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 ($C55H72O5N4Mg$), which is dark green, and chlorophyll b ($C55H70O6N4Mg$), 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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