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Micropropagation of Grape Varieties of Gilgit Baltistan

Reena Batool, Bilquees Fatima*, Muhammad Usman and Sadia Siddiq

Abstract

Micropropagation facilitates the rapid production of disease-free grape plants that are genetically identical to the parent plant. This study was carried out to establish efficient micropropagation in grape varieties viz. Muscat Pink and Early White using nodal segments. For shoot initiation, cultures were established and maintained in Murashige and Skoog media modified by various plant growth regulators including 6-benzylaminopurine (BAP), kinetin (KIN), and indole-3-butyric acid (IBA) in different combinations. Use of BAP 1.5 + KIN 1 and IBA 1 mg/L significantly enhanced shoot and root induction and plant growth attributes in both varieties. Further increment of the PGRs showed a declining growth response highlighting the optimal concentrations of PGRs which enhanced most of the plant growth attributes. Shoot induction and shoot growth responses were better in Muscat Pink whereas root induction and growth responses were greater in Early White indicating a genotype dependent growth attribute in both varieties. Combined use of BAP and KIN effectively induced more shoots and enhanced shoot growth as well. Conclusively the optimized media efficiently enhanced plant growth and multiplication and could be utilized for mass propagation of clean and healthy plant material of commercial varieties.

Keywords: *Tissue culture, in vitro* regeneration, grapes, auxin, cytokinin

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¹Plant Tissue Culture Cell, Institute of Horticultural Sciences, University of Agriculture, Faisalabad.

*Corresponding Authors' email: dr.fatimausman@uaf.edu.pk

Introduction

Botanically, grapes are classified as berries and belong to genus *Vitis* which is a member of the Vitaceae family. *Vitis vinifera* is common grape which is characterized by its flaky bark, weak stem and branches with secondary shoots that grow from the primary long shoots, and tendrils that help it cling to other structures (Akram et al., 2024). It is an excellent source of organic acids, vitamins, minerals, and sugars, containing all components of the human diet that are necessary for healthy growth. Grapes grow globally for several purposes. Mainly they are eaten fresh (27%), used to make wine (71%), and the remaining 2% are dried and consumed. Globally, 21.94 million metric tons of grapes are produced. In Pakistan, grapes are grown on an area of about 15.98 thousand ha with annual production of 196.16 thousand tons (MNFSR, 2025). Balochistan is the dominant grape-producing province of Pakistan, accounting for approximately 92% of the country's total grape production, while the remaining production is mainly contributed by Khyber Pakhtunkhwa and Sindh (MNFSR, 2025). Nowadays, 8,000-10,000 different varieties of *Vitis vinifera* grapes are grown worldwide focused on their table use, raisin, and wine production (Akram et al., 2024).

Grapevine is a crop that is sensitive to the environment. Its growth and development, phenology, output, and quality are all dependent upon environmental factors like temperature, precipitation, and light (Rafique et al., 2024). The disadvantages of sexual propagation and sharing seeds for germplasm exchange are heterogeneity, desiccation, and loss of germinability. Grapes commercial propagation strategies include pitch grafting of rooted rootstock cuttings, softwood cuttings, hard wood cuttings and *in vitro* propagation (Singh and Chauhan, 2020; Kizildeniz et al., 2022). Grafting grapes onto a suitable rootstock is a typical commercial viticulture practice in most parts of the world (Alizadeh et al., 2010). A planted grapevine has a protracted juvenility period, which means it takes four to five years to generate cuttings for multiplication. Thus, to produce the plant material of elite cultivars on a commercial scale and for global germplasm exchange, an alternate technique for protected and rapid multiplication of the healthy plant material is required (Sajid and Zahoor, 2008). In grapes, vegetative propagation strategies result in significant mortality and fewer roots. Grape infections are common these days and infection-related diseases are challenging to diagnose (Alajrami et al., 2019). Hence, *in vitro* techniques must be applied for multiplication and conservation (Ali et al., 2014).

Tissue culture offers enormous opportunities for clonal multiplication of herbaceous and woody crops (Mhatre et al., 2000; Usman et al., 2008; Fatima et al., 2021) planting material in huge quantities while keeping aseptic conditions. Furthermore, it enables the large-scale production of plantlets with high phytosanitary and genetic quality in a limited time and space, regardless of season (Kinf et al., 2017; Shah et al., 2026). Tissue culture aids in production of disease-free grape seedlings, which are identical to the parent plant genetically (Al-Sameen et al., 2021). Micropropagation may allow for the bulk production of elite genotypes that are inexpensive and quickly acclimated based on *in vitro* and genotype circumstances. It also ensures the continuity and preservation of genetic resources (Ekinci et al., 2024). *In vitro* conservation is important for vegetatively propagated and unconventional seed plant species. It allows for the effective use of plant genetic resources while also encouraging plant species conservation. It is an alternative source for large-scale propagation of rare, imperiled, and endangered plants. It has also been demonstrated to multiply many endemics, commercially significant, and medicinally

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significant species *in vitro* (Manokari et al., 2021). Grapes can be micro-propagated by using adventitious bud growth, shoot apical meristems, or axillary buds (Al-Aizari et al., 2020). Grapevine regeneration has been reported using multiple types of explants including shoot tip and node (Mozafari et al., 2016). Micropropagation is also genotype dependent and different plant growth regulators (PGRs) may initiate differential responses in different explants of various horticultural crops (Usman et al., 2012; 2022). Therefore, objective of the study is to establish micropropagation protocol for grapes cultivars Muscat Pink and Early White using different PGRs.

Materials and Methods

The study was conducted during 2024-2025 in the Plant Tissue Culture Cell, Institute of Horticultural Sciences, University of Agriculture, Faisalabad.

Plant Material

The plant material of two grape varieties viz. Muscat Pink and Early White were collected from Gilgit Baltistan and maintained in the germplasm greenhouse at Experimental Fruit Plant Nursery, IHS, UAF. Fresh growing shoots were collected from greenhouse and nodal segments (1-2 cm) were prepared as explants in the lab.

Media Composition and Sterilization

Murashige and Skoog (1962) media was taken as basal media and it was modified with different combinations of plant growth regulators including cytokinin 6-benzylaminopurine (BAP) and kinetin (KIN) and auxin indole-3-butyric acid (IBA). The modified MS media treatments were developed as control (T₀) without PGRs, T₁ (BAP 0.5 + KIN 0.2 + IBA 0.5 mg/L), T₂ (BAP 1 + KIN 0.5 + IBA 0.5 mg/L), T₃ (BAP 1.5 + KIN 1 + IBA 1 mg/L), and T₄ (BAP 2 + KIN 1 + IBA 1 mg/L). 10 ml of media was poured into each glass tube and covered with polyethylene sheet. Media were sterilized by following standardized autoclave conditions at 121°C and 15 PSI for 20 min. The cultural medium was kept under controlled conditions: 25±2°C and 2500 lux fluorescent light intensity (16/8 hr photoperiod) in the growth room.

Explant Sterilization and Culture Establishment

Explants were placed under running tap water to remove dust and dirt. Explants were surface sterilized with 70% ethanol plus 1-2 drops of Tween-20 and rinsed with sterilized distilled water three times. Then, explants were sterilized with 5% NaOCl solution for 5 minutes followed by three rinsing with sterilized dH₂O to remove excessive bleach. The sterilized explants were cultured on modified MS media treatments and immediately covered with polyethylene sheets to ensure asepsis and clean cultures. The cultures were placed in growth room under standard growing conditions including 2500 lux fluorescent light (16/8 hr photoperiod) and 25-26°C temperature for further growth and development. Data were recorded for following parameters.

Shoot Initiation and Shoot-related Traits

The number of days to induce shoots from explants was recorded after culturing the explants on shoot induction medium. The shoot induction percentage was calculated by using the following formula

$$\text{Shoot Induction (\%)} = \frac{\text{Total Number of Shoots per explant}}{\text{To number of Cultures induced shoots}} \times 100$$

The number of induced shoots per explant was recorded after culture on shoot-induction medium. To quantify shoot length, a meter rule was used, and the average was computed.

Root Initiation and Root-related Traits

The number of days to induce roots from explants was recorded after culturing the explants on MS Medium. The root induction percentage was calculated by using the following formula

$$\text{Root Induction (\%)} = \frac{\text{Total Number of roots per explant}}{\text{To number of Cultures induced roots}} \times 100$$

The number of inducing roots per explant was recorded after culture. To quantify root length, a meter rule was used, and the average was computed.

Plant height, Number of leaves per plant, Internodal length and Number of nodes

The height of the plant (from the base to tip of the highest leaf of the plant) and internodal length (from one node to another node) were measured with a standard measuring scale in centimeters (cm) (Raza et al., 2025). The number of leaves per plant and number of nodes per leaf was counted manually (Amjad et al., 2024).

Statistical Analysis

The experiment was laid out in a Completely Randomized Design (CRD) with a two-factor factorial arrangement consisting of two cultivars and five treatments, replicated five times with three explants per replicate. Collected data was analyzed using a two-way Analysis of Variance (ANOVA) and treatment means were compared using the Least Significant Difference (LSD) test ($p \leq 0.05$).

Results

Shoot Initiation Responses

Shoot initiation responses of grapevine nodal segments were significantly influenced by the interactive effects of genotypes and varying levels of exogenous application of cytokinin (BAP, KIN) coupled with auxin (IBA). The quantitative variations recorded across shoot induction percentage, days to shoot induction, number of shoots, and shoot length demonstrated a distinct, dose-dependent response between the two varieties (Figure 1 A, D). The hormone-free control medium (T_0) yielded the lowest shoot induction rates for both Muscat Pink (27.78%) and Early White (16.67%), while requiring the maximum duration to initiate shoots (16.56-17.33 days, respectively). The addition of plant growth regulators (PGRs) progressively enhanced induction rates. Maximum shoot induction percentages were achieved at T_3 (1.5 mg/L BAP + 1.0 mg/L KIN + 1.0 mg/L IBA), where Muscat Pink reached a peak induction rate of 72.22% and Early White reached 36.67%. Concurrently, T_3 notably enhanced shoot induction, reducing the time taken for shoot induction to its lowest values (10.67 days) for Muscat Pink and 9.22 days for Early White (Fig 1A and 1D). Increasing the PGR concentration further to T_4 (2.0 mg/L BAP + 1.0 mg/L KIN + 1.0 mg/L IBA) provoked an inhibitory trend; shoot induction efficiency was reduced to 46.67% in Muscat Pink and 28.89% in Early White, while the time required for induction was delayed upto 13.56 to 11.44 days, respectively (Figure 2).

The number of shoots and shoot length followed a distinct trend. The multiplication rate rose progressively with increasing concentrations of cytokinin, peaking significantly at T_3 with 7.22 shoots for Muscat Pink and 3.67 shoots for Early White. At the highest concentration (T_4), a noticeable reduction in shoot multiplication was observed (4.67 for

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Muscat Pink and 2.89 for Early White). However, the highest PGR concentration (T₄) resulted in the highest shoot length in Muscat Pink (9.53 cm) although lower shoot induction and multiplication efficiency was seen at T₄ as compared to T₃. The contrasting response indicates that the higher PGR concentration caused elongation of the individual shoots, rather than the initiation and proliferation of multiple shoots. However, for Early White, shoot length was marginally larger at T₃ (3.33 cm) than at T₄ (3.20 cm) suggesting that shoot length was influenced by the variety in response to PGR concentration. The minimal elongation rates observed in the control group were 3.11 cm for Muscat Pink and 2.20 cm for Early White (Figure 2).

Root Initiation Responses

The *in vitro* rooting of regenerated grapevine was notably influenced by the interaction between cultivar genotypes and the varying exogenous concentrations of cytokinin (BAP, KIN) and auxin (IBA) in the media. Root induction percentage, days to root induction, number of roots, and root length revealed a distinct, genotype-specific response (Figure 1 B, E). The hormone-free control medium (T₀) completely failed to induce roots for both cvs. Muscat Pink and Early White. The plant growth regulators (PGRs) significantly stimulated rooting processes. Root induction efficiency followed a progressive, dose-dependent upward trend that peaked between the two cultivars. For Muscat Pink, the highest root induction rate was recorded at T₃ (37.78%), whereas Early White achieved its maximum response at T₄ (64.44%) (Figure 1B, E). Specific PGR adjustments also optimized the number of days to root induction. Shoots of Muscat Pink minimized their time to root development down to 31.89 days at T₃ and delayed initiation (46.56 days) when shifted to T₄. Conversely, Early White displayed accelerated rooting at the highest concentration, minimizing its days to root induction down to 30.56 days at T₄, highlighting a faster physiological adaptation to higher hormone concentrations compared to T₁ (54.67 days) and T₂ (45.11 days), as shown in Figure 3.

The number of roots rose consistently across treatments, reaching 3.78 roots for Muscat Pink at T₃ and 2.78 at T₄ treatment. Early White, matching its peak induction rate, produced its maximum root quantity at T₄ with 6.44 roots per explant. Root length also advanced at increased hormonal concentrations. The maximum root length for Muscat Pink was recorded at T₄ (4.12 cm), followed by T₃ (3.57 cm). Early White depicted its longest root development at T₄ (3.53 cm) and T₃ (3.30 cm), whereas lower concentrations of PGRs (T₁) produced only 1.38 cm and 1.63 cm root length for the two cultivars, respectively (Figure 3).

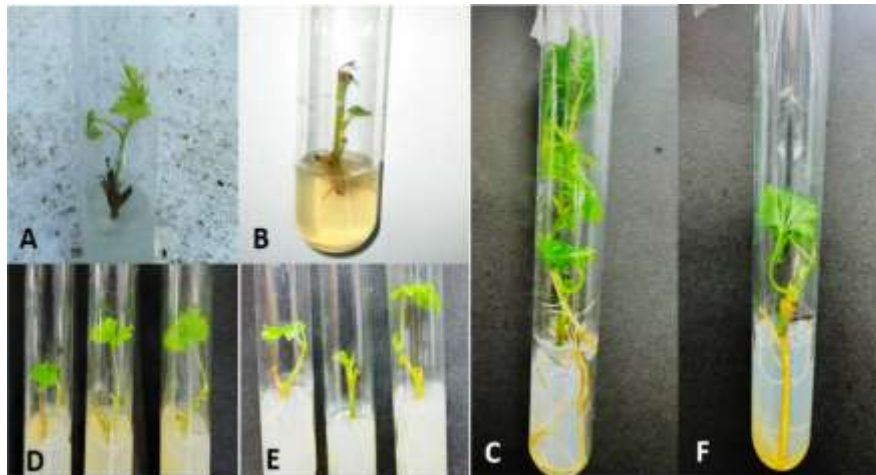
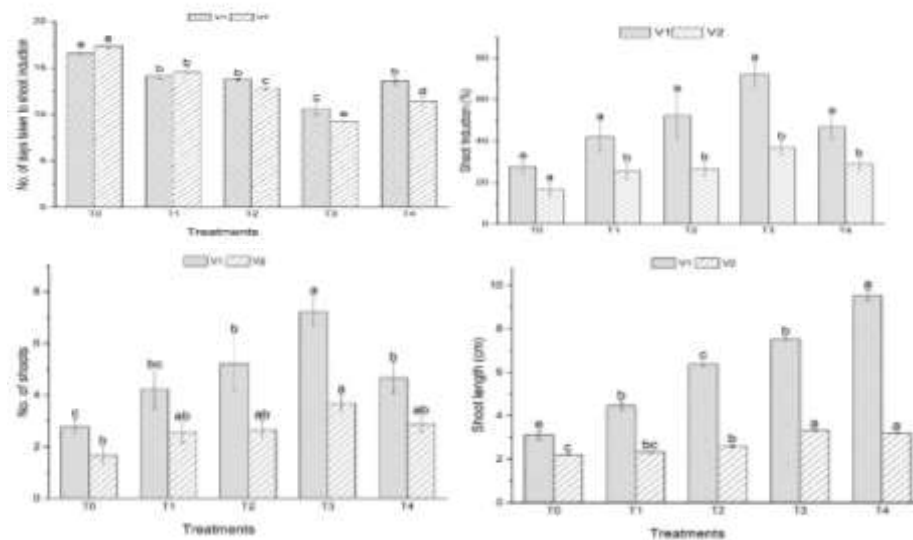
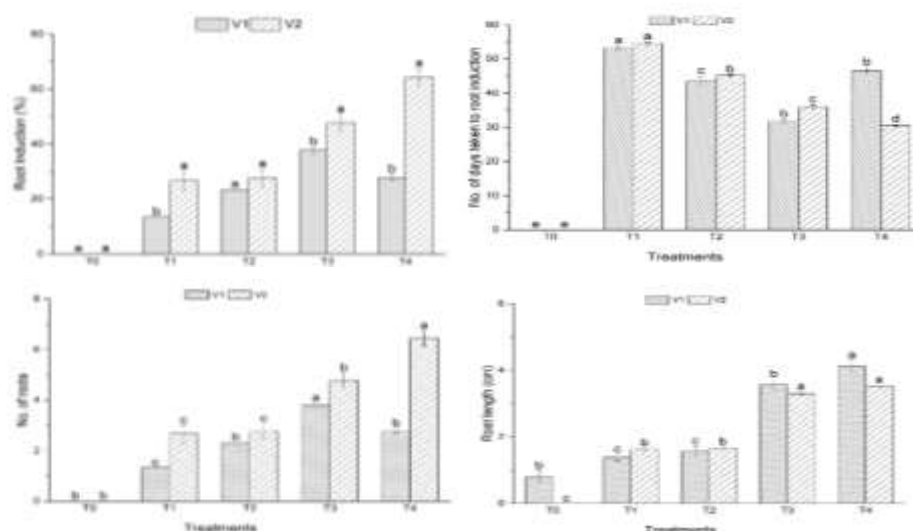


Figure 1. Micropropagation responses in Grape varieties Muscat Pink and Early White. Figures show shoot induction, rooting and plant growth responses in Muscat Pink (A-C) and Early White (D-F) varieties.



Grape varieties Muscat Pink (V1) and Early White (V2). Whereas treatments were T0=control, T1=BAP 0.5 mg/L + Kin 0.2 mg/L + IBA 0.5 mg/L, T2=BAP 1 mg/L + Kin 0.5 mg/L + IBA 0.5 mg/L, T3=BAP 1.5 mg/L + Kin 1 mg/L + IBA 1 mg/L, T4=BAP 2 mg/L + Kin 1 mg/L + IBA 1 mg/L. Means followed by different letters differ significantly by LSD test ($p \leq 0.05$).



Varities Muscat Pink (V1) and Early White (V2). Whereas treatments were T0=control, T1=BAP 0.5 mg/L + Kin 0.2 mg/L + IBA 0.5 mg/L, T2=BAP 1 mg/L + Kin 0.5 mg/L + IBA 0.5 mg/L, T3=BAP 1.5 mg/L + Kin 1 mg/L + IBA 1 mg/L, T4=BAP 2 mg/L + Kin 1

mg/L + IBA 1 mg/L. Means followed by different letters differ significantly by LSD test ($p \leq 0.05$).

Shoot growth and number of leaves

The vegetative growth of grapevine plantlets was significantly influenced by the interactive effects between genotypes and PGRs (Figure 1 C, F). In hormone-free control medium (T₀), vegetative traits remained at minimum baseline, while growth was better in Muscat Pink (4.00 cm, 2.44 nodes, and an inter-nodal distance of 0.48 cm) compared with Early White. The progressive increase of PGRs up to treatment T₃ (1.5 mg/L BAP + 1.0 mg/L KIN + 1.0 mg/L IBA) improved plant growth (Figure 1C, F). Maximum values for plant height, number of leaves, and nodes were achieved at T₃ for both varieties. Muscat Pink attained more plant height (9.58 cm), number of leaves (7.44) and nodes (7.56) compared with Early White (Figure 4). Interestingly, internodal distance showed a similar optimal trend in both varieties. Muscat Pink reached its maximum internodal length at T₃ (2.51 cm), while Early White recorded its maximum internodal length of 2.60 cm at T₃. At T₄ (2.0 mg/L BAP + 1.0 mg/L KIN + 1.0 mg/L IBA) plant height was reduced to 7.20 cm in Muscat Pink (Figure 4).

Discussion

The present study demonstrated that the regeneration efficiency of grapevine was strongly influenced by the interaction between genotype and PGRs composition in the culture medium. Both Muscat Pink and Early White varieties responded positively to the supplementation of BAP, KIN, and IBA in MS medium, although the response varied considerably between varieties. Genotype-dependent variability in morphogenic responses has frequently been reported in grapevine tissue culture due to differences in endogenous

hormone balance, phenolic metabolism, and meristematic competency (Osama, 2022; Pedranzani et al., 2024). Similar cultivar-specific responses were observed in grapevine cvs. Red Globe and Superior, where cytokinin concentration significantly altered shoot proliferation and elongation responses (Osama, 2022). Genotypic responses for shoot induction have also been reported in guava (Usman et al., 2012).

The poor regeneration response observed under the hormone-free control medium confirms that exogenous PGRs are indispensable for successful organogenesis in grapevine explants. In the absence of cytokinin and auxin supplementation, explants exhibited delayed shoot induction, reduced multiplication rates, and poor vegetative development which may be attributed to insufficient endogenous hormone levels to overcome apical dominance and activate dormant axillary meristems. Similar findings were reported by Blazina et al. (1992), who observed minimal shoot proliferation in grapevine explants cultured on hormone-free MS medium. Likewise, Ali et al. (2017) reported that supplementation of cytokinin-containing media was essential for efficient shoot induction and healthy plantlet regeneration in European grape cultivars.

Among the tested treatments, T₃ (1.5 mg/L BAP + 1.0 mg/L KIN + 1.0 mg/L IBA) proved to be the most effective for shoot induction and multiplication in both cultivars which may be attributed to the synergistic interaction between cytokinin and auxins in regulating cell division and meristematic differentiation. BAP is widely recognized as one of the most effective cytokinin for stimulating axillary shoot proliferation because it enhances DNA synthesis and promotes cytokinin-mediated signaling pathways associated with bud break (Mhatre et al., 2000).

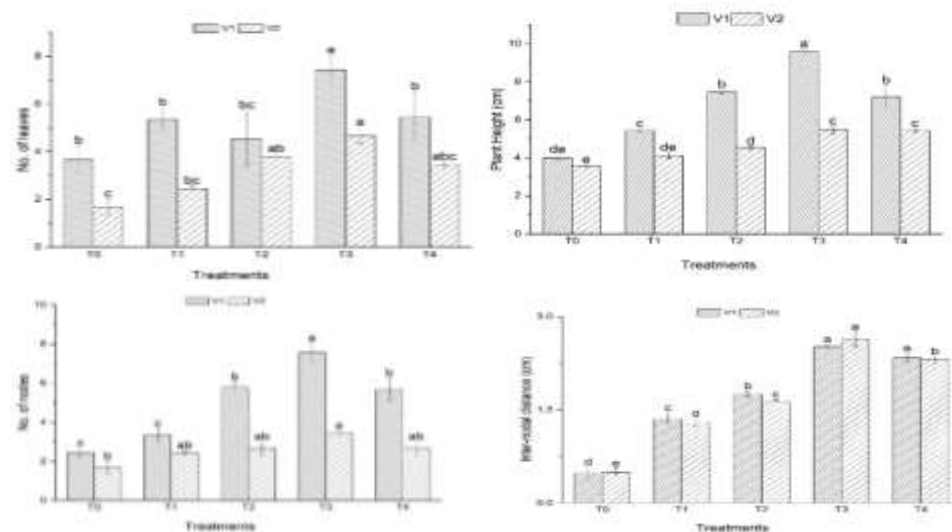


Figure 4. Effect of plant growth regulators (PGRs) on plant height, number of leaves, number of nodes and internodal distances of grape varieties Muscat Pink (V1) and Early White (V2). Whereas treatments were T0=control, T1=BAP 0.5 mg/L+ Kin 0.2 mg/L + IBA 0.5 mg/L, T2=BAP 1 mg/L+ Kin 0.5 mg/L + IBA 0.5 mg/L, T3=BAP 1.5 mg/L +

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Kin 1 mg/L + IBA 1 mg/L, T4=BAP 2 mg/L+ Kin 1 mg/L + IBA 1 mg/L. Means followed by different letters differ significantly by LSD test ($p \leq 0.05$)

Kinetin further complements BAP activity by improving chloroplast differentiation and shoot vigor, whereas low concentrations of IBA help maintain cellular organization and vascular differentiation during organogenesis (Kavitha and Surakhshitha, 2022). Similar synergistic effects of BAP and auxin combinations on grapevine regeneration were also reported by Osama (2022) and Pedranzani et al. (2024).

The enhanced shoot induction percentage and reduced days required for shoot emergence at T₃ suggest that balanced cytokinin-to-auxin ratios accelerate physiological and biochemical processes involved in organogenesis. Cytokinin are known to stimulate RNA transcription, protein biosynthesis, and rapid cell cycle progression, thereby shortening the lag phase between inoculation and bud emergence (Mhatre et al., 2000). The accelerated shoot induction observed in the present study agrees with findings reported by Kavitha and Surakhshitha (2022), who observed improved shoot induction and regeneration efficiency in grape cv. Red Globe cultured on cytokinin-enriched media. Similarly, Pedranzani et al. (2024) reported that optimized cytokinin combinations improved shoot vigor and reduced shoot initiation time in Pinot Noir cultures.

Interestingly, shoot multiplication declined at T₄ despite the higher concentration of BAP. This reduction may indicate inhibitory effects associated with supra-optimal cytokinin accumulation within the tissues. Excessive BAP concentrations are known to induce physiological abnormalities such as hyper-hydricity, hormonal imbalance, and abnormal tissue proliferation, ultimately reducing regeneration efficiency (Mhatre et al., 2000). High cytokinin concentrations may additionally interfere with endogenous auxin transport and impair normal cellular differentiation. Similar inhibitory responses at elevated cytokinin concentrations were reported by Osama (2022), where higher BAP levels reduced shoot quality and multiplication efficiency in grapevine cultivars. Comparable findings were also documented by Theivanai et al. (2020) in grape rootstock Dog Ridge.

A significantly greater number of shoots produced by Muscat Pink compared with Early White further supports the existence of strong genotypic effects on *in vitro* morphogenesis. Differential responses among grapevine cultivars are often associated with variations in endogenous growth regulator concentrations, antioxidant activity, and phenolic exudation (Pedranzani et al., 2024). Genotypes with higher morphogenic competence generally possess greater cellular plasticity and responsiveness to exogenous plant growth regulators. Similar genotype-dependent regeneration responses have previously been documented in Thompson Seedless, Superior, and Dog Ridge grape cultivars (Mhatre et al., 2000; Theivanai et al., 2020).

Root induction responses were also markedly influenced by cultivar and hormone concentration. The complete absence of rooting in the control treatment highlights the indispensable role of auxins in adventitious root formation. Auxins, particularly IBA, are considered highly effective rooting hormones because of their stability, slow degradation, and efficient transport within plant tissues (Ali et al., 2017). IBA stimulates root primordia formation through activation of cambial and pericycle cells, enhancement of cell elongation, and induction of vascular differentiation. The improved rooting response at T₃ and T₄ therefore indicates effective activation of auxin-mediated physiological pathways. Similar observations were reported by Kavitha and Surakhshitha (2022), who found that

IBA supplementation significantly enhanced rooting percentage, root number, and root length in grapevine cultures.

The superior rooting performance of Early White at T₄ suggests that this cultivar possesses a greater physiological requirement or tolerance for elevated hormone concentrations during rhizogenesis. Genotypic variability in rooting ability has been widely documented in grapevine and is often associated with endogenous auxin sensitivity and carbohydrate metabolism (Pedranzani et al., 2024). Mozafari et al. (2016) and Kinfé et al. (2017) also reported substantial differences among grapevine cultivars in rooting efficiency under identical culture conditions, confirming that rooting responses are highly genotype dependent.

Plant growth parameters including plant height, number of leaves, and internodal distance were significantly enhanced at T₃. Improved vegetative growth under balanced cytokinin–auxin combinations may result from enhanced photosynthetic activity, chloroplast development, and nutrient assimilation. Cytokinin promotes leaf initiation and chlorophyll biosynthesis, whereas auxins regulate cell elongation and vascular tissue differentiation (Aazami, 2010). Consequently, balanced hormone combinations improve canopy architecture and overall plant vigor. Similar increase in plant growth traits has been reported in grapevine cultures supplemented with moderate concentrations of BAP and kinetin (Osama, 2022; Theivanai et al., 2020).

The greater number of leaves and nodes observed at T₃ may additionally indicate enhanced meristematic activity and assimilate partitioning within regenerated shoots. Node formation is particularly important in grapevine micropropagation because each node represents a potential propagule for subsequent multiplication cycles. Increased nodal proliferation therefore directly contributes to multiplication efficiency and commercial propagation potential (Theivanai et al., 2020). Similar findings were reported by Pedranzani et al. (2024), who observed improved nodal proliferation and shoot vigor in Pinot Noir cultures supplemented with optimized cytokinin combinations.

Conclusion

Overall, the present study confirms that successful grapevine regeneration through nodal culture largely depends upon optimization of cytokinin–auxin interactions and genotype-specific hormonal sensitivity. The combination of 1.5 mg/L BAP + 1.0 mg/L KIN + 1.0 mg/L IBA effectively promoted shoot induction, multiplication, rooting, and vegetative growth, whereas excessively high cytokinin concentrations exerted inhibitory effects.

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