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Investigation of callus induction in Chardonnay grapevine (*Vitis vinifera* L.)

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Abstract

Callus induction is an important step in plant transformation aimed at improving grapevines, one of the most cultivated fruit crops. Callus induction can be influenced by nutrients, plant growth regulators, physical environments, explant type, infection, and genetic factors. This study investigates the efficiency of callus induction in the Chardonnay grape variety using different nutrient mediums, plant growth regulators, and explant types. Internode and leaf disc parts were selected as explant types and cultured in Murashige and Skoog (MS) and Lloyd and McCown Woody Plant (WP) nutrient medium with 7% agar, containing varying concentrations of plant growth regulators such as NAA, 2,4-D, and BA. Different combinations resulted in various types calli, including friable, compact, shooty, and rooty, with different colors by the end of the 21st day. WP medium consistently yielded the highest callus induction rates across all plant growth regulator combinations. The combination of 0.5 mg/L 2,4-D and 0.2 mg/L NAA in MS medium produced the largest callus area in internode explants. Conversely, leaf discs exhibited lower callus induction rates. Additionally, 1 mg/L BA and 0.2 mg/L NAA in MS medium promoted shoot formation, while the same combination in WP medium facilitated both shoot and root formation in internode explants.

Introduction

Belonging to the genus *Vitis* in the *Vitaceae* family, *Vitis vinifera* is a widely recognized grape species. With a prevalence surpassing that of all other species by 90%, *Vitis vinifera* is readily identifiable and commonly encountered (Parihar and Sharma, 2021). *Vitis vinifera*, commonly known as the European grapevine, holds immense significance in viticulture and winemaking. Among the diverse grape varieties within the *Vitis vinifera* species, Chardonnay stands out as one of the most influential and widely cultivated cultivars (Robinson et al., 2013). Chardonnay grapes are prized for their adaptability to various climates and soil types, contributing to their global prominence in the production of high-quality wines. Chardonnay, a widely favored white wine grape cultivar within *Vitis vinifera*, demonstrates sensitivity to abiotic stresses (Wang et al., 2018).

Plant tissue culture, a sophisticated biotechnological technique, has emerged as a crucial tool in plant biology with diverse applications (Efferth, 2019). This method involves the aseptic culture of plant cells, tissues, or organs in a controlled environment, enabling researchers to manipulate plant growth and development (Hussain et al., 2012). Additionally, plant tissue culture facilitates the production of disease-free plant material by eliminating pathogens, thereby establishing healthy and robust plant stocks (Chadipiralla et al., 2020).

One of the most significant applications of plant tissue culture is callus production. Callus refers to a mass of undifferentiated plant cells that proliferate in tissue

culture (Ikeuchi et al., 2013). This cluster of cells, typically originating from explants such as leaves or stems, lacks specialized structures and exhibits rapid, unorganized growth. Callus formation is crucial in plant tissue culture techniques, serving as a starting point for various applications such as somatic embryogenesis, genetic transformation, and cell suspension culture (George et al., 2008). Researchers use callus cultures to study plant regeneration, manipulate genetic traits, and investigate plant cell behavior under controlled conditions. The versatility of callus cultures has made them invaluable for advancing biotechnological applications and understanding plant development (Gamborg et al., 1968). Callus is cultivated by transferring it to a nutrient medium containing plant growth regulators and a carbon source. This method yields successful results in artificial seed production, somaclonal variation, somatic embryogenesis and organogenesis, gene transfer, and mutation breeding studies (Khan et al., 2015). Research on grapevines has focused on optimizing callus production for applications such as somatic embryogenesis, genetic transformation, and cell suspension culture. Callus culture procedures also provide valuable avenues for conducting breeding in grapevines (Campos et al., 2021). Various plant parts, such as leaf discs, nodes, stem segments, and shoot tips, are used for callus growth studies (Khan et al., 2015).

Plant growth regulators within tissues exhibit pleiotropic effects, in which slight changes in concentration can trigger shifts in gene activation, affecting essential metabolic processes within cells (Teale et al., 2006). Thus, maintaining optimal concentrations of plant growth regulators is crucial for ensuring normal cellular functions (Khan et al., 2015). Plant growth regulators have been utilized to control and enhance plant growth and development, allowing growers to meet the increasing demand for high-quality food driven by global population growth. In citrus cultivation, several plant growth regulators have been evaluated; however, only a select few have been adopted for commercial use. Cytokinin-like growth regulators predominantly include substituted purines, with kinetin and BA (6-Benzylaminopurine) being the most commonly used cytokinins (Gaspar et al., 1996; Kieber and Schaller, 2014). Adenine, adenosine, and adenylic acid are also classified as cytokinins, although they exhibit cytokinin activity to a lesser extent compared to primary cytokinins. Adenine, recognized as vitamin B4, can be applied to induce or enhance responses typically associated with cytokinin action (Li et al., 2021). Frequently employed synthetic auxins in tissue culture include 2,4-dichlorophenoxyacetic acid (2,4-D), commonly used for callus induction and suspension cultures, and 1-naphthaleneacetic acid (NAA) (Gaspar et al., 1996).

This study focuses on optimizing callus induction from grapevine (Chardonnay), involving the testing of various parameters such as plant growth regulators, medium compositions, and plant material parts.

Materials and Methods

Plant materials and growth conditions

The Chardonnay cuttings were purchased from Kavaklıdere A.Ş. and initially placed in the greenhouses of the Manisa Viticulture Research Institute for acclimatization. After observing the leaves, the plants were transferred to growth chambers in the Department of Bioengineering at Manisa Celal Bayar University, where they were maintained under controlled temperature and environmental conditions. The Chardonnay cuttings used in the study were grown in a growth chamber set at day/night temperatures of 25°C/18°C, respectively, with a constant photoperiod of 16 h light and 8 h dark.

Sterilization

The surface-sterilization method was implemented based on a previous study with some modifications (Aguero et al., 2006; Bayraktar et al., 2015). Leaves and shoots were taken from cuttings of Chardonnay grown in the greenhouse. Explants were washed under running water and then surface-sterilized by sequential soaking in 0.64% (v/v) sodium hypochlorite for 30 min and 70% ethanol for 2 min, followed by four washes in sterile distilled water, respectively. After drying on sterile paper, leaf explants were cut into 0.7 cm squares and internode explants into 0.5-1 cm segments. Internode segments were wounded by making surface cuts at three different locations. Leaf disks and internodal segments were used as explants for callus induction.

Callus induction

Callus induction was performed on two different culture media (Murashige and Skoog (MS) (Murashige & Skoog, 1962) and Lloyd and McCown Woody Plant (WPM) (Llyod and McCown, 1980) supplemented with sucrose and different compositions of growth regulators (Table 1). Medium pH was adjusted to 5.8 and solidified by adding the gelling agent (7% agar). Each experimental setup (explant/medium combination) was created as three replicates, and 21 explants (internode segments and leaf discs) were placed in each petri dish (85 mm in diameter), containing 20 ml of medium for induction. All explants were grown aseptically in a growth chamber at 25°C/17°C and a 16h/8h (day/night) photocycle. Cultures were incubated for 21 days, and callus development was recorded after 7, 14, and 21 days.

Table 1. Type and concentration of plant growth regulators included in the culture medium to induce grape callus

Medium		BA (mg/L)	2,4 D (mg/L)	NAA (mg/L)
MS medium supplemented with 3% sucrose and 7% agar	MS1	1	0.5	-
	MS2	1	-	0.2
	MS3	-	0.5	0.2
	MS4	1	0.5	0.2
WPM medium supplemented with 3% sucrose and 7% agar	WPM1	1	0.5	-
	WPM2	1	-	0.2
	WPM3	-	0.5	0.2
	WPM4	1	0.5	0.2

The callus induction rate (%) was calculated after 21 days of induction using the formula below.

$$Callus\ induction\ rate = \frac{Number\ of\ explants\ with\ callus}{Total\ number\ of\ explants} x100$$

The callus proliferation efficiency (CPE) was also calculated based on the callus areas measured using ImageJ software. CPE was estimated using the following formula after 21 days of induction:

$$CPE = \frac{Total\ area\ of\ callus\ generated - Total\ area\ of\ explant\ before\ culture\ *}{Total\ area\ of\ explant\ before\ culture}\ x\ 100$$

* For leaf disc explants, the subtraction of the total area of the explant before culture was not applied.

Data analysis

All experiments were conducted with three replications, and outliers were identified and excluded from the dataset before analysis. A one-way ANOVA was performed to evaluate differences among treatments using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). Subsequent post-hoc analysis using Duncan's multiple range test, with significance set at $p \leq 0.01$, was conducted to determine significant differences between treatments. Data are reported as the mean of the three replicates ± standard error and were visualized using the R package ggplot2 (Wickham, 2011).

Results and Discussion

This study investigated the impact of different combinations of plant growth regulators, media, and explant types on callus induction. Variations were noted in several important parameters, including the duration required for callus initiation, callus induction rate (%), callus proliferation rate (%), and the texture and color of the callus.

Calli first appeared on the 7th and 14th days of culture in internode segments and leaf discs, respectively. Internode segments consistently exhibited a higher callus induction rate than leaf discs across all combinations of growth regulators and media (Table 2). Specifically, MS3, WPM3, and WPM4 resulted in the highest callus induction rates (100%) in internode segments, whereas MS2 showed the lowest but still high rate of 89%. In leaf disc explants, WPM4 yielded the highest callus induction rate (100%), while MS2 had the lowest rate (38%). Overall, WPM medium consistently showed better callus induction rates compared to MS medium across all combinations of growth regulators in leaf discs (Table 2).

Plant growth regulators, auxins and cytokinins, are vital regulators of plant growth and development, encompassing processes such as callus induction and proliferation (Gray, 2004; Su et al., 2011). Both endogenous and exogenous auxins and cytokinins are integral to these processes, and a precise balance between these growth regulators is crucial for successful callus formation (Skoog et al., 1957). Typically, moderate levels of auxins, such as 2,4-D, IAA (Indole-3-acetic acid), and NAA, combined with cytokinins, such as BA, facilitate callus induction (Kotb et al., 2020). In contrast, high ratios of auxin to cytokinin or cytokinin to auxin tend to promote the regeneration of roots and shoots, respectively (Ikeuchi et al., 2013; Skoog et al., 1957;). Incorporating these plant growth regulators into the basal medium can produce diverse effects on callus induction and proliferation, highlighting their significance in plant tissue culture (Kotb et al., 2020; Phillips and Garda, 2019;). This nuanced balance continues to be a central research focus due to its profound influence on callus development and organ regeneration (Ikeuchi et al., 2013).

Previous studies have investigated callus formation using various media supplemented with varying combinations of cytokinins and auxins in different grapevine cultivars (Khan et al., 2015; Ozden, 2024; Pehlivan et al., 2017; Wu et al., 2024). Wu et al. (2024) reported a 100% callus induction rate in Chardonnay leaf discs after 21 days of culture in the B5 medium supplemented with 0.05 mg/L NAA, 0.5 mg/L 2,4-D, 2 mg/L kinetin, and 60 g/L sucrose. In the present study, a similarly high callus induction rate of 100% was achieved using WPM4 medium with 30 g/L sucrose. Notably, our findings indicate that WPM4 medium, incorporating kinetin and lower concentrations of plant growth regulators, produced comparable results to the B5 medium, despite the higher sucrose concentration (60 g/L). This suggests that optimized plant growth regulator concentrations and reduced sucrose levels in WPM4 medium can achieve highly efficient callus induction outcomes. Wu et al. (2024) also reported a 70.48% callus induction rate in PIV (Nitsch and Nitsch 1969) medium with 1 mg/L 2,4-D and 2 mg/L BA, supplemented with 60

Table 2. Effect of different growth media on callus induction rate and proliferation efficiency in internode and leaf disc explants

Medium	Callus induction rate (%)		Callus proliferation efficiency (%)	
	Internode	Leaf discs	Internode*	Leaf discs*
MS1	98.3^{a} ± 2.89	81^{a} ± 26.9	188^{a} ± 10.5	53^{ab} ± 16
MS2	89^{a} ± 19.1	38^{a} ± 0	200^{a} ± 15.5	53^{ab} ± 2
MS3	100^{a} ± 0	90^{a} ± 0	414^{b} ± 53.5	50^{a} ± 0
MS4	98.3^{a} ± 2.89	95^{a} ± 7.07	197^{a} ± 4	52^{ab} ± 14
WPM1	98.3^{a} ± 2.89	88^{a} ± 17	242^{a} ± 87	74^{bc} ± 2
WPM2	98.3^{a} ± 2.89	54.5^{a} ± 30.4	240^{a} ± 26	61.5^{ab} ± 18.5
WPM3	100^{a} ± 2.89	57^{a} ± 33.9	286^{ab} ± 32	47^{ab} ± 1
WPM4	100^{a} ± 0	100^{a} ± 0	287^{ab} ± 13	118^{c} ± 6

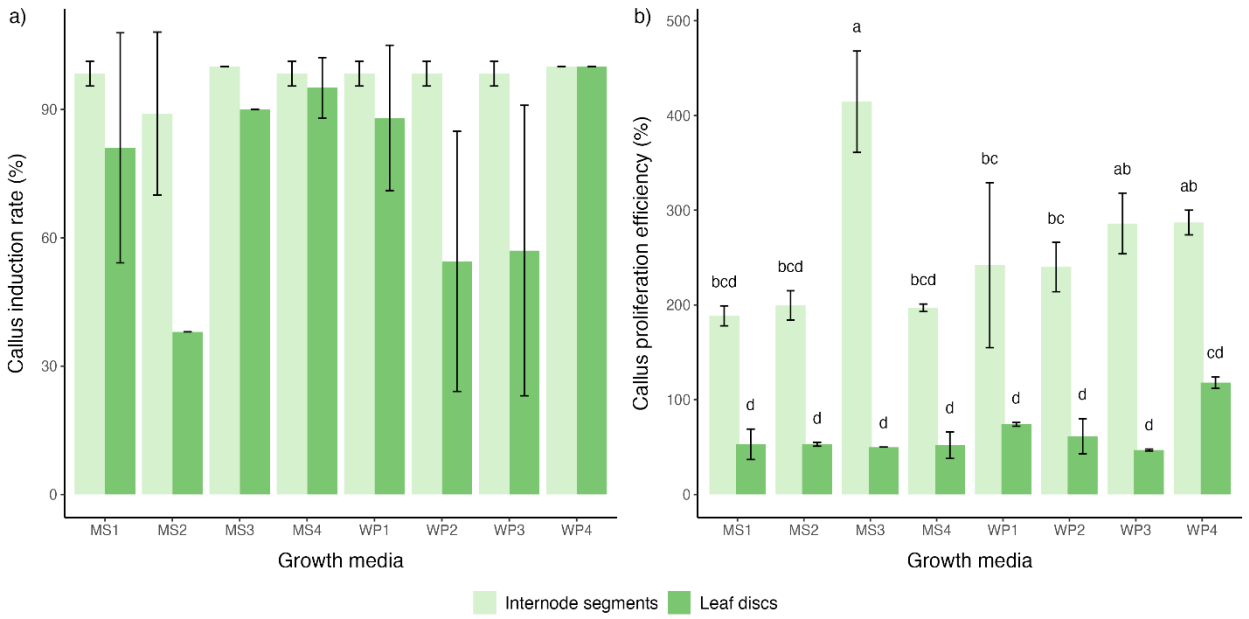

Figure 1. Callus induction rate **a)** and callus proliferation efficiency **b)** for different growth medium. Bars represent mean values ± standard error (SE). In panel **a)**, no significance letters are shown because no significant differences were detected among treatments ($p > 0.05$, Tukey's test). In panel **b)**, different letters above the bars denote statistically significant differences among treatments according to Tukey's HSD test ($p < 0.05$).

g/L of sucrose, from leaf discs. Based on our findings, reducing the sucrose concentration by half, while using the same plant growth regulator combination, resulted in higher callus induction rates of 81% and 88% with MS1 and WPM1 media, respectively.

According to Ozden (2024), varying concentrations of BA (0.0, 0.5, 1.0, and 2.0 mg/L) combined with 0.1 mg/L NAA in MS medium containing 3% sucrose yielded different callus induction rates from petiole explants of Chardonnay and Syrah grape cultivars. Callus formation was observed across all BA treatments. Among these, the combination of 0.5 mg/L BA and 0.1 mg/L NAA was the most effective, achieving a 96% callus induction rate (Ozden, 2024). In comparison, doubling the plant growth regulator concentration in MS2 medium with the same sucrose level resulted in a significantly lower callus induction rate of 38%. These findings suggest that excessive plant growth regulator concentrations, particularly higher levels of BA and NAA, may adversely affect callus induction, as evidenced by the reduced rates observed in Chardonnay leaf discs.

The callus induction rate of MS1 on leaf discs obtained in our study is lower than the results of Pehlivan et al. (2017). While we obtained an 81% , they obtained a 93% callus induction rate from Sultana grapevine leaf discs using an MS medium with 0.5 mg/L BAP and 1 mg/L 2,4-D. Additionally, in our study, the combination of 1 mg/l BA + 0.5 mg/L 2,4-D + 0.2 mg/L NAA resulted in a 95% callus induction rate from leaf disc explants. In contrast, Pehlivan et al. (2017) reported a 70% callus induction rate from node explants using a combination of 2 mg/L 2,4-D + 0.3 mg/L BAP + 0.2 mg/L NAA. The lower callus induction rate observed in their study compared to our results may be due to their use of the same plant growth regulator combinations at higher concentrations or different explants.

Khan et al. (2015) found that 2 mg/L 2,4-D combined with 0.3 mg/L BA in MS medium resulted in the highest callus induction rate (73%) from King's Ruby grapevine leaf discs. Additionally, they reported a 51% callus induction rate with 1.5 mg/L 2,4-D and 0.5 mg/L BAP. In comparison, our study achieved a higher callus induction rate in the Chardonnay cultivar using a lower concentration of 2,4-D and a higher concentration of BA in MS medium. Khan et al. (2015) concluded that reducing the concentration of 2,4-D and increasing the concentration of BAP decreased the callus formation rate in King's Ruby. However, in Chardonnay, a high callus induction rate was observed despite using high concentrations of BA and low concentrations of 2,4-D.

The callus proliferation efficiency (CPE) (%) was calculated as the ratio of the total area of callus generated to the total area of the explant before culture, after 21 days of culture in our study (Figure 1). As shown in Figures 2a and 2b, callus growth primarily began at the incision and wound sites of the internode segments, while leaf discs explant growth mostly started on the surface area. In our study, internode segments were wounded by making surface cuts at three different locations before culturing. A previous report also demonstrated that wounding increased callus formation in *Arabidopsis* (Iwase et al., 2011).

Previous studies on grapevine (Khan et al., 2015; Ozden et al., 2024; Pehlivan et al., 2017; Wu et al., 2024) did not report CPE values. In contrast, Gao et al. (2023) calculated CPE values for peach trees in a manner similar to our approach. Our results show that among internode explants, MS3 exhibited the highest CPE value (414%), while MS1 had the lowest (<200%) (Table 2). For leaf disc explants, WPM4 had the highest CPE value (>100%), and WPM3 had the lowest (<100%). The graph illustrates that internode explants generally have higher CPE

values compared to leaf discs, which aligns with the observed callus induction rates (Figure 2).

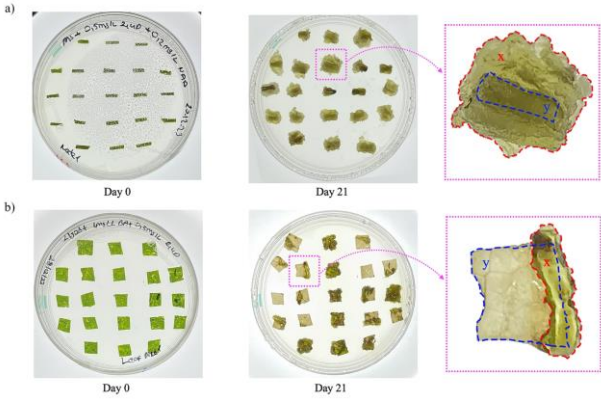

Figure 2. Callus development from different explants: **a)** Internode segments of Chardonnay cultured in MS medium supplemented with 1 mg/L 2,4-D and 0.2 mg/L NAA from day 0 to day 21. Callus proliferation efficiency was calculated as the ratio of callus area (x) to original explant area (y) using ImageJ for internode segments. **b)** Leaf discs of Chardonnay cultured in WPM medium containing 1 mg/L BA and 0.2 mg/L NAA from day 0 to day 21. Callus proliferation efficiency was similarly calculated using ImageJ for leaf discs.

Callus characteristics

Callus cultures with varying textures, sizes, and forms were observed among different growth media in both internode segments and leaf disc explants (Figure 3). Grapevine callus cultures are notably heterogeneous in texture and coloration (Ananga et al., 2013). Previous studies have reported that calli typically display a mix of colorless, yellow, green, and red clusters with a range of textures, including both friable and compact forms (Ananga et al., 2013; Cormier et al. 1996; Qu et al., 2005; Wu et al., 2024).

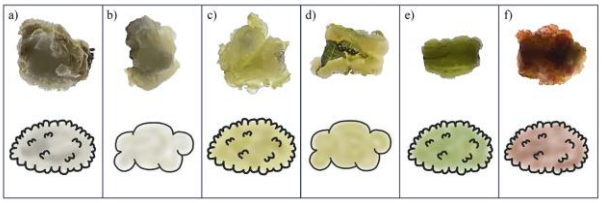

Figure 3. Callus formation, texture, and color from explants of Chardonnay in MS and WPM media supplemented with different plant growth regulators. **a)** White friable from internode on WPM4 and leaf disc on MS2, WPM2, **b)** white compact from internode on MS1 and leaf disc on WPM4, **c)** yellowish green friable from internode on MS3 and leaf disc on WPM1, **d)** yellowish green compact from leaf disc on MS3 and WPM1, **e)** green friable from internode on WPM2 and leaf disc on WPM3, **f)** reddish brown friable from internode on MS2 and WPM2.

In our study, we also observed a variety of colors and textures across all media and plant growth regulator combinations, as shown in Table 3. Predominantly, we obtained yellowish, green, and white callus colors from both explant types, with a generally friable texture. Internode segments exhibited primarily a friable texture (MS2, MS3, WPM2, WPM3, and WPM4), with partial compact areas (MS1, MS4, and WPM1), while leaf disc explants produced either friable (MS2, MS4, WPM1, WPM2, WPM3) or fully compact textures (MS1, MS3, and WPM4) callus texture. Similar to our findings, Wu et al. (2024) also reported yellowish and greenish callus from Chardonnay leaf discs, with textures varying from soft to compact. In our study, reddish/brown callus was exclusively observed in internode explants on MS2 and WPM2 media after two months in culture. Previous studies indicate that reddish coloration is associated with anthocyanin accumulation (Appelhagen et al., 2018; Hayta et al., 2016; Martínez et al., 2018). Carbon source type and concentration significantly influence callus color and texture during induction (Martínez et al., 2018). Additionally, illumination plays a critical role in the expression of anthocyanin production. It was also reported that light enhances anthocyanin production in callus cultures from various grapevine varieties, with pigment levels strongly correlated to the genotype used for callus initiation (Ananga et al., 2013). In our study, the combination of 1 mg/L BA and 0.2 mg/L NAA with MS or WPM medium effectively stimulated shoot formation. Additionally, the same combination with WPM medium promoted root formation. Wang et al. (2018) reported that NAA promoted root formation in tea cuttings. Similarly, in our study, WPM2 (1 mg/L BA + 0.2 mg/L NAA with WPM medium) promoted root formation from internode explants in Chardonnay.

Table 3. Summary of callus characteristics

Medium	Explant type	Callus texture	Callus color
MS1	Internode	Compact but with soft areas	White and green
	Leaf Disc	Compact	Yellowish
MS2	Internode	Shooty with friable areas	Yellowish/ reddish brown
	Leaf Disc	Friable	Yellowish and white
MS3	Internode	Friable	Yellowish green
	Leaf Disc	Compact	Yellowish green
MS4	Internode	Compact with soft areas	Green
	Leaf Disc	Friable	Yellowish
WPM1	Internode	Compact but with soft areas	Green
	Leaf Disc	Friable	Yellowish green
WPM2	Internode	Shooty, rooty/friable	Green and reddish brown
	Leaf Disc	Friable	Yellowish and white
WPM3	Internode	Friable	Yellowish
	Leaf Disc	Friable	Green
WPM4	Internode	Friable	Yellowish and white
	Leaf Disc	Compact	White

Conclusion

This study investigated *in vitro* callus induction using internode and leaf disc explants from Chardonnay. Callus formation was successful across all plant growth regulator combinations in both MS and WPM media. Internode explants yielded more callus than leaf disc

explants, with the highest callus production observed on MS3, WPM3, and WPM4 for internode explants and WPM4 for leaf disc explants. Additionally, different plant growth regulator combinations resulted in varied textures and colors of the callus. These findings suggest that the obtained callus could be valuable for biotechnological applications, including transformation and secondary metabolite production.

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Author Contributions

First Author: Designed, Performed, Writing -review and editing, Second Author: Supervision, Designed, Performed, Analyzed, Writing -review and editing.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Ananga, A., Georgiev, V., Ochieng, J. W., Phills, B. R., & Tsolova, V. (2013). Production of anthocyanins in grape cell cultures: A potential source of raw material for pharmaceutical, food, and cosmetic industries. *InTech*. https://doi.org/10.5772/54592

Aguero, C. B., Meredith, C. P., & Dandekar, A. M. (2006). Genetic transformation of Vitis vinifera L. cvs Thompson Seedless and Chardonnay with the pear PGIP and GFP encoding genes. *Vitis - Journal of Grapevine Research*, 45(1), 1-8. https://doi.org/10.5073/vitis.2006.45.1-8

Appelhagen, I., Wulff-Vester, A. K., Wendell, M., Hvoslef-Eide, A. K., Russell, J., Oertel, A., & Matros, A. (2018). Colour bio-factories: Towards scale-up production of anthocyanins in plant cell cultures. *Metabolic Engineering*, 48, 218-232. https://doi.org/10.1016/j.ymben.2018.06.004

Bayraktar, M., Hayta, S., Parlak, S., & Gurel, A. (2015). Micropropagation of centennial tertiary relict trees of Liquidambar orientalis Miller through meristematic nodules produced by cultures of primordial shoots. *Trees*, *29*, 999-1009. https://doi.org/10.1007/s00468-015-1179-2

Campos, G., Chialva, C., Miras, S., & Lijavetzky, D. (2021). New technologies and strategies for grapevine breeding through genetic transformation. *Frontiers in Plant Science*, 12, 767522. https://doi.org/10.3389/fpls.2021.767522

Chadipiralla, K., Gayathri, P., Rajani, V., & Reddy, P. V. B. (2020). Plant tissue culture and crop improvement. *Sustainable Agriculture in the Era of Climate Change*, 391-412. https://doi.org/10.1007/978-3-030-45669-6_18

Cormier, F., Brion, F., Do, C. B., & Moresoli, C. (1996). Development of process strategies for anthocyanin-based food colorant production using *Vitis vinifera* cell cultures. In F. DiCosmo & M. Misawa (Eds.), *Plant cell culture secondary metabolism toward industrial application* (pp. 167-186). CRC Press LLC. https://doi.org/10.1007/978-1-4615-5919-1_9

Efferth, T. (2019). Biotechnology applications of plant callus cultures. *Engineering*, 5(1), 50-59. https://doi.org/10.1016/j.eng.2018.11.006

Gamborg, O. L., Miller, R., & Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research*, 50(1), 151-158. https://doi.org/10.1016/0014-4827(68)90403-5

Gao, L., Liu, J., Liao, L., Gao, A., Njuguna, B. N., Zhao, C., ... & Han, Y. (2023). Callus induction and adventitious root regeneration of cotyledon explants in peach trees. *Horticulturae*, 9(8), 850. https://doi.org/10.3390/horticulturae9080850

Gaspar, T., Kevers, C., Penel, C., Greppin, H., Reid, D. M., & Thorpe, T. A. (1996). Plant hormones and plant growth regulators in plant tissue culture. *In vitro Cellular & Developmental Biology-Plant*, 32, 272-289. https://doi.org/10.1007/BF02822700

George, E. F., Hall, M. A., & De Klerk, G. J. (2008). Plant propagation by tissue culture (3rd ed.). *Springer*. https://doi.org/10.1007/978-1-4020-5005-3

Gray, W. M. (2004). Hormonal regulation of plant growth and development. *PLoS Biology*, 2(9), E311. https://doi.org/10.1371/journal.pbio.0020311

Hayta, S., Smedley, M. A., Li, J., Harwood, W. A., & Gilmartin, P. M. (2016). Plant regeneration from leaf-derived callus cultures of primrose (*Primula vulgaris*). *HortScience, 51*(5), 558-562. https://doi.org/10.21273/HORTSCI.51.5.558

Hussain, A., Qarshi, I. A., Nazir, H., & Ullah, I. (2012). Plant tissue culture: Current status and opportunities. *Recent Advances in Plant In Vitro Culture*, 6(10), 1-28. https://doi.org/10.5772/50568

Ikeuchi, M., Sugimoto, K., & Iwase, A. (2013). Plant callus: Mechanisms of induction and repression. *The Plant Cell*, 25(9), 3159-3173. https://doi.org/10.1105/tpc.113.116053

Iwase, A., Mitsuda, N., Koyama, T., Hiratsu, K., Kojima, M., Arai, T., Inoue, Y., Seki, M., Sakakibara, H., Sugimoto, K., & Ohme-Takagi, M. (2011). The AP2/ERF transcription factor WIND1 controls cell dedifferentiation in Arabidopsis. *Current Biology*, 21(6), 508–514. https://doi.org/10.1016/j.cub.2011.02.020

Khan, N., Ahmed, M., Hafiz, I., Abbasi, N., Ejaz, S., & Anjum, M. (2015). Optimizing the concentrations of plant growth

regulators for in vitro shoot cultures, callus induction and shoot regeneration from calluses of grapes. *Oeno One, 49*(1), 37-45. https://doi.org/10.20870/oeno-one.2015.49.1.95

Kieber, J. J., & Schaller, G. E. (2014). Cytokinins. *The Arabidopsis Book, 12*, e0168. https://doi.org/10.1199/tab.0168.

Kotb, O. M., Abd EL-Latif, F. M., Atawia, A. R., & Saleh, S. S. (2020). In vitro propagation and callus induction of pear (*Pyrus communis*) Cv. Le-Conte. *Asian Journal of Biotechnology and Genetic Engineering, 3*(2), 1-10.

Li, S. M., Zheng, H. X., Zhang, X. S., & Sui, N. (2021). Cytokinins as central regulators during plant growth and stress response. *Plant cell reports*, *40*, 271-282. https://doi.org/10.1007/s00299-020-02612-1

Lloyd, G., & McCown, B. (1980). Commercially-feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot tip culture. *International Plant Propagation Society, 30*, 421–427

Martínez, M. E., Poirrier, P., Prüfer, D., Gronover, C. S., Jorquera, L., Ferrer, P., ... & Chamy, R. (2018). Kinetics and modeling of cell growth for potential anthocyanin induction in cultures of *Taraxacum officinale* GH Weber ex Wiggers (Dandelion) in vitro. *Electronic Journal of Biotechnology, 36*, 15-23. https://doi.org/10.1016/j.ejbt.2018.08.006

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum, 15*, 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Ozden, M. (2024). Secondary metabolite production in callus cultures of *Vitis vinifera*: influence of genotype and sucrose concentration in the medium on antioxidant activity. *Acta Physiologiae Plantarum, 46*(1), 6. https://doi.org/10.1007/s11738-023-03630-8

Parihar, S., & Sharma, D. (2021). A brief overview on Vitis vinifera. *Scholars Academic Journal of Pharmacy*, 12, 231-239. https://doi.org/10.36347/sajp.2021.v10i12.005

Pehlivan, E. C., Kunter, B., & Royandazagh, S. D. (2017). Choice of explant material and media for in vitro callus regeneration in Sultana grape cultivar (*Vitis vinifera* L.). *Tekirdağ Ziraat Fakültesi Dergisi*. *The Special Issue of 2nd International Balkan Agriculture Congress*, 30-34.

Phillips, G. C., & Garda, M. (2019). Plant tissue culture media and practices: an overview. *In Vitro Cellular & Developmental Biology - Plant, 55*, 242–257. https://doi.org/10.1007/s11627-019-09983-5

Qu, J., Zhang, W., Yu, X., & Jin, M. (2005). Instability of anthocyanin accumulation in *Vitis vinifera* L. var. Gamay Fréaux suspension cultures. *Biotechnology and Bioprocess Engineering*, 10(2), 155-161. https://doi.org/10.1007/BF02932586

Robinson, J., Harding, J., & Vouillamoz, J. (2013). *Wine grapes: a complete guide to 1,368 vine varieties, including their origins and flavours*. *Penguin* UK.

Skoog, F. (1957). Chemical regulation of growth and organ formation in plant tissue cultured in vitro. In *Symposium of the Society for Experimental Biology*. volume.(11), 118-131. https://doi.org/10.1016/B978-0-12-007901-8.50013-X

Su, Y. H., Liu, Y. B., & Zhang, X. S. (2011). Auxin–cytokinin interaction regulates meristem development. *Molecular Plant, 4*(4), 616-625. https://doi.org/10.1093/mp/ssr007

Teale, W. D., Paponov, I. A., & Palme, K. (2006). Auxin in action: Signalling, transport and the control of plant growth and development. *Nature Reviews Molecular Cell Biology, 7*(11), 847-859. https://doi.org/10.1038/nrm2020

Wang, C., He, R., Lu, J., & Zhang, Y. (2018). Selection and regeneration of *Vitis vinifera* Chardonnay hydroxyproline-resistant calli. *Protoplasma, 255*, 1413-1422. https://doi.org/10.1007/s00709-018-1240-2

Wickham, H. (2011). ggplot2. *Wiley Interdisciplinary Reviews: Computational Statistics, 3*(2), 180–185. https://doi.org/10.1002/wics.147

Wu, J., Zhang, J., Hao, X., Lv, K., Xie, Y., & Xu, W. (2024). Establishment of an efficient callus transient transformation system for Vitis vinifera cv. 'Chardonnay'. *Protoplasma, 261*(2), 351-366. https://doi.org/10.1007/s00709-023-01901-2