

EFFECTS OF IBA AND MEDIA ON THE GROWTH PERFORMANCE OF THREE CULTIVARS OF GRAPES (*VITIS VINIFERA* L.)

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Abstract

Annual branches of three grape cultivars, namely 'Xia Hei' grape, 'Eastern Star' grape and 'Giant Rose' grape were first treated with Indole-3-butyric acid (IBA) solution, inserted in different media and then their growth performances were studied. The growth performance of the three grape cultivars were: 'Xia Hei' > 'Eastern Star' > 'Giant Rose'. Media (M4) was the most conducive to improve the survival per cent of grape rooted cuttings. This study provides a reference for choosing a suitable method to grow rooted cuttings taken from grape annual branches, which would furthermore improve the survival rate of young vines.

Introduction

Grapes (*Vitis vinifera* L.) are widely cultivated in China. They have become a significant cash crop and play an important role in the agricultural economy. Grape vegetative propagation is one of the key links to achieve high yields and high-quality cultivation, which directly affects the growth rate and yield per unit area of these plants. Taking cuttings from grape vines is one of the main methods of grape propagation, which retains the genetic characteristics of mother plants; it is a simple low-cost operation (Bobzin *et al.* 2020). Grapes can be propagated from hardwood or greenwood cuttings.

At present, hardwood cuttings are the predominant method for expanding the number of self-rooted grape plants. Hardwood cuttings were used as the propagation method. Many studies have demonstrated that 3-indolebutyric acid (IBA), the major form of auxin in plants, contributes to diverse processes, such as embryonic development, root initiation, leaf formation, phototropism, gravitropism, and apical dominance (Cheng *et al.* 2007, Marsico *et al.* 2021). Application of hormones improved survival to more than 50% (Galavi *et al.* 2013, Meng *et al.* 2014, Daskalakis *et al.* 2017). In addition, the survival rate and grape growth condition with different treatments were different, the per cent rooting of cuttings was 40–50%, and the survival rate of transplants was 50–60% (Adu *et al.* 2016, Li *et al.* 2017, Cai *et al.* 2018, Das and Jha. 2018). This is manifested in many plant hardwood cuttings (Eed 2016, Mukhtar 2019, Grund *et al.* 2020, He *et al.* 2020). Therefore, there is still an issue of a low propagation success. Improving the propagation coefficient of self-rooted vines is a priority for accelerating the development of grape cultivation.

In the present study, the dormant annual branches of three grape cultivars, namely 'Xia Hei', 'Eastern Star' and 'Giant Rose' were selected as test materials. Indole-3-butyric acid (IBA) was used on rooted cutting. Grass soil, vermiculite and pearl rock, along with garden soil from the grape resource nursery were selected as media for grape hardworking cuttings (Sunitha *et al.* 2016, Montenegro *et al.* 2018). Effects of IBA and different media treatment on hardwood cuttings of these three grapes cultivars were studied to provide a reference for selecting suitable methods for development and growth of grape hardwood cuttings, and furthermore improve the survival rate of young vines and plant quality.

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Materials and Methods

'Xia Hei', Eastern Star' and 'Giant Rose' grape (*Vitis vinifera* L.) plants (four-year old) was grown in the grape repository of Henan Institute of Science and Technology (Latitude: 35.1654, Longitude: 113.5550, Altitude: 70 m, Xinxiang, Henan, China). One-year old hardwood cuttings with the same diameter and growth of these three grape cultivars were obtained after a winter of sand storage., and then they were cut into 20 cm sections with two buds. They were obliquely cut at 1.5 cm below the end buds to match the morphology of short canes. A flat cut was made 3 cm above the top bud. These hardwood cuttings were sterilized with carbendazim (1:1000), and rinsed with water to remove the residual carbendazim solution, and soaked in water for 24 hrs. Root end of hardwood cuttings was soaked with 1 mg/l IBA solution, no IBA treatment was given in the control. After soaked for 1 min, these hardwood cuttings were taken out. Grass soil, vermiculite, pearl rock, and soil from the grape resource nursery which were mixed in different ratios to produce five media (M1, M2, M3, M4 and M5) treatments (Table 1). These prepared nutrient media were put into black plastic bowls (18 × 22 cm). All hardwood cuttings in bowls were cultivated in a greenhouse under a 16/8 hrs photoperiod (3000 lx) and relative humidity of approximately 70-80% at 25-28°C.

Table 1. Composition and proportion of different media.

Media	Media formula	Proportion (v/v)
M1	Peat soil: vermiculite: perlite: garden soil	6:6:3:3
M2	Peat soil: vermiculite: perlite: garden soil	4:8:3:3
M3	Vermiculite: perlite: garden soil	2:3:3
M4	Peat soil: vermiculite: perlite: garden soil	8:4:3:3
M5	Peat soil: perlite: garden soil	12:3:3

The growth of below-ground and above-ground parts of each cutting was observed regularly (Fig. 1). A total of 30 cuttings was used. Root callus was assessed 30 days after treatment; the number of rooted cuttings, backbone roots, primary lateral roots, and secondary lateral roots were counted; fresh root weight (g) of new roots was measured. Total root length (cm) and root diameter (mm) were measured. Number of buds, shoots and leaves were counted; shoot diameter (mm) was measured with vernier calipers at the mid-section; Total root length (cm) and plant height (cm) were measured. Fresh weight (g) of leaves was measured using analytical balance; leaf area was measured using leaf area meter.

Some other measurement were done using the following formulae: (i) Each cultivar survival percent of hardwood cuttings % = Each cultivar survival number of hardwood cuttings / total number of hardwood cuttings of each cultivar × 100%; (ii) (No) IBA treatment survival percent of hardwood cuttings % = (No) IBA treatment survival number of hardwood cuttings / total number of hardwood cuttings of each cultivar of (No) IBA treatment × 100%; (iii) Media treatment survival percent of hardwood cuttings % = Media treatment survival number of hardwood cuttings / total number of hardwood cuttings of each cultivar of media treatment × 100%.

The experiment was a 3×2×5 factorial arrangement of treatments in a Completely Randomized Design. ANOVA was done using SPSS20.0.



Fig. 1. The effect of IBA treatments on the growth of grape cuttings (A), the growth of grape above and below ground without IBA treatment (B), and the growth of above ground and below ground with IBA treatment (C).

Results and Discussion

The per cent of rooting of (44.00%), the number of backbone roots (14.52), the number of primary lateral roots (194.85), the number of secondary lateral roots (157.57), the total weight of fresh roots (3.12 g), the total root length (168.11 cm), and the root diameter (1.28 cm) were found to be highest in Xia Hei (Table 2). The root development indices of cuttings of three grape cultivars under 1 mg/l IBA treatment also reached the maximum value, while the indices of

non-IBA treatment were low (Table 2, Fig. 2). The per cent of rooting, the number of backbone roots, the number of lateral roots, the total fresh root weight, the total root length, and the root diameter of the three grape cultivars all reached a maximum in M4 after IBA treatment, meanwhile, these indexes were the lowest in M5 and M1 after IBA treatment (Table 2). All these results all showed that M4 after IBA treatment was the most suitable for inducing the root growth of hardwood cuttings of these three grape cultivars.



Fig. 2. Root development of different grape cuttings with IBA treatment (A), root development of different grape cuttings without IBA treatment (B).

Table 3, showed that the highest survival per cent of hardwood cuttings (24.19%), the number of new shoots (1.85), the diameter of new shoots (3.59 mm), the number of new leaves (7.75), the leaf area (199.32 cm²) and the plant height was (17.49 cm) were obtained in “Xia Hei”. The indexes of new growth and development of cuttings of these three grape cultivars under 1 mg/l IBA treatment also reached a maximum, while the indexes of non-IBA treatment were low. After IBA treatment, the cuttings had a greater number of new shoots, more vigorous growth, thicker new shoots, and stronger and more new leaves, which were better than that with no IBA treatment (Table 3, Fig. 3). After IBA treatment, the values of these indexes in M4 were the highest. Number

Table 2. Effects of cultivars, IBA and media treatments on below-ground indexes of different grape cuttings.

Variable	Number of rooting cutting	Per cent of rooting (%)	No. of backbone roots	No. of primary lateral roots	Number of secondary lateral roots	Total weight of fresh roots (g)	Total root length (cm)	Root diameter (mm)
Cultivar								
Xia Hei	13.28±8.01a ^z	44.00±0.26a	14.52±8.14a	194.85±171.62a	157.57±168.29a	3.12±2.71a	168.11±172.61a	1.28±0.60a
Eastern	9.23±7.82b	29.00±0.26b	10.50±8.11b	137.05±151.17a	94.45±109.62b	1.19±1.29b	51.12±53.10b	0.93±0.60b
Giant Rose	14.25±8.35a	46.00±0.28a	14.20±8.43a	187.52±162.69a	111.35±100.40ab	0.90±0.52b	66.26±49.73b	0.79±0.66b
IBA								
0 mg/l IBA	10.52±7.16a	34.00±0.23b	11.42±7.02b	136.82±124.35b	82.65±92.00b	1.28±1.15b	78.81±114.12a	1.00±0.65a
1 mg/l IBA	13.98±9.01b	46.00±0.31a	14.73±9.28a	209.47±187.80a	159.62±152.64a	2.19±2.53a	111.52±122.94a	1.01±0.65a
Media								
M1	6.20±4.31d	20.00±0.13c	7.00±4.22d	40.29±39.59d	8.91±14.30d	0.57±0.44c	18.158±13.83c	0.52±0.34c
M2	13.54±4.23c	45.00±0.12b	14.41±3.68c	153.50±65.70c	123.41±67.08c	1.57±0.84bc	78.35±36.72b	1.15±0.26b
M3	15.87±4.41b	51.00±0.13b	16.71±3.44b	226.62±118.01b	175.50±131.80b	2.30±2.38b	126.39±109.54b	1.08±0.39b
M4	23.29±3.58a	77.00±0.16a	24.37±4.17a	418.41±97.94a	272.58±125.21a	3.63±2.65a	237.70±156.15a	1.95±0.18a
M5	2.33±1.78e	6.00±0.06d	2.87±2.30e	26.87±26.79d	25.25±41.18d	0.61±0.65c	15.23±17.06c	0.31±0.33d

^zLeast-squared means within columns followed by lowercase letters do not differ at the 1% level of extremely significance. All data were expressed as mean ± standard error.

Table 3. Effects of cultivars, IBA and media treatments on above-ground indexes of different grape cuttings.

Variable		No. of germination cuttings	Survival per cent of hardwood cuttings (%)	No. of new shoots	Diameter of new shoots (mm)	No. of new leaves	Leaf area (cm ²)	Plant height (cm)
Cultivar	Xia Hei	14.12±7.85ab	24.19±31.37a	1.85±1.18a	3.59±0.97b	7.75±3.28a	199.32±97.29a	17.49±6.54a
	Eastern	10.72±7.70b	18.23±26.33a	2.07±1.63a	2.74±0.97c	5.75±2.43b	113.32±82.89b	13.73±8.14b
IBA	Giant Rose	15.95±8.60a	24.60±32.66a	1.57±1.37a	4.59±1.02a	5.97±2.85b	124.02±98.96b	15.55±4.11ab
	0 mg/L IBA	12.27±7.00a	10.41±0.24b	2.28±1.65a	3.65±1.32a	5.83±3.13b	126.70±105.40b	13.86±6.08b
Media	1 mg/L IBA	14.93±9.26a	44.28±29.20a	1.38±0.94b	3.63±1.17a	7.15±2.72a	164.42±91.75a	17.32±6.70a
	M1	7.79±4.79c	12.72±15.40bc	1.54±1.25a	2.93±0.87	4.95±2.19b	73.16±38.26c	11.20±3.29c
	M2	15.04±3.80b	25.47±25.75b	2.04±1.33a	3.95±0.96	5.62±2.01b	126.87±54.76b	16.07±2.68b
	M3	17.20±4.21b	26.45±26.93b	1.62±1.43a	3.81±0.85	5.95±2.11b	132.45±61.43b	16.41±3.82b
	M4	24.41±4.08a	44.28±44.94a	2.00±1.38a	5.05±0.85	10.70±2.25a	312.33±50.35a	25.25±4.18a
M5	3.54±2.18d	2.80±5.34c	1.95±1.68a	2.45±0.78	5.20±2.04b	82.95±45.36c	9.02±3.54d	

^aLeast-squared means within columns followed by lowercase letters do not differ at the 1% level of extremely significance. All data were expressed as mean ± standard error.

of germination cuttings (24.41), survival per cent of hardwood cuttings (44.28%), number of new shoots was 2.00, diameter of new shoots number of new leaves was 5.05 mm, number of new leaves was 10.70, leaf area was 312.33 cm², and plant height was 25.25 cm (Table 3). Figure 3 also showed that grape hardwood cuttings grew vigorously in M4, producing more mature cuttings, more new shoots, more branches, thicker new shoots, and more new leaves. Those results all showed that M4 after IBA treatment was the most suitable for inducing the growth of above-ground of hardwood cuttings of these three grape cultivars.



Fig. 3. The effect of different medias on grape hardwood cuttings: IBA treatment (A), no IBA treatment (B).

The cumulative variance contribution rate of the extracted two principal components was 93.333% (Table 4). The first principal component integrated that survival per cent of hardwood cuttings, new shoot diameter, leaf area, plant height, per cent rooting, backbone root number, primary lateral root number, secondary lateral root number, total fresh root weight, Total root length, and root diameter. Information, the variance contribution rate was 85.102%; the load of the number of new tips and the number of new leaves in the second principal component was larger, and the variance contribution rate was 8.232%. According to the variance contribution rate of each principal component, the comprehensive scores of the growth indicators of the cuttings of different treatments were calculated, and cutting growth was comprehensively evaluated. The comprehensive scores showed that the quality of the cuttings was treated as M4 > M3 > M2 > M5 > M1, indicating that M4 was the most conducive to the development of 'Xia Hei' cuttings (Table 5).

The cumulative variance contribution rate of the extracted two principal components was 88.984% (Table 6). The first principal component integrates variables such as survival per cent of hardwood cuttings, new shoot diameter, plant height, per cent rooting, backbone root number, primary lateral root number, secondary lateral root number, total fresh root weight, total root length, root diameter, etc. Information, the variance contribution rate was 72.741%; the load of the number of new tips and the number of new leaves in the second principal component was larger, and the variance contribution rate was 16.243%. According to the variance contribution rate of each principal component, the comprehensive scores of the growth indicators of the cuttings of different treatments were calculated, and the growth of the cuttings was comprehensively evaluated. The comprehensive scores showed that the quality of the cuttings is treated as M4 > M3 > M2 > M1 > M5, indicating that M4 was the most conducive to the development of 'Eastern Star' cuttings (Table 7).

Table 4. Factor rotation load treatment and variance contribution rate of each factor from 'Xia Hei' grape.

Index parameters	Factor 1	Factor 2
Survival per cent of hardwood cuttings	0.937	0.325
Number of new shoots	0.088	0.948
Shoot diameter	0.929	0.248
Number of new leaves	0.778	0.353
Leaf area	0.924	0.263
Plant height	0.934	0.279
Percent rooting	0.948	0.299
Number of backbone roots	0.936	0.318
Primary lateral root number	0.974	0.032
Secondary lateral root number	0.950	-0.007
Total fresh root weight	0.976	-0.043
Total root length	0.977	0.035
Root diameter	0.978	0.173
Eigen value	11.063	1.070
Variance contribution rate/%	85.102	8.232
Accumulated variance contribution rate/%	85.102	93.333

Table 5. Factor scores and rankings from 'Xia Hei' grape.

Media	Factor 1 score	Factor 2 score	Comprehensive score	Rankings
M1	28.18	-0.05	28.13	5
M2	67.33	-0.30	67.04	3
M3	164.53	-1.19	163.33	2
M4	202.69	-1.28	201.41	1
M5	34.75	-0.16	34.59	4

Table 6. Factor rotation load treatment and variance contribution rate of each factor from 'Eastern Star' grape.

Index parameters	Factor 1	Factor 2
Survival per cent of hardwood cuttings	0.928	0.212
Number of new shoots	0.134	0.619
Shoot diameter	0.970	0.216
Number of new leaves	-0.221	0.882
Leaf area	0.521	0.791
Plant height	0.700	0.514
Percent rooting	0.982	0.067
Number of backbone roots	0.968	0.030
Primary lateral root number	0.918	0.384
Secondary lateral root number	0.952	0.239
Total fresh root weight	0.920	-0.348
Total root length	0.985	0.059
Root diameter	0.971	0.221
Eigen value	9.456	2.112
Variance contribution rate/%	72.741	16.243
Accumulated variance contribution rate/%	72.741	88.984

Table 7. Factor scores and rankings from 'Eastern Star' grape.

Media	Factor 1 score	Factor 2 score	Comprehensive score	Rankings
M1	13.55	6.31	19.86	4
M2	39.93	9.89	49.82	3
M3	48.08	10.96	59.04	2
M4	84.02	27.82	111.84	1
M5	0.28	10.06	10.34	5

The cumulative variance contribution rate of the extracted two principal components was 94.688% (Table 8). The first principal component integrates variables such as survival per cent of hardwood cuttings, new shoot diameter, leaf area, plant height, per cent rooting, backbone root number, primary lateral root number, secondary lateral root number, total fresh root weight, total root length, root diameter, etc. the variance contribution rate was 74.67%; the load of the number of new tips and the number of new leaves in the second principal component was larger, and the variance contribution rate was 11.453%. The load of the total weight of fresh roots in the third principal component was larger, and the variance contribution rate was 8.564%. According to the variance contribution rate of each principal component, the comprehensive scores of the growth indicators of the cuttings of different treatments were calculated, and the growth of the cuttings was comprehensively evaluated. The comprehensive scores showed that the quality of the cuttings is treated as M4 > M2 > M3 > M1 > M5, indicating that M4 was the most conducive to the development of 'Giant Rose' cuttings (Table 9).

Table 8. Factor rotation load treatment and variance contribution rate of each factor from 'Giant Rose' grape.

Index parameters	Factor 1	Factor 2	Factor 3
Survival per cent of hardwood cuttings	0.960	0.257	0.039
Number of new shoots	-0.131	0.104	0.952
Shoot diameter	0.917	0.302	-0.033
Number of new leaves	0.239	0.949	0.171
Leaf area	0.782	0.592	0.107
Plant height	0.891	0.443	-0.005
Percent rooting	0.957	0.192	-0.031
Number of backbone roots	0.947	0.215	-0.023
Primary lateral root number	0.838	0.517	0.106
Secondary lateral root number	0.894	-0.225	0.371
Total fresh root weight	0.643	0.189	0.686
Total root length	0.898	0.283	0.316
Root diameter	0.774	0.353	0.069
Eigen value	9.707	1.489	1.113
Variance contribution rate/%	74.670	11.453	8.564
Accumulated variance contribution rate/%	74.670	86.123	94.688

Table 9. Factor scores and ranking from ‘Giant Rose’ grape.

Media	Factor 1 score	Factor 2 score	Factor 3 score	Comprehensive score	Rankings
M1	75.94	7.44	1.17	84.54	4
M2	501.95	21.02	14.51	537.48	2
M3	387.89	23.03	8.79	419.72	3
M4	834.96	58.29	18.29	911.53	1
M5	55.58	6.45	1.15	63.19	5

In principal component analysis of these three grapes all showed that M4 after IBA treatment was the most suitable for the development of grape cuttings.

Survival percent of hardwood cuttings, shoot number, shoot diameter, new leaf number, leaf area, plant height and root per cent rooting, backbone root number, lateral root number, fresh root weight, total root length, and root diameter showed a very significant positive correlation ($p < 0.01$). Meanwhile visible grape cutting growth and root development showed a close relationship; by affecting hair root and root growth, different treatments affected the growth of new shoots and new leaves above the ground, indicating that well developed roots are the basis for the cultivation of strong hardwood cuttings (Table 10).

Table 10. Correlation analysis between below-ground growth index and above-ground growth index of different grape cuttings.

	Survival per cent of hardwood cuttings	No. of new shoots	Shoot diameter	No. of new leaves	Leaf area	Plant height
Per cent rooting	0.255**	0.111	0.227*	0.133	0.214*	0.170
Number of backbone roots	0.252**	0.141	0.231*	0.141	0.214*	0.166
Primary lateral root number	0.245**	0.113	0.169	0.074	0.104	0.070
Secondary lateral root number	0.274**	0.125	-0.004	0.178	0.298**	0.244**
Total fresh root weight	0.184*	0.171	0.200*	0.224*	0.285**	0.186*
Total root length	0.250**	0.177	0.597**	0.260**	0.284**	0.212*
Root diameter	0.199*	0.055	0.310**	0.042	0.115	0.079

Statistically significant differences are indicated by * $p < 0.05$ and ** $p < 0.01$.

Cuttings are one of the important means of breeding grape plants which were performed by Kakoei and Salehi (2013) using this technique. Song *et al.* (2009) studied the cutting effect of different grape cultivars and demonstrated that the per cent rooting of ‘Zuo hongyi’ grape, ‘Zuo Shan1’ grape, and ‘Shuang hong’ grape were higher. Meanwhile, Wu *et al.* (2001) measured the rooting number and per cent rooting of three grape cultivars; results showed that the ‘Red Fuji’ grape had the highest rooting survival rate, followed by ‘Xia Cun Red Fuji’ grape and the lowest was ‘Kyoho’ grape. Results showed that there were significant differences in rooting quality among different cultivars, and the genetic differences among different cultivars had a significant impact on the occurrence of adventitious roots in hardwood cuttings. In the present research, the

highest effect of cuttings of the three grape cultivars was in 'Xia Hei' and the lowest was in 'Giant Rose'.

This experiment showed that the growth status of grape cuttings grown in different treatment formulations were significantly ($p < 0.01$) different. A significant difference in root development was observed between IBA treatment and non IBA treatment of grape cuttings. After IBA treatment, the root development index of 'Xia Hei', 'Oriental Star' and 'Giant Rose' in M4 and M1 was the highest. Rooting volume of cuttings was the largest, the per cent rooting was the highest, the leaf growth was large, the leaf color was deep green, and the survival per cent of hardwood cuttings was the highest. The growth indexes of shoots and roots were significantly ($p < 0.01$) better than those of other medias. M5 had the lowest per cent rooting and survival per cent of hardwood cuttings. The reason might be that the inclusion of a certain proportion of vermiculite, resulted in a large treatment volume and quality, and the total porosity became larger, which helped to promote root growth. In addition, adding a certain proportion of vermiculite also enhanced the permeability of the treatment, which is beneficial for nutrient absorption and promotion of the growth and development of shoots and leaves.

The correlation between above-ground and below-ground parts of grape hardwood cuttings was further analyzed. Results showed that there was a significant positive correlation between above-ground stem and leaf growth and root growth quality, reinforcing the theory that the growth and vitality of roots directly affected the growth and nutritional status of above-ground parts. This was achieved by optimizing and improving the physical and chemical properties of the treatment, coordinating the air and water environment, creating the best growth environment. In summary, the physical properties of treatments were closely related to cutting quality. A loose and porous treatment with good permeability was most conducive to the rooting of grape cuttings, root system development and cutting growth. While a developed root system was the basis for cultivating strong cuttings. Therefore, for the production of large-scale grape hardwood cuttings, organic and physical treatments such as local low-cost agricultural wastes can be used to further adjust and optimize the physical and chemical properties of cultivation treatments, and to make the most of the advantages of such treatments for grape hardwood cuttings. Under the media of grass soil: vermiculite: pearl rock: garden soil = 8:4:3:3 (v/v), the root growth index and the shoots were significantly ($p < 0.01$) better than under other medias. Correlation analysis showed that root quality directly affected shoot growth, and the two had a very significant positive correlation ($p < 0.01$), indicating that a well-developed root system is the basis for cultivating strong cuttings.

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