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STRATEGIC ADJUSTMENT OF NITROGEN COMPOUNDS IN THE IN VITRO CULTIVATION OF SHIRVAN SHAHI (*Vitis vinifera* L.)

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Abstract. This study investigates the effects of varying concentrations of nitrogen components - specifically potassium nitrate (KNO_3) and ammonium nitrate (NH_4NO_3) - on the *in vitro* growth dynamics of the Shirvan Shahı grapevine cultivar (*Vitis vinifera* L.). Using Murashige and Skoog (MS) medium as the base, different modifications of nitrogen content were tested individually and in combination. The results showed that a 3/4 concentration of combined NH_4NO_3 and KNO_3 significantly enhanced shoot length (5.20 cm), leaf number (7.2) and biomass (560 mg), outperforming the full MS control. In contrast, the lowest concentrations led to decreased vegetative parameters. The findings confirm that not only the presence but also the balance and form of nitrogen are critical for optimal morphogenesis in grapevine micropropagation. The synergy between ammonium and nitrate at moderate levels proved to be the most effective approach for stable *in vitro* development. These results contribute to optimizing nitrogen formulation in culture media, offering practical guidance for improving clonal propagation protocols of sensitive grapevine cultivars.

Keywords: *Vitis vinifera L., Shirvan Shahı cv, ammonium nitrate (NH_4NO_3), potassium nitrate (KNO_3), micropropagation.*

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1. Introduction

Both macro- and micronutrients are essential for plant growth and development and the mineral composition of any nutrient medium must be present in appropriate proportions (George *et al*., 2008). Nitrogen (N) is a critical macronutrient for plant life, being an integral component of proteins and nucleic acids, as well as a constituent of chlorophyll. In grapevines, nitrogen significantly influences both vegetative and reproductive development (Conradie, 2005a; Grechi *et al*., 2007). It is the most demanded element by plants and depending on its concentration in the vine/soil system, it affects both grape yield and wine quality. Excessive nitrogen levels can stimulate vine vigor but may reduce the quality of grapes and wine. Conversely, nitrogen deficiency can severely restrict vine growth, particularly in key developmental stages ranging from budbreak to flowering (Conradie, 1991; Grechi *et al*., 2007). In technical grape cultivars, nitrogen concentration is generally highest in the leaves of lateral (secondary) shoots, followed by those in primary shoots, roots and cane leaves (Grechi *et al*., 2007; Amorós *et al*., 2013).

However, data regarding nitrogen content in the organs of table grape varieties remain limited.

In tissue culture systems, both plant growth and morphogenesis are significantly influenced by the availability and chemical form of nitrogen (N). Nitrogen is typically supplied to culture media in the form of potassium nitrate (KNO_3) and ammonium nitrate (NH_4NO_3). It has been widely proposed that the primary nitrogen sources for plants are nitrate (NO_3^-) and ammonium (NH_4^+) ions (Conradie, 2005b; Amorós *et al*., 2013). Under aerobic soil conditions, nitrate serves as the predominant nitrogen source for most plant species, especially agriculturally important crops (Poothong & Reed, 2016). In addition to serving as a nutrient, nitrate also plays a regulatory role in numerous physiological processes (Ho & Tsay, 2010).

Compared to ammonium uptake, nitrate assimilation is energetically more demanding (Tho *et al*., 2017; Liu *et al*., 2014) and is generally associated with an alkalizing effect in the rhizosphere (Saiz-Fernández *et al*., 2017). In contrast, ammonium assimilation is more energy-efficient due to its lower metabolic cost. Nitrogen metabolism in plants encompasses the uptake, transport and reduction of nitrates (NO_3^-) and ammonium (NH_4^+), as well as their assimilation into organic forms such as amino acids; these processes are regulated by light, phytohormones, carbon status and nitrate-related signaling pathways. Nitrates are reduced to ammonium via the action of nitrate reductase and nitrite reductase enzymes, after which ammonium is incorporated into organic compounds through the glutamine synthetase and glutamate synthase (GS/GOGAT) pathways. In grapevine (*Vitis vinifera* L.), nitrogen plays a crucial role in maintaining the balance between vegetative and reproductive growth, influencing berry quality and supporting the biosynthesis of secondary metabolites; optimal nitrogen levels are essential for achieving high yield and quality. Under in vitro micropropagation conditions, optimizing the NO_3^-/NH_4^+ ratio enhances morphogenesis and shoot formation, with nitrates promoting IPT gene expression, thereby stimulating cytokinin biosynthesis and facilitating cell regeneration (Kiba *et al*., 2011).

However, many crop species exhibit sensitivity to ammonium toxicity, particularly when ammonium is supplied as the sole nitrogen source or in excessive concentrations (typically 0.1-0.5 mM) (Salsac *et al*., 1987). Furthermore, ammonium uptake can lead to acidification of the medium and excessive ammonium supply often causes ionic imbalances, which negatively affect plant growth and development (Guo *et al*., 2007; Stitt *et al*., 1999).

In general, most plant species exhibit optimal growth when both nitrate and ammonium forms of nitrogen are simultaneously available (Britto & Kronzucker, 2002). Accordingly, nitrate (NO_3^-) and ammonium (NH_4^+) are commonly used as inorganic nitrogen sources in plant tissue culture media (Bresinsky *et al*., 2013). The most widely adopted medium for a broad range of plant species is the Murashige and Skoog (1962) medium, which is characterized by a relatively high inorganic nitrogen concentration. In this formulation, the total nitrogen content typically reaches 60 mM, with an approximate nitrate-to-ammonium ratio of 2:1 (George *et al*., 2008).

However, for certain plant species and culture systems, the nitrogen concentration in the MS medium can exceed the actual requirements for optimal *in vitro* development, resulting in unnecessary nitrogen accumulation and wastage. Additionally, in some formulations, the nitrate-to-ammonium ratio may not be optimal for efficient nutrient assimilation or physiological balance in the cultured tissues.

Developing effective plant tissue culture protocols remains a complex and multifactorial challenge, as it requires the simultaneous optimization of numerous interacting variables. These include the source and physiological status of the plant material, physical and chemical culture conditions and media composition - particularly the balance of inorganic and organic nutrients, such as carbohydrates, vitamins and plant growth regulators. The success and quality of any given plant tissue culture outcome are intricately linked to these parameters, each of which must be tailored to the specific requirements of the plant species and the intended micropropagation objective.

In vitro plant tissue culture success is largely dependent on the mineral composition of the culture medium, as these elements play a critical role in supporting optimal morphogenesis and organogenesis (Ramage & Williams, 2002; George *et al*., 2008; Sonnewald, 2013). An imbalance or deficiency of essential mineral nutrients in the medium may result in physiological disorders, toxic effects and reduced survival rates of cultured explants (Bresinsky *et al*., 2013; Nezami-Alanagh *et al*., 2019).

In the case of *Vitis vinifera L*., particularly technical (wine) grape cultivars, the quantity and chemical form of nitrogen in the nutrient medium are decisive factors for successful micropropagation under *in vitro* conditions. Nitrogen is a vital macronutrient involved in key cellular processes such as cell division, protein and nucleic acid biosynthesis and chlorophyll formation, thereby directly influencing morphogenic and organogenic outcomes (Conradie, 2005a; George *et al*., 2008).

However, excessively high or imbalanced nitrogen concentrations *in vitro*, especially in technical grape cultivars, have been associated with adverse effects such as hyperhydricity, poor root development and abnormal callus proliferation (Ramage & Williams, 2002; Nezami-Alanagh *et al*., 2019). Consequently, the optimization of nitrogen components is of crucial importance for achieving efficient clonal propagation of such cultivars in controlled culture systems.

The present study aims to evaluate the impact of different concentrations of nitrogen components (NH_4NO_3 and KNO_3) in Murashige and Skoog (MS) medium on the *in vitro* growth dynamics of the 'Shirvan Shahi' grapevine cultivar.

2. Materials and Methods

Sample Preparation. Grape samples of the *Vitis vinifera L.* cultivar 'Shirvan Shahi' were collected from vineyards located in the Kurdamir region of Azerbaijan. The branches collected from field-grown plants were used in accordance with the in vivo propagation protocols described by Garagözov (2004). The efficient *in vitro* propagation of grapevines has recently become a major focus in the fields of agriculture and biotechnology. Experimental efforts aimed at optimizing morphogenesis and organogenesis were conducted at the Biotechnology Laboratory of the Institute of Molecular Biology and Biotechnology, under the Ministry of Science and Education of the Republic of Azerbaijan. To achieve the study's objectives, modifications were made to the concentrations of the main nitrogen sources - ammonium nitrate (NH_4NO_3) and potassium nitrate (KNO_3) - present in the Murashige and Skoog (MS) medium. Based on these variations, a total of nine distinct nutrient media formulations were prepared (Tables 1, 2 and 3). The standard MS medium served as the control treatment. All media were supplemented with 1 ml BAP, 100 mg/L inositol, 30 g/L sucrose and 0.8% agar, with the pH adjusted to 5.6-5.8 before sterilization at 121°C in an autoclave. This experimental

setup was designed to investigate the stage-specific effects of nitrogen form and concentration on the *in vitro* development of grape cultivars.

Sterilization Procedure. The explants were surface sterilized by immersion in 70% ethanol for approximately 10 seconds under a laminar flow hood, followed by shaking in 5% sodium hypochlorite solution for 20 minutes. Three drops of Tween 20 were added to every 100 mL of hypochlorite solution. After sterilization, explants were rinsed three times with sterile distilled water. Forceps and dissection blades were dipped in absolute ethanol and flamed before and after each use to maintain aseptic conditions.

Nitrogen Modifications. To assess the impact of nitrogen availability, a total of nine nutrient media variants were prepared by varying the concentrations of KNO_3 and NH_4NO_3, both individually and in combination. These variants included the standard MS medium as a control. This approach is of scientific relevance for evaluating how different forms and concentrations of nitrogen affect *in vitro* developmental stages of grapevines.

Culture Conditions. All cultures were incubated under controlled environmental conditions in a growth chamber set to a photoperiod of 16/8 hours (light/dark) with illumination intensity of 1500-2000 lux, provided by white fluorescent lamps. The ambient temperature was maintained at 23 ± 2°C. After an initial incubation period of 28 days, microshoots were transferred to nitrogen-modified nutrient media and cultured for an additional 28 days. Upon completion of this second incubation phase, key morphophysiological parameters were measured and the results were subjected to comparative analysis. For the assessment of fresh biomass (fresh weight), microshoots cultured for 28 days in nitrogen-modified media were carefully removed under sterile conditions. The shoot and leaf structures were gently rinsed with running water to eliminate any residual medium and agar. To avoid inaccuracies due to excess surface moisture, plantlets were placed on sterile filter paper (e.g., Whatman No.1) at room temperature for 3-5 minutes to allow excess water to be absorbed. Each replicate sample was weighed using an electronic analytical balance with a precision of ±0.01 g. The results were recorded in milligrams (mg) and presented as mean ± standard deviation (SD) for each treatment condition.

Table 1. Modifications of Potassium Nitrate (KNO_3) Concentration in the Culture Medium for *In Vitro* Micropropagation of *Vitis vinifera* L. cv. 'Shirvan-Shah'

COMPONENTS	CONTROL	I	II	III
MACROELEMENTS				
NH_4NO_3	1650 mg/L	1650 mg/L	1650 mg/L	1650 mg/L
KNO_3	1900 mg/L	1425 mg/L	475 mg/L	380 mg/L
KH_2PO_4	170 mg/L	85 mg/L	113 mg/L	113 mg/L
$CACL_2 \cdot 2H_2O$	440 mg/L	440 mg/L	440 mg/L	440 mg/L
$MGSO_4 \cdot 7H_2O$	370 mg/L	370 mg/L	370 mg/L	370 mg/L
MICROELEMENTS (COMBINED)	Present	Present	Present	Present
VITAMINS AND ORGANICS (COMBINED)	Present	Present	Present	Present
PLANT GROWTH REGULATOR	0.2 mg/L BAP	0.2 mg/L BAP	0.2 mg/L BAP	0.2 mg/L BAP
SUCROSE	30 g/L	30 g/L	30 g/L	30 g/L
AGAR	8 g/L	8 g/L	8 g/L	8 g/L
REMARKS	Control	**KNO_3** reduced	**KNO_3** reduced	**KNO_3** reduced

Table 2. Modifications of Ammonium nitrate (NH_4NO_3) Concentration in the Culture Medium for *In Vitro* Micropropagation of *Vitis vinifera* L. cv. 'Shirvan-Shah'

COMPONENTS	CONTROL	I	II	III
MACROELEMENTS				
NH_4NO_3	1650 mg/L	1237.5 mg/L	412.5 mg/L	330 mg/L
KNO_3	1900 mg/L	1900 mg/L	1900 mg/L	1900 mg/L
KH_2PO_4	170 mg/L	85 mg/L	113 mg/L	113 mg/L
$CACL_2 \cdot 2H_2O$	440 mg/L	440 mg/L	440 mg/L	440 mg/L
$MGSO_4 \cdot 7H_2O$	370 mg/L	370 mg/L	370 mg/L	370 mg/L
MICROELEMENTS (COMBINED)	Present	Present	Present	Present
VITAMINS AND ORGANICS (COMBINED)	Present	Present	Present	Present
PLANT GROWTH REGULATOR	0.2 mg/L BAP	0.2 mg/L BAP	0.2 mg/L BAP	0.2 mg/L BAP
SUCROSE	30 g/L	30 g/L	30 g/L	30 g/L
AGAR	8 g/L	8 g/L	8 g/L	8 g/L
REMARKS	Control	**NH_4NO_3** reduced	**NH_4NO_3** reduced	**NH_4NO_3** reduced

Table 3. Modifications of NH_4NO_3 and KNO_3 Concentration in the Culture Medium for *In Vitro* Micropropagation of *Vitis vinifera* L. cv. 'Shirvan-Shah'

COMPONENTS	CONTROL	I	II	III
MACROELEMENTS				
NH_4NO_3	1650 mg/L	1237.5 mg/L	412.5 mg/L	330 mg/L
KNO_3	1900 mg/L	800 mg/L	475 mg/L	380 mg/L
KH_2PO_4	170 mg/L	85 mg/L	113 mg/L	113 mg/L
$CACL_2 \cdot 2H_2O$	440 mg/L	440 mg/L	440 mg/L	440 mg/L
$MGSO_4 \cdot 7H_2O$	370 mg/L	370 mg/L	370 mg/L	370 mg/L
MICROELEMENTS (COMBINED)	Present	Present	Present	Present
VITAMINS AND ORGANICS (COMBINED)	Present	Present	Present	Present
PLANT GROWTH REGULATOR	0.2 mg/L BAP	0.2 mg/L BAP	0.2 mg/L BAP	0.2 mg/L BAP
SUCROSE	30 g/L	30 g/L	30 g/L	30 g/L
AGAR	8 g/L	8 g/L	8 g/L	8 g/L
REMARKS	Control	**NH_4NO_3, KNO_3** reduced	**NH_4NO_3, KNO_3** reduced	**NH_4NO_3, KNO_3** reduced

Statistical analysis. Each variant was carried out in three biological replicates, with 60 explants used in each replicate. The statistical analysis was performed using the mathematical mean and standard deviation method. During result analysis, the mean errors and deviations (M±m) were taken into account (Babayev *et al*., 1999). Statistical data processing was conducted using GraphPad Prism (v9.5, GraphPad Software, San Diego, CA, USA) and one-way ANOVA was applied to evaluate differences between groups, followed by the preparation of graphical representations.

3. Result and Discussion

The optimization of mineral components in the culture medium is considered a critical tool for refining microclonal propagation protocols, as it significantly influences the morphological and physiological growth and development of plants. The results obtained in this study demonstrated that the reduction of KNO_3 concentration in the Murashige and Skoog (MS) medium adversely affected the vegetative development of

Vitis vinifera L. cv. 'Shirvan Shahi' plantlets under *in vitro* conditions (Table 4). Specifically, in the full-strength MS control medium, the highest values for stem length (3.85 ± 0.12 cm), number of leaves (5.60 ± 0.24), leaf area (2.15 ± 0.10 cm^2) and fresh biomass (320.5 ± 0.4 mg) were recorded. In contrast, progressive reductions in all these morphometric parameters were observed in media where the KNO_3 concentration was decreased to 3/4, 1/4 and 1/5 of the standard MS formulation. Notably, in the 1/5 KNO_3 variant, stem length decreased to 2.45 cm and leaf area dropped to 1.20 cm^2 (Figure 3).

Table 4. Effect of Varying Concentrations of NH_4NO_3 and KNO_3, Individually and in Combination, on the Growth Dynamics of Microshoots of *Vitis vinifera* L. cv. 'Shirvan-Shah' *In Vitro*

Treatment	Shoot Length (Cm)	No. of Leaves	Leaf Area (Cm^2)	Biomass (Mg)
Control (Ms Full)	3.85 ± 0.02	5. 60 ± 0. 02	2. 15 ± 0.04	320. 5 ± 0.05
3/4 KNO_3	3.42 ± 0. 04	5. 10 ± 0. 02	1. 90 ± 0. 03	285. 2 ± 0.04
1/4 KNO_3	2.97 ± 0. 03	4. 15 ± 0. 04	1. 45 ± 0. 02	210. 8 ± 0.03
1/5 KNO_3	2.45 ± 0. 04	3. 90 ± 0. 03	1. 20 ± 0. 02	185. 4 ± 0.02
3/4 NH_4NO_3	3.90 ± 0. 02	3. 40 ± 0. 02	1. 45 ±0. 02	315.30 ± 0.03
1/4 NH_4NO_3	2.10 ± 0. 03	2. 40 ± 0. 03	1. 20 ±0.04	270.20 ± 0.04
1/5 NH_4NO_3	1.20 ±0.04	1.50 ± 0. 04	0. 80 ±0. 03	200.16 ± 0.02
3/4 NH_4NO_3+KNO_3	5.20 ±0.02	7. 20 ± 0.02	2. 10 ±0.02	560. 0 ± 0.03
1/4 NH_4NO_3+KNO_3	2.90 ± 0. 02	5. 10 ± 0. 03	1. 90 ± 0. 02	340. 0 ± 0.04
1/5 NH_4NO_3+KNO_3	1.90± 0. 04	2. 20 ± 0. 04	1. 20 ± 0. 03	210. 0 ± 0.04

Nitrogen components are among the primary factors necessary for plant cell growth and metabolic activity. A deficiency in these components can lead to suppressed cytoplasmic activity, reduced synthesis of proteins and nucleic acids and ultimately, inhibition of vegetative growth (George *et al*., 2008; Conradie, 2005a).

Based on the findings of this study, it can be concluded that decreasing the concentration of KNO_3 in the MS medium during the *in vitro* cultivation of the 'Shirvan Shahi' grapevine cultivar significantly reduced key morphometric parameters such as stem length, leaf area and biomass. These results reaffirm the critical role of nitrate in promoting vegetative development in grapevines.

Our findings are consistent with those of Nezami-Alanagh et al. (2019), who reported that altering the concentration of KNO_3 (potassium nitrate) in the MS medium had a substantial impact on the morphological and physiological development of plants *in vitro*. Furthermore, nitrogen in the form of nitrate not only serves as a source of energy and carbon skeletons but also plays a regulatory role in fundamental metabolic processes such as photosynthesis and respiration (Grechi *et al*., 2007; Sonnewald, 2013). Compared to elevated levels of ammonium, the nitrate form is considered more stable within plant cells and is crucial for the efficient regulation and utilization of nitrogen (Sonnewald, 2013).

Therefore, maintaining optimal levels of KNO_3 in the MS medium is essential for the healthy development of grapevine plantlets and for improving *in vitro* microclonal propagation protocols (Nezami-Alanagh *et al*., 2019; George *et al*., 2008).

In the subsequent phase, the effects of varying concentrations of ammonium nitrate (NH_4NO_3) on the *in vitro* regeneration of *Vitis vinifera* L. cv. Shirvan Shahi were evaluated. As illustrated in Table 4, the application of reduced doses of NH_4NO_3 (1/4, 3/4 and 1/5 strength) exhibited differential impacts on plant morphogenesis when compared to the full-strength MS control medium. The highest fresh biomass value (320.5 mg) was

recorded in the control treatment, indicating that an optimal nutrient balance plays a crucial role in supporting robust plant development. Interestingly, the greatest shoot length was observed at 3/4 NH_4NO_3 concentration, whereas the highest values for leaf area and number of leaves were recorded in both the 3/4 and 1/4 NH_4NO_3 variants. These findings are consistent with results from similar studies conducted on other plant species (Cardoso & Teixeira da Silva, 2013; Decruse & Seeni, 2002). The optimal vegetative performance observed at 3/4 NH_4NO_3 concentration suggests that a moderate level of ammonium may effectively stimulate cell division and shoot elongation (Greer *et al*., 2009). Conversely, excessive levels of ammonium ions are known to increase medium acidity, disrupt ionic homeostasis and consequently inhibit morphogenetic processes (Rebrov, 2020). Similar observations have been reported in other studies, where high-salt (full MS) media were found to reduce regeneration potential and induce phytotoxic effects (Xie *et al*., 2025). Therefore, a moderate concentration of ammonium nitrate appears to provide a balanced nutrient regime, leading to optimal *in vitro* growth and regeneration in the 'Shirvan Shahi' grapevine cultivar.

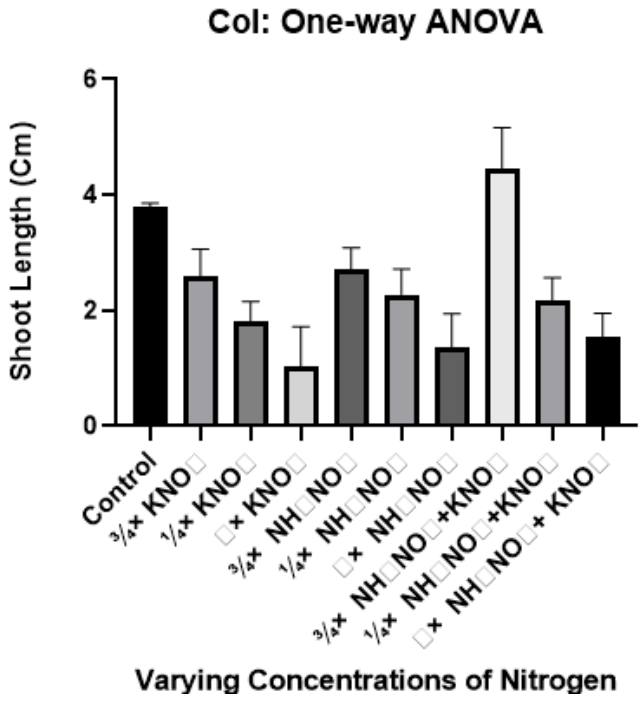

Figure 1. Effect of varying concentrations of NH_4NO_3 and KNO_3, individually and in combination, on the growth dynamics of shoot length of *Vitis vinifera* L. cv. 'Shirvan-Shah' *in vitro*

As clearly illustrated in Figure 1, the maximum shoot length (5.20 cm) was recorded in the medium containing 3/4 NH_4NO_3 + 3/4 KNO_3. The one-way ANOVA results ($F(9, 240) = 170.1$, $P < 0.0001$, $R^2 = 0.8645$) indicated that the application of different nitrogen sources and concentrations had a significant effect on shoot length. This value was significantly higher than that observed in other treatment groups, indicating that the combined application of ammonium and nitrate nitrogen at moderately reduced concentrations enhances shoot development. It is hypothesized that the balanced supplementation of both nitrogen forms provides the plant with a dual-mode nitrogen source - ensuring both rapid and sustained availability - thus creating optimal conditions for growth.

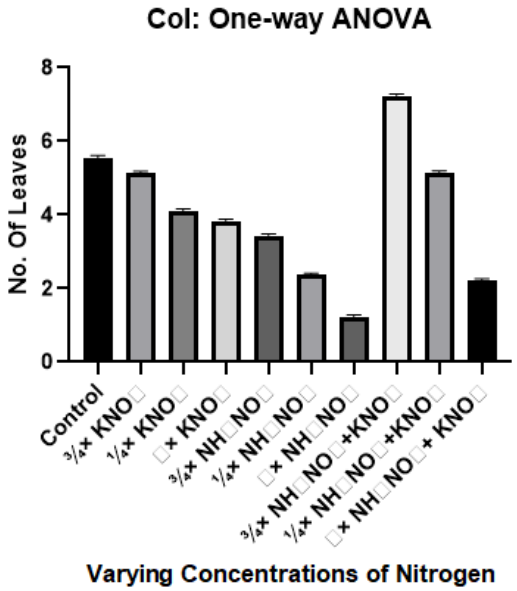

Figure 2. Effect of varying concentrations of NH_4NO_3 and KNO_3, individually and in combination, on the growth dynamics of the number of leaves of *Vitis vinifera* L. cv. 'Shirvan-Shah' *in vitro*

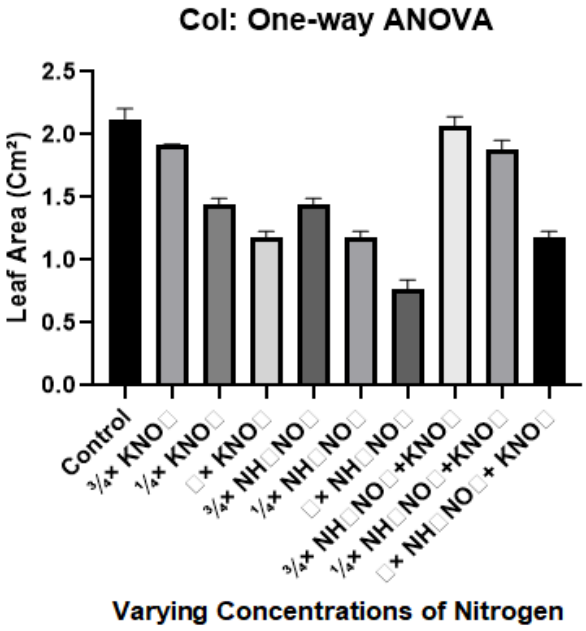

Figure 3. Effect of varying concentrations of NH_4NO_3 and KNO_3, individually and in combination, on the growth dynamics of leaf area of *Vitis vinifera* L. cv. 'Shirvan-Shah' *in vitro*

In the same treatment, the number of leaves (7.2) and leaf area (2.10 cm^2) also reached peak levels, resulting in an expanded photosynthetic surface and an overall improvement in vegetative growth (Figures 2 and 3). Based on the results of the one-way ANOVA for leaf area, the high R^2 value (98.19%) indicates that nearly all of the variation in leaf area can be explained by the applied factor, with random error contributing only a minimal share. These findings suggest that even slight changes in the nitrogen source and dosage can lead to noticeable and consistent differences in leaf area. While some treatments maximize leaf growth, others result in a substantial reduction. The magnitude

of these differences underscores the physiological sensitivity of *Vitis vinifera* to both the form and quantity of nitrogen, which is likely linked to nitrogen's central role in chlorophyll synthesis, cell expansion and the development of the photosynthetic surface. These morphological indicators were directly correlated with increased biomass production, further confirming the beneficial impact of the 3/4 NH_4NO_3 + 3/4 KNO_3 nutrient formulation on the *in vitro* morphogenesis of the 'Shirvan Shahi' grapevine cultivar.

The findings underscore that not only the total nitrogen content but also the form and relative proportion of nitrogen play a decisive role in successful *in vitro* regeneration. While nitrate is essential for amino acid biosynthesis, its uptake is energy-intensive. In contrast, ammonium is more readily assimilated into metabolic pathways but may induce toxicity when applied at high concentrations (Tang *et al*., 2020). The combined use of both forms can mitigate these adverse effects by promoting metabolic stability and maintaining energy balance.

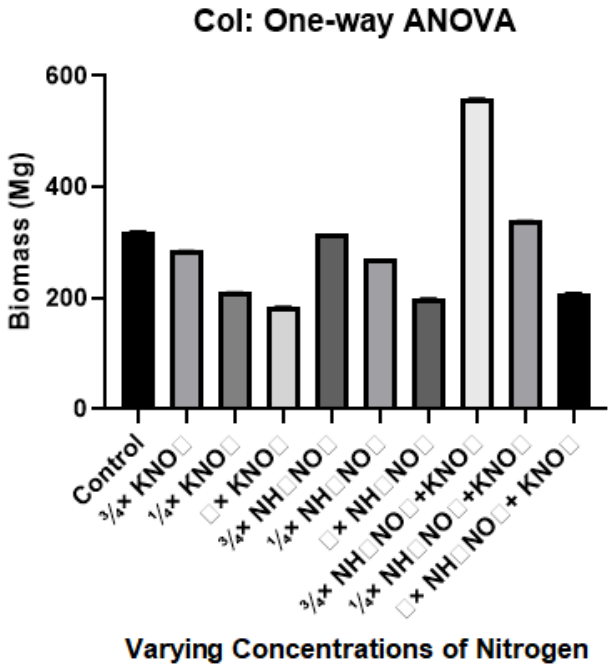

Figure 4. Effect of varying concentrations of NH_4NO_3 and KNO_3, individually and in combination, on the growth dynamics of biomass of *Vitis vinifera* L. cv. 'Shirvan-Shah' *in vitro*

Supporting this conclusion, a similar study conducted by Qin et al. (2021) in *Cyclocarya paliurus* demonstrated that the combined application of nitrate and ammonium led to maximal development of green tissues and biomass (Figure 4). The one-way ANOVA results for biomass indicated an R^2 value of 1.000, representing a maximal fit, which confirms that the variation in biomass was entirely explained by the applied factor, with virtually no influence from random error. The P value of < 0.0001 further confirms that the differences in biomass among the various nitrogen sources and concentrations tested were statistically highly significant. This synergy suggests a complementary effect of mixed nitrogen forms on photosynthesis and nitrogen metabolism. Zhao and Shen (2018) also emphasize the importance of maintaining a proper NH_4^+ to NO_3^- ratio, noting that such a balance is crucial for both morphogenesis and pH regulation of the growth medium. Specifically, ammonium contributes to a

reduction in medium pH, thereby enhancing the uptake of microelements such as phosphorus and iron, while nitrate helps stabilize ion homeostasis and exerts an alkalizing effect on the medium (Grechi *et al*., 2007).

At the same time, the dynamics of all these indicators are influenced by the interaction between the plant's hormonal system and nitrogen. Cytokinins are key hormonal mediators regulating plant responses to nitrogen availability, particularly the presence of nitrates (Wang *et al*., 2024). Nitrate uptake through the roots induces the expression of IPT (isopentenyl transferase) genes, which encode enzymes essential for cytokinin biosynthesis, thereby enhancing the production of active cytokinin forms that are subsequently transported to aerial organs (Sakakibara, 2021). In shoots, cytokinins stimulate cell proliferation, branching and apical meristem activity, whereas in roots they suppress lateral root development, enabling plants to coordinate growth according to nitrogen signals. The transcription factor TCP20 plays a central role in this regulatory network by integrating nitrate, cytokinin and auxin signaling, repressing the transcription of genes (ARR and AUX/IAA) involved in these pathways, thus confirming the presence of a hormonal signaling network linking nitrate perception with morphogenetic responses (Fan *et al.,* 2019). Therefore, nitrates act not only as nutrients but also as signaling molecules that regulate morphogenesis through cytokinin biosynthesis and cross-hormonal interactions, allowing plants to flexibly adjust root and shoot architecture depending on nitrogen supply. This underscores that the impact of nitrates on plant development is closely tied to nitrate concentrations and their integration into the plant's phytohormonal response network.

Thus, the results of the present study are consistent with modern scientific approaches and demonstrate that the combined application of NH_4NO_3 and KNO_3 at optimal doses and in appropriate ratios is a more favorable method for maximizing *in vitro* vegetative growth. This finding aligns with the data obtained by Rebrov (2020), further confirming the importance of a complex approach to nitrogen nutrition in tissue culture. Therefore, the use of a 3/4 concentration mixture of NH_4NO_3 and KNO_3 can be recommended as an effective and promising method for the *in vitro* regeneration of the *Shirvan Shahi* grapevine variety.

4. Conclusion

For the Shahi. This finding constitutes an important outcome for the development of an optimal microclonal propagation protocol, given that nitrogen is one of the most critical components of the Murashige and Skoog (MS) medium. Considering the commercial importance of achieving maximal efficiency in the *in vitro* propagation of grapevine cultivars through the precise optimization of medium components, the results of this study hold significant potential for application in future large-scale and commercially oriented projects.

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