JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2021, VOL. 4 (SPECIAL ISSUE: PLANT NUTRITION IN HORTICULTURE), 1-10

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Effect of Indol-3-butyric acid associated with *Bacillus subtilis* bacteria on rooting of some Prunus spp rootstock hardwood cuttings

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ARTICLE INFO

Original Article

Article history:
Received 27 April 2020
Revised 16 May 2020
Accepted 9 June 2020
Available online 9 July 2020

Keywords:
Cocopeat medium
Greenhouse
Perlite medium
Rooting
Shoots

DOI: 10.22077/jhpr.2020.3335.1141
P-ISSN: 2588-4883
E-ISSN: 2588-6169

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ABSTRACT

Purpose: To enhance the rooting ability of *Prunus* rootstocks by Indol-3-butyric acid (IBA) hormone associated with *bacillus subtilis* bacteria in greenhouse conditions. **Research Method**: Every 20 cuttings of each cultivar were dipped into either powder or liquid IBA with or without *Bacillus subtilis.* Afterward, the cuttings were planted in any of the cocopeat or perlite medium, in a greenhouse. ANOVA was computed to compare the results among *Prunus* cultivars. **Findings:** After 2 months of the experiment, shoots were much better in perlite medium than cocopeat medium. Maxma 14 cuttings did not develop any shoot formation in cocopeat medium, while Garnem cuttings developed the best shoots for liquid IBA and Powder IBA treatment **(**respectively 1.05 and 1 average number of shoots/cutting**)**. The effect of *Bacillus subitilis* bacteria on shoots was significant in perlite medium with more shoots for Garnem, Maxma 14 and Myrobolan 29C cuttings, respectively (1.70, 0.35 and 0.95 average number of shoot/cutting**)**. On the other hand, the highest rooting levels were observed in powder IBA + *Bacillus subtilis* treatment for Garnem and Myrobolan 29C (35% and 20%), while powder IBA treatment induced better rooting level for Myrobolan 29C and Garnem cuttings (respectively 10% and 5% higher compared to the control). **Limitations**: There is a need to do further investigation on the survival rate of the shoots, in field conditions. **Originality/Value:** The association of powder IBA and bacteria (*Bacillus subtilis)* is promissing to increase the rooting of the *Prunus* rootstocks cultivar.

INTRODUCTION

Production of *Prunus* fruit is of a paramount economical importance in the Horticulture sector. Chine, United stated of America, Turkey, Italy and Iran are the top five countries in the world that produce peaches, pears, cherries and plums fruits (Looney, 2018). The common propagation techniques for the *Prunus* fruit trees have been the cutting propagation method, which is also used for many plants, especially hardwood plants (Relf & Ball, 2019). Cutting propagation method varies between type of fruits trees, and from which part of the plant it is taken from. In most cases, the stem is the most widely used; however, stem cutting varies from which part of the plant it is taken from, such as tip stem cutting, leaf-bud cutting, root-cutting and leaf-cutting (Abdullah et al., 2005). There are different methods of taking stem cutting. These are softwood, semi-hardwood and hardwood steels. Cutting is taken with pruning scissors. Stem cutting is taken from the appropriate place of the tree 10-15 cm long and 1-2 cm thick (Gilman, 1990; Tchoundjeu et al., 2006). The cutting taking, the age of the mother plant, the part from which the plant will be taken and the correct cutting takings affect the rooting of the steel. The length and diameter of the cutting taking have a significant effect on rooting. When steel is taken, the loss of water of cuttings plays an important role in rooting. Thus, too much dehydration cutting cannot be rooted well and a successful result is not obtained. The time to take cutting plays an important role in rooting (Dumanoğlu et al., 1999). Rooting of cuttings depends on external and internal factors like rootstocks, the temperature of greenhouse and etc. Planting time plays an important role for the rooting and the growth of pomegranate cuttings (Singh, 2017).

Another factor affecting rooting in steel replication is hormone application. IBA is the best and safest to use than any other rooting hormone. The reason IBA is encouraged by researchers, is because of that it does not have a toxic effect due to the use of different doses (Ahmed et al., 2003; Vejan et al., 2016). Soil contains various microorganisms communities like bacteria and fungi that play a beneficial role in promoting and enhancing plant growth. The interactions of bacteria in rhizo-and endo-sphrere of plants have been discussed, and the rhizosphere is known to host a variety of plant growth-promoting bacteria (PGPB) (Pirlak & Baykal, 2009). The type of plant growing medium has also been documented as another factor to be considered for an excellent rooting of the cuttings. Cocopeat is considered as a good growing medium component with acceptable pH, hydraulic conductivity and other chemical attributes (Tchoundjeu et al., 2006). However, cocopeat has been preferred to have high water holding capacity, which provides air-water circulation. Cocopeat medium also has benefit to roots: affecting the oxygen diffusion to the roots (Awang et al., 2009). Perlite is the most porous and drains water very efficiently and, at the same time, allows oxygen to penetrate**.** Many researchers have reported that the rooting of the rootstock is significantly affected by the mineral concentration of the nutrient medium and by the type of tube sealing material (Dimassi Theriou, 1995; Tchoundjeu et al., 2006). Eed (2016) also studied the effect of rooting on different media of Jojoba (*Simmondsia chinensis)* fruit. According to this study, the highest rooting percentage obtained was 99.7% from peat+vermiculite+perlite media, followed by 99% from cocopeat+sand media.

The rootstocks of Garnem, Myrobolan 29C and Maxma 14 have been selected for their high adaptability in Mediterranean region and for more other specific various advantages (Arioli et al., 2015; Kose & Canli, 2015). 'Garnem'(*P.persica x P. dulcis*), is a hybrid rootstock developed from a cross between 'Garfi' almond (*P. Amygdalus Batsch*, syn. *P. dulcis* (Mill.)) and 'Nemared' peach (*P. persica*) in Spain. Although it was developed for almond, it shows good compatibility with peach, nectarine cultivars and as well as some plum and apricot cultivars. 'Garnem' is characterized by red leaves, middle strength, and easy clonal propagation. It shows good resistance to root-knot nematodes, and adaptation to calcareous soils (Kose & Canli, 2015). MXM clone series was obtained from P. *mahaleb* seeds. The M×M 14 (Maxma14 or maxma delbart14), was observed to be the most dwarf to other MXM rootstocks series (Arioli et al., 2015). In France experiments, it was reported to be resistant to chlorosis while fruit weight was the same as other rootstocks. The start of fruit production is two years earlier than SL 64. Maxma 14 was also resistant to Phytophtora and the bacterial cancer (Spotts et al., 2010). MaxMa 14 shows good scion compatibility and a broad adaptation to soil types and environmental conditions (Spotts et al., 2010; Kishchak & Kishchak, 2019). Myrobolan 29C is the rootstock that is often used in many countries because of its high adaptability to different soil types. The tree growth is also fast with high vigour and satisfactory yield. Indeed, it has been observed that plums propagated by cutting gave better results than sour cherry (Nabı et al., 2000). It also has a good compatibility and tolerates high alkalinity (Arioli et al., 2015). Thus, the present study was to investigate the effects of IBA Auxin, and *Bacillus subtilis* PGPB on those above selected and more adapted *Prunus* spp rootstocks rooting ability in cocopeat and perlite widely used as plantlets growing medium in the Horticulture sector.

MATERIALS AND METHODS

Preparation plant materials and experimental procedure

This study was conducted in a greenhouse at a research farm of the European University of Lefke (EUL) for two months, during spring time. The climate of the Güzelyurt region is semi-arid, with an average annual rainfall of 402.8 mm and average temperature of 27 °C during the experimental period. In this study, Peaches species of Garnem, Cherry species of Maxma 14, Pear species of Fox 11 and Plum species of Myrobolan 29C have been used as the plant materials. The cuttings were taken from two years old clonal orchard. The semi-hardwood has been cut off before the wood hardens and turns brown. Cuttings generally are obtained from new shoots 15 to 20 weeks after a flush of growth. The Cocopeat that was used as a plant growing medium is an agricultural by-product obtained after the extraction of fiber from the coconut husk. No organic matter is present and at least from the start of the trial, only a very low microbial population, was present which could compete for oxygen. Perlite has been giving better response on cutting rooting due to its substrate (Kreen et al., 2002). Additionally**,** Indole Butyric Acid (IBA); an auxin containing product was also used to stimulate the adventitious roots of the rootstock cuttings plant. IBA was reported to be the leading plant hormone used to promote the formation of roots in tomatoes (Rao et al., 2005). In the same way**,** *Bacillus subtilis* strains of bacteria was also used to enhance the fast growth of the rootstock cuttings.

Thus, for the early root formation, the bottom of the cuttings were dipped into 8000 ppm powder IBA rooting hormone on the same day after collecting the cuttings from the rootstock. Moreover, another group of the cuttings were dipped into 3300 ppm liquid IBA rooting hormone on the same day after collecting the cutting from the rootstocks in the orchard. The 5cm of bottom of the cuttings were dipped in the liquid hormone and the cuttings were kept in liquid IBA for 15 seconds. Thereafter, the cuttings were dipped into *Bacillus subitilis* bacteria on the same day after taking the cutting from rootstocks. *Bacillus subitilis* bacteria affects the formation of roots together with cytokinin and auxin hormones. *Bacillus subitilis* bacteria is known to inhibit formation of some fungal diseases. The bottom of the cuttings (5cm) were dipped in the *Bacillus subitilis* bacteria and the cuttings were kept in the bacteria for 10 minutes. Cocopeat and perlite media were prepared to recieve the treated rootstock cuttings. A quarter of the cocopeat medium has been added to perlite for a better drainage. The one-third of the cuttings were inserted in cocopeat or perlite beds. In total, 600 cuttings were established for all varieties and all treatment. Half of the cuttings were planted in cocopeat and the one in perlite media. Powder IBA; powder IBA+*Bacillus subitilis*; liquid IBA; liquid IBA+*Bacillus subitilis* was applied separately to each of the two types of the medium (Fig. 1 & 2). In such way, 130 cuttings were used for each application and 80 cuttings were used in control applications.

Shoot formation has been observed regularly in the greenhouse from planting until the end of the experiment. The determination of the number on the adventitious root (units/cutting) was also investigated. Only the number of cuttings that had remained alive until the end of the experiment was taken into consideration. In the end, while dismantling the cuttings, the one with the longest root has been determined. The longest root has been measure by caliper. In the end, while dismantling the cuttings, the same root diameter was also examined from three different areas. Root diameter has been measured by caliper as well. After data collection and processing into Microsoft excel, shooting rates were come into percentages.

Statistical Analysis

In this factorial experiment, all the treatments were placed in split-plot in the EUL greenhouse, whereby the soil media (cocopeat and perlite) standing for the main factor. The level of significance with different parameters was determined by variance (ANOVA) using SPSS software (16.0 version b). The treatments mean separation was performed by Duncan's multiple range of test (DMRT) at 0.5% level; while the two plant growing media performance (perlite and cocopeat) were compared and analyzed by T-test for independent samples.

RESULTS AND DISCUSSION

Rooting parameters

After two months of the experiment, rooting performance among the treatments varied from one cultivar to another. According to the present findings, the highest formation of adventitious roots was observed from powder IBA+*Bacillus subtilis* (Garnem:12.55; Maxma:14 1.15; Myrobolan 29C:3.15), in the perlite media (Table 1). While there is no root formation in Maxma 14 cuttings grown in cocopeat media, significant differences have been obtained in the perlite media (Table 1, 2 & 3).

Fig. 1. Used cocopeat and perlite.

Fig. 2. Applied rooting chemicals.

Table 1. Effect of the treatments on the number of roots formation

Growing media	Treatments	Average number of adventitious roots		
		Garnem	Maxma 14	Myrobolan 29C
Cocopeat	Control	0.00 c	0.00 c	0.00 c
	Powder IBA	6.30 a	0.00 c	4.80 b
	Powder IBA+*Bacıllus Subitilis*	5.15 ab	0.00 c	0.60 c
	Liquid IBA	6.95 a	0.00 c	2.55 b
	Liquid IBA+*Bacıllus Subitilis*	1.65 c	0.00 c	0.00 c
Perlite	Control	1.00 c	0.25 c	0.00 c
	Powder IBA	11.75 b	0.80 c	1.05 bc
	Powder IBA+*Bacillus subitilis*	12.55 a	1.15 bc	3.15 b
	Liquid IBA	0.00 c	0.00 c	0.00 c
	Liquid IBA+*Bacıllus subitilis*	0.25 c	0.00 c	0.00 c

*Values followed by the same letter or letters for the same growing media are not significantly different according to Duncan's multiple range test ($p<0.05$).

Table 2. Effect of the treatments on root diameter

Growing media	Treatments	Root diameter (cm)		
		Garnem	Maxma 14	Myrobolan 29C
Cocopeat	Control	0.000 c	0.00 c	0.000 c
	Powder IBA	0.129 c	0.00 c	0.132 a
	Powder IBA+*Bacıllus Subitilis*	0.147 c	0.00 c	0.031 bc
	Liquid IBA	0.122 c	0.00 c	0.101 ab
	Liquid IBA+*Bacıllus Subitilis*	0.064 c	0.00 c	0.000 c
Perlite	Control	0.038 c	0.046 c	0.000 c
	Powder IBA	0.381a	0.051 c	0.068 bc
	Powder IBA+*Bacillus subitilis*	0.362 a	0.171 b	0.184 b
	Liquid IBA	0.000 c	0.000 c	0.000 c
	Liquid IBA+*Bacıllus subitilis*	0.033 c	0.000 c	0.000 c

*Values followed by the same letter or letters for the same growing media are not significantly different according to Duncan's multiple range test ($p<0.05$).

Regarding the average root diameter, cuttings in cocopeat media and liquid IBA have had better results than cutting in perlite media (Table 2). Indeed, Özkan & Madakbaş (1995) used Myrobolan GF-31, Marianna GF 8-1, Myrobolan B and Commen Mussel plum clone rootstocks. According to their experimental findings, 2000 ppm dose of IBA was used in Myrobolan GF-31 and Myrobolan GF8-1 varieties for which 80% rooting level was obtained. Differences may be associated with the high rate of hormone application that affects the root formation in some plant varieties. Accordingly, as shown in Table 2, the best root diameter was observed from powder IBA+*Bacillus subtilis* application in perlite media (Garnem: 0.362 mm; Maxma 14: 0.171 mm; Myrobolan 29C: 0.184 mm). In the cocopeat growing media, the best root diameter was observed from powder IBA application (Garnem: 0.129 mm; Maxma: 14 0%; Myrobolan 29C: 0.132 mm).

The results on the effect of the treatment on the average size of the root length of the three prunus rootstock cultivars are presented in Table 3. According to these findings, the longest root formation was observed from powder IBA+*Bacillus subtilis* application in perlite media (Garnem: 16.125 cm; Myrobolan 29C: 7.050 cm; Maxma 14: 3.275cm). In the cocopeat growing media, the best results was powder IBA application (Garnem: 2.971cm; Maxma 14: no rooting; Myrobolan 29C: 3.725cm). Eşitken et al. (2002) found comparable results while investigating the growth response of wild cherry varieties in the perlite growing medium. In this study, 4.07 cm the longest root formation was observed in 750ppm application of IBA. Indeed, though many researchers have supported the positive effects and usefulness of IBA to support the rooting, according to the results by Ahmed et al. (2003), the effect of IBA was at variance at different characters studied with respect to its different concentrations. GF-655 peach rootstock showed a consistent response for rooting final survival of transplanted cuttings. Furthermore, the best doses for GF-655 cutting propagation was 2500 ppm of IBA, while GF-677 was not responsive to IBA, even up to 4000 ppm. In general, as for the effects of different treatments on plant adventive root formation, it has been observed that there are significant differences between applications regardless of the growing media. Accordingly, the powder IBA treatment enhanced the rooting ability much better than (9.02%) powder IBA+*Bacillus subtilis* application (8.85%) compared to the control. However, the mean comaprison revealed that the difference found is not significant between liquid IBA, liquid IBA+*Bacillus subtilis* treatments. However, the effect of bacteria (*Bacillus subtilis*) was significant between powder IBA+ *Bacillus subtilis* to powder IBA. Besides, the highest root formation was also obtained in the perlite media+powder IBA+*Bacillus subtilis* (12.50%) treatment, followed by the perlite media+powder IBA (7.50%) in regard to the control. The root formation for Myrobolan 29C cuttings in cocopeat media was also higher (9.8%) than in the perlite media (8.5%) compared to the control. According to the results, it is observed that the growing media have a significant effect on adventive root formation on Maxma 14 cuttings. Accordingly, the adventive root formation of Maxma 14 cuttings perlite media was on the average 0.440g dry weight of roots per rootstocks, but no results were obtained in the cocopeat media.

Table 3. Effect of the treatments on the root length

Growing media	Treatments	Length of the longest root (cm)		
		Garnem	Maxma 14	Myrobolan 29C
Cocopeat	Control	0.000 c	0.000 c	0.000 c
	Powder IBA	2.971 b	0.000 c	3.725 a
	Powder IBA+*Bacıllus Subitilis*	1.975 b	0.000 c	0.550 c
	Liquid IBA	2.800 ab	0.000 c	3.150 a
	Liquid IBA+*Bacıllus Subitilis*	1.0 bc	0.000 c	0.000c
Perlite	Control	1.125 c	0.550 bc	0.000 c
	Powder IBA	16.750 b	2.375 bc	2.550 c
	Powder IBA+*Bacillus subitilis*	16.125 a	3.275 bc	7.050 b
	Liquid IBA	0.000 c	0.000 c	0.000 c
	Liquid IBA+*Bacıllus subitilis*	0.500 c	0.000 c	0.000 c

*Values followed by the same letter or letters for the same growing media are not significantly different according to Duncan's multiple range test ($p<0.05$).

Table 4. Numbers of stems shoots per type of rootstock

Treatments	Maxma 14	Garnem	Myrobolan
Control+cocopeat	0.000 b	0.000 c	0.000 c
Powder IBA+cocopeat	0.000 b	0.000 c	0.000 c
Powder IBA+*Bacillus subitilis* +cocopeat	0.000 b	0.250 c	0.000 c
Liquid IBA+cocopeat	0.000 b	0.350 c	0.250 c
Liquid IBA+ *Bacillus subitilis* +cocopeat	0.000 b	0.550c	0.400 ab
Liquid IBA+perlite	0.000 b	0.950 bc	0.500 ab
Liquıd IBA+ *Bacillus subitilis* + perlite	0.000 b	1.000 bc	0.550 ab
Control+perlite	0.050 b	1.050 b	0.900 a
Powder IBA+ *Bacillus subitilis* +perlite	0.350 a	1.700 ab	0.950 a
Powder IBA+perlite	0.450 a	2.200 a	1.100 a

*Values followed by the same letter or letters within the same column are not significantly different according to Duncan's multiple range test ($p<0.05$).

The role of different plant growing media on subsequent rooting performance have been documented by Sarropoulou et al. (2012). According to the experimental results in the Table 3, it was observed that the growing media have a significant and different effect on average longest root formations. The average longest root formations of Garnem cuttings in the perlite media was on average 6.69 cm and 1.74 cm in the cocopeat media. The results on the effect of growing media on rooting indicated that the highest adventive root formation was obtained in the perlite media (12.55) of powder IBA+*Bacillus subtilis* application, followed by the perlite media of Powder IBA (11.75). In addition to all these, it was also revealed that the cuttings raised in cocopeat medium were not successful results compared to those grown in perlite medium. The best performance in perlite might be linked to its C/N ratio that may promote rooting (Hartmann et al., 2002). In the same way, these findings are generally in the same line with the findings on semi-wood cutting by Eşitken et al. (2003) stating that the results from the combination of IBA+*Bacillus subtilis* bacteria is highly effective in increasing rooting capacity when compared to the control, or IBA treatments alone.

Inducing stem shoots

According to the findings as presented in Table 4, after two months of the experiment, it was revealed that growing media did not significantly affect the number of shoots plantlets. Accordingly, the average number of shoots of Garnem cuttings grown in perlite media was 0.900 ± 1.580 average number of shoot/cutting, while in the cocopeat media was determined as 0.710 ± 2.066 average number of shoot/cutting.

From the results shown in Table 2, the highest number of shoots was observed from the powder IBA application (0.225), followed by the powder IBA+*Bacillus subtilis* bacteria application (0.175). However, there was no statistical difference between the control application (0.025) and other applications since they did not respond. Indeed, similar results by Ahmed et al. (2003) concluded that cultivars varied in their response to buds or stem sprouts in relation to regime of chemicals applied due to fluctuation of their status of dormancy. Thus, some are responding to low-level chemicals regime, others are for a high level of chemical regimes.

Accordingly, the highest number of shoots was observed from the powder IBA application, followed by the liquid IBA+*Bacillus subtilis* application, and there was no statistically significant difference between the two applications. The other two applications other than these two applications (liquid IBA+*Bacillus subtilis* and liquid IBA) are statistically higher than the numerical control application, 0.40 unit and 0.525 unit, respectively, and the control application (0.175 unit) and there was no significant difference. Thus, there were no differences in the rooting percentages between dilutants or auxin concentrations within a species which is in line with the findings of Beeson Jr (2000).

The results about the interaction effects of growing media, type of auxin and PGB on stem shoots are presented in the Table 2. According to the findings, it was observed that the growing media did not differ significantly on the number of shoots of cuttings. Accordingly, the average number of shoots of Myrobolan 29C cuttings produce in the cocpeat media was 0.64, while in the perlite media was observed as 0.29. According to the results, it is observed that the effect of the growing medium was not significant either for rooting parameters or for the number of shoots of Myrobolan 29C cuttings. The findings are in line for the previous findings reported by Araújo et al. (2001) that the some endophytic bacteria have a stimulatory effects and positive interactions with rooting hormones. The T-test to compare the average performance results of the cultivar between the two plant growing medium did not reveal any significant difference, however, the growth parameters were slightly higher in perlite than in the cocopeat medium.

CONCLUSION

From the present study, wood cuttings were taken from Peach (Garnem), Plum (Myrobolan 29C) and Cherry (Maxma14) rootstocks. These cutting were treated with liquid (3300 ppm) and powder (8000 ppm) concentrations of IBA combined or not with *Bacillus subitilis* bacteria. Stem shoot number, adventitious root number, longest root length root diameter and rooting levels were measured and data adhoc analyzed statistically. All the rootstocks were randomly put and evaluated under the greenhouse conditions. It is thus drawn from the experiment that the application of powder IBA+*Bacillus subtilis* produced better results in the perlite growing medium with all the three varieties rootstocks. The percentage of Garnem and Myrobolan 29C cuttings rooted was significantly greater ($P < 0.05$) in the cocopeat growing media+powder and cocopeat+liquid IBA treatments. However, the findings from the present study revealed that perlite medium developed better results than cocopeat medium; and the combination of powder IBA and bacteria (*Bacillus subtilis)* has effectively promoted rooting compared to the control or IBA treatment alone. Further studies should be conducted to get comprehensive results on optimal and cost-effective treatments for specific rootstock cultivars, field growing conditions for the Prunus cuttings.

Acknowledgment
Special thanks to all European University academic (EUL) staff, especially Prof. Dr. Mehmet Atilla Askin for his esteemed guidance and to Ms. Hatice Serdar for her support in data collection. My special thanks also to the University of Rwanda for the study leave and support along my research work at EUL.

Conflict of interest
The authors have no conflict of interest to report.

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