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Micropropagation of (*Vitis vinifera* L.) cultivar *'Sev Khardji'* using biotechnological approaches and its impact on leaf quality

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ABSTRACT

Background: The grapevine (*Vitis vinifera* L.) is an anciently cultivated plant species with significant economic importance as a fruit crop worldwide. Many studies have revealed that it contains various bioactive compounds. In Armenia, all current, existing vineyards are based on planting materials propagated traditionally, which means they are not free of diseases. Yield losses in plants due to viral-related diseases can reach up to 90.0%. Meristem culture represents the sole method for obtaining virus-free planting material from infected plants.

Objective: The study aimed to establish an effective meristem culture technique for the Armenian aboriginal grapevine (*Vitis vinifera* L.) cv. *'Sev Khardji'* and determine whether this technique influenced leaf quality.

Methods: Apical meristems served as explants for *in vitro* culture. The study employed various sterilizing agents and exposure durations to surface sterilize the explants. For shoot regeneration, the explants were cultured on full-strength Murashige and Skoog (MS) growth medium enhanced with various plant growth regulators (PGRs). In the process of *in vitro* root induction, different concentrations of two auxins, indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA),

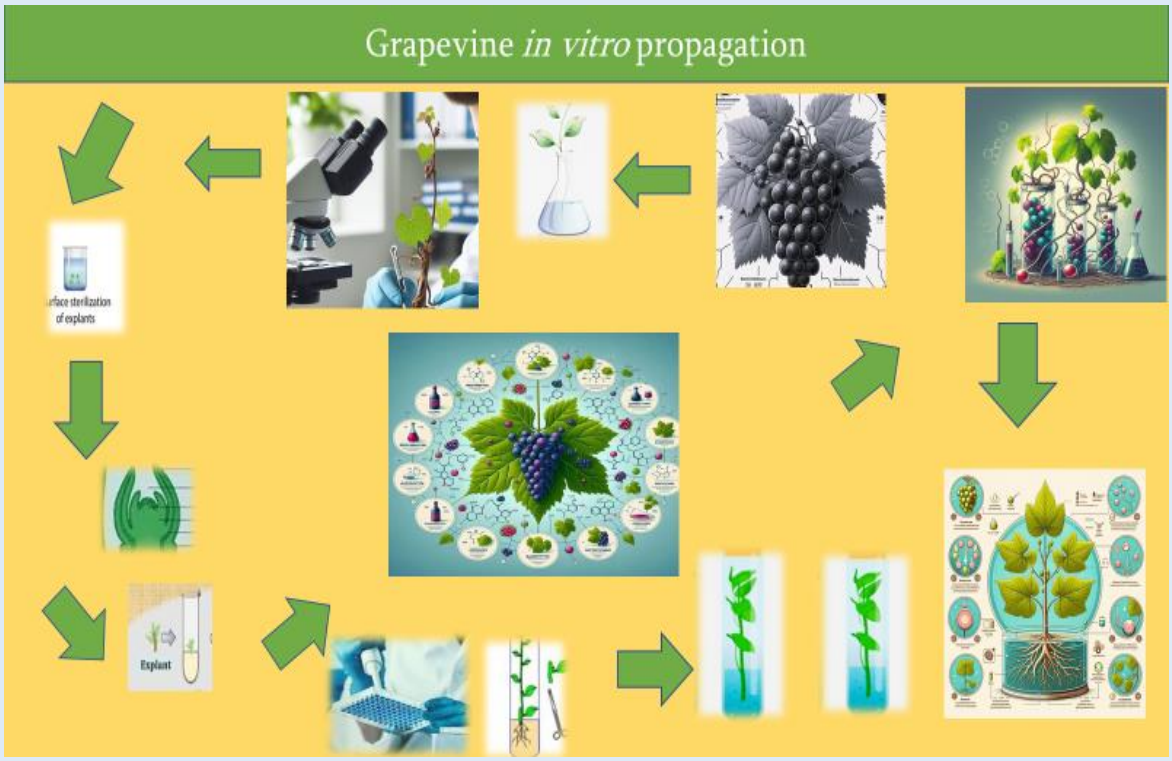
were added to the ½ MS basal medium. The research investigated the levels of sugars, organic acids, vitamin C, and mineral content in fresh grape leaves.

Results: A remarkable explant survival rate of 90.0% was achieved through a co-treatment involving 70% ethanol (v/v) for 10 seconds and 1.0% sodium hypochlorite (NaClO) for 15 minutes. The highest shoot regeneration success rate (100%) was observed on growth medium containing 1.0 mg/l of 6-Benzylaminopurine (BAP), in combination with 0.5 mg/l of Kinetin (Kin) and 1.0 mg/l of gibberellic acid (GA_3). Additionally, a 100% success rate in root development was obtained using the nutrient medium enriched with 1.0 mg/l IBA. *In vitro* plants stored at 18±1°C, under a light intensity of 50 µmol/m²·s and a 12-hour photoperiod, remained viable for 14 months without requiring subculture. Rooted plantlets were acclimatized using a perlite and biohumus substrate mixture (2:1), achieving a survival rate of 92.0%. Improving propagation methods can enhance the biochemical qualities of grapevine leaves, potentially amplifying the health benefits associated with grapes.

Conclusion: For the first time, a successful micropropagation protocol has been developed for the *´Sev Khardji´* grapevine cultivar. This protocol considers the impact of phytohormones and their concentrations on plant regeneration and root formation. Beyond facilitating mass propagation, it serves as a valuable method for *in vitro* preservation. By improving the quality of grape planting material through biotechnological methods, this protocol has the potential to increase the health benefits linked to grape consumption.

Keywords: *´Sev khardji´*, grapevine, explant, *in vitro* propagation, functional foods, micropropagation, virus-free

Graphical abstract: *micr*opagation of grapevine (*Vitis vinifera* L.) cv. *´Sev khardji´*, functional properties.



INTRODUCTION

As people become more aware of the connection between nutrition and health, they seek out products that provide additional benefits beyond basic nourishment. This demand from health-conscious consumers has led to the development of a new food category called functional foods, which are rich in bioactive substances that offer health advantages over simple nutrition and are rising in popularity [1-3].

Functional foods are natural or processed foods that contain biologically active compounds. These compounds, when consumed in specific, effective, and non-toxic amounts, offer clinically proven health benefits by targeting specific biomarkers. They promote optimal health, reduce the risk of chronic or viral diseases, and manage associated symptoms [4–5]. The core principle underlying functional foods is their incorporation of bioactive compounds, which can originate from various sources, including plants, mushrooms, and animals [6].

These bioactive compounds are found in small amounts across various functional foods, have antioxidative effects, and can help prevent diseases through physiological mechanisms [7]. Fruits and vegetables, with their rich content of vitamins, minerals, flavonoids, and anthocyanins, contribute to managing the symptoms of chronic diseases [8, 9]. Grapes and their derivatives are among the most promising sources of functional ingredients [10–11]. The grapevine (*Vitis vinifera* L.) is considered one of the most important crops globally [12]. Grapes are used not only for wine but also to produce fresh fruit, dried fruit, and juice [13]. The nutritional value and health benefits of grapes are undeniable. Naturally sourced grape products have been used for centuries [14–15]. Moreover, grapes are rich in bioactive compounds, such as proanthocyanidins, anthocyanins, flavonols, phenolic acids, stilbenes, and melatonin [16–18]. Most polyphenols, primarily proanthocyanidins, are found in grape seeds [19–20]. Grape skins are notably high in anthocyanins, while grape seeds contain very few anthocyanins [21–22]. Evidence from numerous studies suggests that grapes provide a range of health benefits, including antioxidant, anti-inflammatory, anti-cancer, cardioprotective, anti-asthma, and anti-viral effects [23–27]. *Vitis vinifera* L. leaves have traditionally been used as food in several nations and for treating hypertension [28], diarrhea, varicose veins [29], and diabetic blood glucose levels [30]. Grapevine leaves contain valuable bioactive compounds that contribute to the overall health benefits linked to grapevine consumption [31]. These compounds exhibit antioxidant properties, helping protect against oxidative processes [32].

Viticulture and winemaking in Armenia have a long history dating back thousands of years [33–34] and are among the most developed areas of Armenian agriculture. Armenia boasts a diverse selection of grape varieties, traditionally preserved in gene banks as whole plants in the field. Currently, all existing vineyards are based on planting materials propagated through traditional hardwood cuttings. Consequently, these vineyards are not free of diseases (fungal, bacterial, and viral), and the risk of infection with phylloxera and nematodes is high. Utilizing infected cuttings for the vegetative propagation of grapevines constitutes the primary mechanism for the long-range dissemination of grapevine virus diseases.

There is no chemical control against viruses and viroids globally; the only way to obtain virus-free planting material is through biotechnological methods. Biotechnology can reduce the use of fertilizers and inorganic pesticides in current agricultural production, improving soil, air, and water quality. Leveraging biotechnology can strategically lead to the development of crop varieties that are both high-yielding and stress-

tolerant [35–36]. Plant viruses lead to substantial losses in critical crops worldwide, impacting agricultural yield and product quality [37]. Approximately 80 grapevine viruses have been documented internationally, with varying effects on grapes across different countries [38]. These viruses cause a range of disease symptoms, from mild with little to no economic impact to severe, leading to reduced yield, delayed ripening, and even vine death [39]. The technology of plant tissue culture is widely used for large-scale plant propagation. Other than serving as a research tool, plant tissue culture techniques have recently gained significant industrial importance in plant propagation, disease elimination, plant improvement, and secondary metabolite production [40]. Disease-free plants are a practical application of biotechnology, specifically the micropropagation method [41]. Compared to traditional plant propagation techniques, biotechnological methods offer several advantages. For instance, under controlled conditions, many plants can be produced from a single individual in a relatively short amount of time and using less space [42–43]; additionally, plant propagation can occur year-round, regardless of the season [44].

The primary objective of this study was to create an effective *in vitro* method for propagating and conserving the grapevine cv. *'Sev khardji'* (*Vitis vinifera* L.) and assess its impact on leaf quality.

MATERIALS AND METHODS

The study took place at the Scientific Center of Agrobiotechnology, ANAU, and focused on the *in vitro* propagation and conservation of the grapevine cultivar *'Sev khardji'* from 2018 to 2023.

´Sev Khardji´ is an aboriginal, rare wine grape cultivar, seldom found as single vines within the vineyards of the Yeghegnadzor region of RA. It is characterized by its noir (black) berry skin and can be used to make strong, dessert, and red table wines. The red table wine is distinguished by its taste and pleasing acidity [45].

Green shoots measuring 10–15 cm from the indigenous wine grape cultivar *'Sev khardji'* were collected during the first decade of May for three consecutive years (2018, 2019, 2020). These shoots were obtained from the Armenian national field collection of grapevines, located at geographic coordinates 40.157419°N and 44.291986°E. The apical meristem was used as an explant for shoot culture initiation.

The stem segments were carefully rinsed with a mild detergent for approximately 2 minutes, followed by three washes with distilled water. Subsequently, the explants underwent surface sterilization with the following procedures performed within a laminar airflow chamber: (1) T_1: Immersion in 70% ethanol (v/v) for 10 seconds, followed by soaking in 1.0% sodium hypochlorite for 10 minutes, (2) T_2: Immersion in 70% ethanol (v/v) for 10 seconds, followed by soaking in 1.0% sodium hypochlorite for 15 minutes, and (3) T_3: Immersion in 70% ethanol (v/v) for 10 seconds, followed by soaking in 1.0% sodium hypochlorite for 20 minutes.

Regeneration: Following surface sterilization, the explants were cut into pieces measuring 10-20 mm using a surgical blade and then placed in tissue culture vessels (25 x 150 mm) containing 15 ml of growth medium. Each treatment involved 10 explants and was conducted three times, totaling 30 explants per treatment. After 12 days, data on contamination, mortality, and survival rates were collected. The explants were grown in MS medium enriched with varying concentrations of BAP, Kin, and GA_3, either individually or in a mixture, to promote shoot regeneration. After a six-week period, we recorded the shoot generation percentage, the number of shoots per explant, and the lengths of the shoots. Each treatment

was repeated three times, with 10 explants used for each repetition.

Root formation: Microshoots ranging in size from 3 to 5 cm, obtained from proliferated cultures, were transferred to MS/2 medium without any growth regulators or with varying concentrations of IBA and IAA (0, 0.5, and 1.0 mg/l) to induce root formation. Following six weeks of culture, we measured the root length (in centimeters), the number of roots per shoot, and the rooting percentage. The cultivation vessels were placed in a growth room with a 16/8-hour light/dark cycle at 24±2 °C and 50–60% relative humidity. The experiment was repeated three times, with 10 explants in each repetition. Additionally, we investigated the impact of different environmental conditions on *in vitro* plant growth using MS/2 medium supplemented with 25 g/l sucrose and 50 mg/l ascorbic acid. The plants were subjected to three growth regimes (15°C, 18°C, and 24°C), with a light intensity of 50 µmol/m^2*s and a photoperiod of 12/12 hours. The medium was adjusted to a pH of 5.8 before agar was added, and the media were solidified with 0.6% agar.

Acclimatization: *In vitro* plantlets with fully formed roots were extracted from the culture tubes and placed in 250 ml plastic pots containing: (1) biohumus:perlite (2:1) and (2) biohumus:perlite (1:1). To prevent excessive water loss, a transparent plastic cup was placed on each plantlet. The pots were set up in the acclimatization room with a 16/8 (L/D) light/dark photoperiod, an air temperature of 24±2 °C, and a humidity of 70±5%. Ten to twelve days after planting, when new leaves began to appear, the plastic bags were gradually removed from the pots for proper hardening. Successfully acclimatized plantlets in the culture room were subsequently transferred to the greenhouse.

The research examined the biochemical characteristics of grapevine cv. '*Sev Khardji*' leaves, comparing plants propagated through *in vitro* (virus-free) techniques with those propagated through cutting techniques.

Both groups were grown in an aeroponic system, and the study assessed sugar content, organic acids, and ascorbic acid levels in the leaves collected between 2021 and 2023. Iodine titration was used to determine the ascorbic acid content [46]. Sugars were measured using a modified approach reported by Melgarejo et al. (2000) [47]. Acid measurement was performed using the high-performance liquid chromatography (HPLC) method [48]. The elemental composition of grape leaves was analyzed using the X-ray fluorescence analysis method [49].

Statistical analysis: Data from three different experiments were pooled and shown as mean values. Treatment means were compared using the standard error (SE) of the mean. A Student's t-test was performed to identify significant differences between the means ($P<0.05$).

RESULTS

Explant survival rates were highest in T2 (90.0%) and T3 (75.0%), while T1 exhibited a lower survival rate of 40.0%. No explant regeneration responses were observed when cultured on MS medium without growth regulators (control). The impact of different concentrations of various PGRs on shoot regeneration is documented in Table 1. As shown in Table 1, regeneration occurred in all treatments containing plant growth regulators. Shoot lengths ranged from 1.0 to 2.4 cm, with an average of 1.4 to 3.2 shoots per explant. Increasing the cytokinin concentration from 0.5 mg/l to 1.0 mg/l resulted in more buds per explant and greater shoot height.

Table 1. Effects of different concentrations of various PGRs on shoot regeneration

Concentration of PGRs	Regeneration (%)	Mean number of shoots/explants (Mean ± SE)	Mean of shoot length (cm) (Mean ± SE)
Control (without any growth regulator)	00	-	-
0.5 mg/l BAP	80	1.9 ± 0.1^{d}	1.4 ± 0.1^{c}
0.5 mg/l Kin	85	1.4 ± 0.1^{e}	1.0 ± 0.2^{d}
1.0 mg/l BAP	90	2.2 ± 0.1^{c}	1.8 ± 0.1^{b}
1.0 mg/l Kin	85	1.7 ± 0.1^{d}	1.5 ± 0.2^{c}
0.5 mg/l BAP + 0.5 mg/l Kin + 0.5 mg/l GA_3	95	2.6 ± 0.2^{b}	2.0 ± 0.1^{b}
0.5 mg/l BAP + 0.5 mg/l Kin + 1.0 mg/l GA_3	100	3.2 ± 0.2^{a}	2.4 ± 0.2^{a}

Shoot production per explant ranged from 1.9 to 2.2 with BAP concentrations of 0.5–1.0 mg/l and from 1.4 to 1.7 with Kin at the same concentrations. The most effective direct shoot organogenesis occurred with MS medium supplemented with 0.5 mg/l BAP, 0.5 mg/l Kin, and 1.0 mg/l GA_3 ($P < 0.05$), resulting in 100% shoot formation, 3.2 shoots per explant, and a shoot length of 2.4 cm. This was followed by the combination of 0.5 mg/l BAP, 0.5 mg/l Kin, and 0.5 mg/l GA_3. The *in vitro* rooting experiment demonstrated that among the tested auxin concentrations (ranging from 0.5 to 1.0 mg/l), IBA at 1.0 mg/l was the most effective for promoting rooting, achieving 100% root induction and an average of 5.6 roots per explant.

The rooted *in vitro* plants were successfully acclimatized to a substrate mixture of perlite and biohumus (2:1), presenting the best results with a survival rate of 92.0%. About 86% of the *in vitro* plants were successfully stored for 14 months at a temperature of 18±1 °C, a light intensity of 50 µmol/m²*s, and a 12-hour photoperiod without subculture. The results of the vitamin C content in the leaf of the *in vitro*-derived grape cultivar *´Sev Khardji´*, compared to those propagated by cuttings, are illustrated in Figure 1.

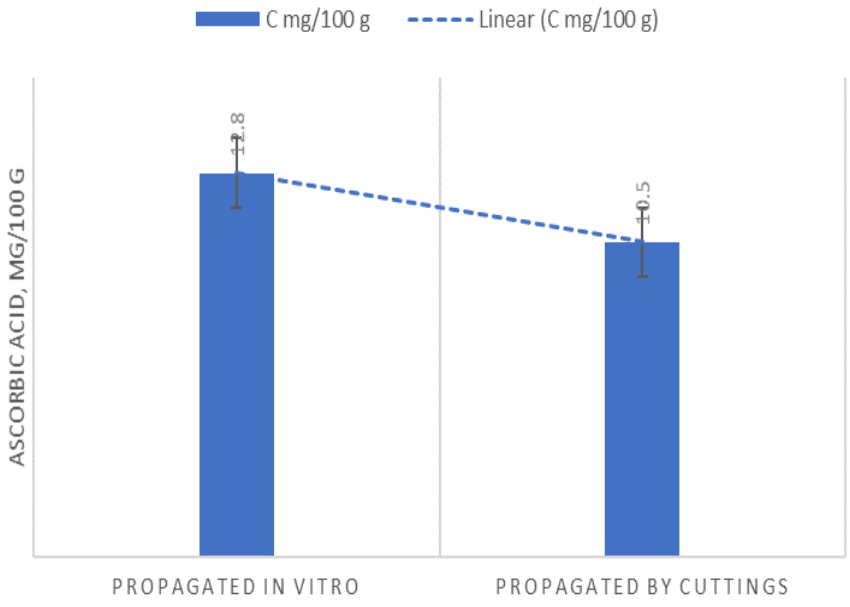


Figure 1. Vitamin C content in leaves of the *´Sev Khardji´* grape cultivar propagated *in vitro* and by cuttings.

As shown in Figure 1, the leaf quality obtained from the *in vitro*-derived grape cultivar ´*Sev Khardji* ´exhibited the highest concentration of ascorbic acid (AA), measured at 12.8±0.4 mg/100 g. Notably, this represents a 21.9% increase in AA concentration compared to the same cultivar propagated by cuttings. Additionally, we analyzed the sugar content in grape leaves of the ´*Sev Khardji*´ cultivar, comparing those propagated *in vitro* with those propagated by cuttings (Figure 2).

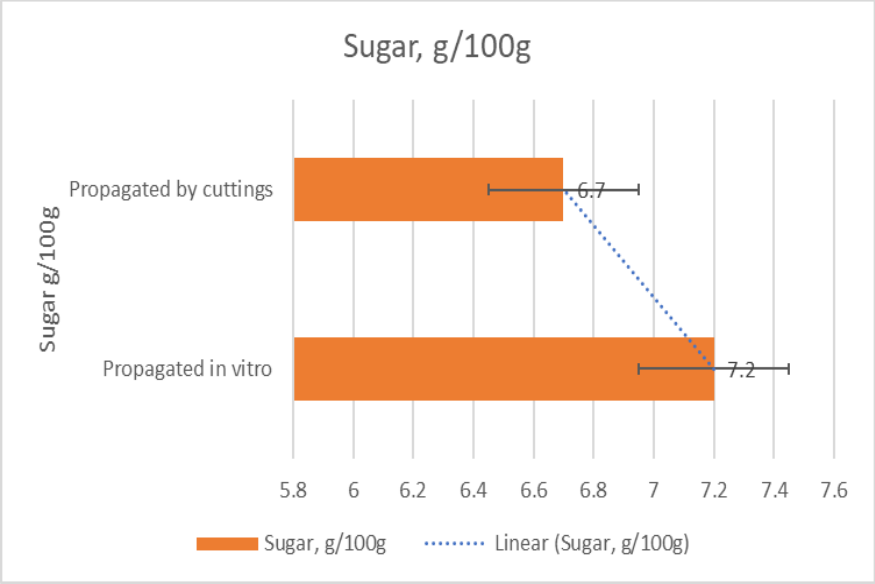


Figure 2. Sugar content in grape leaves of the ´*Sev Khardji*´ cultivar propagated *in vitro* and by cutting.

As illustrated in Figure 2, the leaves of the ´*Sev Khardji*´ grape cultivar propagated *in vitro* had a higher sugar content (7.2 g/100 g) compared to those propagated by cuttings (6.7 g/100 g). Moreover, the content of organic acids in the local wine grape cultivars ´*Sev Khardji*,´ propagated both *in vitro* and conventionally, is detailed in Figure 3.

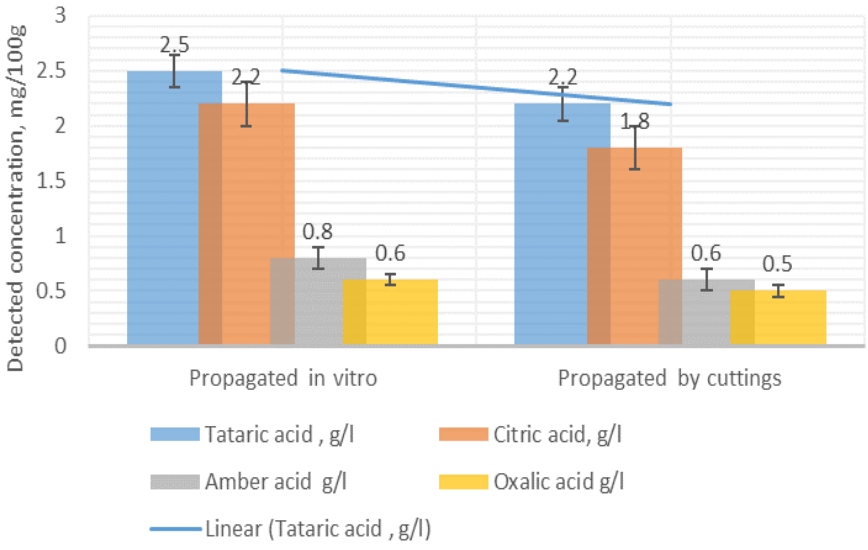


Figure 3. The content of organic acids in the leaves of the local wine grape cultivar ´Sev Khardji´, propagated in vitro and conventionally.

The acid composition of fresh leaves from the grape cultivar *´Sev Khardji´* exhibited significant differences between *in vitro* propagation and propagation by cuttings ($p < 0.05$).

Figure 3 illustrates a comparison of acid content—specifically tartaric acid, citric acid, amber acid, and oxalic acid—in the leaves of the grapevine cultivar *´Sev Khardji´* using two different propagation methods. As seen in Figure 3, grapevine leaves contain the highest amount of tartaric acid, followed by citric acid, with smaller amounts of amber acid and oxalic acid. In comparison to cuttings, *in vitro* propagation results in increased concentrations of all four acids.

Table 2 provides detailed information on the chemical characteristics of fresh leaves from the *'Sev Khardji'* grapevine cultivar. Notably, the mineral composition of these leaves exhibited significant differences ($p < 0.05$), regardless of whether they were propagated *in vitro* or through cuttings.

Table 2. Mineral Composition in Fresh Grapevine Leaves.

Minerals	**Cultivar *´Sev Khardji´***	
	multiplied *in vitro*	**multiplied by cuttings**
Potassium (K)	287.74 ± 2.06	262.74 ± 3.06
Phosphorus (P)	93.25 ± 1.09	85.28 ± 1.10
Calcium (Ca)	339.7 ± 1.30	330.2 ± 1.22
Magnesium (Mg)	94.9 ± 0.50	90.2 ± 0.60
Sodium (Na)	9.32 ± 0.02	8.1 ± 0.03
Iron (Fe)	2.77 ± 0.22	2.47 ± 0.10
Zinc (Zn)	0.68 ± 0.01	0.61 ± 0.01
Manganese (Mn)	2.95 ± 0.01	2.46 ± 0.01
Copper (Cu)	0.46 ± 0.01	0.36 ± 0.01

The results were given in mg/100g.

As shown in Table 2, in vitro propagation significantly influences the mineral composition of grapevines, leading to variations in several essential elements. Analysis of fresh grapevine leaves revealed that potassium (K) was the most abundant mineral across all samples. Leaves from *in vitro*-propagated plants exhibit approximately 25 mg/100g more potassium and 8 mg/100g higher phosphorus content compared to traditionally propagated vines. Additionally, *in vitro*-propagated vine leaves have higher levels of calcium, magnesium, sodium, iron, zinc, manganese, and copper content. These variations influence vine health, fruit quality, and ultimately the resulting wine. For example, higher potassium levels in *in vitro*-propagated vines may affect grape flavor, while differences in iron content can influence color stability.

DISCUSSION

Propagation for the grapevine (*Vitis vinifera* L.) cultivar *´Sev Khardji´* using axillary bud explants was established. The success of biotechnological plant propagation systems depends largely on the control and prevention of microbial contamination. Fungal and bacterial contamination of grapevine explants taken from the field is a serious problem. Sterilization is critical because bacteria and fungi can continuously contaminate and threaten the plant culture during the cultivation period. Therefore, disinfection of the explant surface is the first

main stage in their culture formation. Three different immersion times (10, 15, and 20 min) and the effect of 1.0% NaClO on explant surface sterilization were evaluated: (1) T_1: Explants were briefly treated with a 70% (v/v) ethanol solution for 10 seconds, followed by immersion in a 1.0% sodium hypochlorite solution for 10 minutes, (2) T_2: Similarly, explants underwent a 70% (v/v) ethanol treatment for 10 seconds, followed by a 15-minute immersion in a 1.0% sodium hypochlorite solution, (3) T_3: In this case, explants were treated with a 70% (v/v) ethanol solution for 10 seconds and then immersed in a 1.0% sodium hypochlorite solution for 20 minutes. The highest survival rate (90.0%) was obtained by immersing in 1.0% sodium hypochlorite for 15 minutes after treating with 70% (v/v) ethanol for 10 seconds. This combination proved to be the most successful in preventing contamination. Our results showed that as the exposure time increased, the infection decreased, but some explants died due to prolonged exposure. This result conforms to the report by Birhan et al. 2021 [50], which stated that sodium hypochlorite (1.0%) at 15 minutes of exposure time showed 100% survival of Ethiopian yam explants.

After four weeks of culture, the effects of PGR were measured in terms of the quantity of shoots per explant and the length of the shoots. The investigated plant growth regulators had a considerable impact on the grapevine meristem's *in vitro* regeneration. On MS media treated with several plant growth regulators at varying doses and combinations, the explants displayed a range of responses (Table 1). It is well established that PGRs play a critical role in the formation of an organogenesis-based *in vitro* propagation system in many plant species. Our research revealed that while regeneration happened when PGRs were applied, the explants grown on MS medium without growth regulators—which acted as a control—did not encourage the beginning of axillary buds, which ultimately turned necrotic. On MS medium supplemented with different plant growth regulators at various concentrations and combinations, the explants displayed a variety of responses. It was discovered that the BAP-Kin-GA_3 combination produced the highest mean number of shoots per explant, the highest shoot length, and the largest percentage of organogenesis. Our results showed that BAP and Kin, along with 1.0 mg/l GA_3, enhanced shoot elongation. Many studies have also reported the beneficial impact of GA_3 on shoot growth *in vitro* [51–52]. Compared to Kin, BAP exhibited greater efficacy in direct shoot regeneration, consistent with findings in numerous other plant species [53–55].

The effectiveness of tissue culture relies on the shoot's ability to root. It is widely recognized that the external application of auxins plays a crucial role in rhizogenesis [56–57]. The absence of auxin prevented any rooting from occurring in the grapevine cuttings, indicating that auxin is necessary for rooting to occur. According to the current study's findings, as IBA and IAA concentrations increased from 0.5 to 1.0 mg/l, so did the number of roots and the root length. The percentage of root development is highly influenced by the type and concentration of auxin utilized. The best root responses (100%) were found in ½ MS medium + 1.0 mg/l IBA, with a high mean value of 5.6 roots per explant. The minimum growth method is a suitable strategy for the international exchange of plant germplasm. Using an MS/2 medium, the effects of environmental factors on the growth of *in vitro* plants were examined.

Maintaining *in vitro* plants at 18±1°C, with 50 µmol/m2*s light and a 12-hour photoperiod, sustained their viability for 14 months without requiring subculture. The low percentage of plant acclimatization poses a significant challenge for large-scale commercial *in vitro* reproduction. Ensuring precise control of climatic conditions in adaptation rooms—such as maintaining an effective and high-quality substrate, balanced mineral nutrition, optimal light spectral composition and intensity, and appropriate relative humidity and carbon

dioxide levels—is crucial for obtaining healthy, uniform, and high-quality planting material.

With a survival percentage of 92.0%, the plants that were rooted *in vitro* produced exceptional results after successful acclimation to a substrate blend of perlite and biohumus (in a 2:1 ratio).

The results obtained from grapevine *in vitro* plant acclimatization are consistent with previous studies [58], which found that the perlite substrate is optimal for ensuring high survival rates in many other plant species. All the *in vitro*-derived transplants displayed the same normal development as the mother plants.

In this study, certain plants obtained through *in vitro* and conventional non-sterile cutting methods were transferred to an aeroponic system for leaf biochemical analysis. The aeroponic method facilitates a smoother transition from *in vitro* to *ex vitro* environments, allowing continuous monitoring of physiological processes during seedling growth and development. Moreover, experiments can be conducted under controlled and reproducible conditions [59].

In recent years, the growing interest in healthier eating and the emergence of health-promoting products have brought grapevine leaves into the spotlight. Researchers have described grapevine leaves as an effective antioxidant [60–61]. Ascorbic acid (AA), also known as vitamin C, plays multiple essential roles in the body. It acts as an antioxidant, supports vascular health, reduces inflammation, helps prevent cancer, serves as a co-factor in enzymatic reactions, and is used in the food industry to prevent the formation of harmful compounds [62].

One popular use of grape leaves in food is the ancient Armenian national dish called tolma. Tolma typically consists of ground meat and rice tightly wrapped in grape leaves, which can be either fresh or fermented and salted.

In this study, the vitamin C content of leaves from the *´Sev Khardji´* grape cultivar (both *in vitro* and cutting-grown plants) was investigated under aeroponic conditions. Our findings revealed that the *´Sev Khardji´* cultivar propagated *in vitro* exhibited a higher vitamin C content (12.8 mg/100 g) compared to those propagated by cuttings (10.5 mg/100 g). These results suggest that the *in vitro* propagation method positively influences the vitamin C levels in the grape leaves.

Sugars are produced in leaves through photosynthesis and then transported to the grape berries in the form of sucrose. Any hindrance in the breakdown of sucrose can negatively impact the sugar levels and overall composition of the grape berry, affecting its quality [63]. Our research indicated that *in vitro*-propagated grapes had a higher sugar content compared to cutting-propagated grapes, suggesting that *in vitro* propagation might influence sugar metabolism and composition in grape leaves.

The uptake and accumulation of mineral elements from the substrate not only significantly impact grapevine growth, development, and health but also influence the sensory characteristics of the resulting wines [64].

Organic acids, whether aliphatic or aromatic, exhibit diverse structures and are widely distributed in plants. These compounds play essential roles in various biological and pharmacological processes [65]. In our study, the *in vitro* propagated variant of the *´Sev Khardji´* grapevine exhibited higher levels of acids compared to those propagated by cuttings. These differences may have implications for the health-related properties of grapevine leaves.

It should be noted that propagating grapevines recovered from phytopathogens using meristem technologies offers additional advantages. Many winemakers face challenges associated with the uneven ripening of grapes on a single bush. When grapes ripen unevenly on the bunch and vine, a significant proportion of unripe and/or overripe fruits may be present at harvest time [66–67]. This fruit heterogeneityaffects the

composition of metabolites, potentially impacting the style and quality of wine [68] and thereby creating technical problems for winemakers. These differences may also be important for the properties of grapevine leaves associated with the improvement of planting material. We are convinced that the uniformity of healthy planting material will significantly reduce the heterogeneity of fruit ripening between grape bushes, improving the quality and quantity of fruits.

Our studies found that *in vitro* propagation resulted in higher mineral levels compared to traditional propagation by cuttings. Notably, plants cultured *in vitro* exhibited increased levels of potassium (K), phosphorus (P), calcium (Ca), and magnesium (Mg). The analysis of the mineral composition of the studied grape cultivar provides intriguing insights into how the method of grape propagation impacts the elemental content of the grape leaves.

Different propagation methods can result in differences in the absorption and distribution of mineral elements in the leaves, which ultimately affects their overall composition and quality.

The cultivation of grapes through plantation methods is significantly more efficient when using planting material that is free from phytopathogens and synchronized with growth parameters. This effectiveness is evident in the enhancement of grape leaf quality, where essential macro- and microelements, sugars, vitamins, and other secondary metabolites—beneficial antioxidants for the human body—accumulate.

By optimizing propagation methods, we can enhance the biochemical properties of grapevine leaves, potentially amplifying the health benefits associated with grapes.

One promising avenue for future research involves conducting field studies under various climatic conditions to assess how fruit quality and functional food properties may vary. Additionally, researchers should consider other factors that could impact grapevine leaves, such as the presence of phenolic compounds in *´Sev Khardji´* leaves. By optimizing yield and understanding these contributing factors, we can enhance the overall quality of the final product, leading to increased nutritional value.

CONCLUSION

A method for the successful micropropagation of true-to-type plants of the Armenian grapevine cultivar *'Sev Khardji'* through direct organogenesis has been established for the first time. The specific phytohormones used and their concentrations significantly influenced plant regeneration and root development efficiency. This approach is both practically and theoretically significant, supporting year-round, large-scale production, germplasm conservation, and advancements in *in vitro* culture techniques.

Furthermore, the study demonstrated that leaves from both *in vitro* and conventionally propagated plants of the local *'Sev Khardji'* grape cultivar, grown under aeroponic conditions, contain valuable bioactive compounds. Notably, plants propagated *in vitro* exhibit higher levels of these beneficial compounds. By enhancing grapevine biochemical properties through biotechnological propagation methods, there is potential to enhance fruit quality and yield while reducing pesticide dependency. Given the increasing consumer demand for nutritious, sustainably produced fruits, these findings could benefit both producers and consumers.

List of Abbreviations: ANAU: Armenian National Agrarian University, BAP: 6-Benzylaminopurine, GA_3: Gibberellic Acid, IAA: indole-3-Acetic Acid, IBA: indole-3-butyric acid, Kin: Kinetin, MS: Murashige and Skoog, PGRs: Plant Growth Regulators, NaClO: sodium hypochlorite

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experiments; AB: Assisted with data collection, data analysis, and contributed to the initial manuscript draft; NS: Performed the experiments; KD: Drafted the experimental design and conducted the experiments; KS: Assisted with data collection, data analysis, and contributed to the initial manuscript draft. All authors reviewed and approved the final version of the manuscript.

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