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Development of an Efficient *In vitro* Propagation Protocol for Large Scale Production of *Vitis vinifera* L. cv. 'Black Magic'

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Abstract

This study aimed to standardize a reproducible protocol for direct and indirect micropropagation of the grapevine *Vitis vinifera* L. cv. 'Black Magic' using shoot tips, nodal segments, and young leaf tissues as explants. All experiments were conducted on MS basal medium. Direct shoot induction was found to be the most effective on 1.5 mg/l BAP supplemented medium, producing 86.67% response with 4.00 ± 0.55 shoots per nodal explants and 80% response with 3.20 ± 0.37 shoots per shoot tip explants. Maximum callus induction was obtained on 2.0 mg/l 2,4-D, producing 90% response in nodal explants and 75% in leaf tissues within 18-25 days. Organogenesis from callus was most successful on 1.5 mg/l BAP, producing 86.67% responsive calli with 14.20 ± 0.97 shoots per callus and 3.94 ± 0.41 cm in shoot length. Maximum shoot proliferation (92%) was achieved on 1.0 mg/l BAP + 0.5 mg/l NAA with 3% sucrose, yielding 17.89 ± 1.84 shoots per culture, 4.44 ± 0.29 cm shoot length, and 8.44 ± 0.58 leaves per shoot. The response was further enhanced by the addition of 5% coconut water, resulting in an average of 19.11 ± 1.60 shoots per culture, with a mean shoot length of 4.81 ± 0.71 cm and 9.00 ± 0.60 leaves per shoot. The number of shoots per culture increased from the 1st to the 4th subculture, reaching a maximum of 21.56 ± 2.77 shoots before declining in the 5th cycle. Rooting was appeared to be the highest on half-strength MS medium with 3% sucrose and 1.0 mg/l IBA, achieving 88% rooting, 9.20 ± 2.46 roots per shoot, and a mean root length of 6.14 ± 0.62 cm. Rooted plantlets were transferred to polybags containing garden soil, sand, and compost (2 : 1 : 1), where 83.33% survival rate was recorded.

Introduction

The grapevine (*Vitis vinifera* L.), belonging to the Vitaceae family, is one of the world's most economically important fruit crops cultivated in tropical and temperate regions (Khadatkar et al. 2025). Among the 57 species of the genus *Vitis*, *V. vinifera* accounts for nearly 94% of global grape production and forms the basis of the international grape

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industry (Bavaresco 2019, Bouquet and Torregrosa 2003). Grapes are consumed fresh or processed into raisins, juice, jam, jelly, vinegar, grape seed oil, and wine (Khan et al. 2020). According to the International Organization of Vine and Wine (OIV 2025), approximately 47% of global grape production in 2024 was utilized for wine, must, and juice production, while 46% was consumed as fresh fruit. In Bangladesh, grape consumption has increased significantly, as reflected by the rise in fresh grape imports from 104,000 metric tonnes in 2023 to 125,000 metric tonnes in 2024, mainly imported from China and India (Index Mundi 2024).

V. vinifera L. 'Black Magic' is a recently introduced table grape cultivar in Bangladesh, characterized by its elongated finger-like berries and attractive purple-black appearance. It is an early-budding and early-ripening variety with a high yield potential ranging from 15 to 20 t/ha (Delic et al. 2017, Mohamed 2023). The grape clusters weigh approximately 400-500 g, with individual berries weighing 5-6 g, containing 17.4% sugar and showing excellent resistance to several diseases (Delic et al. 2017). Grape cultivation is gradually expanding in Bangladesh due to favorable soil conditions, increasing market demand, and the potential to reduce import dependency. Preliminary trials conducted by BARI demonstrated that grapevine cultivation is feasible under Bangladeshi agro-climatic conditions (Biswas and Nazrul 1997). At present, commercial cultivation is expanding in regions such as Chuadanga, Jashore, Rajshahi, Jhenaidah, and Naogaon (Seraj 2014). However, grape production is constrained by the scarcity of improved cultivars and certified disease-free planting materials. Conventional vegetative propagation methods such as cuttings, layering, and grafting are slow, inefficient, and associated with a high risk of disease transmission, while the long juvenility period further restricts rapid multiplication (Osman et al. 2008, Bertolini et al. 2010, Chowdhury et al. 2012, Anupa et al. 2016, Hashemi et al. 2020).

In vitro propagation offers an effective alternative for the rapid multiplication of disease-free and true-to-type grapevine plants. Micropropagation techniques, including shoot tip culture, axillary bud proliferation, and adventitious regeneration, have been successfully employed in several *Vitis* genotypes worldwide (Kurmi et al. 2011, Khan et al. 2015). Nevertheless, regeneration efficiency is highly genotype dependent and influenced by culture media composition, growth regulators, and environmental conditions, necessitating cultivar-specific optimization (Kinfe et al. 2017). Despite the increasing popularity of 'Black Magic' in Bangladesh, no standardized *in vitro* regeneration protocol has yet been reported for this cultivar. Therefore, the present study aims to establish and optimize an efficient *in vitro* propagation protocol for *V. vinifera* L. 'Black Magic' for large-scale production of disease-free, true-to-type planting materials.

Materials and Methods

Healthy and actively growing shoot tips, nodal segments, and young leaf tissues were collected as explants from *V. vinifera* L. cv. 'Black Magic'. The explants were washed thoroughly under running tap water for 15-20 min, followed by treatment with a few

drops of Tween-20 in distilled water for 20-25 min, and then rinsed 3-5 times with distilled water. Further sterilization was carried out inside a laminar airflow cabinet using 0.5% Bavistin solution for 5 min, followed by several rinses with autoclaved distilled water. Finally, shoot tips and young leaf explants were surface sterilized with 0.1% $HgCl_2$ for 3 min, while nodal segments were treated with 0.2% $HgCl_2$ for 2 min. Before culture, the $HgCl_2$-exposed cut ends of the explants were aseptically trimmed using sterilized scalpel and forceps.

For direct shoot induction, sterilized shoot tips and nodal segments were cultured individually on MS medium containing various concentrations of BAP (0.0-3.0 mg/l) alone or in combination with NAA (0.5 mg/l). For callus induction, leaf tissue and nodal segments were inoculated on MS medium supplemented with different concentrations of 2,4-D (0.0-3.0 mg/l). Actively growing callus tissues measuring 1.0-2.0 cm^2 were excised and transferred to MS medium containing BAP (0.0-2.5 mg/l) alone or in combination with 0.5 mg/l NAA or 0.5 mg/l Kn for indirect shoot regeneration. Directly and indirectly induced shoots were excised and sub-cultured onto fresh MS media containing different concentrations and combinations of BAP, Kn, TDZ, 2-iP, IBA and NAA to evaluate their effects on shoot proliferation. The influence of subsequent subculture, sucrose concentration (2-4%) and coconut water (5-10%) was also examined to optimize shoot multiplication and growth. Cultures were maintained at 25 ± 2°C under a 16/8 h light/dark photoperiod with a light intensity of 2000-2500 lux.

Elongated shoots were cultured on solidified ½MS medium containing 2-3% sucrose and with different strengths and combinations of IBA and NAA for root induction. Cultures were maintained in darkness for the first 2-3 days and subsequently transferred to standard growth conditions. Well-rooted *V. vinifera* 'Black Magic' shoots were transferred to different potting mixtures to assess the role of suitable substrate in successful adaptation. Finally, they were transferred to larger pots and maintained under shade for 2 weeks before exposure to ambient greenhouse conditions. All experiments were conducted in a completely randomized design (CRD), and data were presented as mean ± SE. Statistical analysis was performed using one-way ANOVA in SPSS version 16.0, and means were compared by DMRT at $p \leq 0.05$.

Results and Discussion

Direct shoot induction was significantly influenced by BAP concentration in MS medium. Both shoot tips and nodal explants showed the best response at 1.5 mg/l BAP, where nodal segments produced higher shoot induction (86.67%) and a greater number of shoots per explant (4.00 ± 0.55) compared to shoot tips (80% response and 3.20 ± 0.37 shoots per explant) (Tables 1&2, Fig. 1 a,b). However, shoots derived from shoot tips were slightly longer (4.84 ± 0.65 cm) than those from nodal segments (4.42 ± 0.54 cm). Lower or higher BAP concentrations reduced shoot induction efficiency. These results are in line with previous reports showing that low BAP concentrations (0.5-2.5 mg/l) effectively promote direct shoot induction from shoot tips and nodal explants in various

V. vinifera cultivars, such as 'Thompson' (Hashemi et al. 2020) and 'Red Globe' and 'Superior' (Osama 2022). In contrast, Alizadeh et al. (2018) reported better responses at a higher BAP concentration (4.0 mg/l) in *V. champinii* 'Dogridge' and *V. vinifera* × *V. labrusca* 'H-144'.

The effect of 0.5 mg/l NAA combined with BAP on shoot induction from nodal segments was also evaluated. However, the addition of NAA did not improve the response compared to 1.5 mg/l BAP alone (Table 2). In contrast, Chowdhury et al. (2012) reported maximum shoot induction in *V. vinifera* 'Zakkao' with 2.0 mg/l BAP + 0.5 mg/l NAA, while Abido et al. (2013) successfully used BAP (0.5-2.0 mg/l) with 0.1 mg/l NAA in *V. vinifera* 'Muscat of Alexandria'.

Table 1. Effects of varying concentrations of BAP on direct shoot induction from *V. vinifera* 'Black Magic' shoot tip explants.

BAP (mg/l)	Responding explants (%)	Shoots / explants (Mean ± SE*)	Shoot length (cm) (Mean ± SE*)
0	20.00	$0.60^{c} \pm 0.40$	$1.48^{b} \pm 0.64$
1.0	50.00	$2.60^{ab} \pm 0.40$	$3.18^{ab} \pm 0.61$
1.5	**80.00**	$\mathbf{3.20^{a} \pm 0.37}$	$\mathbf{4.84^{a} \pm 0.65}$
2.0	60.00	$2.80^{ab} \pm 0.37$	$3.22^{ab} \pm 0.69$
2.5	40.00	$1.80^{bc} \pm 0.58$	$2.64^{b} \pm 0.23$
3.0	30.00	$1.00^{c} \pm 0.45$	$2.12^{b} \pm 0.27$

Values are mean ± SE* (Standard Error) of 5 independent cultures; identical letters within a column denote no significant difference (DMRT, $\alpha = 0.05$).

Table 2. Effects of varying concentrations of BAP alone or in combination with NAA on direct shoot induction from *V. vinifera* 'Black Magic' nodal segments.

BAP (mg/l)	NAA (mg/l)	Responding explants (%)	Shoots / explants (Mean ± SE*)	Shoot length (cm) (Mean ± SE*)
0	0	13.33	$0.40^{e} \pm 0.24$	$1.12^{e} \pm 0.69$
1.0	0	53.33	$3.40^{ab} \pm 0.51$	$2.94^{abcd} \pm 0.54$
1.5	**0**	**86.67**	$\mathbf{4.00^{a} \pm 0.55}$	$\mathbf{4.42^{a} \pm 0.54}$
2.0	0	73.33	$3.00^{abc} \pm 0.55$	$3.04^{abcd} \pm 0.57$
2.5	0	40.00	$2.20^{bcd} \pm 0.20$	$2.22^{cde} \pm 0.28$
3.0	0	33.33	$2.20^{bcd} \pm 0.37$	$1.76^{de} \pm 0.35$
1.0	0.5	60.00	$2.40^{bcd} \pm 0.24$	$3.16^{abcd} \pm 0.50$
1.5	0.5	73.33	$3.40^{ab} \pm 0.68$	$4.22^{ab} \pm 0.62$
2.0	0.5	73.33	$2.00^{bcd} \pm 0.63$	$3.50^{abc} \pm 0.56$
2.5	0.5	53.33	$1.80^{cd} \pm 0.49$	$2.66^{bcde} \pm 0.47$
3.0	0.5	40.00	$1.20^{de} \pm 0.20$	$2.54^{bcde} \pm 0.51$

Values are mean ± SE* (Standard Error) of 5 independent cultures; identical letters within a column denote no significant difference (DMRT, $\alpha = 0.05$).

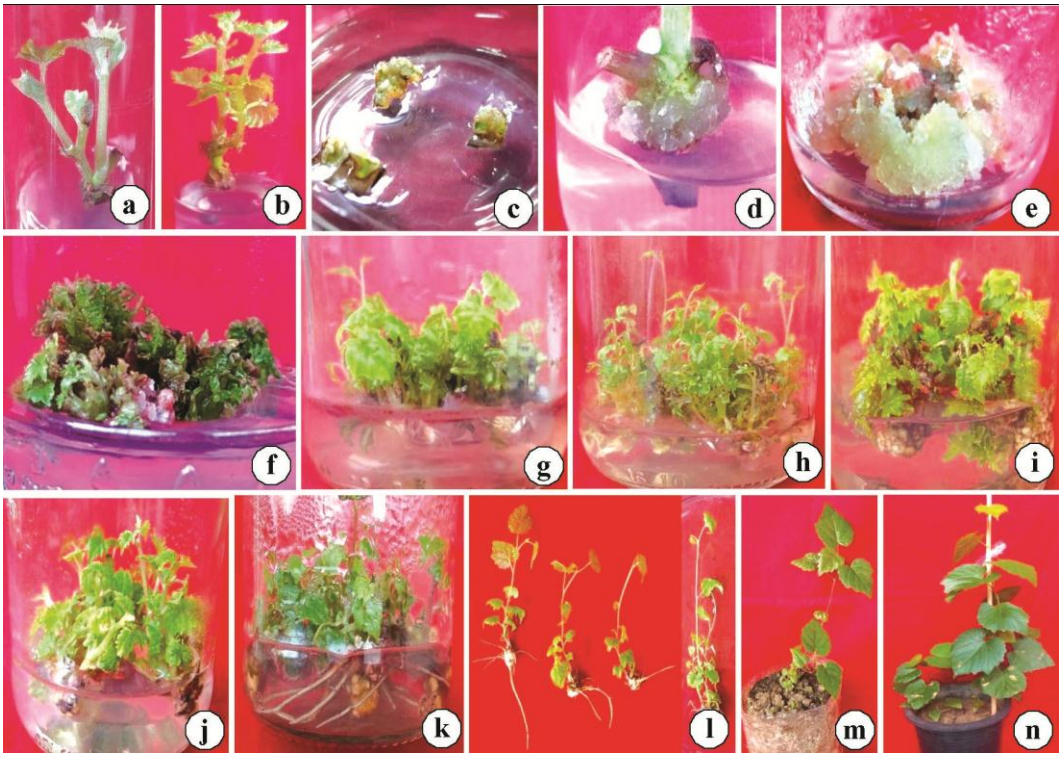

Fig. 1(a-n). Different stages of micropropagation of *V. vinifera* L. cv. 'Black Magic': (a-b) direct shoot inductions from *(a)* shoot tip and *(b)* nodal segment on MS + 1.5 mg/l BAP, (c-d) callus induction from *(c)* leaf tissue and *(d)* nodal segment on MS + 2.0 mg/l 2,4-D, (e) multiplication of the callus, (f-g) shoot regeneration from callus after *(f)* 3-weeks and *(g)* 5-weeks on MS + 1.5 mg/l BAP, (h-i) shoot multiplication on MS + 1.0 mg/l BAP + 0.5 mg/l NAA, after the *(h)* 1st, and *(i)* 4th subcultures, (j) enhanced shoot multiplication on MS + 1.0 mg/l BAP + 0.5 mg/l NAA + 3% sucrose + 5% CW, (k) rooting on solidified ½ MS + 1.0 mg/l IBA + 3% sucrose, (l) complete plantlets, (m) acclimatization in a potting mixture of garden soil, sand and compost (2 : 1 : 1), and (n) Two-month-old acclimatized plant growing in a larger pot.

This study evaluated the effects of 2,4-D on callus induction from leaf and nodal explants of *V. vinifera* 'Black Magic'. The best response obtained with 2.0 mg/l 2,4-D, producing abundant and actively growing callus (Table 3, Fig. 1c-e). Nodal segments showed superior performance, with 90% callus induction and faster initiation (18.20 ± 1.36 days) compared to leaf explants, which showed 75% induction of callus after 24.80 ± 2.87 days. Similar findings were reported across different grape cultivars, where maximum callus induction from nodal explants was achieved with the same 2,4-D concentration (Kurmi et al. 2011, Shaker et al. 2024). The better response of nodal explants over leaf tissues was also observed by Polat and Kaya (2025) in *V. vinifera* cv. 'Chardonnay' using 0.5 mg/l 2,4-D + 0.2 mg/l NAA. In contrast, Pehlivan et al. (2017) reported higher callus induction from leaf disc cultures than nodal explants in *V. vinifera* 'Sultana' using 0.1 mg/l 2,4-D + 1.0 mg/l BAP.

Table 3. Effects of varying concentrations of 2,4-D in MS medium on callus induction from young leaf tissues (YLT) and nodal segments (NS) of *V. vinifera* 'Black Magic'.

2,4-D (mg/l)	Type of explants	CIF (%)	Required days (Mean ± SE*)	Nature of callus	
				Color	Texture
0	YLT	00	-	-	-
	NS	00	-	-	-
0.5	YLT	20.00	$28.00^{bcd} \pm 2.59$	Whitish	Loose
	NS	25.00	$26.40^{bcd} \pm 2.42$	Whitish-green	Friable
1.0	YLT	45.00	$31.20^{cd} \pm 2.73$	Pale yellow	Compact
	NS	55.00	$27.80^{bcd} \pm 3.28$	Creamy	Compact
1.5	YLT	65.00	$28.60^{bcd} \pm 3.39$	Pale yellow	Compact
	NS	70.00	$22.80^{ab} \pm 3.47$	Creamy	Compact
2.0	**YLT**	**75.00**	**$24.80^{abcd} \pm 2.87$**	**Whitish-brown**	**Compact/Granular**
	NS	**90.00**	**$18.20^{a} \pm 1.36$**	**Off-white**	**Brittle**
2.5	YLT	50.00	$32.00^{d} \pm 2.07$	Yellowish-white	Compact with necrotic patches
	NS	60.00	$24.00^{abc} \pm 1.67$	Light green	Friable
3.0	YLT	35.00	$31.00^{cd} \pm 2.10$	Yellowish-white	Compact with necrotic patches
	NS	45.00	$26.00^{bcd} \pm 1.79$	Brownish	Hard

Values are mean ± SE* (Standard Error) of 5 independent cultures; identical letters within a column denote no significant difference (DMRT, $\alpha = 0.05$); CIF: Callus Induction Frequency.

Table 4. Effects of varying concentrations of BAP alone or in combination with Kn or NAA on indirect shoot organogenesis from induced callus of *V. vinifera* 'Black Magic'.

Growth regulators (mg/l)			Responsive callus (%)	Shoots / callus (Mean ± SE*)	Shoot length (cm) (Mean ± SE*)	Leaves / shoot (Mean ± SE*)
BAP	Kn	NAA				
0	0	0	-	-	-	-
1.0	0	0	50.00	$6.40^{bc} \pm 1.12$	$2.42^{bc} \pm 0.32$	$4.20^{de} \pm 0.58$
1.5	**0**	**0**	**86.67**	**$14.20^{a} \pm 0.97$**	**$3.94^{a} \pm 0.41$**	$6.80^{ab} \pm 0.73$
2.0	0	0	63.33	$7.60^{bc} \pm 1.29$	$2.66^{bc} \pm 0.24$	$4.60^{cde} \pm 0.81$
2.5	0	0	46.67	$5.40^{c} \pm 0.87$	$1.70^{cd} \pm 0.29$	$3.40^{e} \pm 0.40$
1.0	0.5	0	56.67	$7.00^{bc} \pm 1.30$	$2.14^{bcd} \pm 0.23$	$6.40^{abc} \pm 0.68$
1.5	**0.5**	**0**	70.00	$8.00^{bc} \pm 0.95$	**$3.86^{a} \pm 0.20$**	**$7.60^{a} \pm 0.75$**
2.0	0.5	0	43.33	$6.20^{bc} \pm 1.11$	$1.32^{d} \pm 0.32$	$3.40^{e} \pm 0.24$
1.0	0	0.5	60.00	$6.80^{bc} \pm 0.73$	$1.72^{cd} \pm 0.39$	$3.80^{de} \pm 0.58$
1.5	0	0.5	76.67	$8.80^{b} \pm 0.86$	$2.80^{b} \pm 0.31$	$5.60^{bcd} \pm 0.75$
2.0	0	0.5	53.33	$7.40^{bc} \pm 0.68$	$1.68^{cd} \pm 0.31$	$3.20^{e} \pm 0.37$

Values are mean ± SE* (Standard Error) of 5 independent cultures; identical letters within a column denote no significant difference (DMRT, $\alpha = 0.05$).

The present study demonstrated that cytokinin is essential for shoot induction from callus tissues, as no adventitious shoot buds formed on hormone-free MS medium and the callus gradually turned brown and died. Among the tested treatments, 1.5 mg/l BAP

proved most effective, yielding the highest responsive callus (86.67%), the greatest number of shoots per callus (14.20 ± 0.97), the longest shoots (3.94 ± 0.41 cm), and a relatively high leaf count (6.80 ± 0.73) (Table 4, Fig. 1f,g). Similarly, Kumsa and Feyissa (2019) reported maximum shoot formation from leaf callus in *V. vinifera* cv. 'Chenin Blanc' and 'Canonannon' using 2.0 mg/l BAP.

In contrast, Polat and Kaya (2025) reported enhanced indirect shoot formation in *V. vinifera* cv. 'Chardonnay' using 1.0 mg/l BAP + 0.2 mg/l NAA. Similarly, Kurmi et al. (2010) achieved high morphogenic callus and plantlet formation with 2.0 mg/l BAP + 0.5 mg/l NAA, while Ibrahim and Soliman (2018) reported shoot organogenesis on medium containing 1.5 mg/l BAP, 0.5 mg/l kinetin, and 0.5 mg/l NAA. However, in our study, combinations of BAP with Kn or NAA did not produce results as satisfactory as those previously reported (Table 4).

This experiment evaluated the effects of different growth regulators on the proliferation of *in vitro* raised shoots of *V. vinifera* 'Black Magic'. Among the treatments, 1.0 mg/l BAP + 0.5 mg/l NAA was the most effective, producing the highest response (92%), maximum shoots per culture (17.89 ± 1.84), longest shoots (4.44 ± 0.29 cm), and highest number of leaves per shoot (8.44 ± 0.58) (Table 5, Fig. 1h). Similar results were reported by Chowdhury et al. (2012), Abido et al. (2013), Khan et al. (2015) and, Arifuzzaman et al. (2016), who found that BAP (1.0-3.0 mg/l) combined with low concentrations of NAA (0.1-0.5 mg/l) effectively enhanced shoot proliferation in *V. vinifera*. In contrast, Kim et al. (2023) observed that NAA inhibited shoot development and promoted callus formation in the grapevine cultivar 'Shine Muscat'.

On the other hand, in the present study, combinations of BAP with IBA, Kn, or TDZ were less effective than BAP alone or BAP with NAA for shoot proliferation (Table 5). In contrast, Pehlivan et al. (2017) and Kinfe et al. (2017) reported optimal shoot proliferation in different *V. vinifera* cultivars using 1.0 mg/l BAP + 0.1 mg/l IBA, while Jamwal et al. (2013) achieved the best response with 1.0 mg/l BAP + 0.5 mg/l Kn in cv. 'Perlette'. Moreover, Kurmi et al. (2011) and Kumsa and Feyissa (2019) found that TDZ combined with NAA enhanced shoot regeneration and increased shoot numbers in several *V. vinifera* cultivars.

This experiment assessed the individual effects of BAP, Kn, and 2-iP on shoot proliferation. BAP was the most effective, with 1.5 mg/l producing the highest regeneration frequency (88%) and the greatest number of shoots per callus (16.33 ± 2.18) (Table 5). Kn improved shoot vigor, while 2-iP showed moderate performance. These results agree with the previous studies reporting the superiority of BAP over Kn, TDZ, and 2-iP in *V. vinifera* cultivars such as 'Shine Muscat' (Kim et al. 2023), 'Red Globe', and 'Superior' (Osama 2022). However, contrasting results were reported in *V. vinifera* 'Marechal Foch', where 2-iP promoted better shoot and leaf development (Ikten and Read 2010).

Table 5. Effects of varying concentrations of growth regulators, applied alone or in combination, on the proliferation of directly and indirectly induced shoots of *V. vinifera* 'Black Magic'.

Growth regulators (mg/l)			Responsive culture (%)	Shoots / culture (Mean ± SE*)	Shoot length (cm) (Mean ± SE*)	Leaves / shoot (Mean ± SE*)
BAP	Kn	2-iP				
1.0	-	-	56	$6.67^{bc} \pm 0.65$	$1.84^{c} \pm 0.13$	$4.11^{def} \pm 0.35$
1.5	**-**	**-**	**88**	$\mathbf{16.33^{a} \pm 2.18}$	$3.20^{ab} \pm 0.25$	$6.78^{b} \pm 0.40$
2.0	-	-	68	$7.11^{b} \pm 0.51$	$2.77^{b} \pm 0.18$	$5.89^{bc} \pm 0.20$
2.5	-	-	52	$5.78^{bc} \pm 0.66$	$2.68^{b} \pm 0.25$	$3.33^{ef} \pm 0.17$
-	1.0	-	40	$2.11^{de} \pm 0.28$	$1.90^{c} \pm 0.16$	$3.22^{ef} \pm 0.28$
-	**1.5**	**-**	48	$3.89^{cde} \pm 0.75$	$\mathbf{3.64^{a} \pm 0.21}$	$\mathbf{8.67^{a} \pm 0.75}$
-	2.0	-	28	$1.44^{e} \pm 0.28$	$3.01^{b} \pm 0.20$	$2.78^{f} \pm 0.28$
-	-	1.0	32	$3.78^{cde} \pm 0.75$	$1.54^{c} \pm 0.19$	$4.56^{cde} \pm 0.75$
-	-	1.5	44	$4.89^{bcd} \pm 0.95$	$2.04^{c} \pm 0.25$	$5.22^{cd} \pm 0.95$
-	-	2.0	36	$4.11^{cde} \pm 0.41$	$2.03^{c} \pm 0.22$	$3.67^{def} \pm 0.41$
BAP	NAA	IBA				
1.0	0.2	-	56	$6.11^{c} \pm 0.48$	$1.01^{cd} \pm 0.08$	$4.56^{c} \pm 0.38$
1.0	**0.5**	**-**	**92**	$\mathbf{17.89^{a} \pm 1.84}$	$\mathbf{4.44^{a} \pm 0.29}$	$\mathbf{8.44^{a} \pm 0.58}$
1.0	1.0	-	40	$3.89^{cd} \pm 0.51$	$0.93^{d} \pm 0.09$	$2.78^{e} \pm 0.22$
1.5	0.2	-	64	$6.44^{c} \pm 0.80$	$1.22^{cd} \pm 0.22$	$4.11^{c} \pm 0.42$
1.5	0.5	-	80	$11.89^{b} \pm 1.51$	$1.61^{bc} \pm 0.23$	$6.78^{b} \pm 0.40$
1.5	1.0	-	60	$6.56^{c} \pm 0.69$	$1.43^{bcd} \pm 0.13$	$3.44^{cde} \pm 0.38$
1.0	-	0.5	24	$1.78^{d} \pm 0.55$	$1.28^{cd} \pm 0.19$	$2.89^{de} \pm 0.26$
1.5	-	0.5	32	$2.22^{d} \pm 0.46$	$2.01^{b} \pm 0.32$	$4.00^{cd} \pm 0.37$
BAP	Kn	TDZ				
1.0	0.5	-	48	$8.44^{abc} \pm 1.11$	$2.24^{a} \pm 0.19$	$5.44^{bc} \pm 0.38$
1.0	1.0	-	72	$11.33^{a} \pm 1.67$	$2.30^{a} \pm 0.08$	$6.22^{ab} \pm 0.46$
1.5	0.5	-	60	$9.89^{ab} \pm 1.57$	$2.49^{a} \pm 0.11$	$7.56^{a} \pm 0.91$
1.5	1.0	-	32	$5.56^{cd} \pm 0.90$	$1.51^{b} \pm 0.26$	$4.11^{c} \pm 0.42$
1.0	-	0.5	36	$5.33^{cd} \pm 0.53$	$1.63^{b} \pm 0.34$	$4.44^{bc} \pm 0.50$
1.0	-	1.0	64	$7.78^{bc} \pm 0.62$	$2.62^{a} \pm 0.13$	$7.67^{a} \pm 0.97$
1.5	-	0.5	68	$7.89^{bc} \pm 0.86$	$2.69^{a} \pm 0.19$	$7.78^{a} \pm 0.89$
1.5	-	1.0	24	$3.11^{d} \pm 0.82$	$1.57^{b} \pm 0.22$	$3.44^{c} \pm 0.38$

Values are mean ± SE* (Standard Error) of 9 independent cultures from 3 replicates; identical letters within a column denote no significant difference (DMRT, $\alpha = 0.05$).

Fresh shoots from the multiplication stage were dissected into segments containing 2-3 shoots and cultured on MS medium supplemented with either 1.5 mg/l BAP or 1.0 mg/l BAP + 0.5 mg/l NAA for periodic subculture to assess their effect on shoot multiplication (Fig. 2). Shoots on 1.5 mg/l BAP showed only slight increases during the first two cycles, whereas those on 1.0 mg/l BAP + 0.5 mg/l NAA steadily increased to peak in the fourth cycle, with 21.56 ± 2.77 shoots per culture averaging 5.03 ± 0.49 cm in length (Fig. 1i, Fig. 2). These results are consistent with those of Mostafa et al. (2015) and

Osama (2022), who reported that grapevine cultivars exhibit peak proliferation and significant variation in shoot length during the third subculture, followed by a gradual decline in subsequent cycles.

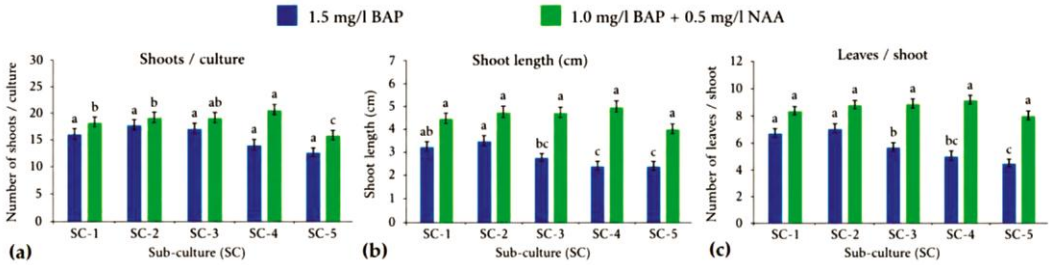

Fig. 2(a-c). Comparative effects of consecutive subcultures on: (a) shoot number, (b) shoot length, and (c) leaf number of *V. vinifera* 'Black Magic' cultured on MS medium with 1.5 mg/l BAP and 1.0 mg/l BAP + 0.5 mg/l NAA.

Table 6. Effects of sucrose and coconut water (CW) on shoot multiplication of *V. vinifera* 'Black Magic' cultured on MS medium with 1.0 mg/l BAP + 0.5 mg/l NAA.

Media supplementation	Shoots / culture (Mean ± SE*)	Leaves / shoot (Mean ± SE*)	Shoot length (cm) (Mean ± SE*)
Sucrose (%)			
2.0	$7.33^{b} \pm 0.68$	**$11.33^{a} \pm 0.87$**	$2.29^{b} \pm 0.10$
3.0	**$17.89^{a} \pm 1.84$**	$8.44^{b} \pm 0.58$	**$4.44^{a} \pm 0.29$**
4.0	$4.22^{b} \pm 1.02$	$5.22^{c} \pm 0.20$	$1.71^{c} \pm 0.12$
CW (%)			
0.0	$17.89^{a} \pm 1.84$	$8.44^{b} \pm 0.58$	$4.44^{a} \pm 0.29$
5.0	**$19.11^{a} \pm 1.60$**	**$9.00^{a} \pm 0.60$**	**$4.81^{a} \pm 0.71$**
10.0	$10.00^{b} \pm 1.57$	$6.11^{b} \pm 0.63$	$3.12^{b} \pm 0.56$

Values are mean ± SE* (Standard Error) of 9 independent cultures from 3 replicates; identical letters within a column denote no significant difference (DMRT, $\alpha = 0.05$).

This study also evaluated the effects of sucrose and coconut water (CW), in combination with 1.0 mg/l BAP + 0.5 mg/l NAA, on shoot regeneration and growth. As shown in Table 6, the number of shoots per culture and shoot length increased with increasing sucrose concentration up to 3%, after which all growth parameters declined. Similarly, supplementation with CW enhanced shoot proliferation up to 5%, beyond which regeneration and growth decreased. The combination of 3% sucrose and 5% CW produced the best results, yielding the highest number of shoots per culture (19.11 ± 1.60), maximum shoot length (4.81 ± 0.71 cm), and the greatest number of leaves per shoot (9.00 ± 0.60) (Fig. 1j). Although reports on the use of CW in *V. vinifera* are limited, the beneficial effect of 3% sucrose is well documented. Tarinejad and Amiri (2019) in *V. vinifera* cv. 'Shahroudi' and Diab et al. (2011) in cv. 'Speryo' reported optimal shoot

proliferation at 3% sucrose, whereas lower (2%) or higher (4%) concentrations reduced regeneration efficiency. Likewise, Kurmi et al. (2011) successfully used 3% sucrose for *in vitro* grape culture.

Newly regenerated shoots rooted most effectively on gelled half-strength MS medium containing 1.0 mg/l IBA and 3% sucrose, producing the highest rooting frequency (88%), an average of 9.20 ± 2.46 roots per shoot, and a mean root length of 6.14 ± 0.62 cm within 22.4 ± 1.33 days (Table 7, Fig. 1k). In contrast, NAA alone or in combination with IBA was less effective for root induction. Similar results were reported by Mostafa et al. (2015), Ali et al. (2017) and, Kumsa and Feyissa (2019), who found that ½ MS medium containing 1.0-2.0 mg/l IBA was optimal for rooting in various *V. vinifera* cultivars. However, Chowdhury et al. (2012) observed maximum root length in *V. vinifera* cv. 'Zakkao' at a higher IBA concentration (3.0 mg/l). In the present study, reducing sucrose to 2% significantly decreased rooting performance even under optimal 1.0 mg/l IBA (Table 7), whereas Kurmi et al. (2011) reported enhanced rooting with 1.0 mg/l IBA and 1% sucrose in three grape cultivars.

Table 7. Influence of auxin and sucrose concentrations in half-strength of MS medium on *in vitro* rooting of micro-shoots of *V. vinifera* 'Black Magic'.

Sucrose (%)	IBA (mg/l)	NAA (mg/l)	Responsive cultures (%)	Required days (Mean ± SE*)	Roots / shoot (Mean ± SE*)	Root length (cm) (Mean ± SE*)
3%	0	0	20.00	$24.80^{abc} \pm 1.20$	$2.80^{c} \pm 0.37$	$2.36^{c} \pm 0.21$
	1.0	**-**	**88.00**	**$22.40^{a} \pm 1.33$**	**$9.20^{a} \pm 2.46$**	**$6.14^{a} \pm 0.62$**
	2.0	-	64.00	$24.00^{abc} \pm 1.52$	$5.60^{abc} \pm 0.68$	$4.30^{b} \pm 0.66$
	-	1.0	56.00	$27.40^{abc} \pm 1.12$	$4.60^{bc} \pm 0.51$	$4.06^{b} \pm 0.72$
	-	2.0	32.00	$27.80^{bc} \pm 2.13$	$4.00^{bc} \pm 0.32$	$3.10^{bc} \pm 0.38$
	1.0	0.5	80.00	$22.80^{ab} \pm 1.85$	$7.40^{ab} \pm 1.25$	$4.44^{b} \pm 0.58$
2%	1.0	-	48.00	$28.20^{c} \pm 1.53$	$7.00^{ab} \pm 1.79$	$2.08^{c} \pm 0.10$
	-	1.0	28.00	$28.40^{c} \pm 1.89$	$3.80^{bc} \pm 0.37$	$2.52^{c} \pm 0.28$
	1.0	0.5	36.00	$25.00^{abc} \pm 1.92$	$4.20^{bc} \pm 0.66$	$2.98^{bc} \pm 0.35$

Values are mean ± SE* (Standard Error) of 5 independent cultures; identical letters within a column denote no significant difference (DMRT, $\alpha = 0.05$).

Well-rooted plantlets of *V. vinifera* cv. 'Black Magic' (Fig. 1l) were transferred to different potting mixtures to evaluate acclimatization success. Among them, garden soil, compost and sand (2 : 1 : 1) produced the highest survival rate (83.33%) (Fig. 1m). Comparable results were reported by Kinfe et al. (2017), who achieved 74-92% survival in grape cultivars using the same substrate composition. Similarly, Jamwal et al. (2013) recorded a 73.33% survival rate in *V. vinifera* cv. 'Perlette' grown in a mixture of soil, sand, FYM, and vermiculture (1 : 1 : 1 : 1). However, Ali et al. (2017) achieved high survival of *V. vinifera* cv. 'Thomson' using a 1 : 1 sand-and-soil mixture for acclimatization. After 30-days, the plants were transplanted into larger pots and

successfully produced new leaves. The acclimatized plants exhibited vigorous shoot and root development and produced a satisfactory number of leaves per plant (Fig. 1n).

The present investigation successfully established an efficient *in vitro* regeneration and propagation protocol for *V. vinifera* L. cv. 'Black Magic' through systematic evaluation of explant type, surface sterilization, growth regulators, carbohydrate and organic supplements, and acclimatization media. Overall, the developed protocol provides an efficient, reproducible, and genotype-specific framework for large-scale propagation of *Vitis vinifera* cv. 'Black Magic'.

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