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Effect of IBA, Brassinosteroid, and Bacterial Applications on Rooting of Some Rosehip (*Rosa canina L.*) Genotypes by Hardwood Cuttings

Muazzez ÇELİKER[1], Aysen KOÇ[2*]

ABSTRACT: Rosehip is a species whose environmental and economic importance is increasing day by day. This study was carried out to determine the effect of auxin, brassinosteroid and bacteria for the adventitious rooting of hardwood cuttings belonging to the SRG 17 and SRK 26 genotypes, which were previously obtained by selection from the province of Yozgat. In the study, hardwood cuttings taken in February were used with single, double and triple IBA (2000 ppm), 24-eBL (0, 0.25, 0.50 and 1.00 ppm) and a commercial bio prepare containing bacteria species *of Pseudomonas fluorescens*, *Paenibacillus polymyx*, *Bacillus megaterium* and *Pantoea agglomerans*. After combined treatment, they were planted in perlite+peat medium and removed after three months. While the highest rooting rate of 40% in SRG17 genotype was obtained from the triple combination of Bacteria + 24-eBL (0.50 ppm) + IBA (2000 ppm), it was obtained from Bacteria application with 43.33% in SRK26 genotype. Bacteria + 24-eBL (1.00 ppm) + IBA (2000 ppm) triple combination in SRG17 genotype in terms of root length and root number provided the highest values with 8.38±4.32 (cm) and 21.00±9.00 pcs, respectively. In the SRK26 genotype, 11.18±1.41 cm root length and 36.00±12.25 pcs were obtained from the 24-eBL (0.50 ppm) + IBA (2000 ppm) binary combination. The viability rates were determined three months after the rooted cuttings were taken into the pots, and the SRG17 genotype provided 100% survival in the binary combination Bacteria + 24-eBL (0.50 ppm) and 50% in the SRK26 genotype in the 24-eBL (0.50 ppm) application.

Keywords: Rosehip, Hardwood Cutting, Propagation, IBA, Bacteria, Brassinosteroid

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INTRODUCTION

Rosehip is a shrub-shaped plant species of *Rosaceae* family, *Rosa* genus, deciduous in winter (Sarıbaş, 1996). Rosehip grows naturally in a wide area including Central and Western Asia, Caucasus, Europe, Northwest Africa, northern and western parts of Iraq and Iran, northern Afghanistan and Pakistan (İlisulu, 1992). One of the most important gene sources of rose hips is Turkey. There are about 100 species of rose hips growing in the world, and 27 species of rose hips are grown in Turkey (Ercişli and Güleryüz, 2005). Rosehip is not a very selective plant in terms of climate and soil requirements. Due to this feature, rosehip species can grow in different soil types, from high altitude plateaus and plateaus to valleys at sea level (Güneş, 2013).

Rosehip plant, which has many different uses, can be preferred as a rootstock for roses, as well as for erosion control, hedge plant and increasing the usability of unused agricultural land due to its adaptability and deep roots. Fruit and other plant parts are very important in terms of nutrition because they are rich in many minerals and vitamins. Ağaoğlu et al. (1987) reported that rosehip fruits had the highest vitamin C ratio among plants. Important studies were carried out on the development and dissemination of standard varieties by cultivating the rosehip bush, which has different usage characteristics, and variety registrations were carried out by making selection studies. In order to expand it, indole acetic acid, indole butyric acid and naphthalene acetic acid and their salts, plant growth regulators, are generally used in studies conducted as rooting promoters (Güneş and Şen, 2001).

It is known that some bacterial species belonging to *Agrobacterium, Bacillus, Streptomyces, Pseudomonas* and *Alcaligenes* genera that increase root growth and regulate plant growth have positive effects on rooting (Goto, 1992; Srinivasan ve ark., 1996; Tripp ve Stomp, 1997). At the same time, it is very important to use bacteria species and genera that are effective and increase rooting in order to ensure sustainability on the ecosystem and to minimize the damage that may ocur (Goto, 1992; Steenhoudt ve Vanderleyden, 2000). It was determined that external application of IBA together with bacteria producing Indole Acetic Acid (IAA) increased rooting (Eşitken et al., 2003).

Brassinosteroids are induced by many auxin signaling genes, auxin and brassinosteroids are involved in root growth and development. Brassinosteroids such as auxin inhibit it at high concentrations while promoting primary root growth at low concentrations (Mussig et al., 2003). They also control lateral root development through a complex exchange process with auxin (Bao et al., 2004; Nemhauser et al., 2004). Brassinosteroids may play a role in the root formation stage because external application of this phytohormone has been shown to promote root elongation in wild plants and more effectively in brassinosteroid-deficient mutants of *Arabidopsis* (Mussig et al., 2003).

Rosehip grows naturally in Yozgat and its products have been loved and consumed by the public for many years. Hardwood cuttings of two rosehip genotypes (SRG 17 and SRK 26), which are determined by selection from Yozgat province and stand out with some physical and chemical properties, were used. These treated with IBA (2000 ppm), 24-eBL (0, 0.25, 0.50 and 1.00 ppm) and a commercial biological preparade containing the bacterial strains *Pseudomonas fluorescens*, *Paenibacillus polymyx*, *Bacillus megaterium* and *Pantoea agglomerans*, singly, a binary and triple combinations. Then they were planted to perlite+peat medium.

MATERIALS AND METHODS

Materials

In the research, hardwood cuttings belonging to the genotypes SRG 17 and SRK 26 from the rosehip garden in Yozgat Bozok University, Gedikhasanlı Agricultural Application and Research Center were used as plant material. The study was established in the cutting replication unit of Bozok Yozgat University, Erdoğan Akdağ East Campus, in the Greenhouses of the Faculty of Agriculture.

SRG 17: It is a very productive type with an average fruit weight of 3.14 g, a fruit pulp ratio of 71.5%, and a total soluble solids (brix,%) amount of 20%.

SRK 26: It is a very productive type with an average fruit weight of 1.8 g, a fruit pulp ratio of 71.6% and a total soluble solids (brix,%) amount of 26%.

Experimental procedure

The research was carried out in 2021. In the research, hardwood cuttings were taken on March 5 and prepared to be 15-20 cm long from the bottom and middle parts of the shoots belonging to the SRG 17 and SRK 26 genotypes were used as control, IBA (2000 ppm), bacteria and 24-eBL (0.25 ppm, 0.50 ppm and 1 ppm) were treated with single, double and triple combinations of applications (Table 1). The applied cuttings were planted in a 1:1 ratio peat: perlite mixture with 90-95% relative humidity, with a temperature of 18-25ºC from the bottom. Cuttings were kept in rooting medium for 3 months (12 weeks).

Table 1. Single, double and triple combinations applied to cuttings

No	Apps
1	Kontrol
2	IBA (2000 ppm)
3	Bakteri
4	24-eBL (0.25 ppm)
5	24-eBL (0. 50 ppm)
6	24-eBL (1.00 ppm)
7	Bakteri + IBA (2000 ppm)
8	Bakteri + 24-eBL (0.25 ppm)
9	Bakteri + 24-eBL (0. 50 ppm)
10	Bakteri + 24-eBL (1.00 ppm)
11	24-eBL (0. 25 ppm) + IBA (2000 ppm)
12	24-eBL (0. 50 ppm) + IBA (2000 ppm)
13	24-eBL (1. 00 ppm) + IBA (2000 ppm)
14	Bakteri + 24-eBL (0.25 ppm) +IBA (2000 ppm)
15	Bakteri + 24-eBL (0. 50 ppm) +IBA (2000 ppm)
16	Bakteri + 24-eBL (1.00 ppm) +IBA (2000 ppm)

Control application: After soaking 2/3 of the cutting in distilled water, it was planted in the rooting medium.

Preparation and application of auxin (IBA): The cuttings were immersed in the prepared 2000 ppm IBA solution for 5 seconds and then planted in the rooting medium.

Preparation of fertilizer containing microorganisms: 5 liters of sterile water and 250 g of granulated sugar as an adhesive were mixed homogeneously and added into 500 ml of solution containing four different bacteria species. Cuttings were kept in the prepared bacterial solution for 6 hours.

Preparation of 24 e-BL: 0.00 (control), 0.25, 0.50 and 1.00 ppm concentrations of 24-eBL [24-epibrassinolide (EBR, Sigma E1641)] were dissolved in ethanol and made up to final volume with

distilled water. 0.02% Tween 20 was added to the solution prepared in this way. Cuttings were immersed in 24-Ebl solutions prepared at these concentrations for 10 minutes and kept.

The cuttings to be treated with Bacteria + IBA were kept in the bacterial solution for 6 hours, and after being immersed for 5 seconds in the 2000 ppm IBA solution prepared, they were planted.

The cuttings to be treated with Bacteria + 24-eBL were kept in the bacterial solution for 6 hours and then immersed in the prepared 24-eBL solution for 10 minutes and then planted. The cuttings to be treated with Bacteria + 24-eBL + IBA were kept in the bacterial solution for 6 hours and then immersed in the prepared 24-eBL solution for 10 minutes, then immersed in the IBA solution for the last 5 seconds and planted.

The following observations and analyzes were made after the hardwood cuttings planted in the rooting medium are kept in perlite + peat medium for 3 months (12 weeks).

Callus formation rate (%): It was determined as % (pcs) by counting only the callus forming cuttings that are not rooted.

Rooting rate (%): It was determined as % by counting the rooted cuttings (pcs).

Root length (cm): In rooted cuttings, the longest 3 root lengths of 3 cuttings per replication were measured and calculated as cm.

Number of roots (pcs/cutting): It was determined by counting the number of roots formed per cutting.

Root fresh weight (g): After evaluating the rooted cuttings for each application, the wet weight of all roots of one cutting was determined for each replication.

Root dry weight (g): The dry root weight of each application was determined by weighing the wet roots after keeping them at +70°C for 48 hours.

Root dry ratio (%): The ratio of root dry weights to root fresh weights was calculated as a percentage. Viability rate of viable cuttings after seedling formation (%): Rooted cuttings, which were measured, were planted in plastic pots and kept for 3 months, and their viability was expressed as %.

In the study, hardwood cuttings of 2 different rosehip genotypes (SRG 17 and SRK 26) were treated, IBA (2000 ppm), 24-eBL (0, 0.25, 0.50 and 1.00 ppm) and containing different bacterial species (*Pseudomonas fluorescens*, *Paenibacillus polymyx*, *Bacillus megaterium* ve *Pantoea agglomerans*) commercial biopreparation as single, double, and triple combinations, used 3 replications and 15 plants in each replication. Angle (arc sin$\sqrt{x}$) transformation was applied to the values (callus formation, rooting and dry root ratio) expressed as a percentage (%) of the results obtained in the experiment, and the actual values were given in the tables. The differences between the data were determined by the Independent two samples t-test in the SPSS 20.0 package program, and the averages were given with ± standard deviation.

RESULTS AND DISCUSSION

Callus formation rate is important in propagation with cuttings. In the study, the callus ratio of the genotypes is given in Figure 1. Considering the genotypes and applications, application no 10 [Bacteria + 24-eBL (1.00 ppm)] gave the highest callus formation in both genotypes (SRK26: 53.33% and SRG17: 46.67%). Güneş and Eraslan (2021) investigated the effects of some growth medium and applications on cuttings in their study with MR-46 genotype rosehip wood cuttings that were selected in Tokat Province. In the study, the average callus rates of the applications were 37.48-42.56%; the callus rates of the growth medium × application interaction varied between 18.44 and 70.08%. In another study, it

was stated that while callus formation was not observed in green and semi-wood cuttings of old garden roses in the Van Lake region, callus formation was observed in hardwood cuttings (Alp et al., 2010).

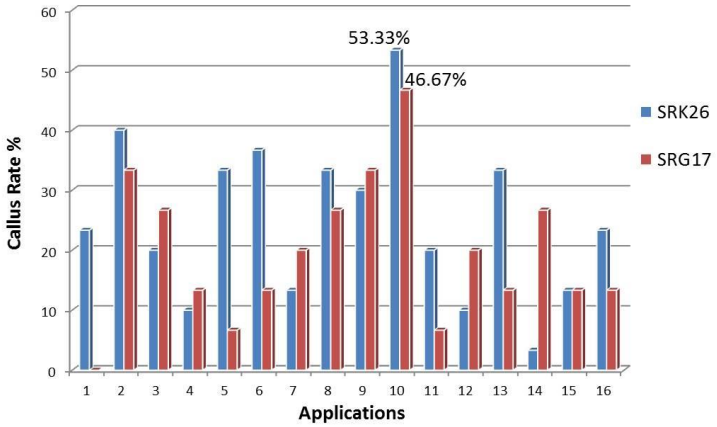

Figure 1. Callus rate (%) of SRG17 and SRK26 genotypes as a result of applications.

While the rooting rate was 43.33% in SRK26 genotype in application no. 3 (Bacteria), 40% rooting rate in SRG17 genotype was achieved in the 15th application [Bacteria + 24-eBL (0. 50 ppm) + IBA (2000 ppm)] (Figure 2). Rooting rates in other studies; Ercişli (1996) are between 3% and 86%, Tansı et al. (1996) 29% from 2% ppm IBA concentration, Ercişli and Güleryüz (1999) between 3.33 - 86.25% in November cutting with 2000 ppm IBA, Güneş and Şen (2001) 90% in October cutting, Kazankaya et al., (2005) 65-70% in the 2500 ppm IBA application in November cutting, Güneş and Eraslan (2021) %22.26-38.33 in IBA, Bacteria, IBA+Bacteria applications were found. Uzunoğlu ve Gökbayrak (2018) were be stated that brassinosteroids might be useful to induce rooting in grapevine rootstocks cuttings.

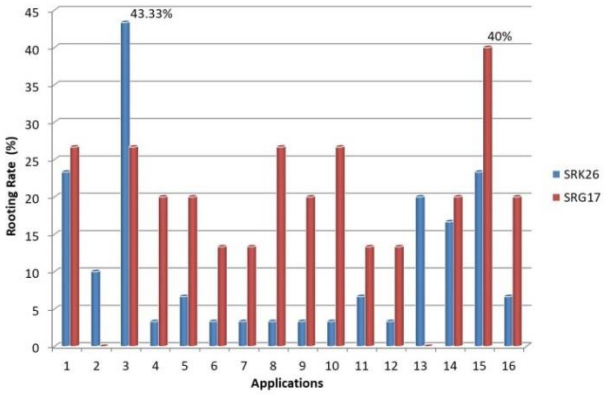

Figure 2. Rooting rate (%) of SRG17 and SRK26 genotypes as a result of applications.

The root dry rate was 67.68% in SRK26 genotype in application no. 6 [24-eBL (1.00 ppm)] while it was 48.96% obtained in application no. 9 [Bacteria + 24-eBL (0.50 ppm)] in SRG17 genotype (Figure 3). Okatar (2019) determined the root dry matter ratio in the selected rosehip genotypes as 72.29% in 1000 ppm application and 64.63% in 2000 ppm application in the first year of the experiment. In the second year of the study, it was determined as 70.78% in 1000 ppm application and 49.23% in 2000 ppm application. Eraslan (2019) determined the highest dry matter rate of 84.75% in the control application in perlite medium and 83.45% in the Bacteria + IBA application in the peat medium.

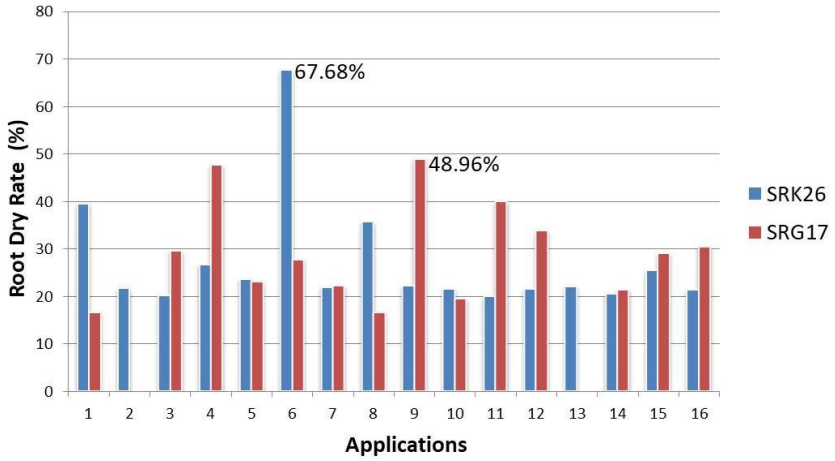

Figure 3. Root Dry Rate (%) of SRG17 and SRK26 genotypes as a result of applications.

While the viability rate of viable cuttings was obtained as 50% in the application no. 5 [24-eBL (0.50 ppm)] in the SRK26 genotype, it was 100% in the application no. 9 [Bacteria + 24-eBL (0.50 ppm)] in the genotype SRG17 (Figure 4). Şenel (2002) determined that the best result in terms of viability rate in white mulberry hardwood cuttings was 43.33% in the bundle planting IBA application and 3.33% in the hydroponic system in the IBA application. While the live steel rate was found to be 26.66% in the row planting control application in the cuttings taken in February, it was obtained at the rate of 6.66% in the IBA application in the hydroponic environment. The best result was found in row planting control application with 10.00% for cuttings taken in March. Zenginbal and Özcan (2013), viability rate of Hayward and Matua kiwi cuttings, 73.1% and 78.6% in 2002 while this value was 84.6% and 89.1% in 2003.

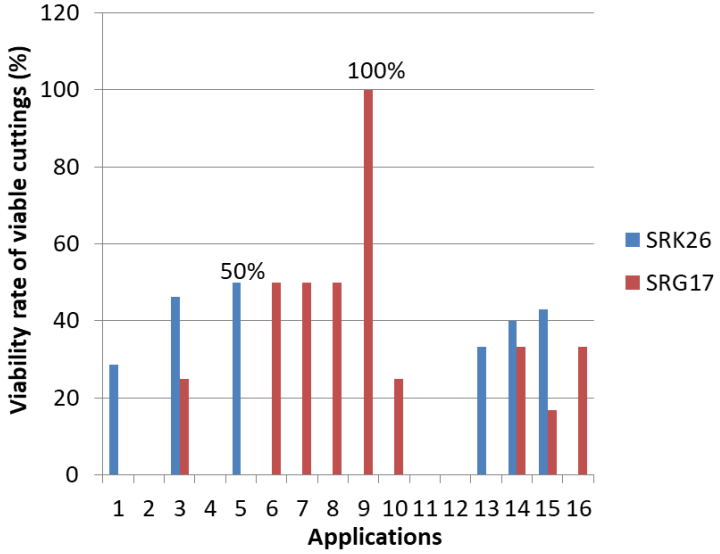

Figure 4. Viability Rate of Viable Cuttings Rate (%) of SRG17 and SRK26 genotypes

Root length, root number, root fresh and dry weight are given in table 2. Root length was obtained as 11.18 cm in SRK26 genotype in application no 12 [24-eBL (0.50 ppm) + IBA (2000 ppm)] while in application no 16 [Bacteria + 24-eBL (1.00 ppm) +IBA (2000 ppm)] in SRG17 genotype was obtained as 8.38 cm. Ercişli (1996) measured 6.59 cm in 1000 ppm IBA application, 7.54 cm in 2000 ppm IBA dose and 8.42 cm in 4000 ppm IBA dose. Kınık and Çelikel (2017) found that bacteria that increase rooting also increase root length. Eraslan (2019) obtained the longest root length with 8.33 cm from IBA

application in peat + perlite medium. The highest root length among the applications was obtained from the IBA application with an average of 7.19 cm. The shortest root length was obtained in cuttings with bacteria applied in perlite medium with 2 cm.

Root number was determined as 36.00 pcs in SRK26 genotype in application no 12 [24-eBL (0.50 ppm) + IBA (2000 ppm)], while in application no 16 [Bacteria + 24-eBL (1.00 ppm) +IBA (2000 ppm)] in SRG 17 genotype was determined as 21.00 pcs. Yıldız et al. (2009) reported that the number of roots increased in 4000 and 6000 ppm IBA doses compared to the control application. Kınık and Çelikel (2017) reported that the average number of roots per cutting varies between 0-2 as a result of bacteria and IBA applications. Yıldız and Koyuncu (2000) reported that the increase in IBA dose caused an increase in the number of roots, and that the cuttings taken in two different periods were insignificant in terms of root numbers. As a result of the rooting of green cuttings of MM106 rootstock in perlite and hydroponic medium by Küçükbasmacı-Sabir and Özkaya (2009), they stated that the maximum 3.19 pieces of IBA application were obtained in the cuttings rooted in perlite medium. Eraslan (2019) determined that the highest root number was obtained with IBA (7.33 (pieces/steel)) planted in peat medium, while it was determined in cuttings planted in perlite medium with the lowest bacteria treatment.

Root fresh and dry weight were obtained as 6.06 g and 1.31 g in SRK26 genotype in application no 12 [24-eBL (0.50 ppm) + IBA (2000 ppm)], while in application no 15 [Bacteria + 24-eBL (0.50 ppm) +IBA (2000 ppm)] in SRG17 genotype was determined as 2.93 g and 0.85 g. Kaplan and Gökbayrak (2012) reported that the effect of brassinosteroid applied at different concentrations varies depending on the rootstock, and the concentration level is effective on the root development level. It was determined that the fresh and dry root weights of 1103 P rootstock were also high depending on the number of roots in the vine, and the fresh root weight was determined at least in 99 R rootstocks. Shahbaz and Ashraf (2007) showed that 24-eBL applications in two different wheat cultivars in saline conditions provided shoot dry and fresh weight increase in S-24 cultivar, while it did not provide a significant increase in shoot fresh and dry weight in MH-97 cultivar. Çoban (2014) determined that the highest root wet weight in mint was obtained in plants that did not contain any salt but were grown in environments containing 24-eBL at 1.5 and 2.5 mg l^{-1} concentrations, and 24-eBL applications at these concentrations increased root growth. It was determined that 24-eBL applications did not make a statistically significant difference on root growth in environments containing salt at 100 and 150 mM concentrations.While there was no statistically significant difference between the root dry weights of the plants in the control group without any application and the plants in which 100 mM salt was applied, it was determined that the average root dry weight of the plants with only 150 mM salt application decreased by 0.12 g and 36.22% compared to the control.

To see whether there are significant differences in the comparison of genotypes and applications, then it was followed by an independent t-test. The results of the comparison of genotypes and applications can be seen in Table 3. Based on statistical tests with an independent t-test presented in Table 3 shows that the significance value of callus formation rate, rooting rate, root dry rate and viability rate of viable cuttings obtained a value of $p > 0.05$. This shows that there were no significant differences in genotypes and applications.

Table 2. Measurement values (Means±Std. deviation) of some root traits of SRK26 genotype and SRG17 genotype

Applications	Root Length (cm)		Number of Roots (pcs)		Root fresh weight (g)		Root dry weight (g)	
	SRK26	SRG17	SRK26	SRG17	SRK26	SRG17	SRK26	SRG17
1	6.03±2.47	4.46±2.03	7.29±4.15	7.75±2.21	1.14±0.83	0.66±0.09	0.22±0.12	0.11±0.00
2	4.51±1.91	0.00	8.33±6.43	0.00	2.49±0.65	0.00	0.54±0.15	0.00
3	4.16±1.90	3.90±2.79	9.38±3.33	8.50±2.65	1.14±0.12	0.27±0.02	0.23±0.02	0.08±0.00
4	2.28±0.93	3.36±1.27	4.00±2.07	8.50±2.12	0.30±0.07	0.63±0.09	0.08±0.01	0.15±0.06
5	4.50±3.73	4.71±1.34	6.50±3.29	6.00±3.80	0.38±0.22	0.39±0.08	0.09±0.03	0.09±0.01
6	9.53±2.51	4.02±1.80	15.00±9.05	6.00±2.65	0.99±0.23	0.72±0.18	0.69±0.15	0.20±0.03
7	4.75±3.22	2.60±1.19	5.00±1.90	7.50±0.71	0.64±0.30	1.17±0.78	0.13±0.04	0.26±0.12
8	3.10±1.27	2.96±1.45	6.00±2.95	6.00±2.94	0.14±0.09	1.38±1.03	0.05±0.02	0.23±0.16
9	1.78±0.33	4.98±2.57	6.00±3.50	13.00±6.19	1.17±0.78	0.96±0.42	0.26±0.12	0.23±0.01
10	7.18±1.85	3.28±2.31	12.00±6.10	7.75±2.08	1.02±0.05	0.72±0.11	0.22±0.12	0.14±0.03
11	6.58±2.45	3.90±1.91	24.50±10.61	5.00±2.83	3.24±1.56	1.05±0.26	0.65±0.22	0.42±0.05
12	**11.18±1.41**	3.81±2.39	**36.00±12.25**	8.50±4.36	**6.06±2.10**	1.36±0.63	**1.31±0.70**	0.46±0.31
13	7.61±5.68	0.00	21.83±14.06	0.00	2.58±0.81	0.00	0.56±0.21	0.00
14	8.29±3.64	7.38±6.11	24.00±11.64	15.33±9.19	1.99±0.44	2.76±2.06	0.41±0.15	0.59±0.49
15	7.65±4.44	5.83±3.51	20.86±13.48	18.67±9.46	2.39±1.18	**2.93±0.70**	0.61±0.21	**0.85±0.37**
16	9.34±5.31	**8.38±4.32**	19.00±13.08	**21.00±9.00**	1.82±1.06	2.20±0.95	0.39±0.16	0.67±0.44

Table 3. The results of the independent t-test of hardwood cuttings of SRG17 and SRK26 genotypes.

Measured/ Counted properties	p- value	Description
Callus Rate (%)	0.254	Not significant
Rooting Rate **(%)**	0.058	Not significant
Root Dry Rate **(%)**	0.734	Not significant
Viability Rate of Viable cuttings **(%)**	0.318	Not significant
Root Length (cm)	0.001	A low level of significant (d=0.35)
Number of Roots (pcs)	0.034	A low level of significant (d=0.42)
Root fresh weight (g)	0.041	A low level of significant (d=0.43)
Root dry weight (g)	0.041	A low level of significant (d=0.32)

Based on statistical tests with an independent t-test presented in Table 3 shows that the significance value of root length, number of roots, root fresh weight and root dry weight obtained a value of $p < 0.05$. This shows that there were a low level of significant differences in genotypes and applications.

CONCLUSION

In this study where effects of IBA, Brassinosteroid and bacteria on roottting and root growth of SRG17 and SRK26 rosehips genotypes supported the fact that adventitious root generation in woody plants is under heredity, hormonal, the concentrations and full-grown hardwood cutting taken control.

The highest rooting rate in SRK26 genotype was obtained from Bacteria application, dry root rate from 24-eBL (1.00 ppm) application, and viable cutting rate from 24-eBL (0.50 ppm) application. The highest values in terms of root length, root number, fresh and dry root weight of SRK 26 genotype were obtained from [24-eBL (0. 50 ppm) + IBA (2000 ppm)] application.

The highest rooting rate in SRG17 genotype was obtained from [Bacteria + 24-eBL (0. 50 ppm) + IBA (2000 ppm)] application, and the dry root rate and viable cuttings rate were obtained from [Bacteria + 24-eBL (0. 50 ppm)] application. In terms of root length and root number, [Bacteria + 24-eBL (1.00

ppm) + IBA (2000 ppm)] application gives the highest values for SRG 17 genotype, while fresh and dry root weight were obtained from [Bacteria + 24-eBL (0. 50 ppm) + IBA (2000 ppm)] application.

As a result of this study, the fact that callus rates were found to be high in cuttings as a result of applications suggests that there may be an increase in rooting rates if the 3-month period generally used in cuttings rooting applications is increased. It was concluded that (bacteri + brassinosteroids + IBA) might give a way to promote rooting in rosehip rooting and possibly in other woody species.

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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