Viticulture Studies (VIS)
2023, 3(2): 57 – 63
https://doi.org/10.52001/vis.2023.20.57.63

Research Paper

Promoting Rooting and Vegetative Growth in 41 B Vine Rootstock Cuttings with Encapsulated Nano Silver and IBA Applications

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How to cite: Kara, Z., Doğan, O., Yazar K. (2023). Promoting Rooting and Vegetative Growth in 41 B Vine Rootstock Cuttings with Encapsulated Nano Silver and IBA Applications. Viticulture Studies (VIS), 3(2): 57 - 63. https://doi.org/10.52001/vis.2023.20.57.63

Article History:
Received: 05.01.2023
Accepted: 10.05.2023
First online: 03.07.2023

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Keywords
Grape rootstock
Rooting of cuttings
Nano promotors
AgNPs
Green synthesis

Abstract

Globally, viticulture is carried on by grafting onto vine rootstocks obtained by hybridizing different vitis species or on their own roots due to their tolerance to biotic and abiotic stresses, especially phylloxera (*Daktulosphaira vitifoliae*). Sustainable regeneration of vineyard areas and new vineyard establishments require significant amounts of vine saplings each year. In addition to hormonal applications, nano products are also tested in the production of high quality vine nursery material. In this study, single nodal cuttings prepared from the vine rootstock 41 B (*Vitis vinifera* L. cv. Chasselas × *Vitis berlandieri*) in the resting period were used as plant material. The effects of encapsulated nano silver particles (AgNPs, 0.5 mg L^{-1} and 1 mg L^{-1}), and IBA (0 and 1 g L^{-1}) applications produced by green synthesis with cv. Öküz Gözü grape seed extract and $AgNO_3$ on promoting rooting and vegetative growth in 41 B vine rootstock cuttings were evaluated in this study. AgNPs and IBA applications promoted rooting of cuttings, and vegetative growth of rooted cuttings. Applications increased shoot length, shoot diameter, number of nodes, fresh and dry weights of roots and shoots, and the highest value among these was achieved by the 1 mg L^{-1} AgNPs application. Leaf area and chlorophyll content (SPAD value) were determined by 1 g L^{-1} IBA application. AgNPs and IBA treatments resulted in increased rooting and vegetative growth compared to control. In conclusion, 1 mg L^{-1} AgNPs was recommended for vine propagation material and sapling producers as nano promotors for rooting and vegetative growth of 41 B rootstock cuttings.

Introduction

The grapevine is very diverse, and globally 25538 grape genotypes and 1432 vine rootstocks are listed in the Vitis Database (VIVC, 2022). In all vineyard regions of the world, about 10 grape rootstocks are used in 90% of the vineyards (Keller, 2020), probably 50% of them are Teleki/Kober selection vine rootstocks. However, viticulture is carried out in areas with different climate and soil conditions all over the world. The few vine rootstock varieties in use today are unlikely to meet the requirements of all viticulture areas (Reynolds, 2015). Moreover, 88% of the rootstocks used consisted of 4 grapevine species [*V. berlandieri* (28%), *V. rupestris* (25%), *V. riparia* (24%) and *V. vinifera* (11%)] or their hybrids (Riaz et al., 2019; VIVC, 2022).

The grapevine is grown on its own roots or grafted onto rootstocks obtained from different grapevine species (Smart et al., 2006). Some rootstocks can change the vegetative and generative development of the grafted variety compared to others (Paranychianakis et al., 2004). Although the resistance level to phylloxera is a critical feature in grape rootstocks, their adaptation to other pathogens, drought, soil water holding capacity, different soil types, as well as the strength of the variety grafted on them and their effects on grape components are also very important (Mullins et al., 1992; Jackson, 2008; Keller, 2015; Zhang et al., 2016).

A quality vine sapling should not be affected by diseases and pests, and should have a good root structure, especially the bottom roots developed all around, the stem length should be normal, and the shoots should develope and mature normally. Rooting stimulating practices should be used to increase the production of vine saplings in sufficient quantities, and to increase the quality and yield of the saplings (Yavaş and Fidan, 1991). 41 B rootstock stands out for reasons such as tolerance to high lime content in the soil, relatively short vegetation period, drought tolerance (Kara and Demirhan, 2005). However, the cuttings of 41 B rootstock are difficult to root (Çelik and Gargın, 2009).

IBA is an auxin used in practice for rooting grapevine rootstock cuttings to stimulate the formation of adventitious roots and increases the rooting rate of cuttings (Galavi et al., 2013). Although auxin is produced naturally in the shoots and young leaves of plants, the use of synthetic auxin is recommended to ensure rooting of cuttings and prevent them from dying (Daskalakis et al., 2019).

Nano-sized silver particles (AgNPs) coated with grape seed extract contain a wide variety of bioactive compounds with biostimulant effects. These products generally improve the productivity of plant nutrients and increase their tolerance to biotic and abiotic stresses (Bulgari et al., 2014; Manda et al., 2014). The extract obtained from the seeds of *Vitis vinifera* L. cultivars provides resistance under stress conditions by increasing the antioxidant activity in plants due to its polyphenol content (Shi et al., 2003; Bita and Preda, 2007; Bita et al., 2009; Manda et al., 2014).

According to Jasim et al. (2017), 1 mg L^{-1} AgNPs applications positively affected root and shoot parameters in fenugreek seedlings. Applications of 30 mg L^{-1} AgNPs from soil to Asian rice improved root growth and branched root system (Mirzajani et al., 2013).

In this study, the effects of IBA (Indole-3 butyric acid) and AgNPs (produced by green synthesis from grape seed extract and $AgNO_3$) on rooting and shoot growth of 41 B rootstock cuttings were investigated.

Material and Methods

In the study, single nodal cuttings of 41 B rootstocks were used as plant material. The Trial Random Plots were arranged according to the trial pattern, with 3 replications and 15 cuttings in each replication. 1 g L^{-1} IBA was applied for 5 seconds as practiced by Galavi et al. (2013) to induce adventitious root formation in single-nodal cuttings.

Grape seed extract used in the experiment was obtained from cv. Öküzgözü. AgNPs synthesis with grape seed extract was performed by green synthesis method (Kara et al., 2021). To 41 B single nodal cuttings, 0.5 and 1 mg L^{-1} AgNPs applications were made by immersing the basal parts of the cuttings (~ 2 cm) for 24 hours before rooting. Control cuttings were also kept in water for the same time. After AgNPs and IBA application, the cuttings were placed in organic pots containing a 3:1 peat: perlite mixture and rooted in the fogging unit in the heated greenhouse.

The effects of applications on 41 B grapevine rootstocks on vegetative growth were evaluated by shoot and root measurements during the vegetation period. For this purpose, changes in vegetative parameters were evaluated.

Results and Discussion

Shoot length

According to the evaluations, the effects of AgNPs and IBA applications on 41 B rootstocks on shoot length were significant (Figure 1a). Shoot lengths obtained from 0 (control), 0.5 and 1 mg L^{-1} AgNPs and 1 g L^{-1} IBA applications to single nodal cuttings were 6.31±0.06 cm, 6.85±0.31 cm, 7.01±0.25 cm and 6.92±0.19 cm, respectively. The longest shoots were obtained in those treated with 1 mg L^{-1} AgNPs, while the shortest shoots were in the control. AgNPs and IBA treatments increased shoot length compared to control.

Shoot diameter

The effects of AgNPs and IBA treatments on 41 B rootstock single nodal cuttings on shoot diameter were significant (Figure 1b). Shoot diameter values were 2.78±0.15, 3.04±0.03, 3.47±0.13 and 3.18±0.17 mm for control, 0.5 and 1 mg L^{-1} AgNPs and 1 g L^{-1} IBA applications, respectively. The highest shoot diameter value was obtained from the 1 mg L^{-1} AgNPs application, while the lowest diameter value was

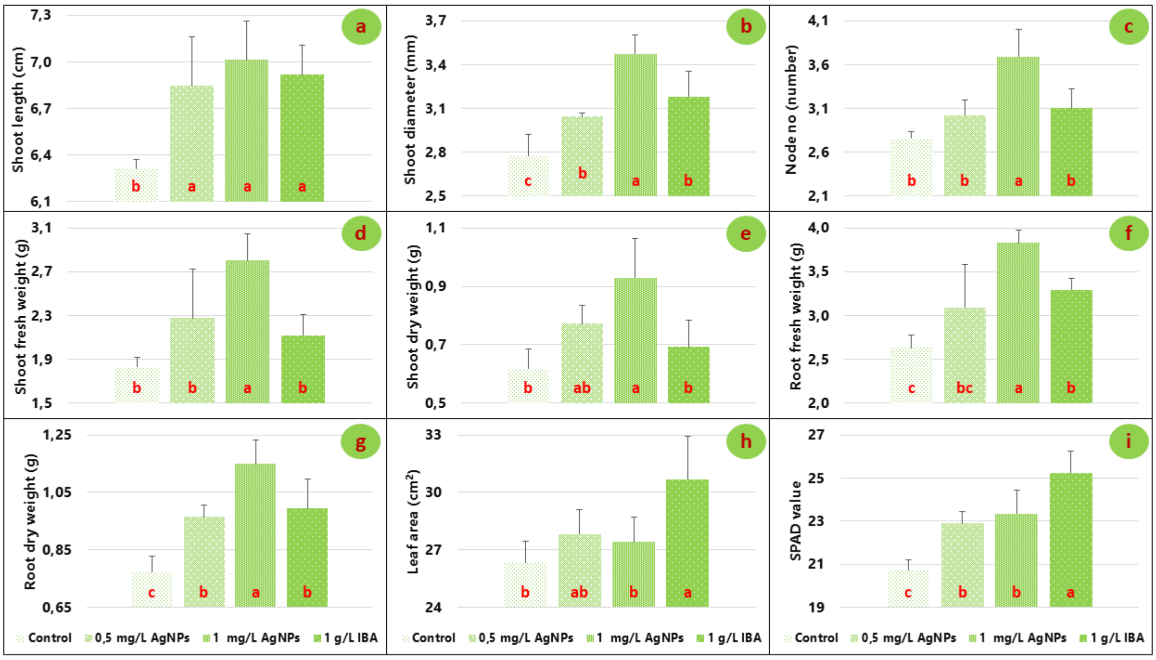

Figure 1. Effects of AgNPs and IBA applications on shoot lenght (a), shoot diameter (b), number of nodes (c), shoot fresh weight (d), shoot dry weight (e), root fresh weight (f), root dry weight (g) leaf area (h) and chlorophyll content (i). LSD (SPSS 24) respectively for: 0.48 cm, 0.28 mm, 0.42 number, 0.61 g, 0.21 g, 0.58 g, 0.16 g, 3.25 cm^2, 1.40 mg kg^{-1}. Each column represents the mean of triplicate determinations with fifteen cuttings for replicate.

in the control. AgNPs and IBA treatments increased shoot diameter compared to control.

Number of nodes

The effects of applications on the number of nodes were significant (Figure 1c). Node numbers determined at the end of the vegetation period in the control, 0.5 and 1 mg L^{-1} AgNPs and 1 g L^{-1} IBA applied cuttings were 2.76±0.08, 3.02±0.18, 3.69±0.31 and 3.10±0.22, respectively. The application of 1 mg of L^{-1} AgNPs increased the number of nodes compared to the control.

Shoot fresh weight

AgNPs and IBA treatments applied to 41 B rootstock single-nodal cuttings had a significant effect on shoot fresh weight (Figure 1d). All treatments increased shoot fresh weight compared to control. Shoot fresh weights were measured as 1.83±0.08 g, 2.28±0.44 g, 2.80±0.24 g and 2.12±0.19 g in control, 0.5 and 1 mg L^{-1} AgNPs and 1 g L^{-1} IBA applications, respectively. The highest shoot fresh weight was recorded in the 1 mg L^{-1} AgNPs application, and the lowest in the control.

Shoot dry weight

Effects of AgNPs and IBA treatments on shoot dry weight of 41B rootstock single-nodal cuttings were significant (Figure 1e). All applications increased the shoot dry weight. Shoot dry weight values obtained from control, 0.5 and 1 mg L^{-1} AgNPs and 1 g L^{-1} IBA treatments were 0.62±0.07 g, 0.77±0.06 g, 0.93±0.13 g and 0.69±0.09 g, respectively. The highest shoot dry weight was determined in the 1 mg L^{-1} AgNPs application, while the lowest was in the control.

Root fresh weight

The effects on root fresh weight of applications to single-nodal cuttings of 41 B vine rootstock were significant (Figure 1f). All treatments increased root fresh weight compared to control. Root fresh weight values obtained from control, 0.5 and 1 mg L^{-1} AgNPs and 1 g L^{-1} IBA applications were 2.63±0.14 g, 3.09±0.49 g, 3.83±0.15 g, and 3.29±0.13 g, respectively. The highest root fresh weight was obtained in the 1 mg L^{-1} AgNPs application, while the lowest value was determined in the control.

Root dry weight

The effects of AgNPs and IBA treatments on root dry weight grown from single nodal cuttings of 41 B vine rootstock were significant (Figure 1g). All treatments increased root dry weight relative to control. Root dry weight values of control, 0.5 and 1 mg L^{-1} AgNPs and 1 g L^{-1} IBA applications were 0.77±0.05 g, 0.96±0.04 g, 1.15±0.08 g and 0.99±0.10 g, respectively. The highest root dry weight was in the 1 mg L^{-1} AgNPs application, and the lowest was in the control.

Leaf area

The effects of applications on 41 B vine rootstock single-nodal cuttings on leaf area were significant (Figure 1h). All treatments increased leaf area relative to control. Leaf area values obtained from control, 0.5 and 1 mg L^{-1} AgNPs and 1 g L^{-1} IBA applications were 26.35±1.11 cm^2, 27.82±1.26 cm^2, 27.41±1.32 cm^2 and 30.70±2.24 cm^2, respectively. The highest leaf area was determined in the 1 g L^{-1} IBA application, while the lowest value was in the control.

Chlorophyll content

The effects of AgNPs and IBA applications on single-nodal cuttings of 41 B vine rootstock on leaf chlorophyll content were significant (Figure 1i). All treatments increased the leaf chlorophyll content compared to the control. Leaf chlorophyll content (SPAD value) data were measured as 20.71±0.51, 22.90±0.56, 23.36±1.07 and 25.25±0.73 in control, 0.5 and 1 mg L^{-1} AgNPs and 1 g L^{-1} IBA applications, respectively. The highest chlorophyll content was determined in the 1 g L^{-1} IBA application, while the lowest value was in the control.

IBA, a growth regulator auxin most widely used as a rooting stimulator, has weak auxin activity, and is degraded very slowly by enzyme systems. The rooting stimulating effect of IBA on cuttings is since it is not easily transported from the applied part but remains there. IBA can be applied as a very concentrated (1000-8000 ppm) and dilute (10-250 ppm) solution (Zenginbal et al., 2006).

AgNPs are thought to positively affect the survival of cuttings in the rooting medium, rooting, and plant growth by preventing tissue damage resulting from emboli or xylem activation (Kara et al., 2021).

AgNPs and IBA doses used in our study increased shoot length and shoot diameter significantly ($p<0.05$) from single nodal cuttings of 41 B vine rootstock. The dose that most stimulated shoot length and diameter values was 1 mg L^{-1} AgNPs.

In a previous study, Bawskar et al. (2021) determined that AgNPs applications significantly increased root and shoot length in *V. radiata*. In another study, 0.5 g L^{-1} IBA extended shoot and internode spacing in willows (Yoon et al., 2021), while 0.8 g L^{-1} IBA maximum shoot growth was achieved in lemons (Kumar et al., 2022). Our results were like the literature.

AgNPs and IBA applications on single-nodal cuttings of 41 B vine rootstocks increased the number of nodes significantly ($p<0.05$). The maximum number of nodes was obtained from the 1 mg L^{-1} AgNPs application. Kara et al. (2021), by applying AgNPs, IBA, and their combination to standard nursery cuttings of 41 B vine rootstocks, obtained the highest number of nodes with 1 mg L^{-1} AgNPs + 50 ppm IBA application. In another study conducted with cv. Isabella under in vitro conditions, the highest number of nodes was obtained with 2 mg L^{-1} IBA application (Ekbiç et al., 2015). Our results were like previous studies in terms of the number of nodes.

As a result of our study, it was determined that the applications significantly increased the shoot fresh and dry weights of 41 B rootstocks growing from single-node cuttings. The highest values in both parameters were determined in the 1 mg L^{-1} AgNPs application. In a previous study, Koç (2020) reported that the 1 mg L^{-1} AgNPs application increased the fresh and dry weight the most in shoots developed from 41 B rootstock standard cuttings.

In a previous in vitro study (Sharma et al., 2022), different doses of AgNPs (0, 2, 4, 6, 8 and 10 mg L^{-1}) significantly increased shoot fresh and dry weight in *Nyctanthes arbor-tristis* L. In another study, Sadak (2019) reported that AgNPs applications increased shoot fresh and dry weight in *Trigonella foenum-graecum* plants. Our study supported previous studies in terms of shoot fresh and dry weight.

In our study, AgNPs and IBA applications significantly ($p<0.05$) increased the fresh and dry weight of roots grown from single nodular cuttings of 41 B rootstock. In a previous study (Koç, 2020), AgNPs and IBA applications were reported to increase root fresh and dry weight. In another previous study, IBA applications at different doses (0, 2, 4 and 6 g L^{-1}) to grapevine cuttings increased root fresh and dry weight (Galavi et al., 2013). IBA applications to Thomson Seedles grape variety cuttings increased root fresh weight to a limited level (Othman and Hawezy, 2022). IBA applications to Ramsey (Mohamed, 2017) and 420 A (Çelik and Gargın, 2009) grapevine rootstocks increased root fresh and dry weight. This study is compatible with the literature in terms of increasing root fresh and dry weight of IBA applications.

In the study, AgNPs and IBA applications significantly increased the leaf area and leaf chlorophyll content ($p<0.05$) on shoots developed from single nodular cuttings of 41 B rootstock. The largest leaf area and the highest chlorophyll (SPAD value) contents were determined in the 1 g L^{-1} IBA application. In a previous study, Patil et al. (2001) reported that IBA application to grapevine rootstocks significantly increased leaf area. In addition, it was reported that AgNPs applications caused a significant increase in rice leaf area (58.8%) (Ejaz et al., 2018). Similarly, Sarropoulou et al. (2016) detected an increase in leaf chlorophyll content in Gisela 6 cherry rootstock, while a decrease in CAB-6P with $AgNO_3$ application. In addition, AgNPs applications in rice caused a decrease in leaf chlorophyll content (Nair and Chung, 2014: Abbas et al., 2019). In a study conducted with a seedless grape variety, leaf chlorophyll content increased significantly with IBA applications (Uddin et al., 2020). While this study was like the literature in terms of leaf area, it differed from some literature in terms of leaf chlorophyll content.

Conclusion

AgNPs and IBA applications to single-nodal cuttings of 41 B vine rootstock increased all parameters compared to control. It was the application of 1 mg L^{-1} AgNPs that increased the shoot length, shoot diameter, number of nodes, shoot fresh and dry weight, root fresh and dry weight data the most. Leaf area and leaf chlorophyll content increased by 1 g L^{-1} IBA at most. Our AgNPs and IBA application results were generally like data from different species.

It was found that 1 mg L^{-1} AgNPs application can be recommended for propagation material and seedling producers as it positively affects the root shoot development from a single node of 41 B vine rootstock.

Conflicts of Interest

The authors declare that there is no conflict of interests.

Author Contribution

The contribution of the authors to all stages of the manuscript is equal.

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