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Enhancing grapevine (*Vitis vinifera* L.) mass propagation: Single-node cuttings vs. traditional approaches

Asma Arab, Mahdi Alizadeh* and Esmaeil Seifi

*Horticulture department, Faculty of Plant Production, Gorgan University of Agricultural Sciences & Natural Resources, Gorgan, Golestan, Iran. *E-mail: mahdializadeh@gau.ac.ir*

Abstract

In the past decade, numerous grape genotypes have been introduced to Iran, many showing desirable traits and adapting well to the region. However, their mass propagation is limited due to their non-native status and the challenges of traditional methods. This research explored single-node cuttings (SNCs) for grapevine propagation as an alternative and compared its effectiveness to traditional hardwood cutting (HWC) techniques. The Perlette and Fakhri grape cultivars were selected and both traditional HWCs (25 cm length, 4-5 nodes) and SNCs (5 cm length, just a single node) were procured from dormant canes. The cuttings were subjected to IBA and inserted into two different rooting substrates in a greenhouse fitted with a misting system. The comparative rooting attributes were measured in both techniques. Both cuttings showed high rooting percentages (more than 95 percent), but the SNC method yielded more plants per square meter. The SNCs planted horizontally rooted earlier and more effectively. While the rooting substrate didn't significantly impact rooting success, the perlite and coco peat mixture (6:1) was easier to manage for SNCs. Overall, we recommend the SNC approach as a creative, quick, cost-effective and efficient method for grapevine mass propagation.

Key words: Multiplication, vegetative propagation, hard wood cutting, rooting, grapevine

Introduction

Propagating plants through cuttings is a common and straightforward method of propagation that is widely utilized. Numerous plant varieties can successfully develop roots from a mere section of the plant. Grapevines, in particular, are known for their ease of rooting, with many grapevine cultivars being commonly propagated worldwide through cutting techniques. Cuttings are typically taken from the previous year's growth of one-year-old wood (Ahmed *et al.*, 2017; Amir *et al.*, 2023).

Propagation of grapes by hard wood cuttings (HWCs) is one of the easiest and best ways to reproduce this plant in large numbers, which preserves the characteristics of the mother plant (Amrajaa *et al.*, 2023). The literature contains numerous reports and reviews on grapevine propagation methods. In most of these research works the auxin application has been found to enhance the histological features like formation of callus and tissue and differentiation of vascular tissue and finally root formation. The most commonly used auxin was also Indole-3-Butyric acid (IBA) with the concentration of 500 to 6000 mgL^{-1}.

The grapevine is an easy-to- root species (Hartmann *et al.*, 2002) and high success can be achieved with dormant stem cuttings. In their 2020 publication, Singh and Chauhan conducted a review on vegetative propagation of grapevines through cuttings. They emphasized that among the factors influencing root induction, the rooting media plays a crucial role in the successful production of rooted cuttings. This factor significantly impacts the rooting and growth of grapevine cuttings. Various grapevine genotypes have been assessed for rooting efficacy utilizing diverse growth media and growth regulators. In *Vitis vinifera*, the combination of sand and garden soil with 2000 mg/L IBA produced optimal root growth (Galavi *et al.*, 2013). Sandy soil and NAA resulted in considerable sprouting and rooting in the Thompson and Crimson Seedless varieties (Ahmed *et al.,* 2017). The *V. champini* rootstock with a peat-perlite mixture demonstrated that PGPR and IBA concentrations, as well as dipping time, influenced rooting success (Işçi *et al.*, 2019). Bidaneh Sefid found variable rooting success with sand, sawdust, and garden soil, and recommended the inverted technique (Khiri and Arshad, 2020). For Punjab Macs Purple, 3000 mg/L of IBA in talcum powder produced the best results (Maninderdeep and Singh, 2022). Black Magic performed best when pre-planted with 2000 ppm IBA and peat moss (Amrajaa *et al.*, 2023). NARC black, Early Round, and Perlette varieties rooted in a 1:1 sand-garden soil mixture with 3000 ppm IBA showed successful rooting and improved root traits (Amir *et al.,* 2023).

In recent decade, various grape cultivars and genotypes have been introduced to Iran from different grape-producing regions around the world. Presently there are more than 1000 local and introduced grape genotypes in the National Grape Collection of the country, located in the vineyard research station in Qazvin province (Alizadeh, personal interview). Some of these imported genotypes exhibit desirable traits and have shown promising adaptation to certain parts of Iran in regional assessments. However, due to their limited numbers and non-native status, their mass propagation through traditional methods is constrained. Tissue culture techniques are potential alternatives, but their high costs and technical challenges pose barriers to their widespread application. Therefore, the need for a simpler and more cost-effective solution to address this challenge is more highlighted.

Hence, in the present research work we examined the propagation of grapevines using single-node cuttings (SNCs), an applied, practically feasible method for grape propagation in situations where plant material is scarce. Additionally, we compared the results obtained from this approach to the traditional hardwood cutting technique.

Materials and methods

Plant materials: Two European grapevine varieties (*Vitis vinifera* L.) known as Fakhri and Perlette were used. The Fakhri is a local cultivar of Semnan province, Iran. It is a seeded and late bearing grapevine with bold and crispy berries. The "Perlette" is a sweet, seedless grape that is white with a mild, aromatic flavor. Its name comes from the French word for "little pearl," reflecting the translucence of the mature fruit. This variety is one of the earliest maturing seedless grapes and was developed by Harold P. Olmo in 1936 in California through the crossbreeding of Reine des Vignes and Sultanine (https://www.plantgrape.fr/en/varieties/fruit-varieties/208). The Perlette grape variety serves as an introduction to Iran, with a total soluble solids (TSS) content of 16-18%. It begins to ripen in the second week of July in the climate of Iran (Alizadeh, personal interview).

Experiment 1: Evaluation of rooting in grapevine SNCs: In this experiment, the rooting success of SNCs using two different rooting substrates was investigated. In early March, healthy dormant canes were pruned from the mother plants and then transferred to the greenhouse. Each cane was carefully cut at the internodes to create SNCs, with each cutting being no longer than 5 cm in length (Fig. 1). To minimize stress during cutting preparation, the SNCs were temporarily placed on a moist cloth

Fig. 1. Size comparison between standard hardwood cutting and single node cutting before (a) and after root production (b). A standard vegetable seedling tray specifically used for inserting single node cuttings (c,d). The arrangement pattern of the cuttings in the tray holes, both vertically (e) and horizontally (f). Rooting in horrizontal and vertical cuttings (g). Very old grapevine branch segments evaluated for rooting (h). Rooted single node cuttings during hardening stage just before potting (i). The potted plantlets raised from single node cutting technique (j).

layer awaiting planting. Two types of rooting substrates were utilized: wood chips and a mixture of fine perlite : cocopeat in a 6:1 ratio. The SNCs were either treated with auxin or left untreated. For the auxin treatment, a solution containing 2000 mgL^{-1} of indole butyric acid (IBA) was applied using the quick dip method for 10 seconds.

In order to facilitate the planting process of SNCs, a custom vegetable seedling tray was employed (Fig. 1. c,d). The cuttings were inserted in two horizontal and vertical patterns in the trays. Following the planting of the SNCs, the trays were transferred to a greenhouse fitted with a misting system. The greenhouse maintained a temperature range of 25/30 (night/day) degrees Celsius. The misting system was programmed to spray water for 30 seconds every hour, providing the necessary moisture for optimal growth conditions. When 50 % of the buds of SNCs were opened, foliar spraying with Rovral® TS fungicide (10 %) was done. Traits such as the time taken for bud break, rooting success rate, loss percentage, number of roots, root length, number of leaves, and new shoot length were quantified. To harden and acclimate the rooted SNCs, they were relocated from the tray to a bed filled with pure coarse perlite for a period of 14 days. The mist system was also programmed to spray water for 40 seconds every 3 hours. Following this acclimatization period, the adapted plantlets were then transferred to plastic pots filled with a mixture of garden soil and leaf mold in a 3:1 ratio (Fig. 1 j) Three weeks after transplanting, the survival rate of the plantlets was recorded.

Experiment 2: Evaluation of rooting in grapevine HWCs: In early March, healthy dormant canes were pruned from the mother plants and then transferred to the greenhouse. The canes were cut as 25-30 cm stem cuttings, each containing 4-5 nodes (Fig. 1 a). The HWCs were then inserted to rooting medium with or without auxin treatment (2000 mgL^{-1} of IBA, quick dip method for 10 seconds). Two types of rooting substrates including wood chips and a mixture of fine perlite and cocopeat in a 6:1 ratio were utilized. The greenhouse was maintained at a temperature of 25/30 degrees Celsius during the night and day. The misting system sprayed water for 40 seconds every three hours to provide necessary moisture. After one week of planting, the misting system was turned off for these standard HWCs, which were instead periodically watered as needed. Furthermore, when 50 % of the buds of the cuttings were opened, foliar spraying with Rovral® TS fungicide (10 %) was also performed. The acclimation process for rooted stem cuttings was similar to that of SNCs. Following acclimation, the adapted plantlets were transplanted into plastic pots filled with a mixture of garden soil and leaf mold in a 3:1 ratio.

Experiment design and data analysis: The present study was structured as a factorial experiment following a completely randomized design. Each experiment comprised three replications, with 10 cuttings (observations) present in each replication. The first experiment considered factors such as cultivar, rooting substrate, vertical and horizontal planting patterns, and auxin treatment. In the second experiment, the factors were akin with the exception that the horizontal planting pattern was omitted.

The data obtained from the experiments were analyzed using the SAS statistical software. Mean comparisons were conducted using Duncan's test at a significance level of 5 % to determine any significant differences among the various treatments and factors studied.

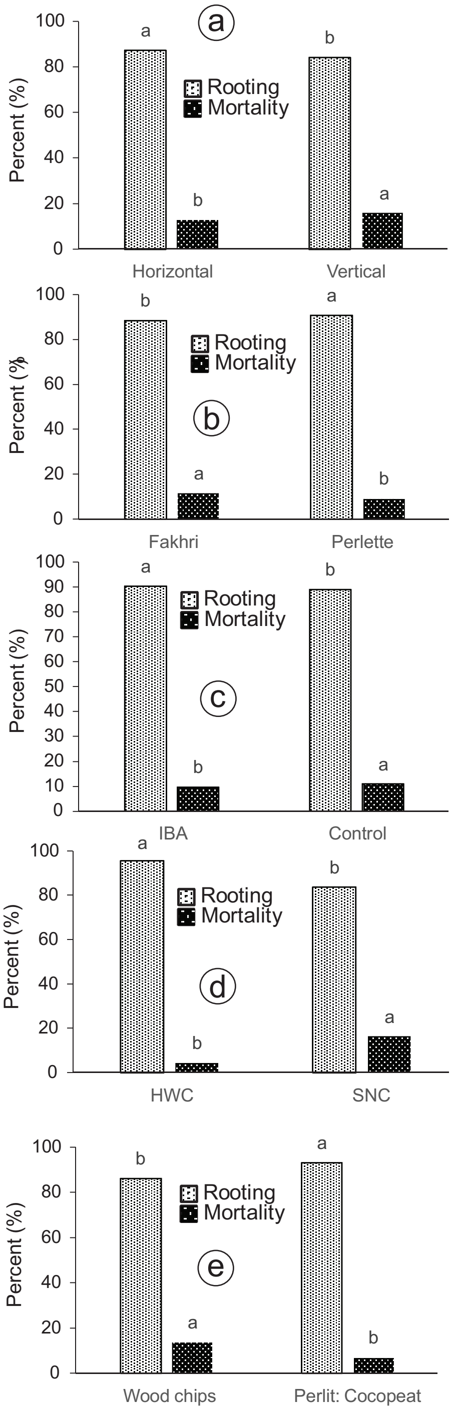

Fig. 2. The influence of insertion pattern (a), cultivar (b), IBA treatment (c), cutting type (d) and rooting substrate (e) on rooting and survival percentages of grapevine cuttings.

Results and discussion

Role of cutting types: The Table 1 displays the results regarding the impact of cutting type on the rooting of grape stem cuttings. The data clearly indicate that HWCs yielded a greater number of roots and longer root lengths. Additionally, they produced more leaves and longer shoots compared to SNCs (Table 1).

The rooting percentages of the cuttings are depicted in Fig. 2. The rooting percentages of the cuttings, clearly indicating that HWCs outperformed single-node cuttings SNCs in rooting success. Across both cultivars, HWCs achieved an impressive 95.6 % rooting rate, compared to 83.7 % for SNCs. Furthermore, the loss percentages were notably lower for HWCs at 4.4 %, while SNCs experienced a loss of 16.6 %.

Influence of cultivar: In our experiment, both Perlette and Fakhri cultivars demonstrated high rooting percentages, which is promising for a grapevine nursery. However, the Perlette cuttings began to show bud break shortly after being planted in the rooting media (Table 1). On average, Perlette cuttings initiated bud break just 9 days following planting, while Fakhri cuttings started after 15 days. Generally, leaves positively influence root emergence. Leaf photosynthesis during rooting of leafy cuttings in hard to root species can contribute to supply carbohydrates to the intensive metabolic processes related to adventurous root formation (Tombesi *et al.*, 2015). Also, a positive relationship between number of roots per cutting and axillary bud growth was found among clones of Stephanotis (Hansen and Kristensen, 2006). They found

Table 1. Effect of cutting type, cultivar, rooting substrate and auxin on the rooting characteristics of grapevine stem cuttings

Treatment	Shoot length (cm)	Number of leaves	Max root length (cm)	Min. root length (cm)	Number of roots	Time to 100 % bud sprouting (day)	Time to 50 % bud sprouting (day)	Time to 1st bud sprouting (day)
Cutting type								
HWCs	16.5[a]	14.6[a]	17.5[a]	2.9[a]	33.9[a]	20.5[a]	16.2[b]	12.2[a*]
SNCs	4.6[b]	3.3[b]	4.32[b]	1.0[b]	8.9[b]	20.9[a]	17.2[a]	12.1[a]
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.1123	<0.0001	0.791
Cultivar								
Perlette	11.9[a]	10.9[a]	11.1[a]	2.1[a]	24.9[a]	17.5[b]	13.1[b]	9.0[b]
Fakhri	9.2[b]	7.0[b]	10.8[a]	1.8[a]	17.9[b]	23.9[a]	20.3[a]	15.3[a]
P value	0.0029	0.0019	0.0271	0.5467	0.2884	<0.0001	<0.0001	<0.0001
Rooting substrate								
Shredded wood	9.9[a]	9.1[a]	10.5[a]	1.8[a]	19.9[a]	20.8[a]	17.0[a]	12.1[a]
Coco peat: perlite	11.1[a]	8.9[a]	11.4[a]	2.1[a]	22.9[a]	20.6[a]	16.4[b]	12.2[a]
P value	0.1408	0.8843	0.3285	0.1542	0.2228	0.4203	0.0037	0.4286
Auxin treatment								
Control	9.9[a]	8.6[a]	11.8[a]	2.1[a]	19.8[a]	20.7[a]	16.7[a]	12.1[a]
IBA (2000 mgL^{-1})	11.2[a]	9.3[a]	10.1[a]	1.8[a]	23.0[a]	20.8[a]	16.8[a]	12.2[a]
P value	0.1261	0.5613	0.3027	0.0079	0.3181	0.6858	0.6577	0.791

* Means followed by the same letter in each column are not significantly different at 5%.

that, with some exceptions, the onset of axillary bud growth is accelerated in cuttings as a result of accelerated root formation and a higher number of roots per cutting. Working with Cotinus cuttings, Cameron *et al.* (2001) also revealed that the use of branched cuttings accelerated root and shoot development and resulted in a finished plant being produced more rapidly than is achieved from conventional, non-branched cuttings. All these reports confirm the positive role of axillary bud or leaf presence on adventitious root development. In case of single node cuttings of Perlette grapevine, the bud break was taken place just 9 days after planting, hence, the rooted plantlets being produced more rapidly than is achieved from Fakhri cuttings. These were ready 20 days earlier than those of the Fakhri cultivar. So, this early readiness was undoubtedly being significant for the economic efficiency of grapevine nurseries.

In the Perlette and Fakhri cultivars, 97.66 % and 96 % of HWCs and 76.3 % and 75.3 % of SNCs successfully rooted, respectively. A similar reverse trend was observed in the loss percentage, with HWCs exhibiting significantly lower losses (Fig. 3). As previously mentioned, grapevines are generally considered easy to root, although certain cultivars, such as muscadines are notoriously difficult to propagate by hard-wood cuttings (Buck *et al.*, 2022). Also, *Vitis rotundifolia* hybrids show low rooting rate and auxin application is important for their cutting propagation (Reinhart and Biasi, 2018).

Influence of rooting substrate: According to previous reports, planting beds have significantly influenced the rooting and vegetative growth of plant cuttings. Growing media should be considered an essential part of the propagation system because rooting competency depends on the type of substrate used. Rooting mixture directly effect on quality and percentage of rooting (Singh and Chauhan, 2020). Regarding the impact of rooting substrate, we tested two different media (Figs. 2 and 3), but there were no significant differences in the rooting traits of either standard or micro-cuttings. However, we found that propagation using HWCs can be easily achieved in both substrates. But, the wood chip substrate was not suitable for propagating SNCs due to difficulties in properly inserting the cuttings and the tendency for the small cuttings to get lost among the shredded woods. Consequently, it is recommended to use only perlite and cocopeat substrates for SNCs propagation. It may be noted that we utilized a blend of 6 parts fine perlite to 1 part cocopeat, but the implementation of a misting system seemed to maintain a higher humidity level in the substrate. This elevated moisture content may have contributed to the increased loss in SNCs, potentially due to fungal contamination. Nevertheless, we employed fungicides (10 % Rovral® TS,) to prevent decay. Additionally, filling the tray

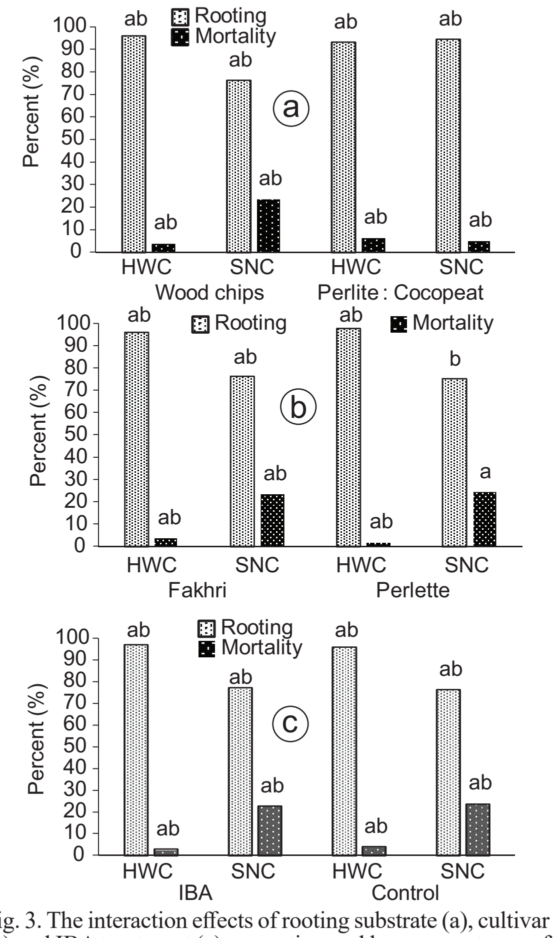

Fig. 3. The interaction effects of rooting substrate (a), cultivar (b) and IBA treatment (c) on rooting and loss percentages of standard and single node grapevine cuttings.

with wood shavings is also not as easy as using other substrates. Considering all these factors, the perlite : cocopeat substrate is recommended as an excellent option for rooting grape SNCs.

Auxin treatment: The use of plant growth regulators to promote rooting in cuttings has been practiced for a long time. Among these regulators, auxins play a crucial role in stimulating root development. Auxins are natural plant hormones that facilitate cell elongation and division, which are essential processes for root formation (Hartmann *et al.*, 2002). Auxin enhances early root induction in cuttings through rapid signaling, localized action at the cut site, promotion of cell division and adventitious root formation, hormonal interactions, and improved nutrient uptake (Adem *et al.*, 2024). The IBA is widely used to induce root formation in various plants (Kohler *et al.*, 2022; Zhao *et al.*, 2014; Hoque et al., 2025).

In this study, the application of IBA to promote root emergence did not have a significant impact on rooting characteristics, as both control and IBA-treated cuttings ultimately rooted without any statistically meaningful differences. This similarity may be due to the natural rooting ease of the two grapevine genotypes examined. However, it was noted that IBA-treated cuttings exhibited longer roots (Table 1).

The literature indicates that the concentration of IBA for root induction ranges from 2000 to 6000 mgL^{-1}. In our study, we opted for a concentration of 2000 mgL^{-1}, as it has been frequently used for grape propagation. For instance, Rao (2004) reported that among various IBA concentrations, 2000 ppm proved to be optimal for hardwood cuttings, while 500 ppm was ideal for softwood cuttings. These concentrations yielded the highest percentage of rooting, the greatest number of roots, and the longest root lengths per cutting in the Dogridge and 1613C grapevine rootstocks. Maninderdeep and Singh (2022) also concluded that IBA @ 3000 ppm when applied as powder (Talcum powder) was found to be best for the induction of roots with all quality parameters. However, in most of the cases observations were at par with concentration of 2000 ppm in liquid form.

Role of insertion pattern: The method of inserting cuttings into the rooting substrate was one of the most intriguing treatments explored in this research. As mentioned earlier, we used vegetable seedling trays to insert grapevine cuttings, which accommodate two different planting patterns. Therefore, we investigated both vertical and horizontal planting arrangements (Fig. 1 e,f). Remarkably, the cuttings planted horizontally activated and rooted significantly higher as well as earlier than those planted vertically. This exploration was particularly interesting, as it has not been documented in prior studies.

Following the results from the horizontal planting of SNCs, we became curious and decided to cut very old grape branches into sections, placing them horizontally in the rooting bed. The buds on these cuttings also demonstrated excellent activation, and subsequently, the cuttings rooted successfully (Fig. a h). However, no statistical data were collected for these cuttings; the focus was solely on observing their responses.

Mass multiplication efficiency: The results demonstrated that HWCs exhibited higher rooting rates and lower mortality compared to SNCs. However, when analyzing the ease of cultivation and evaluating the number of rooted cuttings that can be obtained per square meter, the practical advantages of using SNCs become evident. Despite the lower initial rooting success of SNCs, their efficiency in terms of space utilization and overall productivity is significantly higher. The SNCs allow for a denser planting arrangement, enabling a greater number of cuttings to be planted in a smaller area. This means that while individual SNCs may have lower rooting rates, the volume of rooted cuttings per square meter can surpass that of standard HWCs.

Moreover, the ability to produce a higher mass of grapes per square meter makes SNC technique particularly advantageous for commercial grape propagation (Fig. 1 j).

Each tray contained 105 holes, facilitating the insertion of 105 SNCs. On average, we could fit approximately 6.33 trays in a single square meter of space, allowing for the production of up to 666 rooted grapevine plantlets in just one square meter of the greenhouse. In contrast, commercial nurseries utilizing standard stem cuttings typically yield around 200 rooted plantlets per square meter. This data clearly illustrates the effectiveness of the SNCs compared to standard HWCs.

Based on the data and discussions presented, it is clear that SNCs offer significant advantages over traditional HWCs for grapevine propagation. One of the primary benefits of SNCs is their capacity for higher density planting, which allows producers to maximize space efficiency and enhance productivity in commercial grape production. This efficiency not only leads to a greater number of rooted plantlets per square meter but also improves overall yield and profitability for nurseries.

While SNCs may initially show lower rooting success compared to HWCs, they compensate for this with superior overall output and optimal utilization of available space. This characteristic is particularly beneficial given the limited availability of plant materials for hybrid genotypes and specific introduced cultivars, which often complicates mass multiplication efforts. The single node cutting method allows for the generation of a larger number of plants from a single mother plant, addressing this challenge effectively. So, in overall we recommend the SNCs approach as a quick, simple, and effective method for the mass propagation of grapevine.

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