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Effect of the culture liquid of Antarctic yeast *Nadsoniella nigra* on rooting, growth, and biochemical composition of *in vitro* grapevine (*Vitis vinifera* L.), cultivar '*Karmrahyut*'

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ABSTRACT

Background: Grapevine (*Vitis vinifera* L.) is a globally significant fruit crop known for its nutritional and health benefits, including its role in preventing chronic diseases such as cancer and cardiovascular disorders. The bioactive compounds in grapes contribute to these benefits. Bioactive content changes due to grapevine variety and environmental conditions necessitate ongoing research. *In vitro* propagation provides a controlled environment for grapevine cultivation, minimizing disease risk and ensuring consistent quality. This study investigates the potential of *Nadsoniella nigra (*Nn*)*, an Antarctic yeast, as a natural alternative to synthetic auxins like Indole-3-butyric acid (IBA) in grapevine micro cutting propagation.

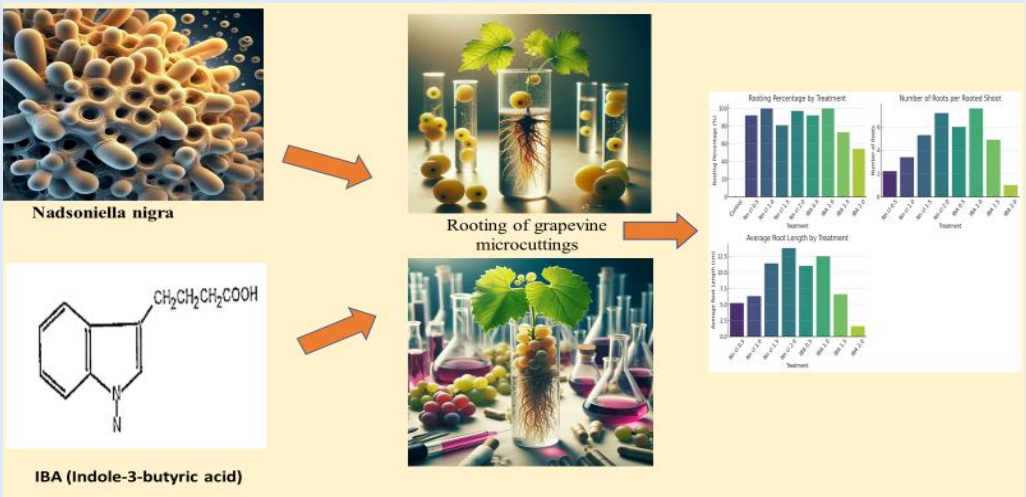
Objective: To evaluate the effectiveness of the culture liquid of Nn as a natural alternative to auxin-class hormones, using IBA as an example, for promoting rooting in grapevine microcuttings and to assess its impact on chlorophyll content, vitamin C levels, and sugar concentrations.

Materials and Methods: *In vitro* propagation of grapevine (*Vitis vinifera* L.) cultivar *'Karmarhyt'* was performed using half-strength Woody Plant Medium (WPM). Nn culture liquid was prepared at concentrations of 0.5, 1.0, 1.5, and 2.0 ml/L, and IBA was used at equivalent concentrations. Microcuttings were cultured on media supplemented with these treatments, with a control group maintained without growth regulators. Rooting was evaluated after 4 weeks by measuring rooting percentage, number of roots per shoot, and root length. Chlorophyll a (Chl a) and Chlorophyll b (Chl b) levels, vitamin C content, and sugar concentrations were assessed. Statistical analysis was performed using standard error and *t-tests*.

Results: Rooting performance of grapevine microcuttings varied with treatment. Nn culture liquid promoted rooting in a dose-dependent manner, with the highest values observed at 2.0 ml/L (97.0% rooting, 7.2 roots, 13.8 cm root length). IBA treatment resulted in peak rooting at 1.0 mg/L (100% rooting) but decreased at higher concentrations, accompanied by callus formation. Chlorophyll content was higher *in* Nn culture liquid treated microcuttings, with a maximum total chlorophyll of 2.20 mg/g at 2.0 mg/L, compared to a peak of 2.05 mg/g in IBA-treated microcuttings at 1.0 mg/L. Sugar and vitamin C content increased with Nn culture liquid concentrations, whereas IBA showed a less consistent effect.

Conclusion: Nn culture liquid was an effective natural alternative to IBA for promoting rooting in grapevine microcuttings. It improved rooting efficiency, chlorophyll content, and increased sugar and vitamin C levels, while IBA led to growth inhibition and callus formation at higher concentrations. Nn culture liquid demonstrated consistent benefits, suggesting potential for sustainable viticulture. As a result of the conducted research, it was established that the mixture of secondary metabolites contained in the liquid culture medium of Nn possesses high biological activity and can be recommended for use in plant biotechnology *in vitro* to promote the growth of both underground and above-ground parts. Future research could explore its use in propagating other plant species.

Keywords: *´Karmrahyut´*, grapevine, culture liquid of *Nadsoniella nigra*. *in vitro* propagation, functional foods



INTRODUCTION

Grapevine (*Vitis vinifera* L.) is a highly valuable fruit crop cultivated globally, prized for its economic and cultural significance [1-2]. Grapes are not only a vital source of nutrition but are also associated with substantial health benefits, including the prevention of chronic diseases such as cancer, cardiovascular disease, hypertension, diabetes, and gastrointestinal disorders like constipation [3]. These health-promoting effects are attributed to their rich composition of essential vitamins, minerals, and bioactive compounds like polyphenols, flavonoids, and antioxidants, which play a key role in reducing oxidative stress and inflammation [4]. These compounds are found in various parts of the grape, including the skin, seeds, leaves, and pulp, and their concentrations can differ depending on the variety and environmental conditions. Grape byproducts, such as grape pomace, leaves, and seeds, are also widely used in the food industry, particularly in functional foods, due to their health-promoting properties [3].

The bioactive content in grapes varies widely depending on factors such as variety, cultivation conditions, and environmental influences, highlighting the need for ongoing research to unlock their full potential [4-5]. Functional foods are crucial for supporting a healthy lifestyle and mitigating risk factors for a range of diseases [6-7]. According to the Functional Food Center, “functional food” encompasses both natural and processed foods that are enriched with biologically active compounds. These compounds are present in specific, safe amounts that contribute to optimal health, reduce the likelihood of chronic or viral illnesses, and help manage their symptoms. The health benefits of these foods have been validated through clinical evidence and precise biomarker analysis [8]. Recently, functional foods have become significantly more popular, primarily due to the growing number of health-conscious consumers seeking food products that provide benefits beyond basic nutrition [9-12].

In recent years, *in vitro* propagation of grapevines has gained popularity as a method for producing healthy plants that are free from viruses and other phytopathogens, ensuring consistent quality in grape production [13-14]. Traditional propagation methods, such as grafting and layering, can carry the risk of transmitting diseases from one generation to the next through plant material, potentially compromising vineyard health and grape yield [15-16]. *In vitro* micropropagation, on the other hand, provides a sustainable alternative by producing disease-free plants, speeding up the release of improved varieties, and preserving germplasm for future use [17].

The use of synthetic hormones like IBA in plant propagation is common, but there is increasing interest in replacing these chemicals with more sustainable, natural alternatives. Biological stimulators derived from the culture liquid of microorganisms play an important role in regulating the growth and development of plants *in vitro*. They can have both direct and indirect effects on the growth rate and morphogenesis of plants. Natural substances rich in auxins and other compounds have shown potential as eco-friendly options for plant propagation [18-19]. The search for effective, biologically derived growth regulators to enhance plant productivity and resilience to biotic and abiotic stress factors is a critical objective in agrobiotechnology. Biological stimulants sourced from the culture fluids of microorganisms, marine algae, or plants play an important role in regulating plant growth and development *in vitro* [20-21]. They can exert both direct and indirect effects on plant growth rate and morphogenesis. Direct growth stimulation occurs through phytohormones and enhanced mineral nutrition of plants. Indirect growth stimulation occurs by

suppressing the development of internal, latent pathogenic fungi and bacteria that inhibit plant growth. Plant growth and development can be stimulated either by altering the rhizosphere or by producing metabolites that directly enhance plant growth and development [22].

Notably, Antarctic yeasts, including *Nadsoniella nigra*, have garnered attention for their unique biochemical properties, shaped by their adaptation to extreme environmental conditions. *Nadsoniella nigra* was validly described in 1916 and later recombined as *Exophiala nigra*. This yeast is characterized by its black pigmentation and belongs to the jeanselmei clade within the genus Exophiala. The genus is known for its ability to produce melanin and other bioactive compounds, which can be beneficial in various biotechnological applications [24]. Originally isolated from Arctic oceanic water, this yeast is recognized for its potential to enhance the synthesis of vitamin C and melanin, both of which offer significant benefits. Vitamin C boosts antioxidant activity, while melanin provides UV protection and has potential applications in cosmetics and pharmaceuticals [25].

Nn has been studied for its unique adaptations, such as producing melanin to protect against UV radiation and surviving freeze-thaw cycles [23-30]. This study aims to investigate the effectiveness of the culture liquid of Nn in promoting *in vitro* rooting of grapevine cultivars. By comparing its effectiveness with that of IBA, we aim to provide alternative potential applications of Nn in grapevine propagation and discuss the broader implications of using natural extracts in plant biotechnology.

MATERIALS AND METHODS

Plant Material: Grapevine (*Vitis vinifera* L.) cultivar '*Karmrahyut*' was selected for the *in vitro* propagation experiments. '*Karmrahyut*' is a late-ripening Armenian wine grape variety, developed through the crossbreeding of the '*Hadisi*' variety and the 15-7-1 hybrid form (*V. amurensis* × *Chernyi Sladkii*). This cultivar is predominantly cultivated in the Ararat Valley and the northeastern viticultural zone, particularly in the Berd region.

Culture Medium: Half-strength Woody Plant Medium (WPM) was employed for all *in vitro* cultures. In this study, we utilized the strain *Nadsoniella nigra* var. *hesuelica*, a coal-black pigmented Antarctic yeast. This natural strain was first isolated by E.L. Ruban in 1957 from a rock soil sample collected by I.P. Ruban during the second Soviet Antarctic expedition (1957-1958) on the island of Heswell. It is registered under VKM number F-2137 in the Russian National Collection of Industrial Microorganisms (VKM) and is preserved in Medium 11 at an incubation temperature of 25°C [31].

Composition of the Medium for Cultivating *Nadsoniella nigra:*

- $(NH_4)_2SO_4$: 5.8 g/L
- K_2HPO_4: 5.2 g/L
- KH_2PO_4: 5.0 g/L
- $MgSO_4 \cdot 7H_2O$: 0.34 g/L
- $CaCl_2$: 0.03 g/L
- $ZnSO_4$: 0.01 g/L
- $CuSO_4$: 0.03 g/L
- Sucrose: 20 g/L
- Peptone: 5%
- Medium pH: 5.5

Cultivation was carried out at 26-27°C. After 96 hours of fermentation, the yeast biomass was filtered from the culture fluid using cold sterilization with 0.22-micron filters, and the biomass was subsequently used in further studies. The sterilized liquid was prepared at

concentrations of 0.5, 1.0, 1.5, and 2.0 mL/L for testing. IBA was included at the same concentrations (0.5, 1.0, 1.5, and 2.0 mg/L) for comparison. Grapevine microcuttings were cultured in media supplemented with either *Nn* culture liquid or IBA at these concentrations, while the control group was grown on half-strength WPM without the addition of any growth regulators.

Experimental Design: After 4 weeks of culture, the following parameters were measured for each treatment group:

1. **Rooting Percentage:** The percentage of microcuttings that successfully developed roots.
2. **Number of Roots:** The average number of roots formed per rooted shoot.
3. **Root Length:** The average length of roots per rooted shoot.

Nutritional and Physiological Analysis:

- **Chlorophyll Levels:** Assessed according to the method described by Lichtenthaler (1987) [32].
- **Ascorbic Acid (Vitamin C) Content:** Quantified using the iodine titration method outlined by AOAC International (2000) [33].
- **Sugar Concentrations:** Measured using a modified procedure adapted from Melgarejo et al. (2000) [34].

Statistical Analysis: Data were analyzed using *t-tests* to compare the differences between treatment groups and the control. Results are presented as means with standard error (*SE*). Statistical significance was determined at a threshold of $p < 0.05$ to identify meaningful variations in rooting parameters, sugar content, vitamin C levels, and chlorophyll content resulting from the different treatments.

RESULTS AND DISCCUSION

The data summarized in Table 1 shows the *in vitro* rooting performance of grapevine microcuttings treated with various concentrations of Nn culture liquid and IBA.

Table 1: *In vitro* Rooting Performance of Grapevine Microcuttings Treated with Nn culture liquid and IBA

Treatment	Concentration ml/L for Nn culture liquid and mg/l for IBA	Rooting Percentage (%)	Number of Roots per Rooted Shoot Mean±SE	Average Root Length (cm) Mean±SE
Control	-	-	-	-
Nn culture liquid	0.5	53.0	2.2±0.2	5.2±0.1
Nn culture liquid	1.0	68.0	3.4±0.1	6.3±0.2
Nn culture liquid	1.5	81.0	5.3±0.2	11.4±0.2
Nn culture liquid	2.0	97.0	7.2 ±0.1	13.8±0.3
IBA	0.5	92.0	6.0±0.1	11.0±0.2
IBA	1.0	100	7.6±0.1	12.5±0.2
IBA	1.5	73.0	4.9±0.2(with callus)	6.6±0.3
IBA	2.0	54.0	1.0±0.1(with callus)	1.6±0.2

Notes: Rooting Percentage (%): Percentage of microcuttings that developed roots. Number of Roots per Rooted Shoot: Average number of roots per plantlet that developed roots. Average Root Length (cm): Average length of roots per rooted shoot.

Rooting Percentage and Root Number: The rooting percentage increased progressively with rising concentrations of Nn culture liquid, from 53.0% at 0.5 mL/L to 97.0% at 2.0 mL/L. For IBA, the highest rooting percentage (100%) was observed at 1.0 mg/L; however, higher concentrations resulted in reduced rooting percentages, with a significant drop to 54.0% at 2.0 mg/L. The number of roots per rooted shoot followed a similar trend, with Nn culture liquid treatments showing an increase in root numbers from 2.2 roots at 0.5 mL/L to 7.2 roots at 2.0 mL/L. IBA treatments peaked at 7.6 roots per rooted shoot at 1.0 mg/L, but higher concentrations led to a decrease, with only 1.0 root at 2.0 mg/L. Additionally, callus formation was observed in IBA-treated microcuttings at concentrations of 1.5 mg/L and 2.0 mg/L.

Root Length: Root length increased consistently with Nn culture liquid concentration, from 5.2 cm at 0.5 ml/L to 13.8 cm at 2.0 ml/L. IBA treatments showed a peak root length of 12.5 cm at 1.0 mg/L, but this decreased significantly at higher concentrations, falling to 1.6 cm at 2.0 mg/L. Table 2 provides data on the chlorophyll content (chl a, chlb, and total chlorophyll) in grapevine microcuttings treated with Nn culture liquid and IBA.

Table 2: Effects of Nn culture liquid and IBA on Chlorophyll Content in *in vitro* Cultured Grapevine Microcuttings

Treatment	**Concentration ml/L for Nn culture liquid and mg/l for IBA**	**Chl a (mg/g FW) Mean±SE**	**Chl b (mg/g FW) Mean±SE**	**Total Chlorophyll (mg/g FW) Mean±SE**
Control	-	1.20 ± 0.05	0.45 ± 0.02	1.65 ± 0.07
Nn culture liquid	0.5	1.35 ± 0.06	0.50 ± 0.03	1.85 ± 0.09
Nn culture liquid	1.0	1.40 ± 0.07	0.55 ± 0.04	1.95 ± 0.11
Nn culture liquid	1.5	1.50 ± 0.08	0.60 ± 0.04	2.10 ± 0.12
Nn culture liquid	2.0	1.55 ± 0.08	0.65 ± 0.05	2.20 ± 0.13
IBA	0.5	1.30 ± 0.06	0.50 ± 0.03	1.80 ± 0.09
IBA	1.0	1.45 ± 0.07	0.60 ± 0.03	2.05 ± 0.10
IBA	1.5	1.30± 0.04	1.48± 0.03	1.78± 0.07
IBA	2.0	1.10 ± 0.04	0.40 ± 0.01	1.50 ± 0.05

Chlorophyll Content: Microcuttings treated with Nn culture liquid showed a concentration-dependent increase in total chlorophyll content, reaching 2.20 mg/g at 2.0 ml/L. IBA treatments showed an increase in chlorophyll content at 1.0 mg/L (2.05 mg/g) but declined at higher concentrations, with the lowest total chlorophyll content of 1.50 mg/g observed at 2.0 mg/L. These results showed that the secondary metabolites of the culture liquid of Nn contributed to a sustained increase in chlorophyll content, while IBA had a peak effect at lower concentrations before declining. The obtained results suggest that, in addition to auxin-like

hormones, there may be other phytohormones from different groups among the secondary metabolites in Nn culture liquid.

Sugar Content: The sugar content in in vitro plants grown in media supplemented with Nn culture liquid and IBA was also measured. The control group exhibited a sugar content of 15.2 mg/g. Treatment with Nn culture liquid resulted in a gradual increase in sugar content, reaching 16.0 mg/g at 0.5 ml/L and 19.0 mg/g at 2.0 ml/L. In contrast, IBA treatments initially increased the sugar content to 16.5 mg/g at 1.0 mg/L, followed by a decline to 14.8 mg/g at 2.0 mg/L (Fig.1).

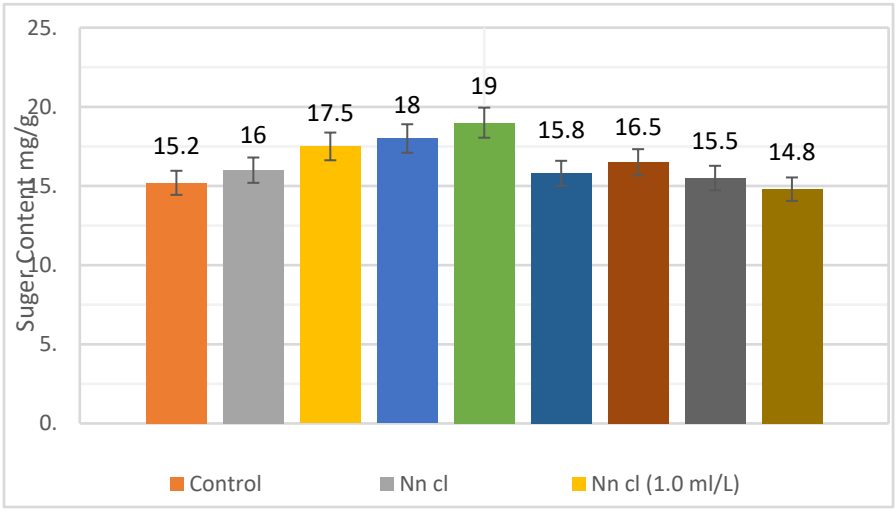


Figure 1. Effect of Nn culture liquid ml/L and IBA (mg/L) on the sugar content (mg/g fresh weight) in in *vitro*-derived grapevine plants. Data represents means ± standard error (n=3).

Vitamin C Content: Vitamin C content, as illustrated in Figure 2, increased progressively with Nn culture liquid treatment, starting at 13.0 mg/100g at 0.5 ml/L and reaching 15.5 mg/100g at 2.0 ml/L. In contrast, IBA treatment showed a variable effect, with vitamin C levels peaking at 13.5 mg/100g at 1.0 mg/L before declining to 12.0 mg/100g at 2.0 mg/L.

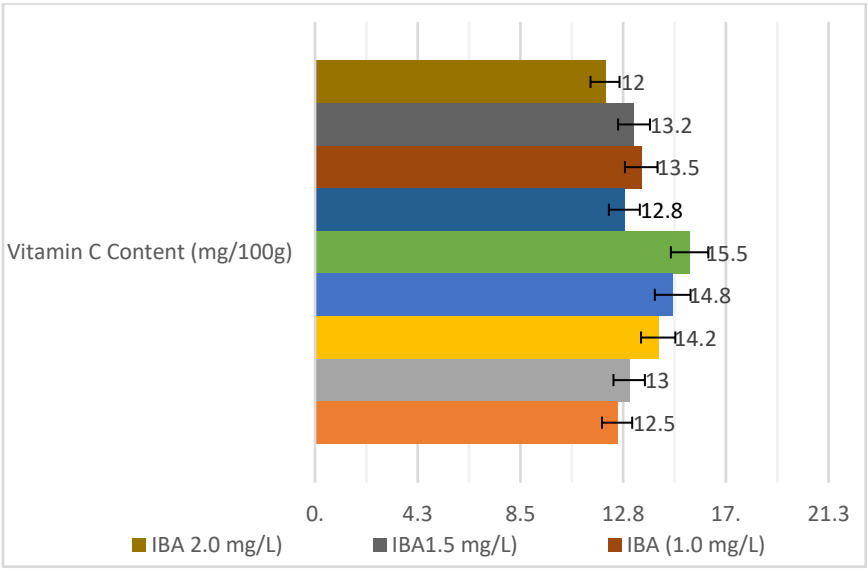


Figure 2. Effect of Nn culture liquid (ml/l) and IBA (mg/L) treatments on the Vitamin C content (mg/100 g fresh weight) in *in vitro*-derived grapevine plants. Data are presented as means ± standard error (n=3).

DISCUSSION

The Antarctic black yeast Nn is renowned for its ability to produce melanin, a pigment that not only offers protection against ultraviolet (UV) radiation but also exhibits strong antioxidant properties. This yeast thrives in cold, extreme environments, demonstrating psychrotrophic characteristics that allow it to grow at low temperatures. Its ecological role in nutrient cycling and organic matter decomposition positions Nn as a crucial organism within its habitat [35].

Melanins are pigments used in the food, cosmetic, and textile industries, produced by extraction from various biological sources, including the yeast *Nn* and *Streptomyces nashvillensis* [36]. Melanins derived from *Nn* are used in traditional medicine as an anti-cancer agent [37]. During the fermentation process, after the separation of melanin, a large amount of liquid remains. Typically, in many productions, after the separation of target components, the culture fluid is not utilized and is disposed of. This practice not only pollutes the environment but is also wasteful, as the culture fluid is known to be rich in biologically active components. It contains melanin-like compounds, peptides, phenolic compounds, free amino acids, sugars, and hormone-like substances. Therefore, it is both possible and beneficial to apply this culture fluid in scientific research, biotechnology, and plant cultivation.

Our study revealed a distinct, dose-dependent improvement in rooting performance as the concentration of Nn culture liquid increased. Rooting percentage, root number, and root length all showed significant improvements, with optimal results achieved at 2.0 ml/L. This suggested that Nn effectively promoted root initiation and elongation, potentially due to bioactive compounds in its culture liquid that might have stimulated plant growth pathways. These findings aligned with earlier studies, which indicated that microbial extracts could enhance root development by encouraging growth and mitigating stress responses in plants [29].

In contrast, IBA was highly effective at lower concentrations, particularly at 1.0 mg/L, where it achieved the highest rooting percentage and root count. However, at concentrations of 1.5 mg/L and above, noticeable callus formation adversely affected root development and elongation. These findings are consistent with previous research [38–42], which has shown that higher levels of auxins, such as IBA, can lead to increased callus formation and diminished rooting efficiency. This suggests that while IBA serves as a potent rooting agent at moderate concentrations, its effectiveness decreases at higher doses due to abnormal tissue growth and disruptions in hormonal balance. Auxin plays a vital role as a plant growth regulator, particularly in initiating new root formation [43].

The superior performance of Nn culture liquid at higher concentrations compared to IBA highlights its potential as an alternative to traditional auxins for the *in vitro* rooting of grapevine microcuttings. Nn culture liquid treated microcuttings achieved robust rooting without callus formation, suggesting it as a more reliable option for improving rooting and root quality, especially when higher concentrations were needed. This supported the growing interest in alternative rooting agents that offered better control over the rooting process.

Melanin and melanin-like substances play significant roles in the metabolism of plants, microbial, and animal cells. Specifically, melanin absorbs energy from light sources, excites electrons, and initiates a process that ultimately leads to the production of chemical energy, like how photosynthesis provides energy to plants. However, the exact role of melanin in this process remains unknown. Studies have shown that the enhancement of electron transfer properties by melanin does not depend on the energy of the incident photons. The authors of the study found in controlled *in*

vivo analyses that melanin has a remarkable ability to convert lower-energy radiation into a more useful form of energy [44].

Chlorophyll a and b are crucial for photosynthesis, providing insight into how efficiently plants convert light energy into chemical energy. Monitoring their levels is particularly important under in vitro conditions, where plants face stress from artificial growth environments. Elevated chlorophyll content often indicates better adaptation and photosynthetic efficiency, while reduced levels might signal stress. In this study, Nn culture liquid demonstrated a dose-dependent increase in chlorophyll content, with the highest values observed at 2.0 mL/L. This suggests that Nn culture liquid might enhance chlorophyll synthesis, thereby improving photosynthetic activity and overall plant growth *in vitro*. These findings are consistent with previous research by Mursalimov et al., 2022 [45] who noted that chlorophyll deficiency delays but does not prevent melanogenesis in barley seed melanoplasts.

In contrast, the response to IBA was more variable. Lower concentrations (0.5 and 1.0 mg/L) of IBA promoted chlorophyll production, peaking at 2.05 mg/g at 1.0 mg/L. However, higher concentrations (1.5 and 2.0 mg/L) resulted in reduced chlorophyll levels, indicating a possible inhibitory effect on chlorophyll synthesis or disruption of physiological processes due to altered hormone balance [46]. This suggested that there was a threshold beyond which IBA's benefits diminished or reversed. The effects of auxins, including IBA, on chlorophyll synthesis could vary significantly depending on concentration, plant species, and environmental conditions.

In recent years, there has been increasing focus on healthier diets and the development of products that promote well-being, which has drawn attention to grapevine leaves. Studies have highlighted the antioxidant properties of grapevine leaves, showing their effectiveness in defending against oxidative stress [47–48].

Vitamin C, or ascorbic acid, is renowned for its multifaceted role in human health, extending beyond its basic function as a vitamin. Its antioxidant properties are well-documented, playing a critical role in neutralizing free radicals and protecting cells from oxidative damage. This antioxidant activity is particularly significant in reducing the risk of chronic diseases, such as cardiovascular conditions and cancer [49]. The ability of Vitamin C to mitigate oxidative stress supports vascular health by maintaining endothelial function and preventing damage to blood vessels, which is crucial for cardiovascular disease prevention.

Furthermore, Vitamin C's anti-inflammatory effects contribute to its therapeutic potential. By modulating inflammatory pathways, Vitamin C helps manage chronic inflammation, a common underlying factor in various health conditions, including arthritis and certain types of cancer. Vitamins' role in cancer prevention and treatment is particularly intriguing. Studies have shown that Vitamin C can enhance the efficacy of certain cancer therapies and potentially reduce treatment side effects [50]. This aspect of Vitamin C's function underscores its potential as an adjunctive treatment in oncology.

The results also showed that the secondary metabolites of Nn culture liquid significantly increased both sugar and vitamin C content. As the concentration increased, both sugar and vitamin C content rose, reaching peak values at 2.0 mL/L. This suggests that the Nn culture fluid contributed to the synthesis and accumulation of these compounds, possibly due to its active components influencing metabolic pathways.

In plants, vitamin C also acts as a powerful antioxidant, absorbing reactive oxygen species that are mainly produced because of oxidative metabolism and photosynthesis. It also participates in the response to various stress factors [51]. In addition, together with

other antioxidant systems, such as the ascorbate-glutathione pair, it participates in regulating plant development processes by manipulating oxidative metabolism. Vitamin C is believed to effectively regulate antioxidant metabolism in plants [52], and endogenous levels of vitamin C can be increased through exogenous applications of ascorbic acid via foliar spraying, seed pre-treatment, or root medium application [53]. The enhancement of vitamin C synthesis may be attributed to key enzymes involved in the biosynthetic pathway, such as L-gulono-γ-lactone oxidase and GDP-L-galactose phosphorylase, which facilitate the conversion of glucose and other precursors into ascorbic acid [54]. Additionally, bioactive compounds like flavonoids and polyphenols, commonly found in fungal metabolites, are known to stimulate antioxidant pathways and enhance vitamin C levels [55]. Similar effects had been observed in other microorganisms, which were known to enhance the synthesis of bioactive compounds in plants [56-57]. Conversely, IBA's impact on sugar and vitamin C content was inconsistent. While initial concentrations (0.5 and 1.0 mg/L) showed beneficial effects, higher concentrations led to decreases in both sugar and vitamin C content. This variability might have indicated that IBA's effects were concentration-dependent or that higher concentrations could have inhibited specific metabolic processes [56-60].

Light intensity and temperature are the main environmental factors affecting plant growth and development [61]. However, under excessive lighting, excess electrons and excitation energy can be transferred to molecular oxygen, generating biologically damaging molecules such as reactive oxygen species (ROS), peroxides, and radicals, leading to photooxidative stress, which can ultimately result in cell death [62]. Under stress conditions, elevated levels of reactive oxygen species (ROS) are produced, which, in addition to their harmful effects, also exhibit signaling functions. In response to increased ROS production, various low-molecular-weight antioxidants and antioxidant enzymes are synthesized. It is possible that the better growth parameters and rhizogenesis of grapevines *in vitro* are associated with the presence of various natural antioxidants (water-soluble melanin, ascorbic acid, etc.).

Our findings indicated that water-soluble melanics and other bioactive compounds in the culture liquid of Nn positively influenced chlorophyll synthesis, photosynthesis, and ascorbic acid content, thereby enhancing the overall growth and development of grapevine microplants *in vitro*. These results aligned with prior studies showing that natural antioxidants could improve plant resilience and promote healthier growth under *in vitro* conditions [63].

Comparison with Other Studies*:* Studies on the biochemical composition of *in vitro* propagated plants often reported similar trends, where natural extracts or bio stimulants enhanced the nutritional quality of the plants.

CONCLUSION

The results of our studies demonstrated that the secondary metabolites from the culture liquid of Antarctic yeast *Nadsoniella nigra* (Nn) exhibited significant biological activity, suggesting their effective application in plant biotechnology *in vitro* for stimulating root and shoot system growth. Nn culture liquid served as a potent natural alternative to synthetic auxins like Indole-3-butyric acid (IBA) for promoting rooting in grapevine microcuttings, enhancing rooting efficiency while maintaining higher chlorophyll content and increasing both sugar and vitamin C levels. Although IBA proved effective at lower concentrations, it inhibited growth and induced callus formation at higher levels. The diverse effects of Nn culture liquid at various concentrations highlighted its potential advantages for

sustainable viticulture methods. Furthermore, in addition to acting as a phytostimulator, the melanin and associated metabolites in the Nn culture liquid may have enhanced the plants' immune responses against phytopathogens, functioning as antioxidants to protect cells from oxidative stress. Future research should focus on optimizing concentrations for enhanced growth and biochemical composition in various crops to deepen our understanding of their practical benefits in sustainable agriculture.

List of abbreviations: ANAU: Armenian National Agrarian University, AOAC: Association of Official Analytical Chemists, Chl a: Chlorophyll a, Chl b: Chlorophyll b, IBA: Indole-3-Butyric Acid, mg/L: Milligrams per Liter, mL/L: Milliliters per Liter, Nn: Nadsoniella nigra, RL: Root Length, RP: Rooting Percentage, SE: Standard Error, UV: Ultra Violet, WPM: Woody Plant Medium.

Competing interests: The authors declare that they have no competing interests.

Authors' contributions: GM, AS, YuM, AB drafted the experimental design. GM, NS, KD, YuM performed the experiments, GM, AB, KD, MZ helped in data collection, data analysis and initial draft of manuscript text. All authors read and agreed with the final version of the manuscript.

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