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MICROPROPAGATION AND PHYTOCHEMICAL ANALYSIS OF LEAF AND FRUIT EXTRACTS OF WILD HIMALAYAN GRAPE (*VITIS JACQUEMONTII*)

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Abstract

Vitis jacquemontii is a wild grape, and indigenous people use it as a substitute for cultivated grapes. Human activities pose a significant threat to the plant's biodiversity. Plants are disappearing due to these activities. Plant tissue culture is recommended for protecting plants through *In vitro* conservation methods. This study aimed to develop an effective method for inducing callus and shoots and also to analyze the chemical constituents in the fruit and leaf extracts. The maximum callus induction response (57%) was achieved from nodal explant weighing (3.12 grams) on an MS medium containing naphthalene acetic acid (NAA 1.5 mg/L). The MS medium supplemented with 1.5 mg/L 6-benzylaminopurine (BA) produced a maximum shoot proliferation of 8.34 per explant. The highest rooting percentage (51.23%) and largest number of roots (5.62) of shoots were obtained on MS medium augmented with 1.0 mg/l NAA. HPLC analysis confirmed eight compounds in the *Vitis jacquemontii* fruit methanol extract (VJFME) and six in the leaf methanol extract (VJLME). Rutine was abundant in the VJLME extract, and quercetin was in the VJFME extract. Quantitative phytochemical analysis revealed higher total tannin (TTC), phenol (TPC), and flavonoids in the VJFME extract than in the VJLME extract. In conclusion, a regenerative system was established to produce materials from *V. jacquemontii* for bioactive compounds.

Key words: Wild grape; Conservation; Micropropagation; Phytochemical profiling

Introduction

People in rural areas utilize wild edible fruit-bearing plants, but these plants are disappearing due to natural and human activities. *Vitis jacquemontii* is wild fruit-bearing plant of the Vitaceae family. Wild edible plants have long been a necessary and well-known source of food particularly for the poor families of developing countries (Mavengahama *et al*., 2013; Yumkham *et al*., 2017). In some rural regions, people play a crucial role in ensuring domestic food safety and nutrition. They utilize wild edible plants to augment essential food, for seasonal food shortages, and to provide extra food in starvation (Soromessa & Demissew, 2002). It is estimated that on worldwide basis wild food is consumed by about 1 million people (Chakravarty *et al*., 2016). The worldwide consumption of wild food is estimated to be by about 1 billion individuals (Lulekal *et al*., 2011). They serve as a source of micronutrient supplements and are rich in nutrients (Awas, 2007) satisfy in the dietary requirements (Feyssa *et al*., 2011).

Generally, grapes are used as table fruits, juice, raisins, and wine (Olmo, 1976). The fermented roots and leaves of the grape plant are used as a diaphoretic and are suggested for high fever (Gaur *et al*., 2010). *Cissus sicyoides* is used in folk medicine to treat indigestion and stomachaches in Jamaica (Asprey & Thornton, 1954). The leaf extract of *V. vinifera* exhibits biological activities, including hypoglycemia and spasmolytic (Orhan *et al*., 2006). The fruit and leaf extracts of *V. jacquemontii* possess anticholinesterase, antioxidant, analgesic, and antidepressant properties (Jan *et al*., 2024). *V. amurensis* has been utilized as a traditional Chinese herb for the cure of stranguria, rheumatoid arthritis, traumatic hemorrhage, chronic arthritis, and nephritis. This species contains oligostilbenes, Saponins, and flavonoids, and possesses antioxidants, cardioprotective, anti-inflammatory, and antibacterial properties (Chen *et al*., 2018).

Medicinally important wild plants are a source of herbal drugs. However, they are vanishing at a high rate for numerous reasons, such as overexploitation for marketing of medicinal plants, fuel, food, agricultural implements, anthropogenic activities, flooding and climatic fluctuation etc. pose severe threats to medicinal plants. Furthermore, the demand for medicinally important plants is increasing tremendously all over the world (Njume & Goduka, 2012). Extreme demand for medicinal plants has put stress on plant species, leading to overexploitation. This constant pressure to discriminate against these plants these days endangers the conservation of their biodiversity, which is essential for future uses (Majid *et al*., 2015).

Safeguarding of plant genetic resources is a crucial component of biodiversity conservation, aimed at preventing future harm to wild plants. Important plant species are being rapidly propagated, and their biomass is produced through plant tissue culture as a mean of control, an important substitute option for a smooth and consistent

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supply of plant active components through tissue culture to handle environmental limitations (Davies & Deroles, 2014). Micropropagation of plants, also known as *In vitro* culture, involves the growing of plant organs or tissues including callus, to reproduce plant material that can produce chemical constituents (Ochoa-Villarreal *et al*., 2016, Atanasov *et al*., 2015). Plant morphogenesis requires *In vitro* growth of plants, a process achieved through plant tissue culture techniques Pullaiah & Subba, 2009). In *vitro* propagation significantly contributes to our understanding of basic research and offers potential applications across different fields. It ensures a sustainable industry by supporting plant-derived compounds' commercial production (Chandran *et al*., 2020; Kumari *et al*., 2021). Since ancient times, people have relied on plants for flavors, medicines, food, and many other usages (Rai *et al*., 2011). Plants are the primary source of herbal medicines in the cosmetics and food industries (Barbulova *et al*., 2014). Overall, plant tissue culture has a widespread application in research and industry (Chandran *et al*., 2020, Rai *et al*., 2011).

There are no reports in the literature on the micropropagation or ex-situ conservation of this species. This study focuses on an efficient regeneration protocol via direct organogenesis using nodal segments, indirect organogenesis using callus culture, and the screening of bioactive compounds in the fruit and leaf extracts of *V. jacquemontii*. This will allow its cultivation, commercial exploitation, and the conservation of wild biodiversity. Wild grapes will be valuable in breeding programs as donors for developing new disease-resistant varieties and potential use in the future pharmaceutical industry.

Methods and Materials

Identification and sterilization: Plant materials were collected in March 2019 from wild populations in Bar Shawar (latitude- 4.9766° N, longitude- 72.2815° E), located in the Swat district, Khyber Pakhtunkhwa (KP), Pakistan. Dr. Nasrullah Khan, Department of Botany, University of Malakand, KP, Pakistan, identified the plant materials. A voucher specimen with the accession number V.NO.H.UOM. 768 was deposited in the Department of Botany University of Malakand, KP, Pakistan. Before inoculation, young branches obtained from the mother plant (*V. jacquemontii*) were sterilized with $HgCl_2$ (0.06%) for 12-14 min. Then, the plant material was rinsed with sterilized distilled water to remove the remaining residue of $HgCl_2$.

Culture conditions: The plant growth regulators such as Indole-3-butyric acid (IBA), Indole-3-acetic acid (IAA), naphthalene acetic acid (NAA), 6- benzylaminopurne (BA), and kinetin, were supplemented in the Murashige & Skoog (MS) (1962) medium. The pH of all culture media was adjusted between 5.5 and 5.6. Agar at 0.6% was used for solidification and sucrose at 3% was a carbohydrate source. Media were autoclaved at 121°C for 13 to 14 minutes at 15 psi. After inoculation of explants, cultured vials were kept at 26 ±2°C in a growth chamber with a 16-hour light period/8-hour dark period using fluorescent electric bulbs.

Shoot induction: For the induction of shoots, nodal explants measuring 2 to 4 cm in length were cultured on media containing BA at 1.0, 1.5 and 2.0 mg/L, Kinetin at 1.0, 1.5, and 2.0 mg/L and IAA (1.0, 1.5 mg/L) individually and in combinations BA + IAA (1.0 + 1.0, 1.5 + 1.0, and 2.0 +1.0 mg/l) and Kinetin + IAA (1.0 +1.0, 1.5 +1.0 and 2.0 +1.0 mg/l). Growth performance was recorded after 25 days of culture, including the percentage of shoot induction, the number of shoots, and their length.

Shoot proliferation through subculture: For enhanced multiplication of shoots, nodes from the *In vitro* regenerated shoots were trimmed and cultured on media containing BA at concentrations of 1.0, 1.5, 2.0, 2.5, and 3.0 mg/L and Kinetin at concentrations of 1.0, 1.5, 2.5, and 3.0 mg/L.

Callus initiation: Nodal explants (2 to 4 cm in length) were cultured on a medium containing NAA, IAA, and NAA at concentrations of 0.5, 1.0 and 1.5 mg/L, respectively, for callus induction.

Root induction: The *In vitro* regenerated shoots were rooted on media augmented with different concentrations of NAA (1.0, 1.5, 2.0, and 2.5 mg/L). After three days of culturing, the time to shoot roots was observed and recorded. Root number and length were recorded after 26 days of culturing.

Sample preparation for phytochemical assessment: Fresh fruits (0.5 Kg) and leaves (1.0 Kg) of *V. jacquemontii* were collected in July 2019 from plants growing wild in Barshawar, Swat district, KP, Pakistan. They were shade-dried for 40 days and ground into fine powder separately. The powder materials (fruits and leaves) were individually immersed in methanol for 2 weeks at 25±2°C for extraction, then filtered. The filtrate was passed through a rotary evaporator for solvent separation at a controlled temperature. Fruit crude extract weighing 25 g and leaf crude extract weighing 48g were collected for HPLC and qualitative analysis.

Assessment of phytochemicals

HPLC assessment: For characterization and quantification of phytochemicals method of Alam (2015) was followed using HPLS. Sample containing fruits and leaves crude extract from *V. jacquemontii* (1g) was mixed with 20 mL of water and methanol in a 1:1 ratio, heated in the water bath at 70°C for one hour, and then centrifuged at 4000 rpm for 10 minutes. After filtration, 2 mL of the sample was transferred into HPLC containers. We used the Agilent 1260 Infinity HPLC system, which included a UV detector, quaternary pump, degasser, and auto-sampler, to detect phenolic compounds. Phytochemicals were separated on an Agilent Zorbax Eclipse XDBC18 column using a gradient system of solvent A containing deionized water: methanol: acetic acid, 180:100:20, v/v and solvent B containing deionized water: methanol: acetic acid, 80: 900:20, v/v. The column temperature was maintained at 25°C. We tested various gradient techniques, leading to the most effective gradient program starting at 100% A at 0 minutes, decreasing to 85% A at 5 minutes, reaching 50% A at 20 minutes, further reducing to 30% A at 25 minutes, and finally switching to 100% B from 30 to 40 minutes. The injection volume was 10 µL, with the UV detector set at 280 nm to analyze the phenolic compounds. Identification of compounds was

achieved by comparing the UV spectra of the unknown sample with those of known standards and their retention times, quantification was conducted by calculating the percentage of the peak height.

Quantitative assessment: Using different methods, total phenolic, flavonoid, and tannin contents of fruit and leaf extracts of *V. jacquemontii* were quantitatively evaluated.

Total phenolic contents (TPC): TPC in fruit and leaf extract was analyzed using the method described by Folin-Ciocalteu (Singleton & Rossi, 1965). The procedure involved dissolving 500 µL of plant extracts in 800 µL of distilled water. Next, 100 µL of Folin-Ciocalteu reagent was supplemented, and the mixture was agitated thoroughly. After a 5-minute incubation, 7% aqueous sodium carbonate (Na_2CO_3) was introduced to the mixture. Following another incubation period, the mixture was diluted with 2 ml of distilled water. The absorbance was then measured at 765 nm using a UV spectrophotometer. TPC was calculated and stated as mg of gallic acid correspondent per g dry weight of extracts (mg GAE g/DW) using a standard curve for reference.

Total tannin content (TTC): TTC in the fruit and leaf extract was estimated using the method described by Julkunen-Titto (1985). Specifically, 50 µl of the leaf and fruit extract was combined with 1500 µl of 4% vanillin solution and mixed thoroughly using a vortex. Afterward, 750 µl of concentrated HCl was added to the mixture and allowed to react for 20 minutes at room temperature. The absorbance of the resulting solution was then calculated at a wavelength of 550 nm using a blank for reference. TTC was determined in milligrams of Gallic acid corresponding to per gram of dry matter, calculated from a calibration curve (mg GAE/g).

Total flavonoid contents (TFC): TFC in fruit and leaf extract was determined using the method of (Ordonez *et al*., 2006). We combined 0.5 mL of the fruit and leaf extract with 100 µL of the 10% aluminum chloride, 1.5 mL of methanol, 100 µL of the 1 M potassium acetate solution, and 2.8 mL of distilled water to measure the TFC. This mixture was incubated at room temperature for 1 h and 30 min. After incubation, the absorbance at a wavelength of 420 nm was measured. The amount of TFC was measured using a standard curve of quercetin, and the results were stated as milligrams of quercetin corresponding to per gram of dry weight of the extract (mg QE/g of DW).

Statistical analysis: Each experiment was performed in triplicate, and data were treated as mean ± SE (n=3). The calculated data was subjected to software (Origin Pro 9.1) for one-way ANOVA with a significance level of $p<0.05$. The data were analyzed for significant differences using SPSS (version 20.0; IBM Company, USA). Further, Tukey's HSD test was applied as a post hoc analysis to identify specific group differences.

Results

Culture initiation and shoot induction: Nodal explants of *V. jacquemontii*, were cultured on media augmented with various concentrations of Kinetin, BA, and IAA, individually and in combination, to initiate shoot induction (Table 1). After 12 to 14 days of cultivation, the buds began to grow (Fig. 1). Statistically significant differences ($p<0.05$) were found in various parameters, including the percentage of sprouting, the average number, and the length of shoots. These differences were noted across all concentrations of BA and Kinetin, whether used individually or in combination (Table 1). At a concentration of 2.0 mg/L, both BA and Kinetin produced the highest number of shoots, with averages of 4.35 and 3.21 shoots per explant, respectively. The bud sprouting rates for this treatment were 74.62% for BA and 65.17% for Kinetin. However, lower concentrations resulted in fewer shoots per explant (Table 1). The maximum shoot length was 3.71cm at 1.5 mg/L of BA, while the maximum shoot length with Kinetin at 1.5 mg/L was 4.73 cm. The addition of IAA into media containing BA and Kinetin did not increase the bud sprouting rate, number and length of shoots. It resulted in a reduction of these parameters. In cases where the media contained only IAA or no hormone, the cultured explants showed no response and survived for 20 to 25 days only.

Shoot proliferation stage: To determine the most effective type of plant growth regulator for large-scale proliferation, nodal explants from the *In vitro* regenerated shoot were subcultured on media comprising various concentrations of BA and Kinetin (Fig. 2). Shoot development was observed within 4 to 7 days of culturing. BA at 1.5 mg/L induced maximum proliferation, with an average of 8.34 per explant and a sprouting percentage of 82.25%. BA at 2.0 mg/L, produced the highest shoot length (6.40 cm). On the other hand, Kinetin formed maximum shoots (3.36) with sprouting percentage (76.34%) at 2.0 mg/L, while elongated shoots (4.08 cm) were achieved at 1.0 mg/L.

Effect of cytokinin and auxin on shoot proliferation: To optimize mass shoot multiplication, various concentrations of NAA were combined with the optimal shoot multiplication concentration of BA (1.5 mg/L) (Table 2). Our findings demonstrated that the combination of NAA and BA decreased the number of shoots, the length of shoots, and the percentage of buds sprouting compared to BA (Table 2). At a high concentration of NAA/BA (3.0/1.5 mg/L), the number of shoots was decreased to 4.21, compared to 8.34 observed with single BA (1.5 mg/L). Similarly, shoot length was decreased to 3.17 with NAA/BA (3.0/1.5 mg/L), compared to 5.17 with BA (1.5 mg/L) only.

Indirect morphogenesis: Sterilized nodal segments were horizontally placed on a medium containing various plant growth regulators to induce callus. The plant growth regulators used affected the rate of callus formation, the amount of fresh weight, and the shape of the callus (Table 3). The induction response for callus formation with different hormones was monitored: 21-29% with IAA, 17-22% with IBA, and 46-62% with NAA. Moreover, the fresh weight yield of callus was determined for different hormone concentrations: 1.04-1.53 g for IAA, 1.19-2.06 g for IBA, and 1.62-3.12 g for NAA. Additionally, the callus varied in morphology using IBA (ranging from white to light brown and friable (Fig. 3c), NAA (ranging from white, light green, compact to nodulated (Fig. 3d, e), and IAA (being white to friable (Fig. 3a,b).

Table 1. Induction of bud sprouting%, number, and length of shoots under the influence of plant growth regulator from nodal explant.

Plant growth regulator	Conce. (mg/L)	Bud sprouting %	Number of shoots ± SE	Length of shoot (cm) ± SE
Control	0.0	0.0	0.0	0.0
BA	1.0	52.26	1.34 ± 0.23 ^{d}	3.13 ± 1.17 ^{b}
	1.5	57.83	2.61 ± 0.15 ^{bc}	3.71 ± 1.14 ^{ab}
	2.0	74.62	4.35 ± 1.53 ^{a}	2.87 ± 1.10^{cb}
KIN	1.0	57.5	1.74 ± 0.46 ^{cd}	3.64 ± 1.76 ^{ab}
	1.5	48.23	1.81 ± 0.32 ^{cd}	4.73 ± 1.96 ^{a}
	2.0	65.17	3.21 ± 0.98 ^{b}	2.52 ± 1.23 ^{cb}
IAA	1.0	—	--	--
	1.5	—	--	--
BA/ IAA	1.0/1.0	35.91	2.65 ± 0.95 ^{bc}	3.11 ± 1.90 ^{b}
	1.5/1.0	55.62	2.3 ± 1.01 ^{c}	3.34 ± 1.58 ^{b}
	2.0/1.0	47.01	3.45 ± 1.53 ^{ab}	2.56 ± 0.68^{cb}
Kin/IAA	1.0/1.0	53.27	0.32 ± 0.13 ^{e}	0.22 ± 0.09 ^{d}
	1.5/1.0	37.45	0.64 ± 0.12 ^{e}	0.56 ± 0.12 ^{d}
	2.0/1.0	62.18	1.96 ± 0.91 ^{cd}	2.42 ± 1.54 ^{cb}

Mean followed by different alphabetical letters are considered statistically significant at the level of $p<0.05$

Table 2. Effects of BA and NAA in the second subculture on shoot multiplication.

Plant hormones	Conce. (mg/L)	Response %	No. of shoot ± SE	Length of shoot (cm) ± SE
NAA/BA	1.0/1.5	47.54	6.29 ± 1.74^{b}	5.12 ± 1.23^{a}
	1.5/1.5	73.12	5.31 ± 1.52^{c}	3.31 ± 1.31^{bc}
	2.0/1.5	53.2	5.17 ± 1.94^{c}	4.51 ± 1.24^{b}
	2.5/1.5	62.5	4.79 ± 1.45^{cd}	2.67 ± 1.34^{c}
	3.0/1.5	48.03	4.21 ± 1.34^{d}	3.17 ± 1.44^{bc}
BA	1.5	82.25	8.34 ± 1.56^{a}	5.17 ± 1.97^{a}

Mean followed by different alphabets are statistically significant at the level of $p<0.05$

Table 3. Effects of auxins on the induction of callus from nodal explants.

Plant growth regulators	Conce. (mg/L)	Explant	Response %	Callus fresh weight (g) ± SE	Callus morphology
IAA	0.5	Node	21	1.04 ± 0.43^{d}	White, friable
	1.0		24	1.53 ± 0.64^{c}	
	1.5		29	1.18 ± 0.52^{cd}	
IBA	0.5		17	1.19 ± 0.43^{cd}	White, and friable, light brown
	1.0		20	2.06 ± 0.98^{b}	
	1.5		22	1.27 ± 0.74^{cd}	
NAA	0.5		46	1.62 ± 0.43^{c}	White, light green, compact and nodulated
	1.0		62	2.49 ± 0.94^{ab}	
	1.5		57	3.12 ± 1.08^{a}	

Mean shown by different alphabets are statistically significant at the level of $p<0.05$

Induction of adventitious shoots from callus: Shoot induction from the callus is an important step in the mass production of plantlets. Selection of the right medium may increase the efficiency of shoot formation. The results shown in Fig. 4 indicated that different hormone concentrations led to significant differences ($p\leq0.05$) in the formation of shoots from the callus. The colour of callus was changed from brownish to pale green when placed in a medium containing BA or Kinetin. After a few days, several green spots appeared on its surface, which ultimately developed into primordial structures. Shoots initiated from these structures when cultured on a medium containing either BA or Kinetin (Fig. 3f). Among the different concentrations of BA, the highest number of shoots (9.73) was observed at 1.5 mg/L, which was significantly greater than the number of shoots produced at other BA concentrations (Fig. 4). The average number of shoots induced with Kinetin at 1.0, 1.5, 2.0, and 2.5 mg/L was 6.83, 5.21, 4.25 and 3.84 respectively.

Induction of roots: The next critical stage in developing a successful *In vitro* regeneration protocol is the rooting of shoots. To induce roots, NAA was applied at different concentrations in the medium (Table 4). The average number of roots produced at 1.0, 1.5, 2.0, and 2.5 mg/L of NAA was 3.75, 3.97, 5.62 and 4.37, respectively. The root length and percent response were affected by the NAA concentration. The average root lengths at 1.0, 1.5, 2.0, and 2.5 mg/L were 3.52, 5.71, 5.33, and 4.84 cm, respectively. The percentage response rates also varied at the different NAA concentrations: 51.23%, 73.18%, 53.58%, and 47.62% at 1.0, 1.5, 2.0, and 2.5 mg/L of NAA, respectively.

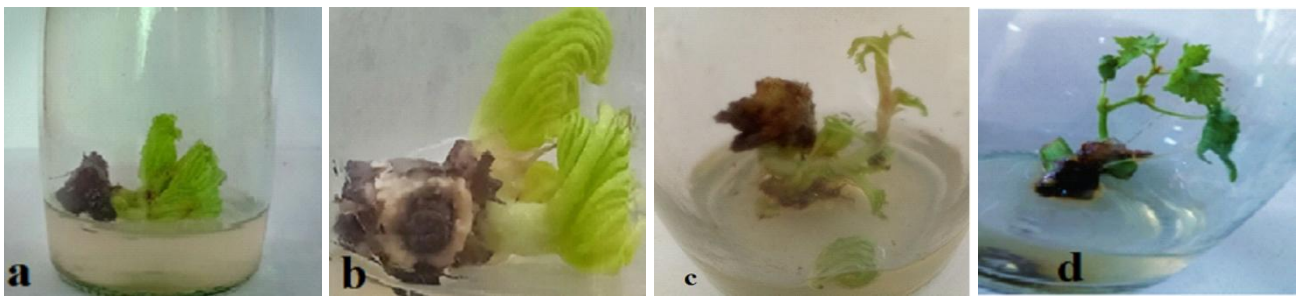

Fig. 1. Proliferation of shoots on MS media: **a)** shoot initiation after 5-9 days, **b)** shoot multiplication after 10-18 days, **c)** shoot elongation after 19-26 days, and **d)** shoot elongation after 28-45 days.

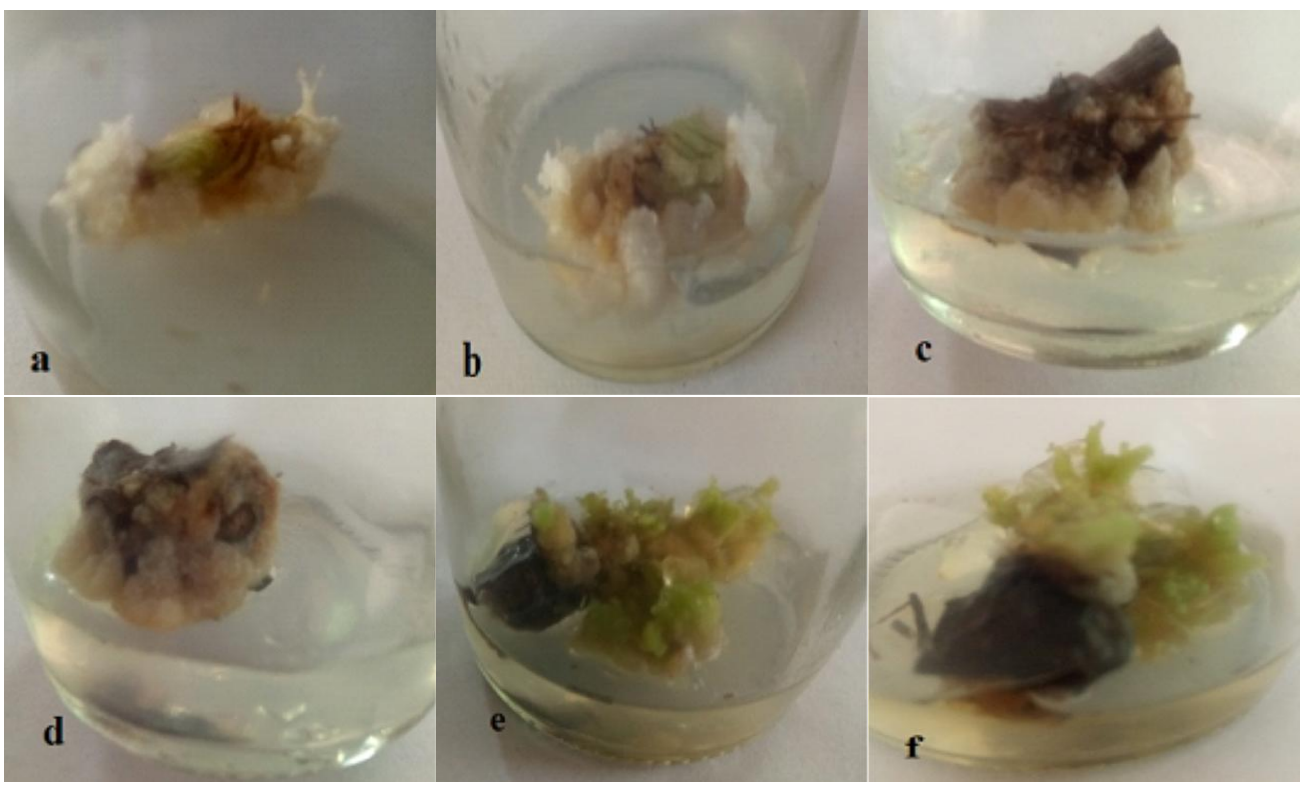

Fig. 3. Different types of Callus: **a, b)** with IAA; **c)** with IBA; **d, e)** with NAA; **f)** adventitious shoot of callus with BA.

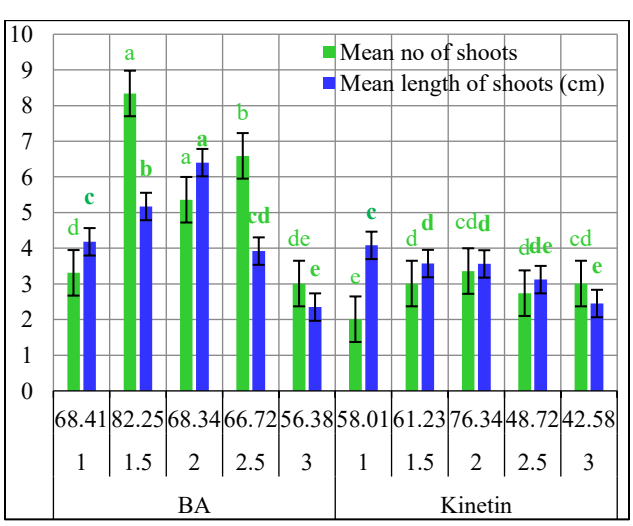

Fig. 2. Effect of BA and Kinetin on the multiplication of shoots during subculture
Key: Alphabets not differing from one another are considered the same, while those that differ significantly at $p<0.05$ are deemed distinct.

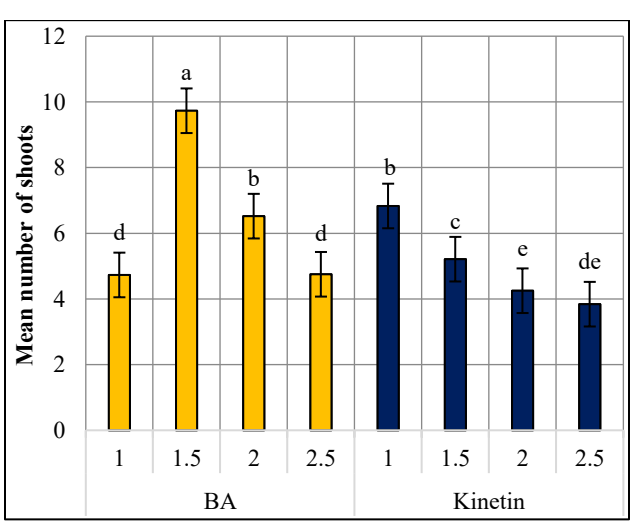

Fig. 4. Adventitious shoots formation from callus with varying concentrations of BA and Kinetin.
Key: The means not significantly different from one another share the same alphabet, while significantly different means are denoted by different alphabets at ($p<0.05$).

Table 4. Effects of NAA on the induction of roots in the *In vitro* shoots.

Plant hormones	Conc. (mg/L)	Response %	No. of root ± SE	Length of root (cm) ± SE
NAA	1.0	51.23	3.75 ± 1.63[c]	3.52 ± 1.88[c]
	1.5	73.18	3.97 ± 1.46[bc]	5.71 ± 1.98[a]
	2.0	53.58	5.62 ± 1.94[a]	5.33 ± 2.01[ab]
	2.5	47.62	4.37 ± 1.89[b]	4.84 ± 1.23[b]
Control	0.0	--	--	--

Mean followed by different alphabets are statistically significant at the level of $p<0.05$

Table 5. Phenolic compounds in the leaf (VJLME) and fruit (VJFME) extracts of *V. jacquemontii*.

VJLME					VJFME				
Peak	Rt (min)	λmax (nm)	Peak height (mAU)	Phenolic compounds	Peak	Rt (min)	λmax (nm)	Peak height (mAU)	Phenolic compounds
1	11.82	320	7.17	Ascorbic acid	1	2.39	320	7.70	Phloroglucinol
2	16.23		1.86	Chlorogenic acid	2	3.92		1.71	Hydroxy bezoic acid
3	33.71		2.71	Quercetin	3	10.62		3.18	Ascorbic acid
4	35.20		18.15	Rutin	4	15.81		34.62	Chlorogenic acid
5	36.26		5.70	Morin	5	32.50		59.46	Quercetin
					6	35.21		17.79	Rutin
6	38.87		2.69	Catechine hydrate	7	36.29		5.71	Morin
					8	38.92		2.75	Catechine hydrate

HPLC profile of bioactive compounds: The HPLC chromatograms of leaf (VJLME) and fruit (VJFME) extracts are shown in Figs. 5 & 6. In these figures, the chromatographic profile of the selected portion of *V. jacquemontii* extracts is reported at 280 nm. The compounds identified in the analyzed extracts are shown in Table 5. In the VJLME of *V. jacquemontii* rutin was the most dominant constituent with a peak height of 18.15 mAU followed by morin with a peak height of 5.70 mAU. Similarly, in the VJFME of *V. jacquemontii* quercetin was the most dominant constituent with a peak height of 59.46 mAU followed by chlorogenic acid with a peak height of 34.62 mAU. Compared to leaf extract (VJLME), the fruit extract (VJFME) contained two additional compounds namely phloroglucinol and hydroxybenzoic acid.

Quantitative profile of bioactive compounds (TTC, TPC, and TFC) analysis: The amounts of tannins, phenolics, and flavonoids varied significantly in the VJLME and VJFME extracts of *V. jacquemontii*. The total tannin contents (TTC) in VJLME and VJFME extracts were 67.03 and 80.21 mg GAE/g, respectively. The results in Table 6 showed a significant difference in total phenolic content (TPC) between the VJLME and VJFME extracts. In the VJLME extract, the TPC was 101.89 mg GAE/g of DW and in the VJFME extract, it was 197.65 mg GAE/g DW. The total flavonoid contents in VJLME and VJFME extracts were 103.20 and 136.89 mg QE/g of DW, respectively.

Table 6. TPC, TFC, and TTC in the fruit and leaf samples.

Compounds group	Samples	
	VJLME	VJFME
TTC (mg GAE/g)	67.03 ± 1.28[b]	92.21 ± 1.03[c]
TPC (mg GAE g/DW)	101.89 ± 1.67[a]	197.65 ± 1.82[a]
TFC (mg QE/g of DW)	103.20 ± 1.21[a]	136.89 ± 1.31[b]

Mean followed by different alphabets are statistically significant at the level of $p<0.05$

Key: TTC: Total tannin contents, TPC: Total phenolic content, TFC: Total flavonoids content, mg: Milligram, GAE: Gallic acid equivalent, QE: Quercetin equivalent

Discussion

This study aimed to develop an efficient micropropagation protocol for *V. jacquemontii*. This species is facing increasing pressure from human activity for its medicinal and to some extent food value, resulting in a decline in its population. A micropropagation protocol is critical as it requires only a small amount of material to initiate cultures and rapidly produce multiple plantlets. This research utilizes the *In vitro* method for plant protection and provides a model for safeguarding and restoring *V. jacquemontii In vitro*. Biotechnological methods for large-scale propagation will aid in conserving plants without harming their natural environment (Rybczynski *et al*., 2004). Re-establishing an unprotected species in its natural environment is expected to accelerate future efforts in species conservation projects. When propagating plants, a node is the best tissue for initiating *In vitro* cultures. The node of *V. jacquemontii* has successfully induced shoots and callus in response to growth regulators. Other studies have also confirmed the effective use of nodes for shoot induction (Abu-Romman *et al*., 2015; Reddy *et al*., 2015). However, culturing the explant on a medium without growth regulators resulted it to turn brown and die. Therefore, the presence of cytokinins (BA-1.5 mg/l or Kineting-1.0 mg/l) is essential for the *In vitro* morphogenesis of this plant. Cytokinins are often used to successfully propagate plants and played a central role in promoting the growth of multiple shoots in *Gymnema sylvestre* (Komalavalli and Rao, 2000) and *Ocimum gratissimum* (Saha *et al*., 2012). It has been shown that the ratio of cytokinin to auxin in the growth medium affects the induction of shoots and roots in tobacco cultures. A high cytokinin to low auxin ratio promotes shoot induction, while the opposite ratio stimulates root formation (Skoog *et al*., 1957). The cytokinin types concentration, and combination are crucial for plant cell division, morphogenesis, and overall growth and development (Srivastava, 2002). In our study, we observed that using an intermediate concentration of BA (1.5 mg/l) resulted in the highest number of shoots. However, the shoots produced were shorter in length. On the other hand, BA at 2.0 mg/l led to

greater shoot length. Conversely, BA at 3.0 mg/l caused a significant decline in sprouting rate, shoot number, and shoot length. Explants cultured on Kinetin-supplemented media displayed the least sprouting rate, number, and length of shoot formation across concentrations. Our research indicates that a concentration of 1.5 mg/l of BA is best for the micropropagation of *V. jacquemontii*. Previous studies demonstrated that BA was effective for the *In vitro* propagation of various species, including *Cirsium hillii* (Sheikholeslami *et al*., 2020) and *Castilleja levisecta* (Salama *et al*., 2018). Kinetin has also been utilized for shoot multiplication in different plant species, including *Limoniastrum monopetalum* (Martini Papafotiou, 2020) and *Cucumis sativus* (Abu-Romman *et al*., 2015). Our study found that combinations of cytokinin (BA) and auxin (NAA) were inhibitory, leading to significantly lower sprouting rates, numbers, and lengths of shoots. It was observed that cytokinins alone prompted the best response overall. These results suggest that BA or Kinetin can more effectively increase shoot multiplication, consistent with previous findings (Jan *et al*., 2020). Applying BA at various concentrations alone stimulates shoot multiplication, regulates cell differentiation, activates RNA synthesis, and enhances the efficiency of proteins and enzymes (Naito *et al*., 2006).

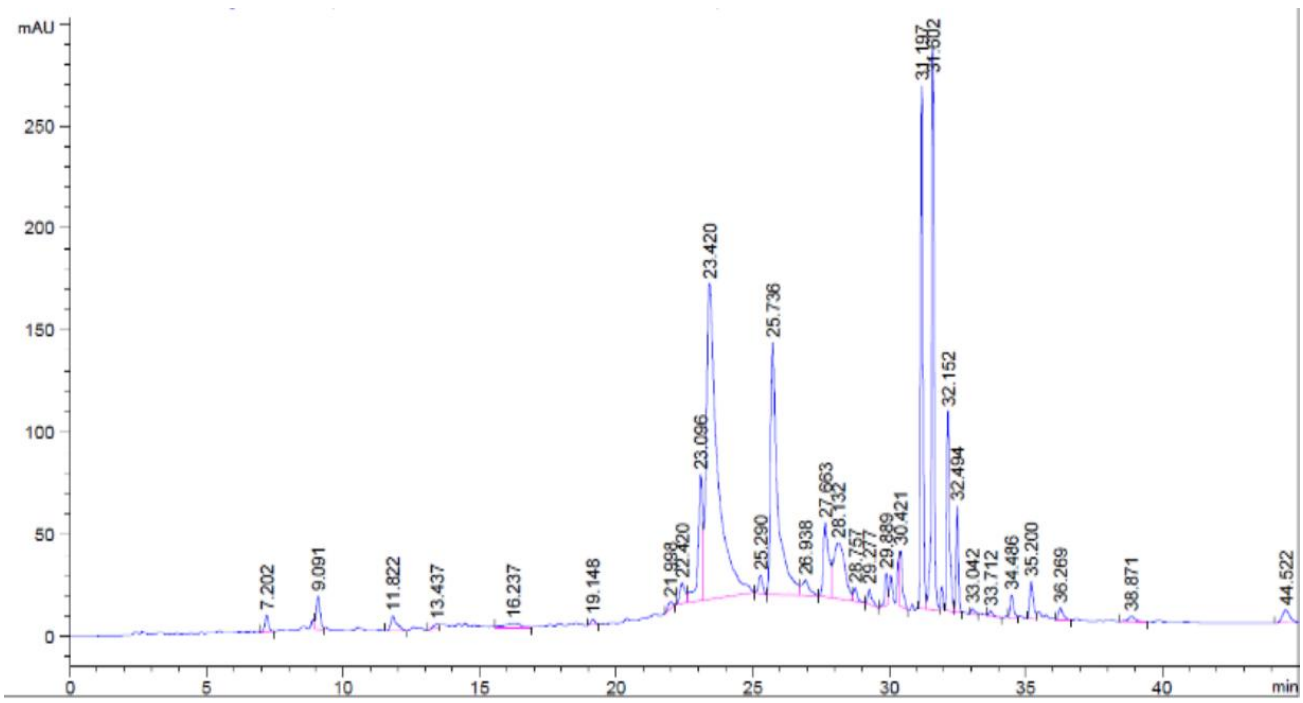

Fig. 5. HPLC chromatogram of the leaf extract of *V. jacquemontii.*

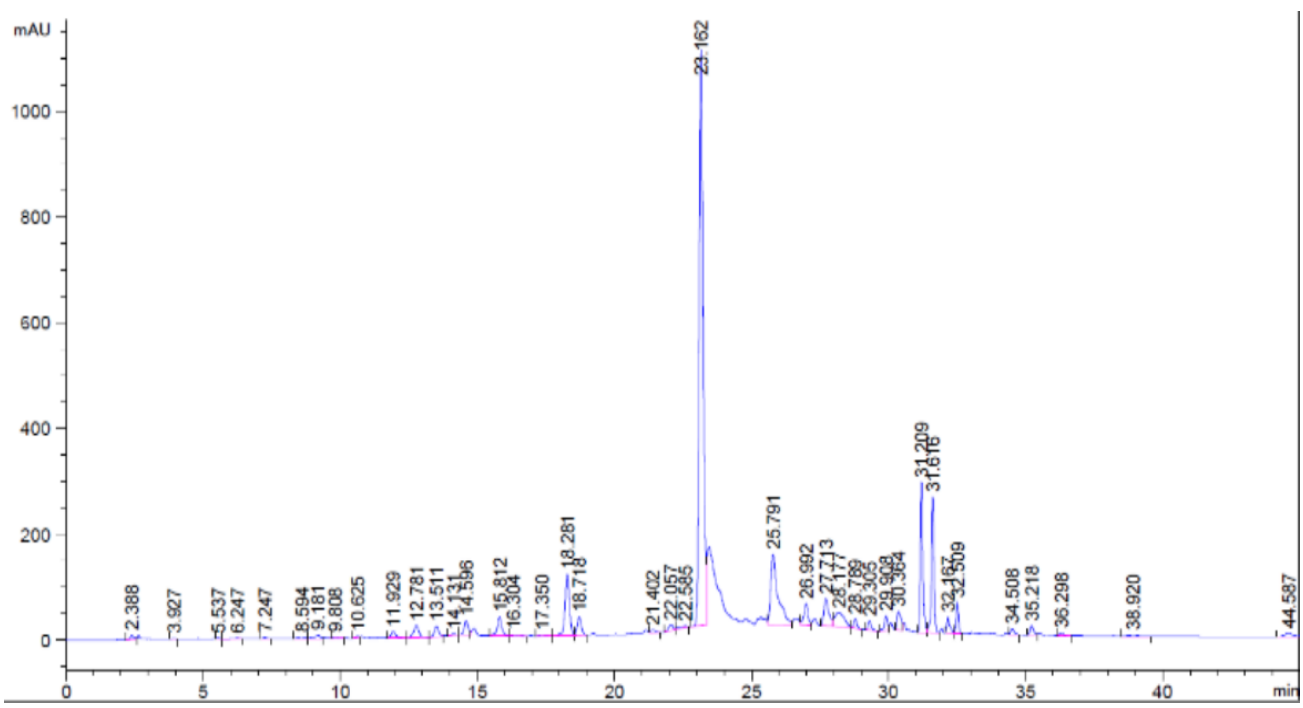

Fig. 6. HPLC chromatogram of the fruit extract of *V. jacquemontii.*

Callus culture is crucial for producing genetically modified plants with altered traits such as resistance to salinity, diseases, and high temperatures. In *V. jacquemontii*, nodal explants form calli on MS mediu with different concentrations of IAA, IBA, and NAA. When the effects of different auxins were compared, it was observed that NAA at a concentration of 1.5 mg/l resulted in a high amount of callus formation. These differences in callus formation ability among the growth regulators can be explained by differences in the biochemical breakdown of the plant cell wall and their sensitivities to cellular mechanisms (Gaspar *et al*., 1996). This effect of NAA is consistent with the findings from other studies, such as in *Pantanus tectorius*, where callus formation was induced on MS medium containing 3.0 mg/l of NAA (Zainal *et al*., 2010), and in *Chenopodium ambrosioides* (Jan *et al*., 2023). The callus induced by NAA, IAA, and IBA displayed various morphologies such as white, light brown, soft, light green, compact, and nodulated. It was noted that brownish callus could only develop rhizogenic potential after turning pale green on a medium containing cytokinins. In the assessment of cytokinins on the formation of adventitious shoots from callus, BA and Kinetin were compared, and it was observed that BA at 1.5 mg/l resulted in the highest number of adventitious shoots compared to Kinetin. The number of adventitious shoot formations from callus was lowest in explants cultured on Kinetin compared to BA across different concentrations. BA was more effective than Kinetin for adventitious shoot formation from the callus of *V. jacquemontii*, previous research also demonstrated its superiority over kinetin for shoot multiplication (Cristea *et al*., 2014; Arikat *et al*., 2004). Auxin has an important role in plants in the control of cell division, elongation, differentiation, and adventitious root formation (Dilworth *et al*., 2017). In our experiments, the NAA concentrations were matched to ensure their impact on the in vitro shoot rooting. The rooting response was observed across all NAA concentrations, ranging from 47.62% to 73.18%. Root number and length were copious at 2.0 mg/l NAA but suppressed as NAA concentration increased. Research by Huang *et al*., (2014) reported that *In vitro*-grown *Gentiana scabra* formed roots on a medium containing NAA (0.3 mg/l) and IAA (0.1 mg/l). The results of Bekheet (2013) demonstrated that supplementation of IAA, IBA, or NAA at 1.0 mg/l induced root in the *In vitro* shoot of *Phoenix dactylifera*.

HPLC is a method used to evaluate the phenolic compounds in plant extracts. In the current investigation, the chemical constituents in the leaves (VJLME) and fruits (VJFME) of *V. jacquemontii* were identified and quantified using HPLC. The fruit (VJFME) extract contained all of the identified compounds from the leaf (VJLME) extract; however, the chemical constituent's phloroglucinol and hydroxy benzoic acid from the fruit (VJFME) extract were not present in the leaf (VJLME) extract. All the identified compounds have therapeutic uses such as rutin, which has anticancer, antioxidant, and antimicrobial properties (Chang *et al*., 2002). Phytochemical assessment is used to determine the predominance of secondary metabolites in plant extracts and to identify bioactive compounds that may be useful in medicinal applications (Silva *et al*., 2016). A literature survey revealed that phenolics, flavonoids, anthocyanins, and tannins play vital roles in the antioxidant potential of grapes (Meng *et al*., 2012). In the present study, the phytochemical evaluation of *V. jacquemontii* revealed that the main constituents of the leaf and fruit extracts were TPC, TFC, and TTC. The TPC, TFC, and TTC concentrations were higher in the fruit extract than in the leaf extract. The varietal alteration in TPC, TFC, and TTC between leaf and fruit extracts advocated that the fruit extract might have a higher antioxidant potential than the leaf extract. The phytochemical study of *Pistacia lentiscus* by Hamad *et al*., (2011) indicated the presence of triterpenes, flavonoids, tannins, and carbohydrates.

Conclusion

In our current study, we have found that nodes are a good source of explants. They are available throughout the year, which is especially beneficial for grapevines when there is a limited selection of explants due to the unavailability of seeds. Nodal explants of *V. jacquemontii* have the potential for quick multiple-shoot regeneration and subsequent micropropagation under the influence of BA. This regeneration system has the potential to produce significant economic benefits by enabling virus-free plant production, which in turn supports the breeding program. The current study results of phytochemical analysis of fruit and leaf extracts may enhance the commercial potential of this *Vitis* species for its antioxidant property.

Author's Contribution: All the authors equally contributed

Conflict of Interest: The authors declare there are no competing interests.

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