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Araştırma Makalesi/Research Article

Influence of IAA, 28-homobrassinolide and 24-epibrassinolide on Adventitious Rooting in Grapevine

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Abstract

In this research, influence of auxin and brassinosteroids on rooting of American grapevine rootstocks (140 Ru and 41 B) was investigated. Hormones and their concentrations used were indole butyric acid (IBA); 0 (control), 1000, 1500, 2000 and 4000 ppm for 5-second dipping, and 22(S),23(S)-homobrassinolide (HBR) and 24- epibrassinolide (EBR); 0 (control), 10, 0,25, 0,50 and 1,00 ppm for 10-minute dipping. In the cuttings allowed to grow and develop in a growth chamber, firstly the ratios of sprouting, rooting and healthy plant were calculated. Later, in the rooted and sprouted cuttings only, root development (root number, longest root length, root development scale, fresh and dry root weights, dry root ratio) and shoot development (primary shoot length, node number, shoot number, auxiliary shoot number) were determined. Applications of 5 second 1500 ppm IBA and 10 minute 1.00 ppm HBR were shown to stimulate rooting and healthy plant ratios in the rootstock 41 B. EBR, on the other hand, did not yield any success. Along with 1000 ppm IBA, 0.50 and 1.00 ppm HBR, all EBR treatments resulted in noticeable increase in the ratios of rooting and healthy plant in 140 Ru. Although 140 Ru had always better growth and development compared to 41 B, IBA applications did not have distinctly significant effects on root and shoot development of the two rootstocks. According to results of this study, it can be stated that brassinosteroids might be useful to induce rooting in grapevine rootstocks cuttings.
Keywords: Auxin, IBA, brassinosteroid, homobrassinolide, epibrassinolide, grapevine, rootstock, rooting

IAA, 28-homobrassinolid ve 24-epibrassinolidin Asmanın Adventif Köklenmesi Üzerine Etkisi

Öz

Bu araştırmada oksin ve brassinosteroidlerin Amerikan asma anaçlarının (140 Ru ve 41 B) köklenmesi üzerine olan etkisi araştırılmıştır. Kullanılan hormonlar ve konsantrasyonları şöyledir: 5 saniyelik daldırma ile indol butirik asit (IBA) 0 (kontrol), 1000, 1500, 2000 ve 4000 ppm ve 10 dakikalık daldırma ile 22(S),23(S)-homobrassinolid (HBR) ve 24- epibrassinolid (EBR); 0 (kontrol), 10, 0,25, 0,50 ve 1,00 ppm. İklim odasında büyümesine ve gelişmesine izin verilen çeliklerde önce sürme, köklenme ve sağlıklı bitki oranları hesaplanmıştır. Daha sonra, köklenen ve süren çeliklerde kök gelişimi (kök sayısı, en uzun kök uzunluğu, kök gelişimi derecesi, taze ve kuru kök ağırlıkları ile kuru kök oranı) ve sürgün gelişimi (primer sürgün uzunluğu, boğum sayısı, sürgün sayısı ve koltuk sürgünü sayısı) belirlenmiştir. 5 saniye 1500 ppm IBA ve 10 dakika 1,00 ppm HBR uygulamalarının 41 B anacında köklenmeyi ve sağlıklı bitki oluşumunu teşvik ettiği gözlenmiştir. Buna karşılık, EBR herhangi bir başarı sağlamamıştır. 1000 ppm IBA, 0,50 ve 1,00 ppm HBR ile birlikte tüm EBR uygulamaları 140 Ru anacında dikkati çeken bir köklenme ve sağlık bitki oranı artışı vermiştir. 140 Ru bütün koşullarda 41 B anacına göre daha iyi büyüme ve gelişme göstermesine rağmen IBA uygulamalarının her iki anacın büyümesi ve gelişmesi üzerine belirgin önemli etkileri tespit edilememiştir. Çalışma sonuçlarına göre brassinosteroidlerin asma çeliklerinin köklendirilmesinde başarılı olabileceği ifade edilebilir.
Anahtar Sözcükler: oksin, IBA, brassinosteroid, homobrassinolide, epibrassinolid, asma, anaç, köklenme

Introduction

Viticulture is based on the *Vitis vinifera* L., the European grape, which includes many commercially valuable cultivars. This species, when own rooted, is particularly susceptible to attack by two soil-borne pests: phylloxera and nematodes. Rootstocks developed throughout intensive works have enabled the grape cultivars to be safely grown and bear fruit for many years. Along with their beneficial aspects, some rootstocks of Vitis spp. come with some shortages, for instance, difficulty in adventitious rooting and high vigor, which translates into hard to propagate and late maturity of the grafted cultivar, respectively.

Overcoming problems associated with acquiring or losing ability of adventitious root development and understanding all related physiological, biochemical or molecular events have been an important subject in propagation and breeding of woody plants. Research showed that adventitious root formation is a hereditary quantitative characteristics controlled by different endogenous and exogenous factors. Among these factors auxin, light, temperature and nutrition are main driving forces (Geiss et al., 2009; Li et al., 2009; da Costa et al., 2013). It has been long recognized that phytohormones play a vital role in the regulation of root growth. They have been shown to interact with each other and environment (Lavenus et al., 2013). Natural auxins and synthetic analogs are the most powerful exogenous stimulants used in rooting of different species. The most studied natural auxins are indole-3-acetic acid (IAA) and indole butyric acid (IBA). Even though IAA is most abundant in plants and is the first to be used in rooting programs, IBA is the one that is preferably used in rooting of cuttings. Exogenously applied IBA stimulates rooting in most grape rootstocks (Coppola and Forlani, 1985; Sabır et al., 2004; Satisha and Adsule 2008; Shagiwal and Jaganath, 2015; Doğan et al., 2016). As new substances, such as brassinosteroids (BRs), are identified in plants, their effects on various aspects of plant growth and development become a focus of research. After their first discovery in rape pollens (Grove et al., 1979), BRs have been found to affect, among other things, seed development, shoot and root growth, vascular differentiation and apical dominancy. These are also under the influence of auxins, which might imply that there is an interaction between these hormones (Halliday, 2004). Bellini et al. (2014) indicated that root development and growth, in which various auxin signaling genes participate, are stimulated by auxins and BRs together. Brassinosteroids have been shown to promote root growth at low concentrations and inhibit at higher concentrations. Also, exogenous applications of BRs were found effective promoting root length in BR-lack mutants of Arabidopsis and wild plants (Müssig et al., 2003).

This study was conducted to determine effects of BRs and IBA on root and shoot development in grapevine rootstocks. The hypothesis was that both plant growth regulators increase rooting percentage and quality in the cuttings of grapevine rootstock.

Materials and Methods

One-year-old dormant cuttings of t140 Ruggeri (*Vitis berlandieri* x *V. rupestris*) and 41 B Millardet Et de Grasset (*V. vinifera* cv. Chasselas *x V. berlandieri*) were obtained from the Viticultural Research Institutes of Tekirdağ and Manisa, Turkey, respectively. These are known for their hard-to-root characteristics. Cuttings of 4-5 buds were kept in a black polyethylene bag at 1-2°C and 80% relative humidity until used.

Cuttings were prepared as two-bud sections with the lower bud removed. Five different concentrations (control, 0.05, 0.10, 0.15 and 0.25 ppm) of both 22(S), 23(S)-homobrassinolide (HBR, Sigma H-1267) and 24-epibrassinolide (EBR, Sigma E1641) was used for 10 min dipping of the bottom part of the cuttings. Additionally, five concentrations of indole butyric acid (IBA) (0 (control), 1000, 1500, 2000 and 4000 ppm) was prepared and the cuttings were dipped into the solutions for 5 seconds. The control group for each substance was dipped in distilled water accordingly. Plastic pots filled with perlite and peat moss (1:2 v/v), were placed in a climatic chamber (24-26ºC and 80% relative humidity) under photoperiod of 16 hrs light and 8 hrs dark. The experiment was ended when the shoot tip of the shoots stopped growing (approximately after 8 weeks).

After finalizing the experiments, the following measurements were taken according to the modified method described by Dardeniz et al. (2008). Firstly, rooted cuttings (%), sprouted cuttings (%) and healthy plants with live shoot and roots together (%) were calculated. Later, in order to determine the quality of root and shoot formed, following aspects of the roots and shoots were determined; 1) root development level on a scale of 0 to 4 (root formation; 0: no roots, 1:on one side, 2: on two sides, 3: on three sides, and 4: on four sides); 2) fresh and dry weights of root(g); 3) dry root ratio (%) obtained by dividing dry root weight to fresh root weight in an expression of percentile; 4) number of roots at least in 1 mm length; 5) primary shoot length (cm); 6) node number on the main shoot; 7) number of auxiliary shoots on primary shoot.

The study was conducted in a completely randomized trial design, with three replicates and 15 cuttings per replicate. The data obtained was evaluated with MINITAB (Release 13.1, Minitab Inc.) for one-way ANOVA for the treatments in a rootstock, and the differences were tested with Duncan's multiple range test.

Results

Influence of the plant growth regulators on rooting potential of the two American grape rootstocks indicates that the response is genotype specific. The rootstock cultivar 140 Ru had always higher values compared to the cultivar 41 B in all the characteristics evaluated. In the 41 B rootstock, the treatments did not exert any significant effects on the parameters except for rooting, sprouting and healthy plant ratios (Table 1, Figure 1). Considering the mean values, control cuttings were higher only in numbers of nodes and axillary shoots. The highest numbers of roots above average were obtained with all IBA concentrations and 1.00 ppm HBR applications. However, the quality of roots was preserved with 1500 ppm IBA and 1.00 ppm HBR. Shoot growth characteristics also seem to be more supported with the same applications. Rooting ratio in 41 B cuttings took benefit from the hormone applications although the extent was too great to safely exclude the control group. However, IBA concentrations of 1000 and 1500 ppm and HBR concentrations of 0.50 and 1.00 ppm gave the highest values in the order of 1500 ppm IBA, 1.00 ppm HBR, 1000 ppm IBA and 0.50 ppm HBR. 0.25 ppm HBR had the worst rooting ratio. The highest IBA concentrations cause a dramatic decline in rooting and also in sprouting. EBR applications, on the other hand, always resulted in poorest part of rooting. Interestingly, negative influence of EBR was not observed in the sprouting ratio. Sprouting ratio was greatly depended on the type and the concentration of the hormones. As was the case with 4000 ppm IBA, 0.50 ppm HBR and EBR resulted in the lowest sprouting in the cuttings of 41 B. Healthy plant ratio was ranged between 6.67-24.4%, being the lowest with 0.25 ppm EBR and the highest with 1.00 ppm HBR, which was followed by 1500 and 1000 ppm IBA applications. The linear rooting slope shown in Figure 1 indicates that IBA improved rooting more than brassinosteroid applications, especially more than epibrassinolide treatments. Applying 1000 or 1500 ppm IBA for 5 seconds or 0.50 or 1.00 ppm HBR for 10 minutes to the freshly cut basal parts of the 41 B cuttings proved to be useful in improving rooting and obtaining healthy plants. One other result to take home was that brassinosteroids might play an important role in supporting shoot growth.

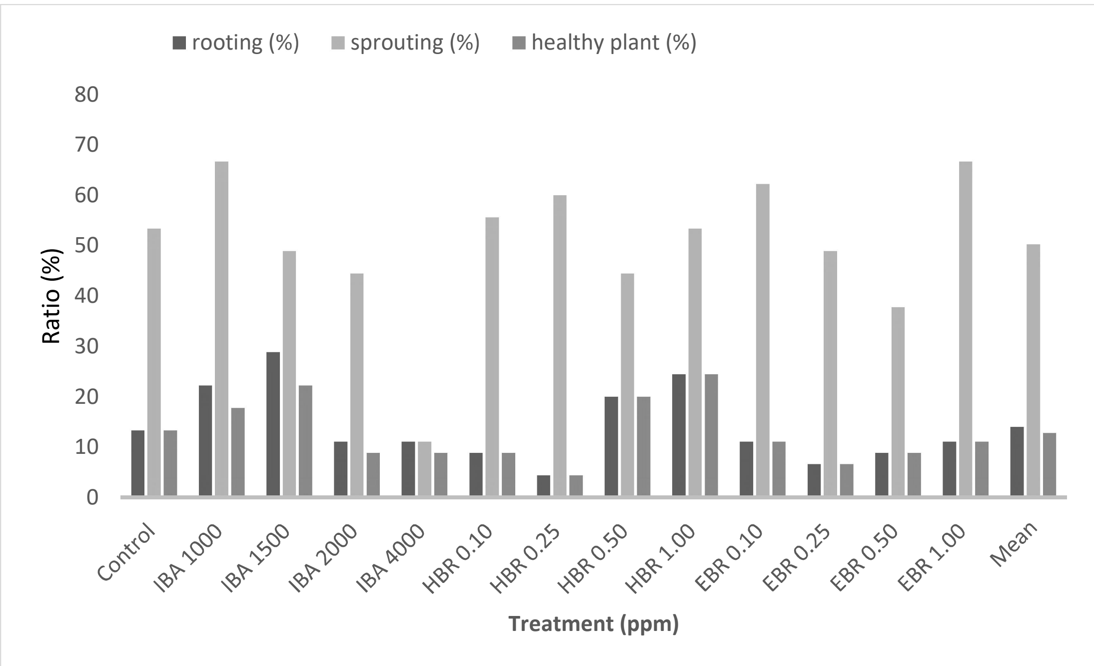

Figure 1. Ratios of rooting, sprouting and healthy plants in American grape rootstock, 41B. (IBA: indole butyric acid, HBR: 28-homobrassinolide, EBR: 24-epibrassinolide)

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Table 1. Effects of hormone treatments (IBA: indole butyric acid, HBR: 28-homobrassinolide, EBR: 24-epibrassinolide) on root and shoot features of 41 B American grape rootstock

Treatment (ppm)	root number (n)	longest root length (cm)	Fresh root weight (g)	dry root weight (g)	primary shoot length (cm)	node number (n)	axillary shoot number (n)	rooting (%)	sprouting (%)	healthy plant (%)
Control	0.93	7.18	0.58	0.06	5.14	3.88	0.56	13.33 ab	53.33 a	13.33 ab
IBA 1000	2.19	10.90	2.65	0.23	7.58	4.58	0.24	22.22 ab	66.67 a	17.78 ab
IBA 1500	1.85	13.28	2.42	0.25	5.82	3.33	0.26	28.89 a	48.89 a	22.22 ab
IBA 2000	2.17	5.28	1.34	0.10	5.32	3.36	0.31	11.11 ab	44.44 a	8.89 ab
IBA 4000	1.50	6.11	1.57	0.19	6.71	2.08	0.42	11.11 ab	11.11 b	8.89 ab
HBR 0.10	1.33	9.47	1.24	0.10	6.22	3.83	0.57	8.89 ab	55.56 a	8.89 ab
HBR 0.25	0.17	8.10	1.07	0.08	6.12	3.46	0.47	4.44 b	60.00 a	4.44 b
HBR 0.50	0.73	13.91	4.55	0.50	11.36	4.53	0.24	20.00 ab	44.44 a	20.00 ab
HBR 1.00	2.48	15.04	3.55	0.30	8.66	4.68	0.39	24.44 ab	53.33 a	24.44 a
EBR 0.10	1.11	9.47	0.72	0.06	4.67	4.19	0.68	11.11 ab	62.22 a	11.11 ab
EBR 0.25	1.00	5.67	0.14	0.01	5.50	3.97	0.85	6.67 ab	48.89 a	6.67 ab
EBR 0.50	0.67	6.16	0.92	0.11	5.44	3.30	0.27	8.89 ab	37.78 ab	8.89 ab
EBR 1.00	1.33	9.70	0.86	0.07	6.12	4.07	0.43	11.11 ab	66.67 a	11.11 ab
Mean	1.34	9.25	1.66	0.16	6.51	3.79	0.44	14.02	50.26	12.82

*small letters in a column indicate significant differences among treatments ($p \leq 0.05$).

140 Ru cuttings responded better to the applications of the hormones compared to the 41 B cuttings (Table 2). Hormones did not have any significant effects on parameters other than the longest root length, fresh root weight, and ratios of rooting and sprouting. Root number per cuttings ranged between 2.29 (0.25 ppm HBR) and 4.62 (1.00 ppm HBR), which depended on the concentration of IBA and HBR. However, as the concentration of EBR increased, the number of roots developed decreased. The longest roots (longer than 11 cm) were obtained from 4000 ppm IBA and 1.00 ppm EBR. The cuttings also showed a tendency to develop roots from two sides, especially with 1500 ppm IBA and 0.10 ppm EBR. Fresh root weight of the cuttings indicated some important differences. All EBR applications greater than 0.10 ppm and HBR applications of 0.50 and 1.00 ppm resulted in heavier roots compared to the other treatments. On the other hand, these differences disappeared on the dry weight and dry root ratio of the roots. Shoot growth did not significantly change with the applications. Compared to the mean value (15.50 cm), most of the treatments provided longer shoots. Most HBR concentrations remained below. The shoots had more than 6 nodes and developed more than one shoot. Axillary shoot growth (more than 2) was supported with 1000 ppm IBA, and with 0.10 and 1.00 ppm HBR.

Rooting percentage was between 42.22 (0.10 ppm HBR) and 75.56 (0.10 ppm EBR) (Table 2, Figure 2). Although significant differences were not observed among the applications, 0.10 ppm EBR, 0.50 ppm HBR and 1000 ppm IBA resulted in the higher ratios, respectively. Sprouting was more responsive to the concentrations (Figure 2). The lowest and the highest ratios were obtained with IBA applications of 2000 and 1000 ppm, respectively. Healthy plant ratio changed between 40% and 69% and ratios higher than the control group were obtained with 1000 ppm IBA, 0.50 and 1.00 ppm HBR and all of the EBR concentrations (Figure 2). The slope of linear rooting ratio inclined from IBA to EBR treatments.

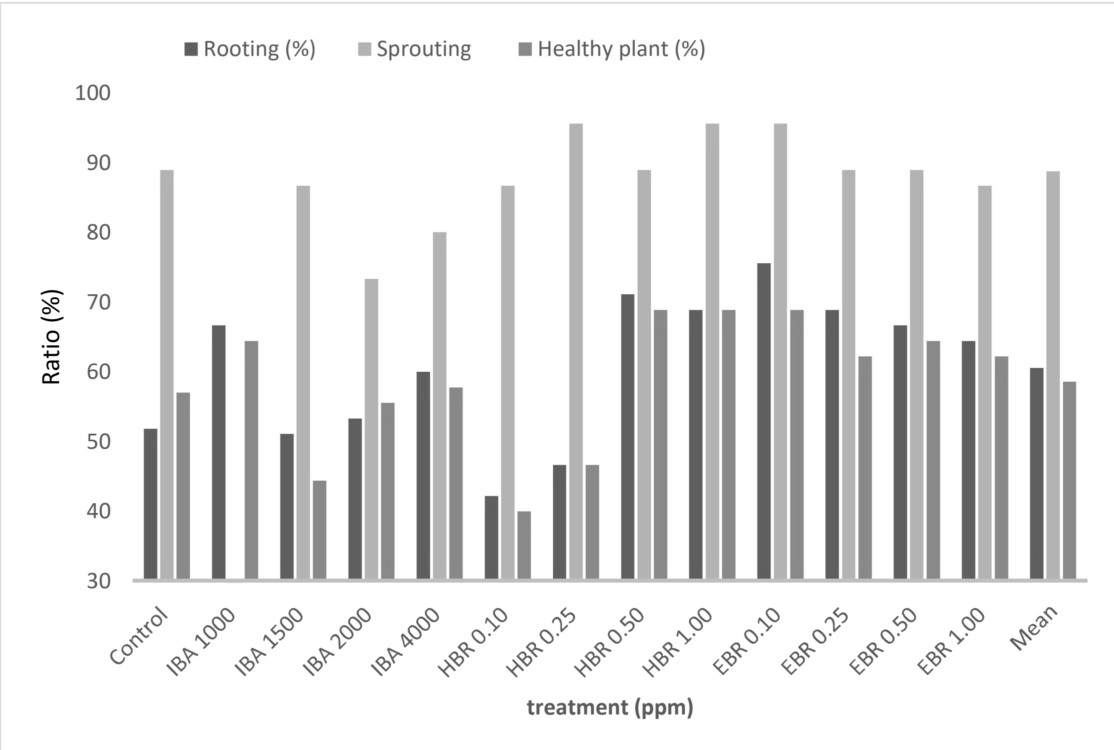

Figure 2. Ratios of rooting, sprouting and healthy plants in American grape rootstock, 140 Ru. (IBA: indole butyric acid, HBR: 28-homobrassinolide, EBR: 24-epibrassinolide)

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Table 2. Effects of hormone treatments (IBA: indole butyric acid, HBR: 28-homobrassinolide, EBR: 24-epibrassinolide) on root and shoot features of 140 Ru American grape rootstock

Treatment (ppm)	root number (n)	longest root length (cm)	Fresh root weight (g)	dry root weight (g)	primary shoot length (cm)	node number (n)	axillary shoot number (n)	Rooting (%)	Sprouting (%)	Healthy plant (%)
Control	3,49	9,29 abcd*	6,08 ab**	0,67	16,51	7,22	1,35	51,85 ab	88,89 abc *	57,04
IBA 1000	3,19	7,98 abcd	1,65 b	0,14	16,66	7,10	2,06	66,67 ab	97,78 a	64,44
IBA 1500	3,62	9,81 abcd	2,39 ab	0,48	15,30	6,85	1,82	51,11 ab	86,67 abc	44,44
IBA 2000	3,13	8,05 abcd	2,05 b	0,37	17,82	7,04	1,50	53,33 ab	73,33 c	55,56
IBA 4000	3,63	11,21 a	3,41 ab	0,54	18,37	7,54	1,30	60,00 ab	80,00 bc	57,78
HBR 0.10	3,08	6,65 cd	1,89 b	0,19	10,72	7,77	2,01	42,22 b	86,67 abc	40,00
HBR 0.25	2,29	6,20 d	1,51 b	0,20	10,63	5,84	1,50	46,67 ab	95,56 ab	46,67
HBR 0.50	4,03	8,90 abcd	7,12 ab	0,68	15,76	7,06	1,89	71,11 ab	88,89 abc	68,89
HBR 1.00	4,62	10,32 abc	6,62 ab	0,60	13,93	7,89	2,32	68,89 ab	95,56 ab	68,89
EBR 0.10	4,01	6,85 bcd	2,50 ab	0,38	15,69	6,74	0,75	75,56 a	95,56 ab	68,89
EBR 0.25	3,87	10,62 ab	8,24 a	0,88	15,62	7,03	1,31	68,89 ab	88,89 abc	62,22
EBR 0.50	3,50	10,38 abc	6,97 ab	0,82	18,84	7,69	1,64	66,67 ab	88,89 abc	64,44
EBR 1.00	3,35	11,00 a	6,75 ab	0,79	15,71	7,45	1,45	64,44 ab	86,67 abc	62,22
Mean	3,52	9,02	4,40	0,52	15,50	7,17	1,61	60,57	88,72	58,58

small letters in a column indicate significant differences among treatments (: $p \leq 0.05$ and ** $p \leq 0.01$).

Discussion

Although literature indicate many researches on the effects of auxins on rooting of grape cuttings, the results are mostly dependent on source of propagation material, genotype of the plant, and the concentrations. The results of this study indicated that 41 B and 140 Ru, both hard to root species, reacted differently and 140 Ru always had the better and higher values of root and shoot growth characteristics. Alley (1979) reported that collection time of the cuttings of Salt Creek and Dog Ridge, hard to root species, caused variations in rooting. In the present research, time of collection was not factored in; however we agree that the results might have been affected by it. Kracke et al. (1981) indicated that 140 Ru contained low level of auxin and very high level of rooting inhibitors. This might be the reason for differential response to various levels of IBA of the 140 Ru cuttings. Coppola and Forlani (1985) showed that water treatment for 24 hours followed by 2000 ppm IBA caused an increase in rooting of 140 Ru. In this research, 2000 ppm generally provided poorer results. In a study with 140 Ru and 41 B. Sağlam et al. (2005) reported positive effects of 2000 and 4000 ppm IBA on increasing rooting ratio, respectively. This contradicts our finding that 1000 and 1500 ppm IBA provided the best results, respectively in the same rootstocks.

Studies involving effects of brassinosteroids on rooting of woody plants are rare and even rarer in grapevine. Even though a synergistic effect was detected between auxins and brassinosteroids (Marquardt and Adam, 1991), influence on rooting have been found to be various. For instance, in hypocotyl cuttings of mung bean plants (*Phaseolus aureus* Roxb.) adventitious root formation was induced by auxins and inhibited by brassinosteroids. In the present research such effect of brassinosteroids were not observed (Guan and Roddick, 1988). On the other hand, 24-epibrassinolide applied to hypocotyl segments of soy bean improved rooting at low concentrations (Sathiyamoorthy and Nakamura, 1990). Effect of epibrassinolide was genotype dependent in our study, in which rooting was not effected in 41 B, however, improved in 140 Ru. Roddick and Guan (1991) indicated that high concentrations of four different brassinosteroid compounds (brassinolide; 22,23,24-trisepibrassinolide, 24-epibrassinolide and 28-homobrassinolide) inhibited root development in tomato. This effect was not observed in the current study. Swamy and Rao (2006, 2010) reported improving effects of 24-epibrassinolide and 28-homobrassinolide on rooting in geranium and Coleus plants. Kaplan and Gokbayrak (2012) showed that homobrassinolide supported rooting in 1103 P and 99 R grape rootstocks with varying results with the genotype.

Conclusion

In this study where effects of IBA, epibrassinolide and homobrassinolide on root formation and shoot growth of 140 Ru and 41 B grape rootstocks supported the fact that adventitious root formation in woody plants is under genetic and hormonal control. 140 Ru always had better responses to the hormones. Although the IBA, the auxin most widely used for promoting rooting in plants, did not exert significant and distinct effects on root and shoot development in both of the rootstocks, dipping 41 B cuttings for 5 seconds in 1500 ppm IBA and 10 minutes in 1.00 ppm 28-homobrassinolide was determined to increase rooting and healthy plant ratio. Epibrassinolide on the other hand did not support rooting. For rooting 140 cuttings, 1000 ppm IBA was found better. Success of homobrassinolide relied on the concentrations and rooting was higher with 0.50 and 1.00 ppm. Epibrassinolide, in contrast to 41 B, was supportive of rooting and healthy plant ratios independent of the concentrations.

It was concluded that brassinosteroids might give a way to promote rooting in grapevine rooting and possibly in other woody species.

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