Greenhouse Plant Production Journal

Effects of Explant Type on In Vitro Growth and Morphogenic Responses of *Vitis vinifera* L. cv. Mondrop

Ali Asghar Saeid[a], Zahra Pakkish*[a], and Fatemeh Nasibi[b]

[a]*Department of Horticultural Science, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran*
[b]*Department of Biology, Shahid Bahonar University of Kerman, Kerman, Iran*

Original Article

Use your device to scan and read the article online

Citation: Saeidi, A.A., Pakkish, Z. and Nasibi, F. 2026. Effects of Explant Type on In Vitro Growth and Morphogenic Responses of *Vitis vinifera* L. cv. Mondrop. Greenhouse Plant Production Journal 3(4): 53-66.

https://doi.org/10.22034/jgpp.2026.2108236.1126

KEYWORDS

Explant
Hyperhydricity
Micropropagation
Mondrop
Vitis vinifera

ABSTRACT

The selection of an appropriate explant type is a critical factor influencing the efficiency of grapevine micropropagation. Nevertheless, comparative information on explant-dependent responses remains limited for several commercially valuable grape cultivars. This study evaluated three explant types of *Vitis vinifera* L. cv. Mondrop, focusing on growth, biomass accumulation, and culture-related abnormalities. Shoot tips, nodal segments, and buds developing near callus tissue were cultured on Murashige and Skoog (MS) medium. Following establishment, developing shoots were transferred to MS medium supplemented with 0.5 mg L^{-1} benzyladenine (BA) for multiplication. The experiment was conducted using a completely randomized design with three replicates. Shoot length, leaf and node number, fresh and dry weight, hyperhydricity, and shoot-tip necrosis were evaluated. Explant type markedly affected in vitro performance. Nodal explants produced the longest shoots (3.75 cm), highest leaf (4.03) and node (3.48) numbers, and greatest fresh (320 mg) and dry (102 mg) biomass. In contrast, buds developing near callus tissue showed the lowest values for these traits, with shoot length, leaf number, node number, fresh weight, and dry weight of 2.74 cm, 2.38, 2.32, 190 mg, and 55 mg, respectively. Nodal explants also exhibited the lowest hyperhydricity (1.01%) and shoot-tip necrosis (0.90%), whereas buds near callus tissue showed the highest incidences (5.11% and 3.12%, respectively). Overall, nodal explants provided the most favorable combination of growth, biomass accumulation, and culture quality under the tested conditions. These findings highlight the importance of explant selection for efficient grapevine micropropagation and provide a practical basis for developing a propagation system for cv. Mondrop.

ARTICLE HISTORY

Received: 07 October 2026
Revised: 27 October 2026
Accepted: 30 November 2026

* Corresponding author: Z. Pakkish
E-mail address: zhrapakkish@uk.ac.ir

Publisher: Arak University

1. Introduction

Grapevine (*Vitis vinifera* L.) is one of the most economically and culturally important perennial fruit crops worldwide, supporting diverse value chains that encompass fresh-market grapes, raisins, juice, wine, and numerous processed products. Beyond its economic significance, grapevine represents a genetically and phenotypically diverse crop in which cultivar characteristics are closely associated with fruit quality, yield potential, adaptation, and end-use value. Recent characterization of table grape germplasm has further emphasized the substantial agronomic and genetic diversity present among grape cultivars and the importance of preserving and efficiently exploiting this diversity for sustainable grape production (Guliyeva *et al*., 2026). However, the perennial and highly heterozygous nature of grapevine make the production of genetically uniform planting material through conventional propagation relatively slow and, depending on the propagation material and production system, potentially associated with the accumulation or dissemination of pathogens. Consequently, the development of efficient clonal propagation systems capable of producing uniform, vigorous, and healthy plants is a major component of modern grapevine production and germplasm management.

In vitro culture and micropropagation provide an important alternative to conventional propagation because they allow the rapid multiplication of selected genotypes under controlled and aseptic conditions. Particularly in grapevine, tissue culture has been used for the production of disease-free planting material, rapid multiplication of elite cultivars, conservation of valuable genotypes, and establishment of platforms for genetic transformation and advanced biotechnology. For example, efficient protocols have recently been developed for large-scale *in vitro* propagation of grapevine cultivars such as 'Black Magic' and for the mass propagation of disease-free 'Shine Muscat', demonstrating the practical potential of tissue culture for rapid multiplication of commercially valuable grapevine material (Alam *et al*., 2026; Kim *et al*., 2023). Similarly, shoot-tip culture has been successfully applied to several grape cultivars, confirming the usefulness of meristematic tissues as starting material for grapevine micropropagation (Özdemir Memiş & Sağlam, 2024). Beyond conventional multiplication, grapevine tissue culture has become an essential component of more advanced biotechnological approaches, including somatic embryogenesis and genetic transformation (Carra *et al*., 2024; Capriotti *et al*., 2022, 2023), while recent work on protoplast isolation, transformation, and callus induction further illustrates the expanding role of in vitro systems in grapevine biotechnology (Kolasinliler *et al*., 2025).

Despite these advantages, successful micropropagation cannot be reduced to the selection of a suitable basal medium or growth-regulator combination. The response of plant tissues *in vitro* is strongly dependent on the biological characteristics of the explant, the physiological status of the donor material, the genotype, and the culture environment. Differences among grapevine genotypes in their responses to in vitro propagation have recently been demonstrated, emphasizing that a protocol optimized for one cultivar cannot necessarily be transferred directly to another (Bahrehmand *et al*., 2026). Likewise, differences in culture-media composition can substantially influence shoot development in grapevine, highlighting the interaction between the nutritional environment and the inherent regenerative capacity of the cultured tissue (Ekinci *et al*., 2024). More broadly, tissue-culture responses may also vary with donor age and plant growth-regulator regime, indicating that the physiological background of the explant can influence subsequent morphophysiological performance (Martins *et al*., 2024). Such variability reinforces the need to consider the explant itself as a critical experimental factor rather than merely as a source of starting material.

The importance of explant selection is particularly evident in comparative studies of plant tissue culture. Different explant types may differ substantially in their developmental competence and in their capacity to initiate organized growth, callogenesis, or shoot regeneration. Comparative evaluations involving different explant sources have demonstrated distinct morphogenic responses in a range of species, including *Physalis angulata*, *Lupinus montanus*, *Taraxacum belorussicum*, *Boscia senegalensis*, and *Annona muricata* (Mastuti & Munawarti, 2018; Ramírez-González *et al*., 2015; Gałuszka *et al*., 2019; Daffalla *et al*., 2011; Hamad & Majid, 2017). Similar evidence has been reported for other horticultural and crop species, in which explant type interacts with growth-regulator concentration, medium composition, or physiological status to determine the magnitude and direction of the in vitro response (Huda *et al*., 2020; Sheykh Abdollahzadeh Mamaghani *et al*., 2025; Okao *et al*., 2025). These findings collectively indicate that the identity and developmental status of the explant can substantially influence the subsequent trajectory of in vitro growth and morphogenesis.

For grapevine specifically, explant-related responses have received attention in studies addressing callus induction, shoot regeneration, and micropropagation. Shaker *et al*. (2025), for instance, demonstrated that explant type and growth-regulator treatment influence callus induction in *V. vinifera*, while Özdemir Memiş and Sağlam (2024) showed the applicability of shoot-tip culture across several grapevine varieties. The importance of optimizing the physiological and hormonal conditions surrounding shoot development is also evident from studies

addressing shoot multiplication and associated abnormalities. Efficient optimization of plant growth-regulator regimes has improved *in vitro* multiplication of grapevine (Kim *et al*., 2023), whereas recent work has demonstrated that boron and cytokinin combinations can modulate shoot-tip necrosis and improve subculture efficiency in grapevine micropropagation (Yazar, 2025). Thus, although considerable progress has been made in optimizing grapevine micropropagation, the biological response of different explant categories remains an important source of variation that deserves more direct attention.

This issue is particularly relevant because successful micropropagation is not defined solely by the production of shoots. High-quality in vitro growth requires a balance between shoot elongation, node and leaf formation, biomass accumulation, and the maintenance of normal tissue development. Undesirable physiological abnormalities, including hyperhydricity and shoot-tip necrosis, can compromise the quality and subsequent establishment of regenerated plantlets. Hyperhydricity is a widespread problem in plant tissue culture and is associated with pronounced morphological and physiological abnormalities that can reduce the quality of regenerated material (Isah, 2015). Shoot-tip necrosis, in particular, represents a recurrent limitation in micropropagation and has been associated with a complex interaction of nutritional, hormonal, environmental, and physiological factors (Teixeira da Silva *et al*., 2020). In grapevine, shoot-tip necrosis has been specifically reported as a constraint during the *in vitro* propagation of 'Red Globe', and optimization of the culture conditions can substantially alleviate its occurrence (Surakshitha *et al*., 2019). Comparable efforts to reduce shoot-tip necrosis in other species further demonstrate that the expression of this disorder is closely connected to the conditions under which shoots are initiated and multiplied (Kundan Kishore *et al*., 2015; Liu *et al*., 2024). These observations suggest that explant selection should ideally be evaluated not only in terms of its capacity to produce vigorous shoots but also in relation to the frequency of undesirable developmental responses.

The optimization of explant choice is therefore a fundamental step toward developing reliable, cultivar-specific micropropagation protocols. However, much of the existing grapevine literature has focused on growth-regulator optimization, culture-media composition, shoot-tip culture, callus induction, somatic embryogenesis, or genotype-specific responses rather than directly comparing distinct explant types on a common basis for their subsequent vegetative growth and morphogenic performance. Even studies that examine explant effects have frequently emphasized callus induction or regeneration rather than simultaneously evaluating shoot elongation, leaf and node production, biomass accumulation, and physiological abnormalities within a single comparative framework (Shaker *et al*., 2025). Moreover, the documented influence of genotype on tissue-culture behavior indicates that conclusions derived from one grapevine cultivar cannot automatically be extrapolated to another (Bahrehmand *et al*., 2026). This cultivar-specific dimension is especially important for *V. vinifera* cultivars intended for commercial production, for which efficient multiplication must combine high regenerative performance with stable and healthy shoot development.

An additional challenge is that different explant sources can represent distinct developmental states. Shoot tips contain actively developing apical tissues, nodal segments contain axillary meristematic regions with established positional identity, whereas buds developing in association with callus represent a morphologically distinct source of regenerating tissue. Such differences may translate into substantial variation in shoot establishment, subsequent elongation, biomass production, and the occurrence of culture-associated abnormalities. Previous studies using nodal explants have demonstrated their effectiveness as a source of organized shoot development in other species (Trivedi & Joshi, 2021), while studies involving multiple explant types have repeatedly shown that morphogenic competence is not uniform among tissues originating from the same plant (Ramírez-González *et al*., 2015; Mastuti & Munawarti, 2018; Gałuszka *et al*., 2019). The broader development of optimized tissue-culture protocols has also benefited from systematic approaches to identifying the most effective combinations of biological and cultural factors (Hesami & Jones, 2020). Nevertheless, a direct comparison of shoot tip, nodal, and bud-near-callus explants under the same culture conditions remains insufficiently documented for grapevine, particularly for cultivar-specific micropropagation of *V. vinifera*.

Against this background, the present study was designed to address a fundamental but insufficiently resolved question in grapevine micropropagation: does the type of explant itself determine the subsequent quality and vigor of in vitro shoot development when the culture environment is kept constant? To answer this question, shoot-tip, nodal, and bud-near-callus explants of *Vitis vinifera* L. cv. Mondrop were comparatively evaluated under standardized in vitro conditions. Shoot length, number of leaves, number of nodes, fresh weight, dry weight, hyperhydricity, and shoot-tip necrosis were assessed to provide an integrated measure of both productive growth and culture-associated abnormalities. To the best of our knowledge, the literature considered in the present study does not provide a direct comparative evaluation of these three explant types in cv. Mondrop with simultaneous assessment of vegetative growth, biomass accumulation, and major in vitro abnormalities. The study therefore

seeks to establish an evidence-based explant preference for the efficient micropropagation of this cultivar and, more importantly, to clarify whether explant identity can serve as a practical determinant of both growth performance and morphogenic quality in grapevine tissue culture. Such information can provide a useful foundation for cultivar-specific protocol development and for the production of vigorous and morphologically normal planting material of 'Mondrop'.

2. Materials and Methods

The plant material used in this study consisted of shoots of *Vitis vinifera* L. cv. Mondrop (Vitaceae). Shoots were collected from four-year-old mother plants of grapevine cv. Mondrop grown in Mahan, Kerman Province, Iran. The donor plants were selected based on their healthy appearance and active vegetative growth. Healthy, actively growing shoots were collected from the mother plants and transferred to the laboratory for surface sterilization and subsequent in vitro establishment and multiplication. To remove surface debris and initial microbial contaminants, the collected shoots were thoroughly washed under running tap water. The shoots were then cut into suitable segments and subjected to surface sterilization under aseptic conditions. The explants were initially treated with 70% ethanol and subsequently rinsed with sterile distilled water. They were then immersed in a 15% sodium hypochlorite solution for 5 min. Following sodium hypochlorite treatment, the explants were rinsed three times with sterile distilled water to remove residual disinfectant.

2.1. In vitro establishment and shoot multiplication

Following surface sterilization, nodal explants obtained from the mother shoots were initially cultured on basal Murashige and Skoog (MS) medium without plant growth regulators. After establishment and the initiation of shoot growth, nodes derived from newly developed shoots were excised from the mother tissues and transferred to MS medium supplemented with 0.5 mg L^{-1} benzyladenine (BA) for further multiplication. To obtain uniform and actively growing plant material for the main experiment, the explants were subjected to four to five consecutive subculture cycles under identical culture conditions. After sufficient multiplication and development of healthy shoots, the explants required for the main experiment were prepared.

2.2. Explant preparation and experimental treatments

Three types of explants were evaluated in the main experiment: shoot tips (ST), nodal explants (N), and buds developing near callus tissue (B). Shoot tip explants (ST) consisted of the apical meristem and adjacent young tissues. Nodal explants (N) consisted of single- or two-node segments excised from actively growing shoots. Buds developing near callus tissue (B) were defined as specify the actual morphological characteristics and origin of these buds. These explants were obtained from [specify the culture stage and procedure through which the buds developed]. At the time of excision, the buds were selected at a comparable developmental stage, based on their visible morphological characteristics. The presence or absence of callus tissue attached to the excised buds was recorded and standardized across explants, as applicable to the experimental procedure.

To minimize variation attributable to explant dimensions, explants within each category were selected and prepared using consistent morphological criteria. Explant size was standardized by specify the actual length, dimensions, or other measurement criterion used, and visibly damaged or developmentally atypical explants were excluded before inoculation. All explant types were cultured on Murashige and Skoog (MS) basal medium supplemented with 0.5 mg L^{-1} benzyladenine (BA). All explant types were cultured under identical nutritional and environmental conditions to allow comparison of their in vitro growth and morphogenic responses. All explant preparation, transfer, and inoculation procedures were performed under aseptic conditions. Cultures were maintained in a controlled growth room at 25°C under a 16-h photoperiod followed by 8 h of darkness. The cultures were maintained under these conditions for 32 days, after which the explants and regenerated shoots were evaluated for growth characteristics and tissue health.

2.3. Assessment of morphological traits

At the end of the 32-day culture period, the explants were evaluated for vegetative growth and tissue quality. The measured traits included shoot length, number of nodes, number of leaves, fresh weight, dry weight, hyperhydricity, and shoot tip necrosis (STN).

2.4 .Shoot length

Shoot length was measured at the end of the culture period from the point of shoot attachment to the explant to the shoot apex using a ruler. The results were recorded in centimeters.

2.5. Number of nodes and leaves

The number of nodes developed on each regenerated shoot was determined by direct counting. The number of fully developed leaves on each shoot was also recorded by direct counting.

2.6. Fresh weight

For fresh-weight determination, the explants and regenerated shoots were carefully removed from the culture medium at the end of the culture period, and any adhering medium was gently removed from the tissue surface. Each explant was then weighed individually using a digital balance (Sartorius, model BPSIID), and fresh weight was recorded in grams.

2.7. Dry weight

Following fresh-weight determination, the samples were placed separately in aluminum foil and dried in a forced-air oven at 70°C for 72 h to remove tissue moisture. After complete drying, the samples were reweighed using the same digital balance (Sartorius, model BPSIID), and dry weight was recorded in grams.

2.8. Assessment of hyperhydricity

Hyperhydricity was assessed visually after 32 days of culture based on the characteristic morphological changes observed in the regenerated tissues. These changes included a watery or translucent appearance, a glossy or glass-like surface, soft and spongy tissue texture, increased tissue fragility, and pale or chlorotic leaves. The extent of visibly hyperhydric tissue was estimated separately for each explant and expressed as a percentage of the tissue assessed. The individual estimates were subsequently averaged within each experimental replicate, and the resulting replicate-level values were used for statistical analysis.

Accordingly, hyperhydricity was quantified as the estimated proportion of visibly affected tissue rather than as the incidence of explants exhibiting hyperhydricity. This distinction is important because the percentage of affected tissue describes the apparent extent of the disorder within an explant, whereas incidence represents the proportion of explants affected within a treatment. The reported hyperhydricity values therefore refer to replicate-level mean estimates of affected tissue and should not be interpreted as percentages calculated directly from the total number of explants per treatment.

2.9. Assessment of shoot tip necrosis

Shoot tip necrosis (STN) was evaluated after 32 days of culture by visually examining the shoot apical region of each explant. STN was identified by characteristic symptoms, including browning or blackening of the shoot apex, cessation of apical growth, and visible deterioration or death of apical tissues. Each explant was classified as either exhibiting or not exhibiting STN, based on the presence or absence of these symptoms. The incidence of STN was calculated as the percentage of explants exhibiting necrosis relative to the total number of explants assessed within the corresponding experimental unit:

$$\text{STN}\ (\%) = \frac{\text{Number of explants exhibiting STN}}{\text{Total number of explants assessed}} \times 100$$

Where percentages were calculated separately for individual culture vessels, the vessel-level values were summarized within each experimental replicate before statistical analysis. The experimental replicate, rather than each individual explant considered independently, was used as the basis for statistical inference.

2.10. Experimental design and statistical analysis

The experiment was conducted using a completely randomized design (CRD) with three replicates per explant type. Each replicate consisted of 10 explants, resulting in a total of 30 explants per explant type. Three explants were cultured per vessel. After 32 days of culture, shoot length, number of leaves, number of nodes, fresh weight, dry weight, hyperhydricity, and shoot-tip necrosis (STN) were assessed. Observations were recorded for the individual culture vessels and summarized at the replicate level before statistical analysis. Data were analyzed using SAS software (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was performed to evaluate the effect of explant type, and treatment means were compared using Duncan's multiple range test at the 5% significance level ($P \leq 0.05$).

3. Result

3.1. Effect of explant type on in vitro growth and morphogenic traits

3.1.1. shoot length

The analysis of variance indicated that explant type affected the in vitro growth and morphogenic performance of *Vitis vinifera* L. cv. Mondrop, with the magnitude and direction of the response varying among the evaluated

traits (Table 1). Overall, nodal explants showed the most favorable growth performance, whereas buds developing near callus tissue generally exhibited lower growth and greater incidence of culture-related abnormalities. Shoot length was significantly affected by explant type (Figure 1). Nodal explants produced the longest shoots, with a mean shoot length of 3.75 cm, compared with 3.11 cm for shoot-tip explants and 2.74 cm for buds developing near callus tissue. Thus, shoots originating from nodal explants were approximately 20.6% longer than those obtained from shoot tips, whereas the response of buds near callus tissue was 11.9% lower than that of shoot tips. The highest shoot elongation observed in nodal explants indicates that this explant type provided a more favorable basis for shoot development under the conditions of the present experiment.

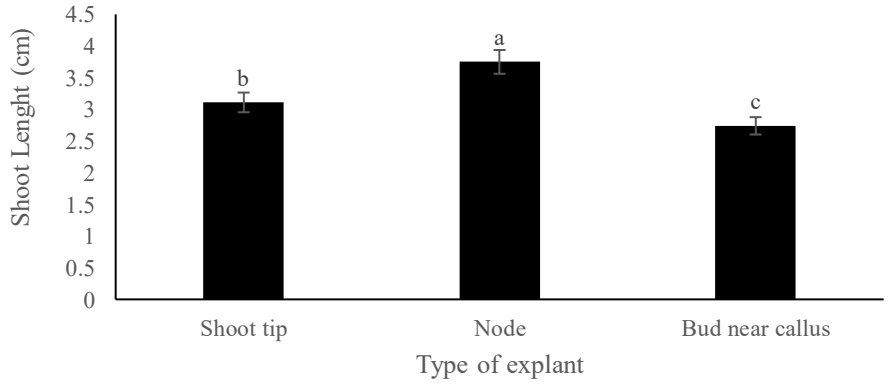


Figure 1. Effect of explant type on shoot length of *Vitis vinifera* L. cv. Mondrop under in vitro conditions. Means followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% probability level ($P \leq 0.05$).

3.1.2. Number of leaves

The number of leaves was significantly affected by explant type (Duncan's multiple range test, $P \leq 0.05$). (Table 1). Nodal explants produced the highest number of leaves, with an average of 4.03 leaves per shoot, representing a 13.84% increase compared with shoot-tip explants (3.54 leaves per shoot). Shoot-tip explants showed an intermediate response, whereas buds developing near callus tissue produced the fewest leaves, with an average of 2.38 leaves per shoot, corresponding to a 32.77% reduction relative to shoot-tip explants. Notably, the number of leaves produced by nodal explants was 69.33% higher than that of buds near callus tissue. Overall, nodal explants exhibited the most favorable response in terms of leaf production under the in vitro conditions evaluated.

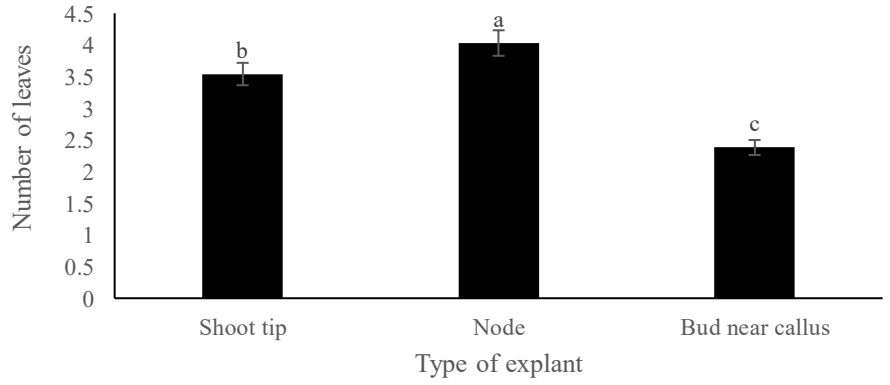


Figure 2. Effect of explant type on the number of leaves per shoot of *Vitis vinifera* L. cv. Mondrop under in vitro conditions. Means followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% probability level ($P \leq 0.05$).

3.1.3. Number of nodes

The number of nodes per shoot was significantly affected by explant type (Duncan's multiple range test, $P \leq 0.05$). (Table 1). Nodal explants produced the highest number of nodes, with an average of 3.48 nodes per shoot, representing a 15.61% increase compared with shoot-tip explants (3.01 nodes per shoot). Shoot-tip explants showed an intermediate response, whereas buds developing near callus tissue produced the lowest number of nodes, averaging 2.32 nodes per shoot, which was 22.92% lower than that observed in shoot-tip explants. Direct comparison between the two extreme responses showed that nodal explants produced 50.00% more nodes per

shoot than buds near callus tissue. Overall, nodal explants exhibited the most favorable response for node formation under the in vitro conditions evaluated.

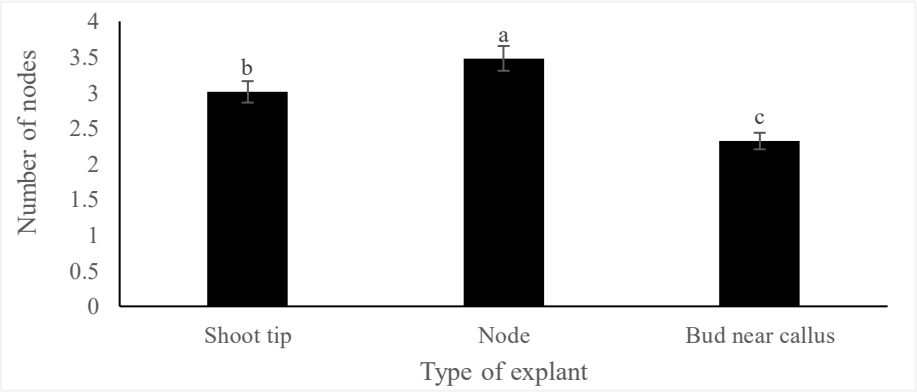


Figure 3. Effect of explant type on the number of nodes per shoot of *Vitis vinifera* L. cv. Mondrop under in vitro conditions. Means followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% probability level ($P \leq 0.05$).

3.1.4. Fresh weight

Explant type significantly affected the fresh weight of in vitro-derived shoots (Duncan's multiple range test, $P \leq 0.05$). (Table 1). Nodal explants produced shoots with the highest fresh weight, reaching 320 mg, which represented a 14.29% increase compared with shoot-tip explants (280 mg). Shoot-tip explants showed an intermediate response, whereas buds developing near callus tissue produced shoots with the lowest fresh weight (190 mg), corresponding to a 32.14% reduction relative to shoot-tip explants. A direct comparison between the two extreme responses showed that shoots derived from nodal explants had 68.42% greater fresh weight than those originating from buds near callus tissue. Overall, nodal explants showed the most favorable response in terms of shoot fresh biomass under the in vitro conditions evaluated.

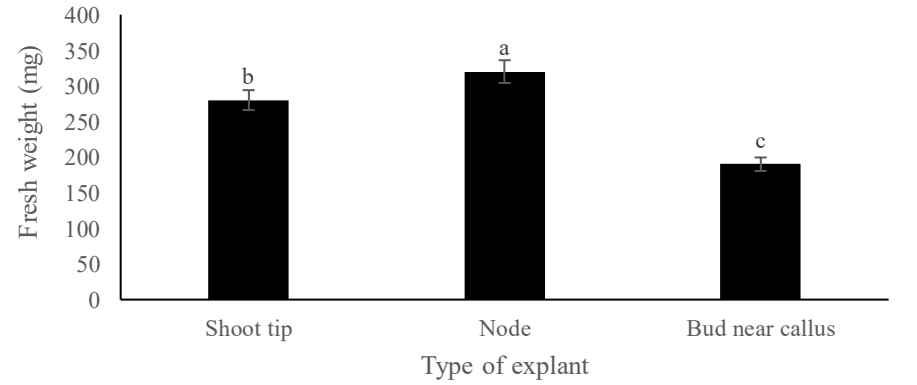


Figure 4. Effect of explant type on fresh weight of in vitro-derived shoots of *Vitis vinifera* L. cv. Mondrop. Means follow ed by the same letter are not significantly different according to Duncan's multiple range test at the 5% probability level ($P \leq 0.05$).

3.1.5. Dry weight

Explant type significantly affected the dry weight of in vitro-derived shoots (Duncan's multiple range test, $P \leq 0.05$). (Table 1). Nodal explants produced shoots with the highest dry weight, reaching 102 mg, which represented a 15.91% increase compared with shoot-tip explants (88 mg). Shoot-tip explants showed an intermediate response, whereas buds developing near callus tissue produced shoots with the lowest dry weight (55 mg), corresponding to a 37.50% reduction relative to shoot-tip explants. A direct comparison between the two extreme responses showed that shoots derived from nodal explants had 85.45% greater dry weight than those originating from buds near callus tissue. Overall, nodal explants exhibited the most favorable response in terms of dry biomass accumulation under the in vitro conditions evaluated.

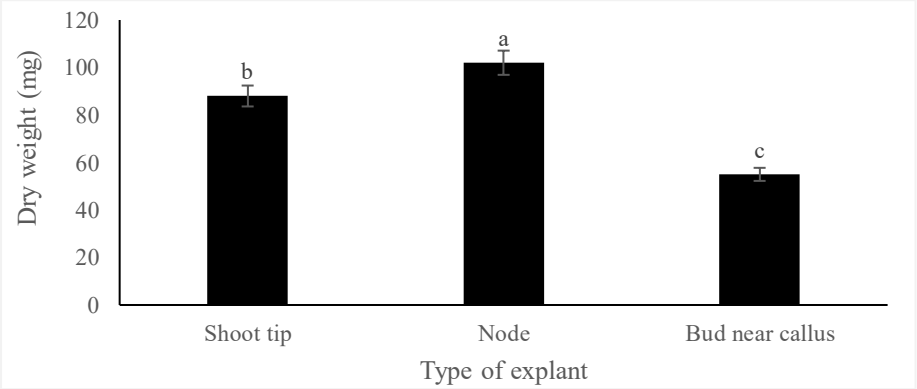


Figure 5. Effect of explant type on dry weight of in vitro-derived shoots of *Vitis vinifera* L. cv. Mondrop. Means followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% probability level ($P \leq 0.05$).

3.1.6. Hyperhydricity

Explant type significantly affected the incidence of hyperhydricity in the in vitro-derived shoots (Duncan's multiple range test, $P \leq 0.05$). (Table 1). The lowest incidence of hyperhydricity was recorded in shoots derived from nodal explants (1.01%), which was 58.78% lower than that observed in shoot-tip explants (2.45%). Shoot-tip explants showed an intermediate level of hyperhydricity, whereas buds developing near callus tissue exhibited the highest incidence, reaching 5.11%. This represented a 108.57% increase compared with shoot-tip explants. Notably, the incidence of hyperhydricity in shoots derived from buds near callus tissue was 405.94% higher than that recorded for nodal explants. Overall, nodal explants showed the lowest incidence of hyperhydricity among the explant types evaluated.

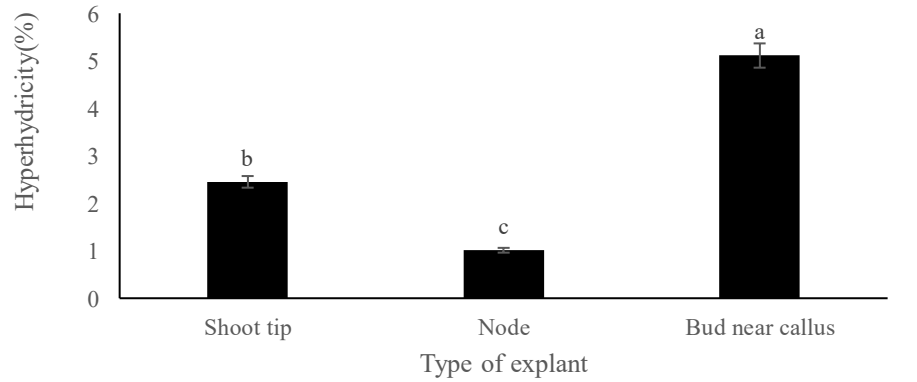


Figure 6. Effect of explant type on the incidence of hyperhydricity in *Vitis vinifera* L. cv. Mondrop under in vitro conditions. Means followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% probability level ($P \leq 0.05$).

3.1.7. Shoot tip necrosis (STN)

Explant type significantly affected the incidence of shoot tip necrosis (STN) (Duncan's multiple range test, $P \leq 0.05$). (Table 1). The lowest incidence of STN was recorded in shoots derived from nodal explants (0.90%), which was 11.76% lower than that observed in shoot-tip explants (1.02%). In contrast, buds developing near callus tissue exhibited the highest incidence of STN, reaching 3.12%, corresponding to a 205.88% increase compared with shoot-tip explants. Direct comparison of the two extreme responses showed that the incidence of STN in shoots derived from buds near callus tissue was 246.67% higher than that recorded for nodal explants. Overall, nodal explants exhibited the lowest incidence of shoot tip necrosis, whereas buds developing near callus tissue showed the highest incidence under the in vitro conditions evaluated.

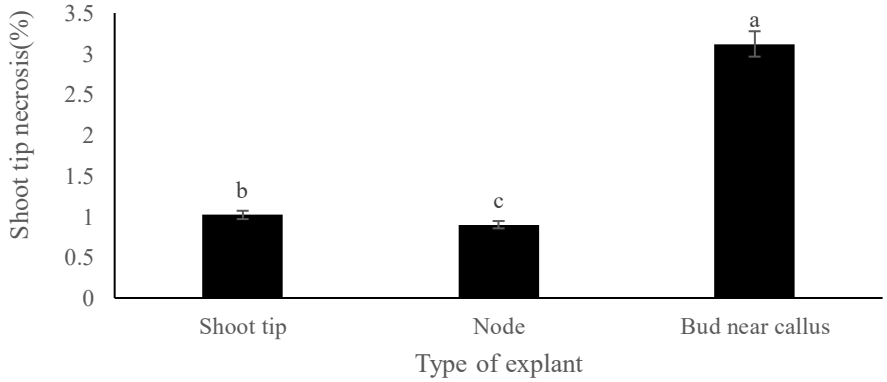


Figure 7. Effect of explant type on the incidence of shoot tip necrosis in *Vitis vinifera* L. cv. Mondrop under in vitro conditions. Means followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% probability level ($P \leq 0.05$).

Table 1. Analysis of variance for the effects of explant type on the in vitro growth and morphogenic responses of grapevine (Vitis vinifera L. cv. Mondrop)

Source of variation	df	Shoot length (MS)	Number of leaves (MS)	Number of nodes (MS)	Fresh weight (MS)	Dry weight (MS)	Hyperhydricity (MS)	Shoot tip necrosis (MS)
Explant type	2	1.842**	1.126*	0.984*	5625.33**	1842.67**	12.684**	4.216**†
Error	6	0.084	0.173	0.128	214.50	73.33	0.846	0.384
F-value	—	21.929	6.509	7.688	26.225	25.128	14.993	10.979
Approximate P-value	—	0.0017	0.0314	0.0221	0.0011	0.0012	0.0046	0.0099
CV (%)	—	7.82	10.46	9.37	5.14	6.73	9.38	6.49

MS, mean square; df, degrees of freedom; CV, coefficient of variation. * and ** indicate statistically significant effects at $P < 0.05$ and $P < 0.01$, respectively; ns indicates a non-significant effect ($P \geq 0.05$). Treatment means were compared using Tukey's honestly significant difference (HSD) test at the 5% significance level.

3.1.8. Multivariate relationships among growth and morphogenic traits

Multivariate analysis provided a complementary description of the relationships among growth, biomass accumulation, and culture-related abnormalities in *Vitis vinifera* cv. Mondrop. Pearson correlation analysis based on the mean values of the three explant types showed strong positive associations among shoot length, leaf number, node number, fresh weight, and dry weight ($r = 0.927–1.000$). In contrast, hyperhydricity showed strong negative associations with these growth- and biomass-related traits ($r = −0.948$ to $−0.999$), while shoot-tip necrosis was also negatively associated with most growth and biomass traits ($r = −0.809$ to $−0.970$). These relationships were descriptive and indicated a consistent multivariate pattern in which explant types associated with greater vegetative growth and biomass accumulation generally exhibited lower frequencies of culture-related abnormalities. Principal component analysis further summarized the multivariate pattern among the measured traits. PC1 accounted for 97.10% of the total variance, while PC2 explained an additional 2.90%, together accounting for the variance represented in the two-dimensional ordination. PC1 was primarily aligned with shoot length, leaf number, node number, fresh weight, and dry weight, whereas hyperhydricity and shoot-tip necrosis were oriented in the opposite direction. The ordination differentiated the three explant types according to their overall morphogenic profiles. Nodal explants were positioned toward the growth- and biomass-related traits, whereas buds developing near callus were more closely associated with hyperhydricity and comparatively lower growth performance. Shoot-tip explants occupied an intermediate position. Overall, the multivariate pattern was consistent with the univariate results and indicated that nodal explants exhibited the most favorable combination of vegetative growth and biomass-related traits under the conditions tested (Fig 8).

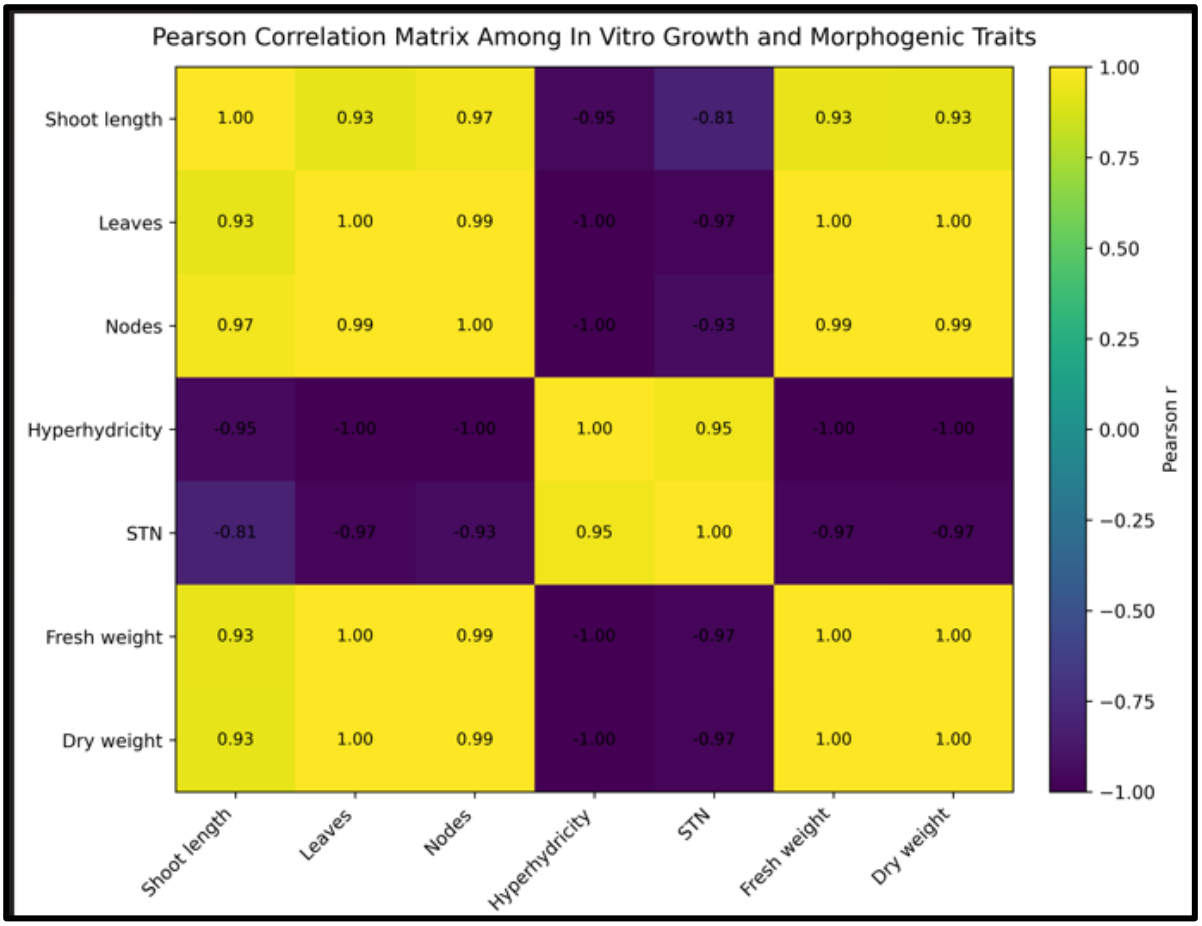


Figure 8. Pearson correlation matrix showing the relationships among in vitro growth, biomass accumulation, and morphogenic abnormality traits in different explant types of Vitis vinifera cv. Mondrop.

3.1.9. Principal Component Analysis of In Vitro Growth and Morphogenic Traits in Different Explant Types of 'Mondrop' Grape

The PCA biplot summarizes the multivariate variation in growth, biomass accumulation, and culture-related abnormalities among the three explant types. The first principal component (PC1) accounted for 97.1% of the total variance, while the second principal component (PC2) explained the remaining 2.9%, together representing 100% of the total variation. PC1 primarily separated the bud explants developing near callus from the nodal and shoot-tip explants. The nodal explants were positioned in the negative region of PC1 and the positive region of PC2, showing a closer association with shoot length and node number. In contrast, shoot-tip explants occupied the negative regions of both components and were positioned toward the fresh- and dry-weight vectors. Buds developing near callus were located in the positive region of PC1 and were more closely associated with hyperhydricity and shoot-tip necrosis (STN). The opposing orientations of the growth- and biomass-related vectors relative to the abnormality-related vectors indicate contrasting multivariate patterns among these groups of traits. Overall, the biplot highlights the distinct morphogenic profiles of the explant types and supports the comparatively favorable growth response of nodal explants under the tested in vitro culture conditions(Fig 9).

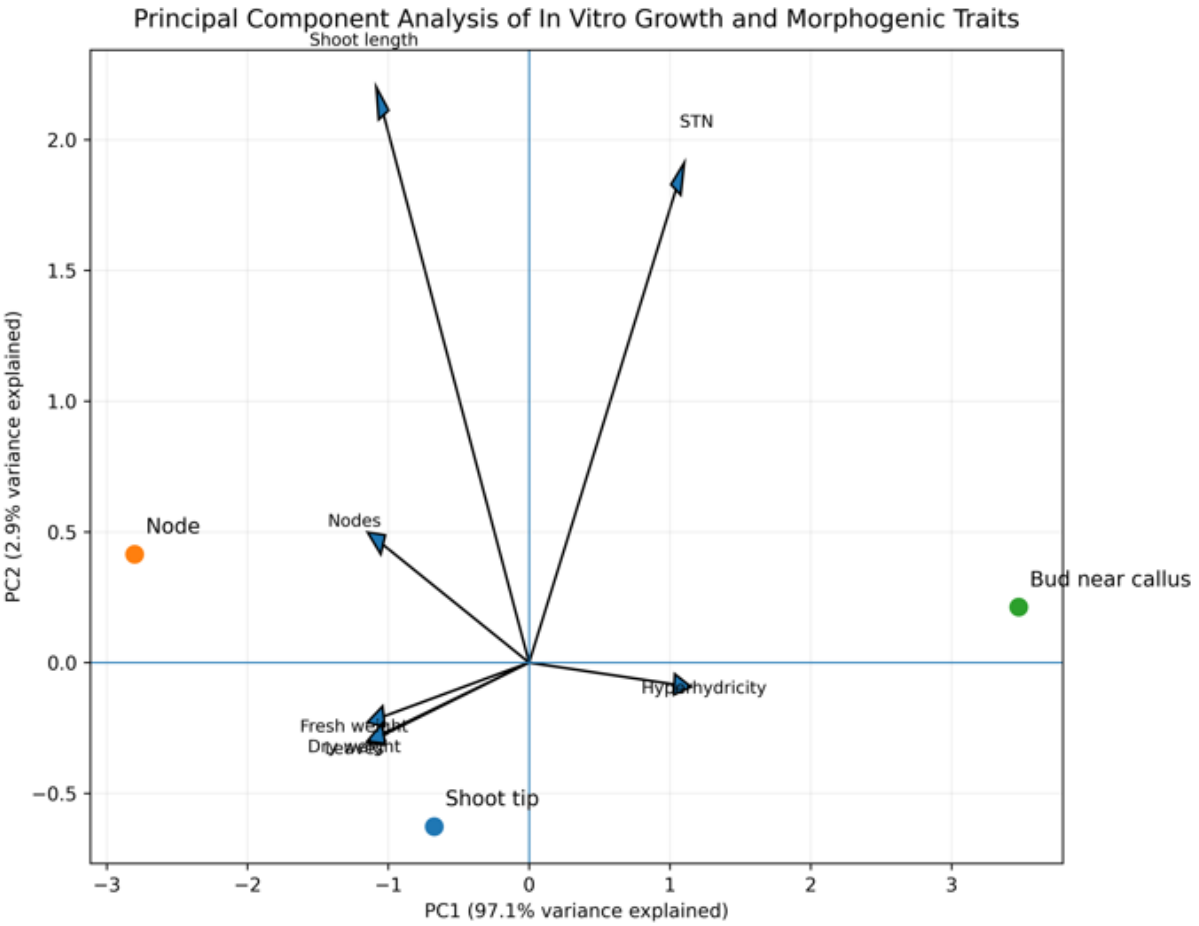


Figure 9. Principal component analysis (PCA) biplot illustrating the relationships between in vitro growth, biomass-related, and morphogenic abnormality traits and the distribution of different explant types in *Vitis vinifera* cv. Mondrop.

4. Discussion

The present study shows that explant source is a major determinant of the in vitro performance of Vitis vinifera L. cv. Mondrop, influencing shoot growth and biomass accumulation as well as the occurrence of culture-related disorders. Nodal explants performed best across the growth and biomass traits and showed comparatively low hyperhydricity and shoot-tip necrosis (STN), indicating that the choice of starting material can shift the balance between productive morphogenesis and developmental abnormalities during grapevine micropropagation. This agrees with the view that in vitro regeneration depends on the physiological and developmental status of the explant, the genotype, and the culture environment, and not on medium composition alone (Capriotti *et al*., 2022; Carra *et al*., 2024; Bahrehmand *et al*., 2026). Because genotype-specific establishment and shoot development have been documented across grapevine cultivars (Bahrehmand *et al*., 2026), the response reported here should be regarded as a cultivar-specific outcome under the tested conditions and not as a universal ranking of explant types.The advantage of nodal explants is biologically plausible. Nodal tissue carries pre-existing axillary meristems that provide a relatively direct route to shoot development, whereas tissues requiring greater cellular reprogramming may undergo a more pronounced developmental transition before sustained growth is achieved. Our data support this interpretation only at the phenotypic level, as hormonal, cellular, and anatomical parameters were not measured. The pattern is consistent with grapevine studies showing that explant source alters regeneration efficiency and that different tissues may require different combinations of basal salts and plant growth regulators (Capriotti *et al*., 2022; Ekinci *et al*., 2024). Capriotti *et al*. (2022) reported pronounced differences among floral explants and genotypes in somatic embryogenesis, underlining the joint contribution of explant identity and genotype. Explant-dependent morphogenesis has likewise been described in Physalis angulata, Lupinus montanus, Boscia senegalensis, and Taraxacum belorussicum (Mastuti & Munawarti, 2018; Ramírez-González *et al*., 2015; Daffalla *et al*., 2011; Gałuszka *et al*., 2019).

From a propagation standpoint, shoot elongation alone is an inadequate indicator of explant quality; a useful explant must also yield structurally normal and physiologically stable shoots. In nodal explants, greater elongation coincided with greater leaf and node production and higher biomass, suggesting that their advantage reflected overall shoot development and not elongation only. The positive associations among these traits in the exploratory

correlation analysis support this coherence. However, as the correlations were computed from the means of three explant types, they describe relationships among treatment means and cannot be taken as independent evidence of causality. Comparable emphasis on coordinated shoot development appears in recent grapevine studies, where medium and growth regulator regimes strongly affected shoot formation, leaf production, and elongation (Ekinci *et al*., 2024; Kim *et al*., 2023). Kim *et al*. (2023) identified BA as an effective cytokinin for shoot formation in 'Shine Muscat', while inappropriate hormonal conditions shifted development toward callus formation, which reinforces the need to consider explant identity and hormonal environment together.

Buds developing near callus tissue were the weakest explant type. Their reduced growth was accompanied by the highest levels of hyperhydricity and STN, so the response was marked by more frequent abnormalities as well as lower productivity. Callus-associated tissue may be a developmentally heterogeneous starting material, and its morphogenic behavior may be less predictable than that of tissue with an established axillary meristem. Because cellular origin, endogenous hormone levels, and bud anatomy were not examined, explanations based on altered hormonal balance, cellular reprogramming, or heterogeneity remain hypothetical. Changes in callus status and explant physiology can strongly influence morphogenic competence, but their underlying causes cannot be resolved from morphological data alone (Capriotti *et al*., 2022; Carra *et al*., 2024). Work in other species similarly shows that explant type can determine whether tissues proceed toward callogenesis, organogenesis, or other developmental pathways (Daffalla *et al*., 2011; Mastuti & Munawarti, 2018; Ramírez-González *et al*., 2015; Gałuszka *et al*., 2019).

Evaluating explants through undesirable responses as well as growth traits gives a more complete assessment of their value. Hyperhydricity is a particular concern in commercial micropropagation, because excessive tissue hydration and the associated morphological abnormalities can compromise shoot quality and acclimatization. The experiment was not designed to identify its physiological basis. Nevertheless, its inverse association with growth and biomass indicates that the explant type with the most vigorous development also tended to show fewer quality problems. This is in line with the literature emphasizing that explant condition, medium composition, growth regulators, and culture environment must be optimized to limit abnormal development (Isah, 2015; Teixeira da Silva *et al*., 2020). An efficient protocol should maximize multiplication while maintaining morphological quality, which makes the low hyperhydricity of nodal explants practically relevant.

STN is a multifactorial physiological disorder that can occur during multiplication, elongation, or rooting and has been linked to mineral nutrition, growth regulators, medium strength, subculture interval, and vessel conditions (Surakshitha *et al*., 2019; Teixeira da Silva *et al*., 2020). In Mondrop, STN incidence was low overall (0.90–3.12%), yet it was consistently lower in nodal explants than in callus-associated buds. This suggests that the source tissue may influence shoot susceptibility under the conditions tested. Surakshitha *et al*. (2019) showed that adjusting calcium and boron supply, lowering BAP concentration, and shortening the subculture interval markedly reduced STN in 'Red Globe', and that elevated calcium in half-strength MS improved recovery. Their findings indicate that low STN is not an intrinsic property of an explant but arises from the interaction between explant identity and the nutritional and hormonal environment. Studies in other woody and herbaceous species lead to the same conclusion: modifying the culture environment alleviated STN in one woody species (Kundan Kishore *et al*., 2015), and STN management was successfully integrated into a regeneration system in Trichosanthes cucumerina (Liu *et al*., 2024). Although these results cannot be extrapolated directly to Mondrop, they support the view that STN control requires attention to both explant biology and culture conditions. Accordingly, the low STN of nodal explants marks them as a favorable starting material here, but it does not demonstrate inherent resistance.

All explant types were compared in the same multiplication medium (MS with 0.5 mg L^{-1} BA), so the observed differences are unlikely to result from differences in medium. Because BA concentration was not varied, however, these data do not establish 0.5 mg L^{-1} as optimal for Mondrop. Cytokinin responses in grapevine are genotype- and condition-dependent: Kim *et al*. (2023) found that 2.0 µM BA promoted shoot formation in 'Shine Muscat', whereas NAA reduced shoot growth and favored callus, and Alam *et al*. (2026) obtained effective direct shoot induction in 'Black Magic' with 1.5 mg L^{-1} BAP, with nodal explants responding particularly well. Such differences show that cytokinin concentrations cannot be transferred between cultivars without validation.

These findings have practical implications for protocol design. Grapevine micropropagation is moving from generic procedures toward cultivar-specific optimization, as genotypes differ in establishment, proliferation, rooting, and acclimatization (Bahrehmand *et al*., 2026), and as basal medium performance varies among local varieties (Ekinci *et al*., 2024). The present study extends this principle to the explant level: within a single cultivar, the choice of starting tissue led to markedly different outcomes. For Mondrop, nodal material should therefore be prioritized when establishing multiplication cultures, particularly when vigorous shoots with few culture-associated abnormalities are required. The relevance also extends beyond clonal multiplication. Reproducible regeneration underpins germplasm conservation, genetic transformation, and genome editing, and recalcitrance, which depends on genotype and the developmental identity of the tissue, remains a limitation in some grapevine genotypes (Capriotti *et al*., 2022; Carra *et al*., 2024). Although axillary shoot multiplication rather than somatic embryogenesis was evaluated here, the principle is the same: the developmental state of the explant influences the

efficiency and predictability of morphogenesis. Nodal explants thus provide baseline information for future work on rooting, acclimatization, genetic fidelity, and more advanced regeneration systems.

The multivariate analysis integrates these observations. The PCA separated the explant types along a single dominant axis (PC1, 97.10% of the variance), contrasting growth and biomass traits, with which nodal explants were associated, with hyperhydricity and low growth, where callus-associated buds were positioned; shoot-tip explants were intermediate. Because the PCA and correlations were based on the means of only three explant types, they are exploratory and descriptive, and their value lies in showing that the univariate responses form a coherent pattern. This pattern agrees with evidence that explant identity, genotype, basal medium, growth regulators, and culture management jointly determine micropropagation success in grapevine (Ekinci *et al*., 2024; Kim *et al*., 2023; Capriotti *et al*., 2022; Surakshitha *et al*., 2019; Bahrehmand *et al*., 2026) and in other plant systems (Daffalla *et al*., 2011; Gałuszka *et al*., 2019; Mastuti & Munawarti, 2018; Ramírez-González *et al*., 2015; Sheykh Abdollahzadeh Mamaghani *et al*., 2025).

In conclusion, explant selection should be treated as an early optimization step in developing a Mondrop micropropagation system, and nodal tissue is the most promising starting material under the conditions tested. This conclusion is specific to the media, cytokinin level, and culture regime used. Future studies should examine the interaction of nodal explants with cytokinin concentration, mineral nutrition, and subculture interval, and should determine whether the favorable growth–quality balance persists through rooting and acclimatization.

Overall, the present study provides cultivar-specific evidence that explant identity is closely associated with both morphogenic productivity and culture quality in V. vinifera cv. Mondrop. The most favorable response of nodal explants, expressed simultaneously through enhanced shoot growth and biomass accumulation and reduced hyperhydricity and shoot-tip necrosis, supports their use as the preferred explant source under the tested conditions. Importantly, the results also demonstrate that evaluating micropropagation success solely through shoot proliferation is insufficient; the quality and developmental stability of the regenerated shoots should be considered alongside quantitative growth performance. This integrated perspective provides a stronger basis for designing efficient and reproducible micropropagation protocols for commercially important grapevine cultivars.

Rererences

Alam, T.B., Urbi, S.I., Raju, M.R.I. and Hossain, M.T., 2026. Development of an efficient in vitro propagation protocol for large scale production of Vitis vinifera L. cv. 'Black Magic'. *Plant Tissue Culture and Biotechnology*, 36(1), pp.269-281. https://doi.org/10.3329/ptcb.v36i1.91184

Bahrehmand, S., Zakizadeh, H., Zarghami, R., Naderpour, M. and Karimi, R., 2026. Genotype-specific responses of six grapevine cultivars to in vitro propagation. *Scientific Reports*, 16, Article 64381. https://doi.org/10.1038/s41598-026-64381-3

Capriotti, L., Limera, C., Mezzetti, B., Ricci, A. and Sabbadini, S., 2022. From induction to embryo proliferation: Improved somatic embryogenesis protocol in grapevine for Italian cultivars and hybrid Vitis rootstocks. *Plant Cell, Tissue and Organ Culture*, 151, pp.221-233. https://doi.org/10.1007/s11240-022-02346-w

Capriotti, L., Ricci, A., Molesini, B., Mezzetti, B., Pandolfini, T., Piunti, I. and Sabbadini, S., 2023. Efficient protocol of de novo shoot organogenesis from somatic embryos for grapevine genetic transformation. *Frontiers in Plant Science*, 14, 1172758. https://doi.org/10.3389/fpls.2023.1172758

Carra, A., Wijerathna-Yapa, A., Pathirana, R. and Carimi, F., 2024. Development and applications of somatic embryogenesis in grapevine (*Vitis* spp.). *Plants*, 13(22), 3131. https://doi.org/10.3390/plants13223131

Daffalla, H.H., Abdellatef, E., Elhadi, E.A. and Khalafalla, M.M., 2011. Effect of growth regulators on in vitro morphogenic response of Boscia senegalensis (Pers.) Lam. Poir. using mature zygotic embryos explants. *Biotechnology Research International*, 2011, 710758. https://doi.org/10.4061/2011/710758

Ekinci, H., Rastgeldi, İ., Şaşkın, N., Ak, B.E. and Korkmaz, Ş., 2024. Evaluation of performance of different culture media in in vitro shoot propagation of local grape varieties. *Applied Fruit Science*, 66, pp.641-648. https://doi.org/10.1007/s10341-023-00993-7

Gałuszka, A., Gustab, M. and Tuleja, M., 2019. In vitro morphogenetic responses from obligatory apomictic Taraxacum belorussicum Val. N. Tikhikh seedlings explants. *Plant Cell, Tissue and Organ Culture*, 139, pp.505-522. https://doi.org/10.1007/s11240-019-01694-4

Guliyeva, A., Salimov, V., Huseynova, A., Röckel, F., Aprile, A., Rehman, S., Rossetti, G. and Rustioni, L., 2026. Agronomic and genetic characterization of table grape cultivars in Azerbaijan: Implications for domestication-related yield traits. *Agriculture*, 16(14), 1559. https://doi.org/10.3390/agriculture16141559

Hamad, M.S. and Majid, N.B., 2017. Influence of explants and growth regulators in the induction of callus of Annona muricata in vitro. *Euphrates Journal of Agricultural Science*, 9(4), pp.1-12.

Hesami, M. and Jones, A.M.P., 2020. Application of artificial intelligence models and optimization algorithms in plant cell and tissue culture. *Applied Microbiology and Biotechnology*, 104(22), pp.9449-9485.

Huda, M.F., Indriyani, S. and Widoretno, W., 2020. Effect of benzyl adenine concentration on callus induction of geranium plants (Pelargonium graveolens L'Her) from petiole and leaf explants. *Journal of Experimental and Life Sciences*, 10(1), pp.20-23.

Isah, T., 2015. Adjustments to in vitro culture conditions and associated anomalies in plants. Acta Biologica Cracoviensia. *Series Botanica*, 57. https://doi.org/10.1515/abcsb-2015-0026

Kadhim, Z.K. and Abdulhussein, M.A., 2021. Minimal media strength for in vitro conservation of strawberry (Fragaria ananassa) cultures. *Basrah Journal of Agricultural Sciences*, 34(2), pp.1-9.

Kim, S.-H., Zebro, M., Jang, D.-C., Sim, J.-E., Park, H.-K., Kim, K.-Y., Bae, H.-M., Tilahun, S. and Park, S.-M., 2023. Optimization of plant growth regulators for in vitro mass propagation of a disease-free 'Shine Muscat' grapevine cultivar. *Current Issues in Molecular Biology*, 45, pp.7721-7733. https://doi.org/10.3390/cimb45100487

Kolasinliler, G., Akkale, C. and Kaya, H.B., 2025. Establishing a reliable protoplast system for grapevine: Isolation, transformation, and callus induction. *Protoplasma*, 262, pp.1401-1417. https://doi.org/10.1007/s00709-025-02069-7

Kundan Kishore, K.K., Samiksha Patnaik, S.P. and Shukla, A., 2015. Optimization of method to alleviate in vitro shoot tip necrosis in Trichosanthes dioica Roxb. *Indian Journal of Biotechnology*, 14, pp.107-111.

Liu, C.-L., Deng, J.-M., Yan, H.-M. and Huang, H.-Y., 2024. In vitro cluster buds regeneration and control of shoot tip necrosis in tissue cultures of *Trichosanthes cucumerina* L. Plant Cell, *Tissue and Organ Culture (PCTOC),* 159, 24. https://doi.org/10.1007/s11240-024-02883-6

Martins, J.P.R., Wawrzyniak, M.K., Kalemba, E.M., Ley-López, J.M., Mendes, M.M., Naskręt-Barciszewska, M.Z., Barciszewski, J. and Chmielarz, P., 2024. Differential morphophysiological and epigenetic responses during in vitro multiplication of Quercus robur depending on donor age and plant growth regulators. Plant Cell, *Tissue and Organ Culture*, 159, 62. https://doi.org/10.1007/s11240-024-02914-2

Mastuti, R. and Munawarti, A., 2018. In vitro morphogenesis responses of various explants in Physalis angulata L. *Journal of Tropical Biodiversity and Biotechnology*, 3(2), pp.71-77.

Okao, M., Bharati, R. and Fernández-Cusimamani, E., 2025. Effect of explant physiology and media composition on callogenesis of Vitellaria paradoxa leaf explants. *Horticulturae*, 11(9), 1127. https://doi.org/10.3390/horticulturae11091127

Özdemir Memiş, S. and Sağlam, H., 2024. Shoot tip culture of Bilecik İrikarası, Sarı Üzüm, Kartal Çavuş and Razakı grape varieties grown in Bilecik province. *International Journal of Agriculture Environment and Food Sciences*, 8(3), pp.729-735. https://doi.org/10.31015/jaefs.2024.3.26

Ramírez-González, G., Rodríguez-de la O, J.L., Arreola-Ávila, J.G. and Álvarez-Moctezuma, J.G., 2015. Morphogenic responses of three explants of Lupinus montanus (H.B.K.) cultured in vitro. *Revista Chapingo Serie Ciencias Forestales y del Ambiente*, 21(1), pp.17-28. https://doi.org/10.5154/r.rchscfa.2013.07.022

Shaker, F.K., Kadhim, Z.K. and Almukhtar, S.A., 2025. Effect of growth regulators and explant types on the callus induction in grape vine (Vitis vinifera L.). *SABRAO Journal of Breeding and Genetics,* 57(3), pp.1283-1291. https://doi.org/10.54910/sabrao2025.57.3.39

Sheykh Abdollahzadeh Mamaghani, A., Pazhouhandeh, M. and Tarinejad, A., 2025. Effects of explant types and phytohormones on Azarshahr red onion micropropagation. *Agrotechniques in Industrial Crops*, 5(3), pp.189-199. https://doi.org/10.22126/ATIC.2025.11448.1178

Surakshitha, N., Soorianathasundaram, K., Ganga, M. and Raveendran, M., 2019. Alleviating shoot tip necrosis during in vitro propagation of grape cv. Red Globe. *Scientia Horticulturae*, 248, pp.118-125. https://doi.org/10.1016/j.scienta.2019.01.013

Teixeira da Silva, J.A., Nezami-Alanagh, E., Barreal, M.E., Kher, M.M., Wicaksono, A., Gulyás, A., Hidvégi, N., Magyar-Tábori, K., Mendler-Drienyovszki, N. and Márton, L., 2020. Shoot tip necrosis of in vitro plant cultures: A reappraisal of possible causes and solutions. *Planta*, 252, pp.1-35. https://doi.org/10.1007/s00425-020-03449-4

Torres-Silva, G., Resende, S.V., Lima-Brito, A., Bezerra, H.B., de Santana, J.R.F. and Schnadelbach, A.S., 2018. In vitro shoot production, morphological alterations and genetic instability of Melocactus glaucescens (Cactaceae), an endangered species endemic to eastern Brazil. *South African Journal of Botany*, 115, pp.100-107. https://doi.org/10.1016/j.sajb.2018.01.001

Trivedi, D.R. and Joshi, A.G., 2021. Synergistic effect of cytokinins on in vitro propagation of Stereospermum suaveolens using nodal explants. *Environmental and Experimental Biology*, 19, pp.131-139. https://doi.org/10.22364/eeb.19.13

Yazar, K., 2025. Boron and cytokinin combinations modulate shoot tip necrosis and improve subculture efficiency in grapevine micropropagation. *International Journal of Agriculture, Environment and Food Sciences*, 9(3).