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PRODUCTION OF TETRAPLOID AMUR GRAPE (*Vitis amurensis*) Via *In Vitro* COLCHICINE APPLICATION AND INITIAL CHARACTERIZATION OF THE INDUCED TETRAPLOIDS

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ABSTRACT

Polyploidy breeding is a promising strategy to enhance stress tolerance and functional quality in fruit crops. Tetraploid *Vitis amurensis* (*V. amurensis*) may offer superior advantages over its diploid counterpart, including increased biomass, improved antioxidant capacity, and greater adaptability to environmental stress. This study investigated the effects of colchicine concentration and treatment duration on tetraploid induction in *Vitis amurensis* and analyzed the initial growth characteristics of colchicine-induced tetraploids. Nodal explants were treated with four colchicine concentrations (0.01–0.2%) for three durations (8, 16, and 24 hours), and ploidy levels were determined using flow cytometry. Morphological and physiological traits were evaluated after ex vitro acclimatization. The highest induction efficiency was achieved with 0.1–0.2% colchicine for 16 hours, optimizing tetraploid production while maintaining a 50% survival rate. Higher colchicine concentrations and longer exposure times significantly reduced survival rates, indicating a dose-dependent cytotoxic effect. Tetraploid *V. amurensis* exhibited a 2.7-fold increase in the average total length of the main shoot and a 1.93-fold increase in stomatal size compared to diploid *V. amurensis* regardless of genotypes.. Antioxidant properties were markedly improved, with phenolic and flavonoid contents up to 2.5 times greater than in diploids, alongside increased DPPH radical scavenging activity and Ferric reducing Antioxidant power values, reflecting better oxidative stress mitigation. These improvements can be attributed to chromosome doubling, which enhances cellular size, secondary metabolite production, and metabolic efficiency. This study underscores the importance of balancing colchicine efficacy with toxicity for successful tetraploid induction. Tetraploid *Vitis amurensis* presents strong potential as a climate-resilient and functional grape cultivar, with superior growth and stress tolerance traits. Further *in vivo* and *ex vitro* research is needed to confirm these findings and explore mechanisms underlying stress resilience for sustainable viticulture.

Key words: Antioxidant properties; Grapevine; Polyploidy; Leaf characteristics

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INTRODUCTION

Climate anomalies are increasingly disrupting grape production worldwide, creating significant challenges for agricultural sustainability and economic stability (Van Leeuwen *et al*., 2024). The International Organisation of Vine and Wine reported a 7% decline in global wine production in 2023, the lowest level since 1961, attributed to extreme climatic events such as early frosts, prolonged droughts, and intense rainfall. These environmental stresses have caused severe losses in major wine-producing regions, particularly in Europe and the Southern Hemisphere, highlighting the urgent need for the development of grape varieties resilient to environmental extremes.

In Korea, grape cultivation depends heavily on a narrow selection of dominant varieties, including 'Shine Muscat' (43.9%), 'Campbell Early' (29.3%), 'Kyoho' (17.0%), and 'Muscat of Bailey A' (6.3%), which collectively occupy 96.5% of the total grape cultivation area of 14,706 hectares in 2023. The main grapevine cultivation area in Korea is Gyeongsangbuk-do, a region characterized by a continental climate with hot summers and cold winters. The combination of genetic uniformity and specific growth conditions amplifies the vulnerability of the industry to climatic stresses. For example, 'Shine Muscat' is highly susceptible to cold stress and pest infestations, while 'Campbell Early' is prone to fungal diseases like black rot and anthracnose under humid conditions. Similarly, 'Kyoho' and 'Muscat of Bailey A' exhibit poor cold tolerance. Climate change and erratic weather patterns exacerbate these vulnerabilities, leading to reduced yields, diminished fruit quality, and higher production costs in grape production (Jogaiah, 2023). These challenges underscore the urgent need for innovative, adaptive breeding strategies to ensure the

sustainability and resilience of grape production.

Wild grape species provide a promising genetic reservoir for breeding climate-resilient cultivars. Having evolved under diverse environmental conditions, wild species demonstrate inherent resistance to abiotic and biotic stresses, making them valuable for genetic improvement programs (Mao *et al.*, 2023). Among Korea's native species, *Vitis amurensis* (*V. amurensis*) stands out for its exceptional adaptability. As a diploid species, *V. amurensis* thrives in forest-edge environments with high sunlight exposure and withstands extreme cold temperatures down to –40°C (Liu and Li, 2013). Moreover, it exhibits robust resistance to common grape diseases such as grape white rot, anthracnose, and downy mildew, as well as tolerance to drought, salinity, and high humidity. These attributes make *V. amurensis* a highly resilient candidate for open-field cultivation under challenging environmental conditions (Xu *et al*., 2014; Hu *et al*., 2022). However, its commercial potential remains underutilized due to limitations such as low propagation efficiency, sour fruit flavor, and suboptimal productivity (Park *et al*., 2005; Kwon *et al*., 2019).

Polyploid breeding offers a transformative approach to overcoming these limitations. Chromosome doubling through polyploidization has been shown to improve fruit size and enhance the accumulation of functional compounds compared to diploid plants, as polyploids frequently exhibit larger cell sizes and elevated DNA content (Basit and Lim, 2024; Li *et al*., 2024; Zhang et al., 2024). In addition, polyploidization has emerged as a promising strategy for enhancing abiotic stress tolerance, with tetraploid individuals of leguminous crops and *Dendranthema nankingense* exhibiting greater resistance to salt and/or drought stress than their diploid counterparts (Liu *et al.*, 2011; Mangena and Mushadu, 2023). These findings underscore the physiological and agronomic advantages that polyploidy can offer to *V. amurensis*.

Despite the well-documented stress tolerance and ecological adaptability of *V. amurensis,* the application of polyploid breeding strategies to this species remains largely underexplored. This research gap is particularly critical given the species' inherent potential as a genetic resource for developing climate-resilient grapevine cultivars. To address this gap, the present study investigates the efficacy of colchicine-induced tetraploid induction in *V. amurensis* and evaluates whether tetraploid plants exhibit enhanced morphological and physiological traits compared to their diploid counterparts. The findings offer foundational insights into the early developmental advantages conferred by polyploidization in *V. amurensis*, providing a valuable basis for future breeding programs aimed at enhancing sustainability and resilience in viticulture under intensifying climatic stress.

MATERIALS AND METHODS

Production of tetraploid *V. amurensis* via *in vitro* colchicine treatment: Healthy 1-year-old potted diploid *V. amurensis* cv. 'Cheongpung' grapevines were used as the plant source. Node segments (~2 cm in length) containing axillary buds were excised and used as initial explants. These explants were cultured on Murashige and Skoog (MS) medium solidified with 8 g/L agar, supplemented with 30 g/L sucrose and 5 μM benzyladenine (BA), and adjusted to pH 5.7. To generate sufficient plant material for the experiment, explants were subcultured twice at 4-week intervals on the same medium. To induce tetraploidy, node segments (~1 cm) were subjected to colchicine treatment. The experimental design consisted of a completely randomized design with four colchicine concentrations (0.01%, 0.05%, 0.1%, and 0.2%) and three exposure durations (8, 16, and 24 hours), resulting in 12 treatment combinations. For each treatment, 22 explants were used, and all treatments were performed in triplicate, yielding a total of 792 explants. Colchicine treatments were applied in sterile liquid MS medium under dark conditions. After treatment, explants were thoroughly rinsed three times with distilled water and transferred to MS medium supplemented with 1 μM indole-3-butyric acid (IBA) to promote rooting. The cultures were incubated at 25 ± 1 °C under a light intensity of 1,500 lux, with a 16-hour photoperiod, in the tissue culture room of Gangneung-Wonju National University, Korea.

Five weeks after treatment, survival rate and morphological characteristics were recorded to assess the phytotoxic effects of colchicine. Rooted plantlets were transplanted into containers filled with a 1:1 (v/v) mixture of peat moss and commercial horticultural soil and cultivated in a growth chamber at 25°C with frequent misting to maintain 90% relative humidity (DS-51GLP-F, Dasol Scientific, Hwaseong, Korea). Hardened plants were subsequently transplanted into 18 cm pots and grown in a glasshouse at Gangneung-Wonju National University, where the temperature was maintained at approximately 25 °C under natural sunlight. Tetraploid plants were identified three months after ex vitro acclimatization using flow cytometry, a widely accepted method for ploidy analysis. Flow cytometry provides a rapid, non-destructive, and reproducible estimation of ploidy level, and is extensively used in ploidy analysis of grapevines and other horticultural crops (Sliwinska, 2018). Leaf samples (~1 cm^2) were prepared according to the CyStain UV Ploidy protocol (Sysmex Partec, Görlitz, Germany). Nuclear DNA content was measured using a CyFlow Ploidy Analyzer (Partec, Görlitz, Germany) and analyzed with CyView software. Diploid 'Cheongpung' and tetraploid 'Kyoho' served as internal controls. Relative fluorescence intensity was calculated against the diploid standard, and individuals showing approximately

double the fluorescence of diploid controls were identified as tetraploids. Flow cytometry was chosen over traditional chromosome counting due to its efficiency, non-destructive nature, and proven accuracy in polyploid plant screening.

Early growth characteristics of diploid and tetraploid *V. amurensis*: After three months of acclimatization, two tetraploid *V. amurensis* lines (Cheongpung 4x-1 and 4x-2) showing outstanding growth patterns were propagated and transplanted into experimental plots at Gangneung-Wonju National University in April 2024. Plants were cultivated for six months, during which key growth parameters such as branch number, total stem length, stem diameter, node count, leaf dimensions, leaf weight, leaf area, stomatal size, and chlorophyll content were measured. Field measurements included branch number, total stem length (from the branch base to the terminal bud), and stem diameter (measured at the base using calipers). Six representative leaves were selected to measure leaf length, width, and weight. Stomatal size was determined using impressions made with clear nail polish, which were examined under a microscope (Kern Optics, Balingen, Germany).

Stomatal size was calculated as: $A = (\text{length} \times \text{width}) \times \prod/4$.

Chlorophyll content was quantified using freeze-dried leaf samples ground into powder. One gram of powdered sample was mixed with 3 mL of 80% acetone, sonicated (CPX3800H-E, Emerson, Missouri, USA), and centrifuged (1580R, Labogene, Gimpo, Korea) at 7000 RPM for 10 minutes. Absorbance at 665 nm and 649 nm was measured by Uv-vis spectrophotometer (Mobi, MicroDigital, Seongnam, Korea) and used to calculate chlorophyll content as follows:

Chlorophyll A = $12.21 \times A - 2.81 \times B$, Chlorophyll B = $20.13 \times B - 5.03 \times A$

Total Chlorophyll = Chlorophyll A + Chlorophyll B

Important weather data for April through September 2024—the grapevine growing period—were obtained from the Korea Meteorological Administration for the Gangneung region. During this period, the average monthly temperatures were 15.8°C (April), 19.5°C (May), 24.3°C (June), 27.9°C (July), 28.7°C (August), and 23.1°C (September). Corresponding monthly cumulative solar radiation values were 504.13, 649.98, 618.53, 472.88, 565.58, and 343.86 $MJ \cdot m^{-2}$, respectively. No abnormal climatic events that could have affected grapevine growth were recorded during these six months.

Antioxidant properties in leaves of diploid and tetraploid *V. amurensis* : Powdered samples (0.1 g each) were prepared in triplicate for extraction, and analyses of total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity, and Ferric Reducing Antioxidant Power (FRAP) were performed based on the method described by Geleta *et al*. (2023). For extraction, 0.1 g of sample powder was mixed with 7 mL of 100% methanol, emulsified using a sonicator (CPX3800H-E, Emerson, Missouri, USA), and centrifuged at 4°C, 4500 RPM for 20 minutes (1580R, Labogene, Gimpo, Korea). The supernatant was collected, and 3 mL of 80% methanol was added to the remaining pellet for a second extraction under the same conditions. The final combined extract (10 mL) was stored at –20°C. For TPC analysis, 0.2 mL of sample extract was mixed with 2.5 mL of 10% Folin reagent, 1.25 mL of distilled water, and 0.75 mL of 20% sodium bicarbonate. The mixture was incubated in the dark for 30 minutes, and absorbance was measured at 765 nm using a spectrophotometer (Mobi, MicroDigital, Seongnam, Korea). For TFC analysis, 0.2 mL of sample extract was mixed with 1.5 mL of methanol, 0.1 mL of 10% $AlCl_3$, 2.8 mL of 1 M sodium acetate, and 3.1 mL of distilled water. The mixture was incubated in the dark for 30 minutes, and absorbance was measured at 415 nm using a spectrophotometer. For DPPH radical scavenging activity, 0.2 mL of sample extract was mixed with 3 mL of DPPH solution, incubated in the dark for 30 minutes, and absorbance was measured at 517 nm using a spectrophotometer. For FRAP analysis, 0.2 mL of sample extract was added to 2.8 mL of FRAP reagent prepared by mixing acetic acid buffer, TPTZ solution, and $FeCl_3$ solution in a 5:2.5:2.5 ratio. The mixture was incubated at 37°C for 4 minutes, and absorbance was measured at 593 nm using a spectrophotometer.

Statistical analysis: The data from this study were statistically analyzed using SPSS software (Version 28; IBM, New York, USA). All analyses were conducted in at least triplicate. Statistical significance was assessed using one-way analysis of variance (ANOVA), and mean comparisons were performed with Duncan's test to evaluate differences for the measurements ($p \leq 0.05$)

RESULTS

The regeneration of buds from colchicine-treated *V. amurensis* explants occurred primarily within two weeks of culture on regeneration medium. These regenerated buds then exhibited continuous growth, with root development initiated approximately two weeks later. Although statistical significance was not observed, colchicine-treated explants tended to show delayed regeneration and suppressed growth compared to untreated controls, indicating a potential inhibitory effect of colchicine on early plant development. The effects of colchicine treatment on explant survival and tetraploid induction efficiency are presented in Table 1. Survival rates declined progressively with increasing colchicine concentration and duration of exposure. At 0.01% colchicine, the survival rate was 90.91%, while at 0.2%, it dropped to 31.82%. Similarly, 24-hour treatments

resulted in approximately 20% lower survival than 8-hour treatments at the same concentrations, demonstrating the dose- and time-dependent toxicity of colchicine. Of the colchicine-treated explants that survived, 92.36% remained diploid, while 7.64% were induced to tetraploid status. The proportion of tetraploids among surviving explants increased with both higher colchicine concentrations and longer exposure durations (Table 2). The highest tetraploid induction rate (33.33%) was achieved with 0.2% colchicine applied for 24 hours. In contrast, minimal induction was observed at 0.01% colchicine for 8 hours. When considering the overall efficiency of tetraploid production (i.e., the proportion of total explants that became tetraploid), values ranged from 4.55% to 13.64%. Optimal results were obtained with 0.1% and 0.2% colchicine concentrations combined with 16-hour exposure durations, balancing tetraploid induction with explant viability. For example, although 0.2% colchicine for 8 hours resulted in a 10% tetraploid induction rate among surviving explants, the low survival rate reduced overall efficiency to 4.55%.

Early growth characteristics of diploid and tetraploid *V. amurensis* are summarized in Table 3. Notable differences in vegetative development were observed. The diploid cultivar 'Cheongpung' had an average stem count of 2.33 and a mean plant height of 120.00 cm. In contrast, tetraploid line 'Cheongpung 4x-1' produced 5.33 stems and reached a height of 422.67 cm, approximately 3.52 times taller than the diploid. Stem thickness was also significantly higher in tetraploids (11.90 mm) compared to diploids (9.12 mm). As shown in Table 4, leaf morphology differed between ploidy levels. Tetraploid leaves were larger, with 'Cheongpung 4x-1' exhibiting a mean length and width of 8.30 cm and 9.02 cm, respectively, compared to 5.88 cm and 7.47 cm in diploids. The total leaf area of tetraploids reached 60.26 cm², markedly larger than that of diploids. Stomatal size measurements revealed that tetraploids had significantly larger stomata. In 'Cheongpung 4x-1', the average stomatal area was 7.83 μm², approximately 2.26 times greater than in diploids (3.47 μm²). In terms of chlorophyll content (Table 5), tetraploids showed increased levels of chlorophyll A, chlorophyll B, and total chlorophyll compared to diploids. The highest total chlorophyll content was observed in 'Cheongpung 4x-1' (37.31), while 'Cheongpung 4x-2' exhibited lower values (17.97) than the diploid 'Cheongpung' (25.04). Antioxidant properties were significantly enhanced in tetraploid *V. amurensis*. Dried and powdered leaves were analyzed for total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity, and ferric reducing antioxidant power (FRAP). Tetraploid line 'Cheongpung 4x-1' exhibited the highest TPC (18.77 mg/g) and TFC (17.67 mg/g), corresponding to 2.54-fold and 2.37-fold increases, respectively, over diploid levels (7.38 mg/g and 7.45 mg/g). DPPH scavenging activity reached 76.61% in tetraploids compared to 25.45% in diploids. FRAP values also increased markedly, from 1.82 μM/g in diploids to 6.87 μM/g in tetraploids. Statistical analysis confirmed that genotype significantly affected antioxidant compound levels ($p \leq 0.001$), independent of leaf size.

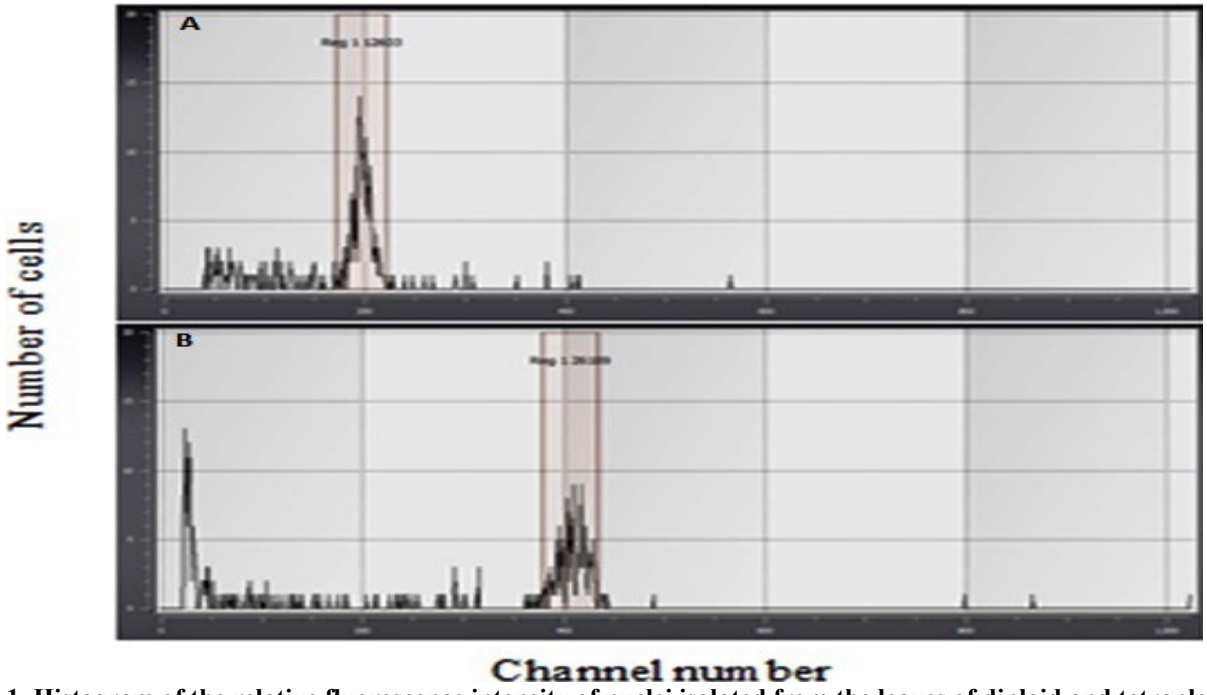

Fig. 1. Histogram of the relative fluorescence intensity of nuclei isolated from the leaves of diploid and tetraploid *V. amurensis* plant. (a) The control diploid plant of *V. amurensis* and (b) a tetraploid plant of *V. amurensis*.

Table 1. Evaluation of survival and basal growth in *V. amurensis* explants following colchicine treatment

Colchicine concentration (%)	Duration of colchicine treatment (hours)	Number of shoot surviving (survival rate, %)	Length of main shoot (cm)	Number of axillary buds[z]
0.01	8	20(90.9%)	1.97±0.07ab	7.0±0.2a
	16	19(86.4%)	2.04±0.10a	7.1±0.3a
	24	16(72.7%)	1.82±0.08abcd	6.8±0.3a
0.05	8	19(86.4%)	1.89±0.08abc	6.9±0.3a
	16	19(86.4%)	1.71±0.07bcd	6.7±0.3ab
	24	15(68.2%)	1.62±0.10cdf	6.1±0.3abc
0.1	8	17(77.3%)	1.75±0.08abcd	6.5±0.3abc
	16	15(68.2%)	1.55±0.09dfg	5.9±0.3abc
	24	13(59.1%)	1.31±0.09g	5.6±0.4bc
0.2	8	12(54.6%)	1.66±0.11cdf	6.3±0.4abc
	16	13(59.1%)	1.41±0.13fg	5.5±0.6c
	24	7(31.8%)	1.37±0.11fg	5.6±0.5bc

[z] Different letters in a column indicate significant differences between the mean at $p \leq 0.05$. ± indicates standard error.

Table 2. Efficiency of tetraploid induction via colchicine treatment in *V. amurensis.*

Colchicine concentration (%)	Duration of colchicine treatment (hours)	Number of successfully acclimatized plants	Number of tetraploid individuals	Ratio of tetraploid generation relative to surviving individuals	Tetraploid generation efficiency relative to explants (%)
0.01	8	19	0	0.0	0.0
	16	16	0	0.0	0.0
	24	15	0	0.0	0.0
0.05	8	17	0	0.0	0.0
	16	18	1	5.6	4.6
	24	12	0	0.0	0.0
0.1	8	14	0	0.0	0.0
	16	13	3	23.1	13.6
	24	8	2	25.0	9.1
0.2	8	10	1	10.0	4.6
	16	9	3	33.3	13.6
	24	6	2	33.3	9.1

Table 3. Initial growth characteristics of diploid and tetraploid *V. amurensis*

Grapevine	Number of stems	Total length of main shoot (cm)	Node thickness of main shoot (cm)	Number of nodes
Cheongpung	2.3±0.6b[z]	120.00±66.70b	9.12±0.29b	18.3±9.5b
Cheongpung 4x-1	5.3±2.1a	422.67±102.08a	11.90±1.29a	48.7±11.9a
Cheongpung 4x-2	3.0±1.0b	246.68±190.01ab	7.59±0.27b	35.1±18.8ab

[z] Different letters in a column indicate significant differences between the mean at $p \leq 0.05$. ± indicates standard error.

Table 4. Leaf characteristics of diploid and tetraploid *V. amurensis*

Grapevine	Length of leaves (cm)	Width of leaves (cm)	Weight of leaves (g)	Leaf area (cm^2)	Stomata size (μm^2)
Cheongpung	5.88±2.11b[z]	7.47±2.90a	0.72±0.42a	41.42±25.40ab	3.47±0.57c
Cheongpung 4x-1	8.30±3.09ab	9.02±3.34a	1.20±0.85a	60.26±38.91ab	7.83±3.48a
Cheongpung 4x-2	6.30±2.50b	6.84±2.82a	0.64±0.51a	29.22±20.92b	5.59±0.97b

[z] Different letters in a column indicate significant differences between the mean at $p \leq 0.05$. ± indicates standard error.

Table 5. Chlorophyll content measured in leaves of diploid and tetraploid *V. amurensis*

Grapevine	Leaf size	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll content (mg/g)
Cheongpung	Large-sized leaf	17.06±2.70ab	7.98±1.02a	25.04±3.72ab
	Medium-sized leaf	16.86±0.17a	5.87±0.17a	22.74±0.25a
	Small-sized leaf	12.88±0.29c	5.67±0.09a	18.55±0.27b
Cheongpung 4x-1	Large-sized leaf	21.62±4.51a	15.69±9.40a	37.31±13.90a
	Medium-sized leaf	16.19±1.44a	8.48±7.86a	24.67±9.07a
	Small-sized leaf	18.64±1.09b	5.66±0.70a	24.30±0.59a
Cheongpung 4x-2	Large-sized leaf	12.69±1.62b	5.28±1.04a	17.97±2.58b
	Medium-sized leaf	17.85±0.97a	6.84±1.74a	24.69±2.52a
	Small-sized leaf	20.63±0.68a	5.78±2.37a	26.40±1.80a

[z] Different letters in a column indicate significant differences between the mean at $p \leq 0.05$. ± indicates standard error.

Table 6. Antioxidant properties measured in leaves of diploid and tetraploid *V. amurensis*

Grapevine	Leaf size	Total phenolic content (mg/g)	Total flavonoid content (mg/g)	DPPH radical scavenging activity (%)	Ferric Reducing Antioxidant Power (μM/g)
Cheongpung	Large-sized leaf	7.38±0.74d[z]	7.19±0.67c	25.5±2.3d	1.82±0.06d
	Medium-sized leaf	6.00±0.97c	7.45±0.50c	26.3±1.4d	1.56±0.21c
	Small-sized leaf	5.74±0.05d	5.70±0.24c	28.8±3.6d	1.58±0.23c
Cheongpung 4x-1	Large-sized leaf	18.77±0.54a	17.67±1.42a	76.6±2.9a	6.87±0.10a
	Medium-sized leaf	14.06±0.79a	14.84±1.16a	67.5±1.4a	5.45±0.39a
	Small-sized leaf	15.57±0.08a	16.92±1.64a	72.2±2.7a	5.69±0.19a
Cheongpung 4x-2	Large-sized leaf	9.69±0.58c	12.52±1.72b	37.4±1.5c	3.34±0.28c
	Medium-sized leaf	12.22±0.45b	15.94±1.28a	46.5±2.9c	4.47±0.19b
	Small-sized leaf	9.95±0.45c	14.02±1.58b	37.7±2.8c	2.62±0.04b

[z] Different letters in a column indicate significant differences between the mean at $p \leq 0.05$. ± indicates standard error.

DISCUSSION

Colchicine-induced chromosome doubling is a widely utilized method for inducing polyploidy in horticultural crops and has been successfully applied to a variety of species, including grapes, bananas, grapefruits, and jujubes (Kara, 2022; Zhan *et al*., 2023). Colchicine functions by disrupting spindle fiber formation during mitosis, thereby preventing proper chromosome segregation and resulting in cells with duplicated chromosome sets (Manzoor *et al*., 2023; Widoretno *et al*., 2023). This mechanism effectively enables the generation of polyploid cells. In the present study, colchicine treatment was shown to be a viable approach for inducing tetraploidy in *V. amurensis*. Explants containing axillary buds, known for their high mitotic activity, were particularly effective for colchicine-induced chromosome doubling. The observed increase in tetraploid induction rates with higher colchicine concentrations and prolonged exposure aligns with previous findings in grapevines and other crops (Wu *et al*., 2020; Lin *et al*., 2023). However, these treatments also led to increased cytotoxic effects, reflected in reduced survival rates and delayed growth, consistent with the known toxicity of colchicine at elevated levels (Eng and Ho, 2019). These results underscore the importance of optimizing colchicine treatment conditions to balance induction efficiency and explant viability. While high concentrations and extended exposure times improved induction rates, they also compromised plant regeneration. The observed optimal induction efficiency at 0.1–0.2% colchicine for 16 hours suggests a critical threshold beyond which cytotoxicity outweighs the benefits of polyploid induction.

Tetraploid *V. amurensis* plants exhibited enhanced vegetative growth, including greater plant height, increased stem thickness, and expanded leaf area, compared to diploids. These morphological changes are likely due to cellular and physiological alterations associated with polyploidy, such as increased cell size and modified gene expression (Heslop-Harrison *et al*., 2023; D'Agostino and Fasano, 2024). Larger leaf size in tetraploids also suggests an expansion of the photosynthetic surface area, which could support higher rates of photosynthesis and biomass accumulation. Chlorophyll content was higher in tetraploid line, though variability between lines indicates that genetic or epigenetic factors may influence pigment accumulation. Increased chlorophyll levels, in turn, support enhanced photosynthetic capacity, contributing to improved growth

performance (Bharati *et al*., 2023; Su *et al*., 2024). Additionally, the enlarged stomata observed in tetraploid plants are likely to facilitate greater gas exchange, further enhancing photosynthetic efficiency under variable environmental conditions (Zhang *et al*., 2021; Nirala *et al*., 2023). In line with these physiological improvements, tetraploid *V. amurensis* plants exhibited significantly higher antioxidant activity. The elevated levels of phenolic and flavonoid compounds, along with increased DPPH and FRAP values, suggest that chromosome doubling may enhance secondary metabolism. This is consistent with prior research indicating that polyploidy stimulates the biosynthesis of bioactive compounds by altering metabolic pathways (Zhou *et al*., 2015; Kong *et al*., 2017). These findings also highlight the potential of tetraploid lines in stress resilience. Enhanced antioxidant content is crucial for mitigating oxidative stress caused by environmental factors such as drought, salinity, and temperature extremes (Celi *et al*., 2023; Rahman *et al*., 2024). The improved physiological and biochemical traits observed in this study support the use of colchicine-induced tetraploids as a valuable resource for breeding climate-resilient grapevine cultivars with superior growth and functional properties.

Recently, Hu *et al*. (2021) reported that artificially induced tetraploids of *Averrhoa carambola* L. exhibit significantly enhanced vegetative growth and superior fruit characteristics, including improved shape, quality, and weight, compared to their diploid counterparts. These observations suggest that such improvements in fruit traits may be, at least in part, attributed to increased photosynthetic efficiency associated with enhanced growth vigor in certain tetraploid individuals. Accordingly, extended evaluations under diverse environmental conditions are warranted to assess abiotic stress tolerance and to perform comprehensive phenotypic analyses of fruit quality in polyploid genotypes. These efforts will be instrumental in determining the breeding potential and agronomic value of colchicine-induced tetraploidy in *V. amurensis*.

Conclusions: In conclusion, this study demonstrates the effectiveness of in vitro colchicine treatment in inducing tetraploidy in *V. amurensis*, thereby providing a refined protocol that contributes meaningfully to the advancement of polyploid breeding in grapevine improvement. The induced tetraploid lines exhibited substantial enhancements in key vegetative traits—such as stem number, height, diameter, and leaf size—as well as elevated chlorophyll content and secondary metabolite production, indicating their potential for improved photosynthetic capacity and adaptive performance. However, several limitations must be acknowledged. The enhanced stress-related characteristics were inferred indirectly from biochemical and morphological indicators, without direct evaluation under abiotic stress conditions. Since plant stress responses are highly context-dependent, further validation under both controlled and field environments is necessary. In addition, fruit quality and yield parameters essential for commercial utilization were not assessed in this study and should be prioritized in future evaluations. Despite these limitations, the promising vegetative and physiological traits of tetraploid *V. amurensis* highlight their potential value in modern viticulture. Given the commonly limited genetic diversity and environmental sensitivity of polyploid grapevines, tetraploid *V. amurensis* could serve as a valuable genetic resource for developing resilient polyploid cultivars, provided their stress tolerance is confirmed through future studies. These findings lay the groundwork for utilizing *V. amurensis* tetraploids both as high-performing cultivars and as breeding parents in sustainable grapevine improvement programs.

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REFERENCES

Basit, A. and K.B. Lim (2024). Systematic approach of polyploidy as an evolutionary genetic and genomic phenomenon in horticultural crops. Plant Sci. 348: 112236. doi.org/10.1016/j.plantsci.2024.112236

Bharati, R., A. Gupta, P. Novy, L. Severová, K. Šrédl, J. Žiarovská and E. Fernández-Cusimamani (2023). Synthetic polyploid induction influences morphological, physiological, and photosynthetic characteristics in *Melissa officinalis* L. Front. Plant Sci. 14: 1332428. doi.org/10.3389/fpls.2023.1332428

Celi, G., P. Gratão, M. Lanza and A. Reis (2023). Physiological and biochemical roles of ascorbic acid on mitigation of abiotic stresses in plants. Int. J. Plant Physiol. Biochem. 202: 107970. doi.org/10.1016/j.plaphy.2023.107970

D'Agostino, N. and C. Fasano (2024). Genetics and genomics of polyploid plants. Genes 15: 1377. doi.org/10.3390/genes15111377

Eng, W. and W. Ho (2019). Polyploidization using colchicine in horticultural plants: A review. Sci. Hortic. 246: 604-617. doi.org/10.1016/j.scienta.2018.11.010

Geleta, B., J.C. Lee and J.Y. Heo (2023). Antioxidant activity and mineral content in unripe fruits of 10 apple cultivars growing in the northern part of Korea. Horticulturae 9: 114. doi.org/10.3390/horticulturae9010114

Heslop-Harrison, J., T. Schwarzacher and Q. Liu (2023). Polyploidy: its consequences and enabling role in plant diversification and evolution. Ann. Bot. 131: 1-10. doi.org/10.1093/aob/mcac132

Hu, Y., D. Sun, H. Hu, X. Zuo, T. Xia and J. Xie (2021). A comparative study on morphological and fruit quality traits of diploid and polyploid carambola (*Averrhoa carambola* L.) genotypes. Sci. Hortic. 277: 109843. doi.org/10.1016/j.scienta.2020.109843

Hu, Y., H. Zhang, B. Gu and J. Zhang (2022). The transcription factor VaMYC2 from chinese wild *Vitis amurensis* enhances cold tolerance of grape (*V. vinifera*) by up-regulating VaCBF1 and VaP5CS. Int. J. Plant Physiol. Biochem. 192: 218-229. doi.org/10.1016/j.plaphy.2022.10.011

Jogaiah, S. (2023). Vineyard management strategies in scenario of climate change–A review. Grape Insight 1: 11-22. doi.org/10.59904/gi.v1.i1.2023.7

Kara, Z. (2022). Induction of polyploidy in grapevine (*Vitis vinifera* L.) seedlings by *in vivo* colchicine applications. Turk. J. Agric. For. 46: 152-159. doi.org/10.55730/1300-011X.2967

Kong, D., Y. Li, M. Bai, Y. Deng, G. Liang and H. Wu (2017). A comparative study of the dynamic accumulation of polyphenol components and the changes in their antioxidant activities in diploid and tetraploid *Lonicera japonica*. Int. J. Plant Physiol. Biochem. 112: 87-96. doi.org/10.1016/j.plaphy.2016.12.027

Kwon, J.H., Y.S. Park, S.H. Kim and J.Y. Heo (2019). Evaluation of genetic stability and effects of plant growth regulators for in vitro propagation of underutilized *Vitis amurensis* 'Cheongsan'. Not. bot. Horti. Agrobot. Cluj-Napoca. 47: 987-994. doi.org/10.15835/nbha47311599

Li, X., L. Zhang, X. Wei, T. Datta, F. Wei and Z. Xie (2024). Polyploidization: a biological force that enhances stress resistance. Int. J. Mol. Sci. 25: 1957. doi.org/10.3390/ijms25041957

Lin, J., B. Zhang, J. Zou, Z. Luo, H. Yang, P. Zhou, X. Chen and W. Zhou (2023). Induction of tetraploids in Paper Mulberry *(Broussonetia papyrifera* (L.) L'Hér. ex Vent.) by colchicine. BMC Plant Biol. 23: 574. doi.org/10.1186/s12870-023-04487-2

Liu, L. and H. Li (2013). Research progress in amur grape, *Vitis amurensis* Rupr. Can. J. Plant Sci. 93: 565-575. doi.org/10.4141/cjps2012-202

Liu, S., S. Chen, Y. Chen, Z. Guan, D. Yin and F. Chen (2011). *In vitro* induced tetraploid of *Dendranthema nankingense* (Nakai) Tzvel. shows an improved level of abiotic stress tolerance. Sci. Hortic. 127: 411-419. doi.org/10.1016/j.scienta.2010.10.012

Mangena, P. and P.N. Mushadu (2023). Colchicine-induced polyploidy in leguminous crops enhances morpho-physiological characteristics for drought stress tolerance. Life 13: 1966. doi.org/10.3390/life13101966

Manzoor, A., T. Ahmad, M. Naveed, A. Rehman, M. Bashir, R. Ahmad and N. Akhtar (2023). Assessment of blological damage and toxic potency of colchicine in gladiolus (*Gladiolus grandifloras*) plants. J. Agric. Sci. 5: 72-92. doi.org/10.56520/asj.v5i2.259

Mao, H., C. Jiang, C. Tang, X. Nie, L. Du, Y. Liu, P. Cheng, Y. Wu, H. Liu, Z. Kang and X. Wang (2023). Wheat adaptation to environmental stresses under climate change: Molecular basis and genetic improvement. Mol. Plant. 16: 1564-1589. doi.org/10.1016/j.molp.2023.09.001

Nirala, D., J. Tiwari, N. Sahu and S. Sinha (2023). Morphological and physiological changes (variation) in colchicine induced tetraploids of spine gourd (*Momordica dioica* Roxb.) in comparison to their diploid counterparts. Plant Physiol. Rep. 28: 481-489. doi.org/10.1007/s40502-023-00758-0

Park, Y.S., J.Y. Heo, I.J. Kim, S.J. Heo, K.H. Kim, B.C. Jeong and S.M. Park (2005). Growth and fruit characteristics of *Vitis amurensis* Rupr., collected in Gangwondo. Korean J. Med. Crop Sci. 13: 226-233

Rahman, M., P. Ghosh, M. Akter, M. Al Noor, M. Rahman, S. Keya, M. Roni, A. Biswas and M. Bulle (2024). Green vanguards: harnessing the power of plant antioxidants, signal catalysts, and genetic engineering to combat reactive oxygen species under multiple abiotic stresses. Plant Stress 13: 100547. doi.org/10.1016/j.stress.2024.100547

Sliwinska, E. (2018). Flow cytometry-a modern method for exploring genome size and nuclear DNA synthesis in horticultural and medicinal plant species. Folia Hortic. 30: 103. doi.org/10.2478/fhort-2018-0011

Su, X., X. Wang, R. Li, C. Zhou, L. Chen, S. Chen, N. Cai and Y. Xu (2024). Identification and

evaluation of diploid and tetraploid *Passiflora edulis* sims. Plants. 13: 2603. doi.org/10.3390/plants13182603

Van Leeuwen, C, G. Sgubin, B. Bois, N. Ollat, D. Swingedouw, S. Zito and G. Gambetta (2024). Climate change impacts and adaptations of wine production. Nat. Rev. Earth Environ. 5: 258-275. doi.org/10.1038/s43017-024-00521-5

Widoretno, W., R. Azriningsih, D. Sukmadjaja and M. Rosyidah (2023). *In vitro* induction and identification of polyploid *Amorphophallus muelleri* blume plants by colchicine treatment. Agrivita 45: 87-97. doi.org/10.17503/agrivita.v45i1.3992

Wu, J., Y. Sang, Q. Zhou and P. Zhang (2020). Colchicine *in vitro* tetraploid induction of *Populus hopeiensis* from leaf blades. Plant Cell Tissue Organ Cult. 141: 339-349. doi.org/10.1007/s11240-020-01790-w

Xu, W., R. Li, N. Zhang, F. Ma, Y. Jiao and Z. Wang (2014). Transcriptome profiling of *Vitis amurensis*, an extremely cold-tolerant Chinese wild *Vitis* species, reveals candidate genes and events that potentially connected to cold stress. Plant Mol. Biol. 86: 527-541. doi.org/10.1007/s11103-014-0245-2

Zhan, N., L. Li, L. Zhang, W. He, Q. Yang, F. Bi, G. Deng, A. Kiggundu, G. Yi and O. Sheng (2023). Transcriptome and metabolome profiling provide insights into hormone-mediated enhanced growth in autotetraploid seedlings of banana (*Musa* spp.). Front. Sustain. Food Syst. 6: 1070108. doi.org/10.3389/fsufs.2022.1070108

Zhang, X. and J. Gao (2021). Colchicine-induced tetraploidy in *Dendrobium cariniferum* and its effect on plantlet morphology, anatomy and genome size. Plant Cell Tissue Organ Cult. 144: 409-420. doi.org/10.1007/s11240-020-01966-4

Zhang, R., S. Rao, Y. Wang, Y. Qin, K. Qin and J. Chen (2024). Chromosome doubling enhances biomass and carotenoid content in *Lycium chinense*. Plants. 13: 439. doi.org/10.3390/plants13030439

Zhou, Y., L. Kang, S. Liao, Q. Pan, X. Ge and Z. Li (2015). Transcriptomic analysis reveals differential gene expressions for cell growth and functional secondary metabolites in induced autotetraploid of chinese woad (*Isatis indigotica* Fort.). PLoS One. 10: e0116392. doi.org/10.1371/journal.pone.0116392.