]. The dry matter (DM) content in the tubers was determined following the AOAC method (1995) [22]. Total starch (TS) content was measured using the method developed by McCready et al. (1950) [23]. Ascorbic acid content was assessed according to the procedures in the CIP manual for chemical analysis of potato and sweet potato samples [24].

Data from three separate experiments were combined and presented as average values. The treatment averages were compared using the standard error (SE) of the mean. A *Student's t-test* was conducted to determine significant differences between the means ($P<0.05$).

RESULTS AND DISCUSSION

The effect of varying melanin concentrations on *in vitro* shoot multiplication in the *Nevsky* and *Impala* varieties of potato was investigated, focusing on the mean number of roots per shoot and mean shoot length. 15 plant explants were utilized for each treatment, and each treatment was replicated three times.

For the *Nevsky* variety, both the mean number of roots per shoot (Table 1 and Fig. 1) and shoot length (Table 1 and Fig. 3) increased progressively with rising melanin concentrations up to 0.028%. At 0% melanin, the mean number of roots per shoot was 2.29±0.3, and the shoot length was 3.85±0.2 cm. With an increase to 0.028% melanin, there was a significant enhancement in shoot multiplication, with the mean number of roots per shoot rising to 12.30±0.6 and the mean shoot length reaching 16.91±0.5 cm.

Table 1. The effect of different concentrations of melanin on *in vitro* shoot multiplication in the *Nevsky* and *Impala* varieties of potato.

Variety	Melanin (%)	Mean number of roots/shoots (Mean ± SE)	Mean shoot length (cm), (Mean ± SE)
Nevsky	0	2.29±0.3	3.85±0.2
	0.007	3.67±0.1	5.83±0.3
	0.014	5.12±0.1	7.87±0.4
	0.028	12.30±0.6	16.91±0.5
	0.042	8.54±0.4	8.41±0.6
	0.056	3.67±0.1	5.83±0.3
Impala	0	1.78±0.1	3.90±0.3
	0.007	3.10±0.2	6.03±0.3
	0.014	4.20±0.1	8.01±0.3
	0.028	10.30±0.3	14.71±0.4
	0.042	7.95±0.4	7.41±0.3

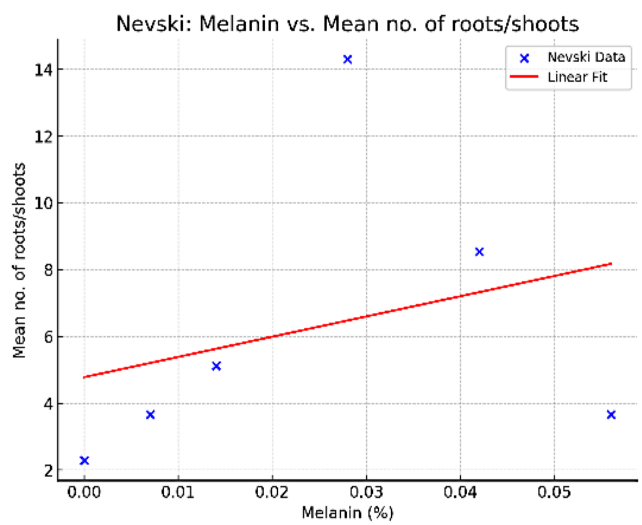


Figure 1: *Nevsky* Variety Melanin vs. Mean Number of Roots/Shoots

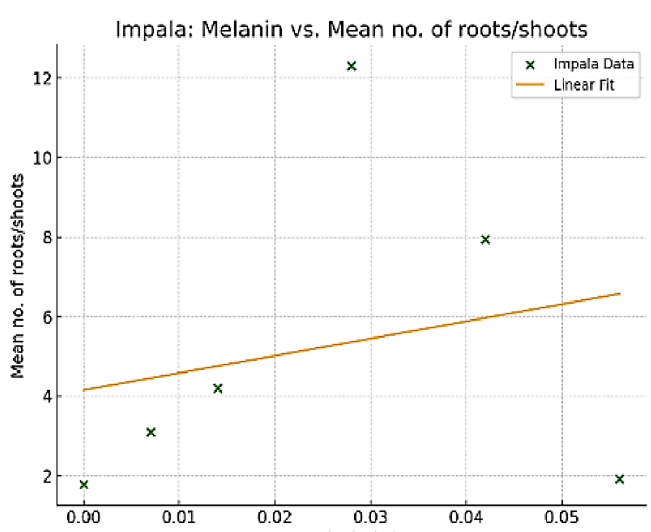


Figure 2: *Impala* Variety Melanin vs. Mean Number of Roots/Shoots.

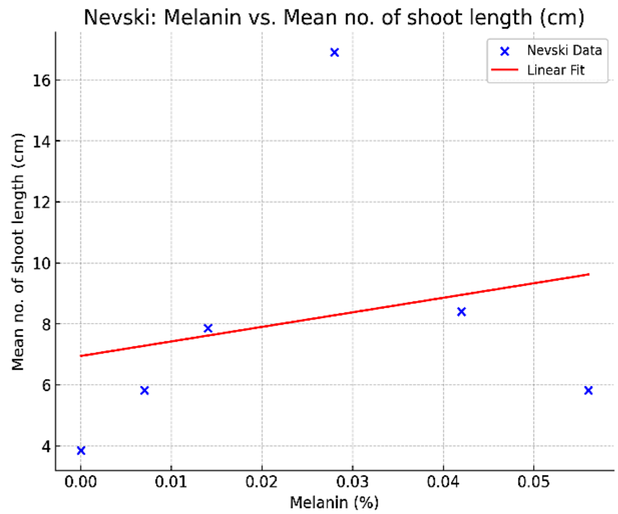


Figure 3: *Nevsky* Variety Melanin vs. Mean Shoot Length (cm)

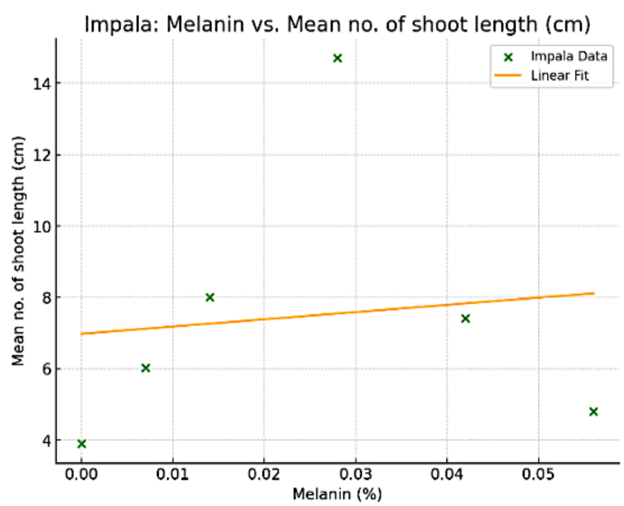


Figure 4: *Impala* Variety Melanin vs. Mean Shoot Length (cm)

However, beyond this concentration, both parameters declined. At the highest concentration of 0.056%, the mean number of roots per shoot decreased to 3.67±0.1, and the mean shoot length reduced to 5.83±0.3 cm.

A similar trend was observed in the *Impala* variety. At 0% melanin, the mean number of roots per shoot was 1.78±0.1 (Table 1 and Fig. 2), and the mean shoot length was 3.90±0.3 cm (Table 1 and Fig. 4). These values increased with higher melanin concentrations, peaking at 0.028%, where the mean number of roots per shoot was 10.30±0.3 and the mean shoot length was 14.71±0.4 cm. As with the *Nevsky* variety, further increases in melanin concentration led to a decline in shoot multiplication. At 0.056% melanin, the mean number of roots per shoot dropped to 1.92±0.1, and the mean shoot length fell to 4.80±0.3 cm.

The chlorophyll content of potato leaves for the *Nevsky* and *Impala* varieties under varying melanin concentrations (0%, 0.007%, 0.014%, 0.028%, 0.042%, and 0.056%) is summarized in Tables 2 and 3, respectively. The data is presented as mean values with (±SE) for chlorophyll a, chlorophyll b, and total chlorophyll content (µg/g FW).

Table 2. Chlorophyll content of potato leaves of *Nevsky* variety *in vitro* by melanin influence.

Variety	Melanin Concentration (%)	Chlorophyll a (µg/g FW) Mean ±S E	Chlorophyll b (µg/g FW) Mean ± SE	Total Chlorophyll (µg/g FW) Mean ± SE
Nevsky	0	1.106±0.005	0.600±0.01	1.713±0.01
	0.007	1.220±0.047	0.630±0.02	1.85±0.01
	0.014	1.361±0.05	0.650±0.03	2.016±0.02
	0.028	1.50±0.01	0.713±0.01	2.216±0.02
	0.042	1.37±0.01	0.663±0.02	2.04±0.02
	0.056	1.123±0.03	0.610±0.04	1.73±0.04

***Nevsky* Variety:** Chlorophyll a concentration increased with rising melanin levels, peaking at 0.028% with a maximum concentration of 1.50 µg/g FW, an increase of approximately 35% compared to the control (1.106 µg/g FW). However, at higher concentrations (0.042% and 0.056%), chlorophyll a content slightly decreased to 1.37 µg/g FW and 1.123 µg/g FW, respectively. A similar trend was observed, with the highest concentration of chlorophyll b (0.713 µg/g FW) recorded at 0.028% melanin, an increase of approximately 19% compared to the control (0.60 µg/g FW). Beyond this concentration, a slight reduction in chlorophyll b content was noted, with values decreasing to 0.663 µg/g FW at 0.042% and 0.61 µg/g FW at 0.056%. The total chlorophyll content followed the same pattern as individual chlorophyll a and chlorophyll b, with the highest total chlorophyll content observed at 0.028% melanin, reaching 2.216 µg/g FW, an increase of approximately 29% from the control (1.713 µg/g FW). After this peak, total chlorophyll content decreased slightly to 2.04 µg/g FW at 0.042% and 1.73 µg/g FW at 0.056%, which is comparable to the control (Table 2).

Impala Variety: The concentration of chlorophyll an increased progressively with rising melanin concentrations up to 0.028%, where the maximum concentration of 1.613 µg/g FW was observed, representing an increase of approximately 25% compared to the control (1.286 µg/g FW). At higher concentrations of melanin (0.042% and 0.056%), chlorophyll a content decreased to 1.26 µg/g FW and 1.256 µg/g FW, respectively, which is close to or slightly lower than the control. Chlorophyll b content showed a similar pattern, increasing with melanin concentrations up to 0.014%, where a peak concentration of 0.726 µg/g FW was observed, approximately 22% higher than the control (0.593 µg/g FW). A slight decrease was noted at 0.028% melanin (0.69 µg/g FW), followed by further reductions at higher concentrations, reaching 0.62 µg/g FW at 0.056%. Total chlorophyll content was highest at 0.028% melanin, with a concentration of 2.30 µg/g FW, approximately 22% higher than the control (1.88 µg/g FW).

Table 3. Chlorophyll content of potato leaves of *Impala* variety *in vitro* by melanin influence.

Variety	Melanin Concentration (%)	Chlorophyll a (µg/g FW) Mean±SE	Chlorophyll b (µg/g FW) Mean±SE	Total Chlorophyll (µg/g FW) Mean±SE
Impala	0	1.286±0.01	0.593±0.02	1.880±0.02
	0.007	1.361±0.09	0.610±0.02	1.963±0.09
	0.014	1.420±0.20	0.726±0.03	2.140±0.03
	0.028	1.613±0.01	0.690±0.01	2.300±0.01
	0.042	1.260±0.01	0.670±0.01	1.926±0.01
	0.056	1.256±0.01	0.620±0.04	1.81±0.03

After this peak, total chlorophyll content decreased to 1.926 µg/g FW at 0.042% and 1.81 µg/g FW at 0.056%, slightly lower than the control (Table 3).

The adaptation of potato varieties *Nevsky* and *Impala* was evaluated at various melanin concentrations using a 1:3 ratio of perlite to compost.

Table 4. Adaptation of Potato Variety *Nevsky* in a 1:3 (Perlite: Compost) Ratio at Different Melanin Concentrations.

Melanin concentration, %	Root development	Shoot Development	Viability during acclimatization
0	Poor	Moderate	50%
0.007	Moderate	Moderate	70%
0.014	Good	Good	85%
0.028	Robust	Robust	96%
0.042	Less robust	Less robust	80%
0.056	Poor	Poor	60%

Nevsky Potato Variety (Table 4): Root development improved as the melanin concentration increased from 0% to 0.028%. At 0% melanin, root development was poor, while at 0.028% melanin, it was robust. However, at higher concentrations of 0.042% and 0.056%, root development decreased to less robust and poor levels, respectively. Similar to root development, shoot development improved with increasing melanin concentration, reaching robust levels at 0.028%. Higher concentrations resulted in moderate and poor shoot development. The viability of the *Nevsky* variety during acclimatization increased with melanin concentration, peaking at 96% at 0.028% melanin. Viability declined at higher melanin concentrations, dropping to 80% at 0.042% and 60% at 0.056%.

Table 5. Adaptation of Potato Variety *Impala* in a 1:3 (Perlite: Compost) Ratio at Different Melanin Concentrations

Melanin concentration, %	Root development	Shoot Development	Viability during acclimatization
0	Poor	Moderate	60%
0.007	Moderate	Moderate	73%
0.014	Good	Good	80%
0.028	Robust	Robust	89%
0.042	Good	Less robust	74%
0.056	Poor	Poor	63%

Impala Potato Variety (Table 5): Root development in the *Impala* variety showed a similar trend to the *Nevsky* variety, with gradual improvement as melanin concentration increased, peaking at 0.028% where root development became robust. However, root development declined at higher concentrations of 0.042% and 0.056%. Shoot development also improved with increasing melanin concentrations, reaching robust levels at 0.028%. Beyond this concentration, shoot development decreased, becoming less robust at 0.042% and poor at 0.056%. The viability during acclimatization was highest at 89% with 0.028% melanin. At higher concentrations, viability declined to 74% at 0.042% and 63% at 0.056%.

The biochemical composition of potato tubers was analyzed to determine the impact of melanin treatment on key nutritional parameters. This analysis aims to understand how different concentrations of melanin influence the quality and nutritional content of potato tubers, specifically focusing on dry matter, total sugars, starch, and ascorbic acid levels. For this study, a melanin concentration of 0.0028% was used, as it was identified as the optimal concentration for *in vitro* plant growth and development. Table 6 summarizes the biochemical composition of tubers from the *Nevsky* and *Impala* varieties treated with 0% and 0.028% melanin concentrations. The data are presented as mean values with SE, providing a clear comparison of the effects of melanin treatment on tuber quality.

Nevsky Variety: Dry matter content increased by 11.85% (from 21.1% to 23.6%). Total sugars increased by 8.86% (from 0.79% to 0.86%). TS content increased by 6.96% (from 15.8% to 16.9%). Ascorbic acid content increased by 9.85% (from 13.2 mg% to 14.5 mg%).

Table 6. Biochemical Analysis of Tubers from *Nevsky* and *Impala* Potato Varieties Derived from *in vitro* Plants Treated with 0.028% Melanin Concentration, post-harvest.

Melanin Concentration (%)	Variety	DM (%)	TSS (%)	TS (%)	AA (mg/%)
	Nevsky				
0		21.1[b]	0.79[b]	15.8[b]	13.2[b]
0.028		23.6[a]	0.86[a]	16.9[a]	14.5[a]
	Impala				
0		18.21[b]	0.71[b]	13.1[b]	15.5[b]
0.028		19.91[a]	0.80[a]	13.9[a]	16.1[a]

Impala Variety: Dry matter content increased by 9.34% (from 18.21% to 19.91%). Total sugars increased by 12.68% (from 0.71% to 0.80%). Starch content increased by 6.11% (from 13.1% to 13.9%). Ascorbic acid content increased by 3.87% (from 15.5 mg% to 16.1 mg%).

The results of this study demonstrate that melanin concentration significantly affects shoot multiplication in both *Nevsky* and *Impala* potato varieties. The optimal concentration for promoting shoot multiplication was identified as 0.028% melanin. Beyond this concentration, both the number of roots per shoot and shoot length decreased, indicating a threshold above which melanin may have inhibitory effects. These findings are supported by statistical analysis, with significant differences confirmed by *SE and t-tests*, and further validated by *ANOVA* results (p-values < 0.05). Optimizing melanin levels is crucial for enhancing plant development. At the 0.028% concentration, the highest number of roots per shoot and the greatest shoot length were observed, suggesting that melanin at this level positively influences these growth parameters. However, concentrations exceeding 0.028% resulted in reduced growth, potentially due to the disruption of metabolic processes or altered light absorption in chloroplasts.

Chlorophyll in higher plants includes two main types: chlorophyll a ($C_{55}H_{72}O_5N_4Mg$), which is dark green, and chlorophyll b ($C_{55}H_{70}O_6N_4Mg$), which is light green [25]. Chlorophyll is essential for photosynthesis, which converts light energy into chemical energy, fundamental for plant growth and energy production [26-30]. It also has notable health benefits, including counteracting free radicals, supporting detoxification, and potentially reducing inflammation [31-32].

***Nevsky Variety*:** In the *Nevsky variety*, lower melanin concentrations (0.007%–0.028%) significantly enhanced chlorophyll a, b, and total chlorophyll, with the peak effect at 0.028%. This enhancement is likely attributed to melanin's auxin-like properties, which may promote chlorophyll synthesis. However, at higher concentrations (0.042% and 0.056%), chlorophyll content declined, suggesting an inhibitory threshold. *Impala* exhibited a similar trend, albeit with slightly lower overall chlorophyll levels compared to *Nevsky,* indicating a differential varietal response to melanin. These findings suggest that melanin can modulate chlorophyll synthesis, potentially impacting the photosynthetic efficiency of potato plants. Further research is warranted to elucidate the biochemical pathways through which melanin influences chlorophyll synthesis and to determine whether these effects are consistent across different plant species or varieties. Understanding these mechanisms could provide valuable insights into the application of melanin in agricultural practices, particularly in improving plant growth and productivity.

In vitro plant adaptation is a critical phase in plant tissue culture, especially when transitioning from controlled laboratory conditions to external growing environments. This phase is crucial for ensuring the survival, establishment, and successful development of plants outside the sterile *in vitro* conditions [33-38]. Successful *in vitro* adaptation is closely linked to the development of robust root and shoot systems, which are vital for the plant's ability to absorb nutrients and water, anchor itself, and continue growth post-transfer. High survival rates during the acclimatization phase indicate effective in vitro adaptation.

The results demonstrate that melanin concentration influences the adaptation of *Nevsky* and *Impala* potato varieties. In *Nevsky*, root and shoot development improved with melanin levels up to 0.028%, likely due to its bioactive properties enhancing growth and stress resistance. The peak viability at this concentration suggests it is optimal for *Nevsky*'s growth and establishment. However, higher concentrations led to a decline in development and viability, indicating potential inhibitory effects due to altered metabolic balance or nutrient absorption. The *Impala* variety showed a similar trend, with growth peaking at 0.028% melanin and declining at higher levels. The slightly lower overall viability during acclimatization compared to *Nevsky* suggests a differential sensitivity to melanin, indicating that the optimal concentration may vary between potato varieties. These findings underscore the potential of melanin as a bioactive compound to enhance the adaptation and growth of potato plants under controlled conditions. The results also highlight the importance of determining optimal melanin concentrations to avoid potential inhibitory effects at higher levels.

The primary components of potato tubers include carbohydrates, fiber, nitrogen compounds, fats, and ash elements. Carbohydrate metabolism plays a crucial role in potato plants, influencing both productivity and the quality of the resulting yield. The primary carbohydrate found in tubers is starch [39-40].

In our research, we treated *in vitro*-derived plants of the *Nevsky* and *Impala* potato varieties with melanin concentrations of 0% and 0.028%. Following adaptation and growth, we harvested the tubers for biochemical analysis. The parameters analyzed included dry matter content, total sugars, starch content, and ascorbic acid levels—key indicators of tuber quality.

In the *Nevsky* variety, dry matter content increased from 21.1% to 23.6% with the addition of 0.028% melanin, suggesting that melanin may contribute to better overall growth or stress adaptation. A similar trend was observed in the *Impala* variety, with dry matter content increasing from 18.21% to 19.91%. While the increase is less pronounced compared to *Nevsky*, it still indicates a positive effect of melanin on dry matter content.

The accumulation of dry matter and starch in potato tubers is influenced by the genetic characteristics of the variety, organomineral nutrition, and soil-climatic conditions. The nutritional value of potatoes is determined not only by starch and dry matter content but also by vitamins and other biologically active substances, including ascorbic acid (vitamin C) [41].

In our research, the TSS of the *Nevsky* variety increased from 0.79% to 0.86% with melanin treatment, indicating that melanin may enhance carbohydrate accumulation or alter metabolic processes in the plant. A similar trend was observed in the *Impala* variety, where TSS content rose from 0.71% to 0.80%, reflecting a consistent effect of melanin across both varieties. Additionally, TS content in the *Nevsky* variety increased from 15.8% to 16.9%, suggesting that melanin may influence starch synthesis or storage mechanisms. A comparable increase was noted in the *Impala* variety, with starch content rising from 13.1% to 13.9%, though the increase was slightly less pronounced compared to *Nevsky*. Melanin treatment led to an increase in ascorbic acid content in the *Nevsky* variety, rising from 13.2 mg% to 14.5 mg%. Similarly, in the *Impala* variety, ascorbic acid content increased from 15.5 mg% to 16.1% with melanin treatment. These findings suggest that melanin may improve the nutritional quality of the tubers.

Solanum tuberosum tubers have been reported to contain up to 46 mg of ascorbic acid per 100 g tuber on a fresh weight basis, and their availability is dependent on the variety, maturity status, and environmental conditions under which the crop is grown [42]. For centuries, the significance of vitamin C in preventing scurvy has been recognized. However, recent research has revealed broader health benefits and clinical applications beyond scurvy prevention [43].

Overall, this study highlights the multifaceted role of melanin in influencing plant growth, chlorophyll synthesis, *in vitro* adaptation, and tuber quality in potato varieties. The differential responses observed between *Nevsky* and *Impala* indicate that melanin's effects are variety-specific, emphasizing the need for further research to optimize its application in different cultivars. The insights gained from this study could inform future agricultural practices aimed at enhancing crop productivity and nutritional value using bioactive compounds like melanin.

The novelty of this research lies in its investigation of plant-derived melanin's influence on both growth parameters and biochemical composition in vitro. Our findings demonstrated a clear threshold effect at 0.028%, beyond which growth and chlorophyll content declined. These results provide new insights into optimizing melanin concentrations for enhancing crop productivity and nutritional value, laying the groundwork for future agricultural applications.

CONCLUSION

Melanin concentration significantly affected the growth and biochemical properties of the *Nevsky* and *Impala* potato varieties. An optimal concentration of 0.028% melanin promoted the best shoot multiplication and chlorophyll content. Higher melanin levels resulted in reduced growth and chlorophyll, indicating a threshold effect. This concentration also improved *in vitro* adaptation and enhanced the nutritional quality of potato tubers by increasing dry matter, sugars, starch, and ascorbic acid. These findings suggested that melanin could be beneficial for optimizing potato growth and tuber quality, though further research was needed to fully understand its mechanisms and applications.

List of Abbreviations: AA: Ascorbic Acid, ANAU: Armenian national Agrarian University, CC: Chlorophyll Content, DM: Dry Matter, MS: Murashige Skoog, FW: fresh weight, SPS**:** Scientific and Production Center, SE: standard error, TSS: Total Soluble Sugars, TS: Total Starch.

Conflict of interest: The authors have no conflict of interest to declare.

Author Contributions: AA, AM, HM, GM drafted the experimental design. AA, AM, GM performed the experiments, GM, AM helped in data collection, data analysis and initial draft of manuscript text. All authors read and approved with 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 in the scope of the 21T-4D086 project.

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