

## COMPARISON OF THE EFFICIENCY OF SYNTHETIC AUXINS AND BIOSTIMULANTS AND TWO TYPES OF SUBSTRATE IN ROOTING OF SHOOT CUTTINGS IN ‘PI-KU 1’ ROOTSTOCK

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### ABSTRACT

The low-cost propagation of semi-dwarf cherry rootstock is an essential issue in the production of maiden trees of this species. Among the promising rootstocks is ‘Pi-ku 1’, obtained in Germany. However, the possibility of its propagation using shoot cuttings has not been investigated. It was the purpose of this study. Two ways of cuttings treatment before placing them in the substrate were assessed. One used two preparations in powder (Rhizopon AA and Ukorzeniacz AB), and the second used two alcoholic auxin solutions (IAA and IBA). Instead of synthetic auxins, foliar spraying with two biostimulants was performed (Goteo and Bispeed). All the applied treatments increased the rooting percentage of ‘Pi-ku 1’ rootstock cuttings compared to the control from 5% for Rhizopon AA to 18.2% for auxin IBA. The exception was the lack of a positive effect of the Bispeed biostimulant (less than 5.2%). The use of synthetic auxins increased the number of cutting roots more than three times (IBA) or almost twice (Ukorzeniacz AB) and their length more than twofold (IAA, Rhizopon AA) in proportion to the control. The effect of synthetic auxin treatments on the remaining growth parameters under study was also positive. Foliar treatment of cuttings with biostimulants did not change their growth. Only in one of the two years of the study did the fresh mass of cuttings improve after using Goteo biostimulant (7.5%). As part of the experiment, the effect of two substrate types – peat mixed with perlite and peat with sand – was also tested. During the two years of research, the cuttings were rooted several percent better in peat and sand (2.7% – 2018 and 4.4% – 2019). Using peat with sand as a rooting substrate significantly improved the number and length of roots (11.90 and 125.10) of Pi-ku 1 rootstock cuttings compared to the second one (9.23 and 109.08, respectively). All treatments applied to cuttings, except two biostimulants, increased the amount of chlorophyll in the leaves.

**Key words:** sweet cherry, semi-dwarf rootstock, propagation, softwood cuttings, rooting stimulants, substrate

### INTRODUCTION

The cultivation of sweet cherry trees is very popular in Poland and worldwide. However, rootstock that reduces the growth vigor of the trees is required to intensify the cultivation of this species. Additionally, some rootstocks are difficult to obtain and can be propagated using the *in vitro* method [Štefančič et al.

2007, Sedlak et al. 2008], which increases the production cost of sweet cherry trees in nurseries. Therefore, research is necessary to improve the methods of obtaining the rootstock, e.g., with the use of softwood cuttings. According to Bourrain and Charlot [2014], one of the most interesting rootstocks is “Pi-ku 1”

(*P. avium* × *P. canescens* × *P. tomentosa*). According to some authors [Cmelik et al. 2004, Gyeviki et al. 2008, Usenik et al. 2008, Franken-Bembenek 2010, Spornberger et al. 2015, Bassi 2016], this rootstock, due to its low soil requirements and high yielding capacity, can be planted in places unsuitable for more demanding rootstocks. Moreover, 'Pi-ku 1' rootstock positively affects the size of cherry fruit [Usenik et al. 2005, Gyeviki et al. 2008, Spornberger et al. 2015]. It is one of the best rootstocks for growing cherry trees [Gyeviki et al. 2008, Hrotkó et al. 2009a, 2009b], especially in the northern part of Europe [Sansavini and Lugli 2009]. So far, rootstocks for sweet cherries have been propagated using hardwood cuttings or the *in-vitro* method [Dessy et al. 2004, Bourrain and Charlot 2014, Eremin et al. 2017, Gergoff Grozoff et al. 2018, Aysanov et al. 2019]. According to many authors [Mezey and Leško 2014, Markovski et al. 2015, Sharma and Kumar 2019], rootstocks that are difficult to propagate rooted more effectively with hardwood cuttings than softwood ones. Additionally, the propagation of some sweet cherry rootstocks by softwood cuttings is very effective and gives 85% to 90% of rooted cuttings [Drabudko et al. 2016]. Of the rootstocks obtained by this method, 70%, after further cultivation in greenhouse conditions, acclimatize well in the nursery even in a relatively dry spring [Myndra et al. 2010]. There are no publications on the propagation efficiency of 'Pi-ku 1' rootstock using softwood cuttings.

When rooting shoot cuttings, synthetic auxins are used as substances that stimulate the growth of the roots. IBA is the best form of auxin that improves the rooting efficiency of cuttings [Skůpa et al. 2014]. There are many results on synthetic auxins' effect in supporting cuttings' rooting [Dinkova et al. 2006, Raju and Prasad 2010, Pacholczak et al. 2013]. However, in recent years, synthetic compounds have been replaced by biostimulants. Of particular interest are biostimulants produced from seaweed extracts, including from brown algae. Seaweed products sometimes contain many phytohormones necessary for plants, including auxins, gibberellins, and cytokinins, which increase their development [Wang et al. 2016]. So far, such preparations have been used mainly for propagating various species of ornamental shrubs [Pacholczak et al. 2012, 2016, Monder 2019, Loconsole et al. 2022]. Biostimulants increase the ability of plants to use nu-

trients, improve tolerance to stress factors, contribute to better root system growth, and increase the efficiency of photosynthesis and other metabolic processes in plants [Calvo et al. 2014, Rouphael et al. 2018]. The potential of biostimulants is emphasized; however, their efficiency differs depending on the species and varieties of plants and other factors such as climatic or soil conditions [Murawska et al. 2017, Dorobek et al. 2019]. So far, biostimulators included in this experiment have been used in other experiments [Pacholczak et al. 2016, Pacholczak and Nowakowska 2017, Malinowska et al. 2018, Świerczyński 2023] obtaining not always positive results.

In order to determine the effectiveness of the propagation of the 'Pi-ku 1' rootstock, this experiment was carried out using softwood cuttings with the application of powdered synthetic auxins and their alcoholic solution as well as by foliar application of biostimulants. The two tested biostimulants were selected for research based on previous positive results of our experiments and those of other authors indicated above. Additionally, the usefulness of two substrates for rooting these cuttings was compared.

## MATERIAL AND METHODS

The cuttings were taken from four-year-old mother plants without visual disease symptoms and rooted in 2018–2019. Stem cuttings (10 cm long) were obtained in the first days of June and consisted of 4 internodes. After harvesting, the cuttings were treated with the formulations listed below (Tab. 1) and then placed in plastic trays filled with substrate. Auxin application in the form of an alcohol solution took place in a room with limited access to light.

Two substrates were used in the experiment. The first was high peat and sand river 2 : 1 (pH 6.0). The second one was prepared as a mixture of ready-made substrate TS1 (Klasmann-Deilmann) with the addition of perlite in proportion 2 : 1 (pH 6.5). Both substrates were supplemented with a compound fertilizer PG Mix (1 kg·m<sup>-3</sup>) (N 14%, P 16, K 18%, and microelements). The cuttings were rooted in a low tunnel with an automatic fogging system inside. During the cuttings' rooting period, the tunnel's air temperature was maintained at 18–28°C, and air humidity was 80–85%. The cuttings were protected against fungal

**Table 1.** Treatments of cutting used in the experiment

Treatments	Method of application
Control	Three-spray treatment with distilled water
Ukorzeniacz AB (0.2% NAA; 0.1% IBA; 0.1% amid NAA) powder	One treatment and three-spray treatment with distilled water
Rhizopon AA (0.2% IBA) powder	One treatment and three-spray treatment with distilled water
IAA ( $2 \text{ g} \cdot \text{L}^{-1}$ ), the auxins were dissolved in pure ethanol and filled up with water to obtain 1%	Quick-dipped for about 5 s and three-spray treatment with distilled water
IBA ( $2 \text{ g} \cdot \text{L}^{-1}$ ), the auxins were dissolved in pure ethanol and filled up with water to obtain 1%	Quick-dipped for about 5 s and three-spray treatment with distilled water
Goteo 0.2%	Three-spray treatment
Bispeed 0.5%	Three-spray treatment



**Fig. 1.** The appearance of 'Pi-ku 1' rootstock cuttings after treatment (from left to right): Control, Rhizopon AA, Ukorzeniacz AB, IAA, IBA, Goteo, Bispeed

diseases by spraying with fungicides at one-week intervals. Five spraying treatments were carried out, alternating with Amistar 250SC 0.1% and Switch 62,5 WG 0.1%.

The experiment consisted of six treatments with various preparations stimulating the rooting of the cuttings and a control one without additional treatments. The names of the preparations, their concentrations, and the application method are presented in Table 1. The experiment included fourteen treatments (seven cutting

treatments and two substrates). Thirty cuttings were subjected to each treatment, ten in three repetitions.

Three foliar treatments with biostimulators were applied at intervals of two weeks – the first one right after placing the cuttings in the substrate. The biostimulant concentrations corresponded to the manufacturer's recommendations (Tab. 1). Bispeed biostimulant contains three groups of nitrophenolates: potassium 4-nitrophenolate (potassium para-nitrophenolate) 0.25–0.30% m/m; potassium 2-nitrophenolate

nolate (potassium ortho-nitrophenolate) 0.14–0.20% m/m; potassium 5-nitroguaiacolate (potassium 2-methoxy-5-nitrophenolate) 0.07–0.10% m/m. According to the manufacturer, this biostimulant can be used as foliar fertilizers in agricultural, vegetable, ornamental, and fruit crops grown in the ground and under cover. It stimulates plant growth, supports the action of natural auxins, and increases the ability of the roots to absorb mineral compounds. Goteo biostimulant contains GA142-biologically active extracts from *Ascophyllum nodosum*, 13.0% phosphorus pentoxide, and 5.0% potassium oxide soluble in water. The manufacturer recommends using the Goteo to produce cuttings of many species of ornamental and fruit plants. Goteo improves the development of the root system and the growth of hairy roots, providing a more effective absorption of minerals from the substrate.

At the end of October 2018–2019, all leaves from ten randomly selected cuttings for each treatment were picked, and their fresh weight (g) was determined. Using the 'Skwer' program (IksmodaR, Poland), the total area of leaf blades (cm<sup>2</sup>) of previously scanned leaves (Brother DCP-9020CDW) was obtained. Moreover, the number of rooted cuttings in proportion to those placed in the substrate was calculated. After the leaves fell, all cuttings were removed from the containers, the number of shoots was counted, the length of the shoots on the cutting was measured, and the number of roots was determined.

At the beginning of August 2019, the level of chloroplast pigments in leaves was measured. Leaves for testing were taken from the middle part of the side shoot without visible disease symptoms. The procedure for determining the pigment content was based on the method of Hiscox and Israelstam [1979], using dimethyl sulfoxide without crushing the leaves. The content of pigments was calculated according to the modified Arnon formulas [Hiscox and Israelstam 1979]. The content of chlorophyll pigments was determined using a spectrophotometer Specol type (Carl Zeiss, Jena, Germany).

The results obtained in the experiment were compared using the Statistica 13.1 program (StatSoft, Poland). Duncan's test was used, with a probability level of  $p = 0.05$ . The percentage results (rooted cuttings) were recalculated by arc sine transformation. Plant growth parameters were subjected to a two-factor

analysis of variance (treatment, substrate). Calculations were made separately for two years of research. Fresh weight and leaf blade area of cuttings, chlorophyll, and carotenoid contents were analyzed by one-way analysis of variance (treatment).

## RESULTS

In the first year of the study, the percentage of rooted cuttings of 'Pi-ku 1' rootstock for Ukorzeniacz AB preparation, auxin IBA, and Goteo biostimulant was the highest (Tab. 2). Lower values were obtained for auxin IAA, followed by Rhizopon AA. The lowest number of rooted cuttings was obtained for the Bispeed biostimulant and the control. In the second year of the study, the best result was obtained for auxin IBA, followed by, in decreasing order and with different results, Goteo biostimulant, auxin IAA, Rhizopon AA, and Ukorzeniacz AA. The lowest percentage of rooted cuttings was observed for Bispeed biostimulant, and the control was better. In the first and second years of the experiment, the average result for the peat and sand substrate was better than for peat with perlite (Tab. 2).

Concerning the fresh weight of cuttings, the best result was obtained for the two auxins and Rhizopon AA preparation, which did not differ from Ukorzeniacz AB and Goteo biostimulant (Tab. 2). The lowest fresh weight of cuttings was recorded for the Bispeed biostimulant and the control. In the second round of the experiment, the best value of the weight of cuttings was recorded for auxin IBA, followed by the second type of auxin IAA. The results of other treatments did not differ significantly. In both rounds of the experiment, the average results for the substrates were not different (Tab. 2).

In 2018, the most significant number of side shoots on a cutting was obtained for auxin IBA, followed by auxin IAA, Rhizopon AA, and Ukorzeniacz AB (Tab. 3). The other treatments resulted in a lower number of side shoots, not significantly different. The composition of the substrate did not affect the number of shoots. In the second year of the study, a significantly lower number of shoots was obtained for the two biostimulants and the control compared to the other treatments. More shoots were obtained when rooting the peat and perlite substrate cuttings than rooting the peat with sand (Tab. 3).

**Table 2.** Percentage (%) and fresh mass (FM) of rooted softwood cuttings of 'Pi-ku 1' rootstock depending on treatments and substrates in the years 2018–2019

Treatment	2018 (%)	2019 (%)	2018 FM (g)	2019 FM (g)
Control	76.95 ab	75.86 b	2.03 a	2.02 a
Ukorzeniacz AB	94.11 d	79.77 c	2.34 bc	2.32 ab
Rhizopon AA	78.22 b	82.61 c	2.54 cd	2.31 ab
IAA	84.68 c	87.41 d	2.74 d	2.52 b
IBA	94.18 d	92.61 f	2.80 d	2.96 c
Goteo	93.29 d	89.78 e	2.34 bc	2.26 ab
Bispeed	73.81 a	70.71 a	2.22 ab	2.09 a
Average for year	84.96	82.68	2.43	2.35
Standard deviation	4.25	3.47	0.56	0.67
Average for substrate	PS 87.20 b PP 84.92 a	85.15 b 81.39 a	2.48 a 2.38 a	2.40 a 2.31 a

PS – peat with sand, PP – peat with perlite

Data followed by the same letters do not differ significantly at  $p = 0.05$  for each parameter, according to Duncan's test.

**Table 3.** Number of shoots (NS) and a sum of shoots length (SSL) of softwood cuttings of 'Pi-ku 1' rootstock depending on treatments and substrates in the years 2018–2019

Treatments	2018 NS	2019 NS	2018 SSL (cm)	2019 SSL (cm)
Control	0.00 a	0.00 a	0.00 a	0.00 a
Ukorzeniacz AB	0.27 b	0.50 b	2.00 ab	7.50 c
Rhizopon AA	0.30 b	0.53 b	1.83 ab	4.47 b
IAA	0.67 c	0.63 b	3.90 b	5.93 bc
IBA	1.40 d	0.57 b	14.97 c	5.37 bc
Goteo	0.03 a	0.03 a	0.73 a	0.73 a
Bispeed	0.00 a	0.00 a	0.00 a	0.00 a
Average for year	0.38	0.32	3.35	3.43
Standard deviation	0.56	0.54	6.65	5.93
Average for substrate	PS 0.31 a PP 0.45 a	0.13 a 0.51 b	3.90 a 2.79 a	1.10 a 5.75 b

PS – peat with sand, PP – peat with perlite

Data followed by the same letters do not differ significantly at  $p = 0.05$  for each parameter according to Duncan's test.

**Table 4.** Number of roots (NR) and a sum of roots length (SRL) of softwood cuttings of 'Pi-ku 1' rootstock depending on treatments and substrates in the years 2018–2019

Treatments	2018 NR	2019 NR	2018 SRL (cm)	2019 SRL (cm)
Control	5.40 a	5.90 a	72.4 a	79.77 a
Ukorzeniacz AB	9.90 b	14.63 b	105.6 b	148.17 b
Rhizopon AA	14.40 c	13.93 b	155.67 c	142.17 b
IAA	15.63 cd	20.33 c	179.2 d	191.10 c
IBA	16.73 d	26.40 d	142.43 c	209.80 c
Goteo	6.30 a	6.83 a	86.87 ab	78.87 a
Bispeed	5.60 a	6.40 a	77.47 a	79.80 a
Average for year	10.57	13.49	117.09	132.81
Standard deviation	5.82	8.99	58.04	68.48
Average for substrate	PS 11.90 b PP 9.23 a	13.68 a 13.30 a	125.10 b 109.08 a	136.46 a 129.16 a

PS – peat with sand, PP – peat with perlite

Data followed by the same letters do not differ significantly at  $p = 0.05$  for each parameter according to Duncan's test.

**Table 5.** Fresh mass (FM) and leaf blade area (LBA) of softwood cuttings leaves of 'Pi-ku 1' rootstock depending on treatments in 2018–2019

Treatments	2018 FM (g)	2019 FM (g)	2018 LBA (cm <sup>2</sup> )	2019 LBA (cm <sup>2</sup> )
Control	0.93 a	0.86 a	37.51 a	35.01 a
Ukorzeniacz AB	1.01 b	1.01 cd	43.29 ab	45.79 c
Rhizopon AA	1.13 c	1.00 c	43.66 b	41.16 b
IAA	1.17 cd	0.93 b	45.16 b	40.16 b
IBA	1.19 d	1.07 d	46.89 b	41.89 bc
Goteo	0.97 ab	0.93 b	42.31 ab	37.31 ab
Bispeed	0.96 ab	0.89 ab	41.75 ab	38.25 ab
Average for year	1.05	0.96	42.94	39.94
Standard deviation	0.11	0.80	4,25	4.23

Data followed by the same letters do not differ significantly at  $p = 0.05$  for each parameter according to Duncan's test.

In the first stage of the experiment, the best sum of shoot lengths of the 'Pi-ku 1' rootstock cuttings was obtained for auxin IBA, and a significantly lower sum was noticed for IAA (Tab. 3). The values obtained for other treatments did not differ. The averages for the substrates were also similar. Applying the Ukorzeniacz AB agent resulted in the most extended shoots in the second year of the study; this result did not differ from the results obtained using the two types of auxins and Rhizopon AA. Lower values of the parameter under consideration were observed concerning the three other treatments. Peat with perlite as a substrate increased the sum of shoot lengths compared to peat and sand in the second year of the experiment (Tab. 3).

Considering the outcome of the individual treatments, the best number of roots of the cuttings was obtained for auxin IBA and IAA. The result of the latter was not different from Rhizopon AA (Tab. 4). The number of roots was the lowest for the control and the two biostimulants and the result was significantly better for Ukorzeniacz AB. A better type of substrate turned out to be a mixture of peat and sand than the second used. As in the first year of the study, a higher number of roots was obtained for Auxin IBA. Subsequently, a significantly lower value was obtained for the second auxin – IAA. The lowest number of roots was found for the control and the two biostimulants. Compared to those treatments, significantly higher

values were found for Ukorzeniacz AB and Rhizopon AA. In the experiment's second year, the substrate type did not influence the number of roots (Tab. 4).

The sum of root lengths was the best for auxin IAA and significantly lower for Rhizopon AA and the second auxin IBA (Tab. 4). The lowest value of that parameter was obtained for the control and the two biostimulants. The average result for Ukorzeniacz AB was significantly higher than for the control and Bispeed preparation. The mixture of peat and sand was a better substrate than the second one. In the following year of observation, the most significant length of roots was found for the two auxins; the roots were significantly shorter in the case of Ukorzeniacz AB and Rhizopon. The lowest sum of root lengths was observed in the control plants and plants treated with two biostimulants. The average result for the given type of substrate did not differ significantly (Tab. 4).

In 2018, the fresh weight of leaves of 'Pi-ku 1' rootstock cuttings was significantly higher for auxin IBA (Tab. 5). It was followed in descending order by auxin IAA, Rhizopon AA, and Ukorzeniacz AB. The lowest fresh weight of leaves was recorded for the control, which did not differ significantly from the values obtained for the two biostimulants. In the second year of the research, the best result regarding the fresh weight of leaves of the cuttings was obtained for auxin IBA, Ukorzeniacz AB, and Rhizopon AA. Signifi-

**Table 6.** Content of chlorophyll A, B, A + B, and carotenoids in leaves of 'Pi-ku 1' rootstock cuttings depending on the treatments in the year 2019

Treatments	Chlorophyll A	Chlorophyll B	Chlorophyll A + B	Carotenoids (mg·g <sup>-1</sup> fresh mass)
Control	2.17 a	0.48 a	2.65 a	5.98 a
Ukorzeniacz AB	2.36 bc	0.57 c	2.93 bc	6.50 ab
Rhizopon AA	2.41 c	0.56 c	2.97 c	6.41 ab
IAA	2.36 bc	0.53 bc	2.89 bc	6.55 ab
IBA	2.41 c	0.62 d	3.02 c	6.67 b
Goteo	2.27 ab	0.51 ab	2.78 ab	6.17 ab
Bispeed	2.22 a	0.49 a	2.71 a	6.29 ab

Data followed by the same letters do not differ significantly at  $p = 0.05$  for each parameter according to Duncan's test.

cantly lower values were obtained for auxin IAA and Goteo preparation. The lowest fresh weight of leaves was obtained for the control, which did not differ from the Bispeed biostimulant (Tab. 5). The appearance of the cuttings, depending on the treatments, is shown in Figure 1.

In the first year of the experiment, the leaf area of cuttings of 'Pi-ku 1' rootstock was significantly larger after applying IBA and IAA auxins and Rhizopon AA preparation concerning control (Tab. 5). In the second year of the study, Ukorzeniacz AB and auxin IBA increased the leaf area of the cuttings most significantly, subsequently, Rhizopon AA and auxin IAA. The smallest leaf area was observed for the control and two biostimulants (Tab. 5).

Higher chlorophyll A and A + B contents were noticed for Rhizopon AA, auxins IBA and IAA, and Ukorzeniacz AB (Tab. 6). The values obtained for Goteo and Bispeed preparations and the control were significantly lower. The highest chlorophyll B content was observed for auxin IBA treatment, followed by Ukorzeniacz AB, Rhizopon, Auxin IAA, and Goteo. The lowest value was obtained for Bispeed preparation and the control. Only for auxin IBA was a higher level of carotenoids in the leaves of the cuttings determined compared to the control (Tab. 6).

## DISCUSSION

Due to the lack of available research on the rooting of 'Pi-ku 1' rootstock shoot cuttings, the results were compared to other types of rootstock and woody plant species treated with auxins and biostimulants. In the experiment, significant differences in the percentage of rooted cuttings depending on the treatments were found, which was 84% for all treatments, on average. Applying the rooting stimulants significantly improved the rooting percentage of the cuttings. Compared to the control, better results were noticed for all treatments with synthetic auxins and Goteo biostimulant. The best results were obtained for both auxins applied in the form of an alcoholic solution, especially IBA. Better rooting of cuttings was obtained after using auxin IBA, which other authors did not confirm. No differences in the efficiency of the two tested auxins (IBA, IAA) applied on rooting of 'GiSela5' rootstock cuttings were found by Štefančič et al. [2005,

2006]. It may have been due to the very good rooting conditions of the cuttings (a fogging system was used) and the young age of the mother plants, as indicated by the authors mentioned above. The research conducted by Markovski et al. [2015] also confirmed no significant difference in the effect of IBA 2% and NAA 0.2% auxins on the rooting of softwood and hardwood cuttings of various types of sweet cherry rootstocks. However, the results obtained by those authors for GiSela 4 and GiSela 5 rootstocks, which are difficult to root, differed only by a few percent. Similar results to those obtained in the considered experiment for *in vitro* propagation of GiSela 5 rootstock were obtained by Kumar et al. [2020], where auxin IBA turned out to be better than NAA and IAA. Other researchers also proved the positive effect of auxin IBA on the rooting of cuttings of different types of woody plants [Nasri et al. 2015, Otiende et al. 2017]. Sharma and Kumar [2019], when propagating plum rootstock using softwood cuttings, despite a deficient rooting percentage while using auxin IBA, obtained a better result than the control, where the cuttings did not root.

In the conducted experiment, the use of synthetic preparations in the form of powder gave better rooting results compared to the control. Opposite results were obtained by Nečas and Krška [2013], who rooted softwood cuttings of different types of *Prunus* rootstocks and did not obtain a higher percentage of rooting of the cuttings using Rhizopon AA compared to the control. It could have been due to the use of bottom heat ground for their research, which was not the case in the experiment under consideration and stimulated rooting of the cuttings. For the rootstock under study, applying Goteo biostimulant had a positive effect. In the first year, the results obtained with its use were the same as for the best synthetic auxin treatment, and in the second year, only the results obtained with the use of auxin IBA were better. The positive effect of Goteo and Rhizopon AA on the number of rooted dogwood cuttings was confirmed by the research conducted by Pacholczak et al. [2016]. Also, Szabó et al. [2016] demonstrated the significant effect of foliar spraying of cuttings of *Prunus mahaleb* rootstock with a marine algae biostimulant compared to the treatment with only auxin IBA. Similarly, when rooting ground cover roses, Pacholczak and Nowakowska [2020] found out that the application of auxin IBA and Goteo biostimu-



lant resulted in a similar level of rooting. However, using the second biostimulant Bispeed in the experiment did not improve the rooting of the cuttings.

Considering most of the tested growth parameters of the cuttings after rooting, the best results were obtained for alcohol solutions of auxins. It was particularly noticeable in the number of roots, three times higher in the first year and four times higher in the second year, compared to the control. The above is consistent with the results of the experiments conducted by Štefančič et al. [2005, 2006], who obtained greater root length for GiSela 5 rootstock cuttings for both auxins (IBA and IAA) at the same concentration. The positive effect of IBA on the number and length of roots of softwood cuttings of GiSela 5 rootstock was also confirmed by Trobec et al. [2005]. However, it was only when the cuttings were taken from the upper parts of the growth over a given year, not from the lower part of the plant, which, in their opinion, was already partially woody. Likewise, Gergoff Grozeff et al. [2018], when propagating the Ferdor Julior rootstock, obtained a two-fold increase in the number of roots of woody cuttings treated with auxin IBA compared to the control.

The experiment found no improvement in the cuttings' rooting level after the treatment with the tested biostimulants. The opposite correlation was observed by Loconsole et al. [2022], who treated shoot cuttings of two species of ornamental shrubs with Goteo foliar biostimulant and noticed a more significant number of roots and shoots. Kapczyńska et al. [2020] also proved the positive effect of Goteo preparation on the length of roots of *Pennisetum purpureum* cuttings. Similarly, in the case of propagation using cuttings of *Robinia pseudoacacia*, the application of microalgae extract improved the growth parameters of cuttings [Kaviani et al. 2016]. In turn, the results are consistent with the outcomes of the research conducted by Traversari et al. [2022], who, when rooting rose cuttings, used IBA together with NAA and obtained higher root length compared to Phylgreen biostimulant. It can be concluded that the results of applying biostimulants depend on many factors, including the type of propagated species and the rooting conditions of the cuttings.

When the two auxins under study were applied, the highest fresh weight of 'Pi-ku 1' cuttings was obtained. It confirms the efficiency of their applica-

tion when rooting cuttings of that type of rootstock. Similarly, after applying auxin IBA, Sarropoulou et al. [2015] obtained a higher fresh and dry weight of rootstock cuttings for sweet cherries. When analyzing the effect of the tested biostimulants on the parameter in question, a greater weight of cuttings while using Goteo biostimulant was recorded for only one year compared to the control. Similarly, Szabó et al. [2016] found no increase in the fresh weight of *Prunus mahaleb* cuttings when applying a similar biostimulant. However, the fresh weight of leaves for that biostimulant was higher in the second year of the study. The number and length of new shoots did not differ for two biostimulants compared to the control. That observation is inconsistent with the results obtained by Pacholaczak et al. [2016], who observed more shoots of ninebark stem cuttings after foliar treatment using Goteo preparation. The fresh weight and leaf area of the cuttings after applying the Goteo biostimulant were better than the control for one parameter and one year. It is inconsistent with the result obtained by Loconsole et al. [2022], who recorded a more significant number of leaves and generally larger leaf area for cuttings of two species of ornamental shrubs treated with that biostimulant.

According to Bondarenko [2019], an increase in photosynthetic activity in plants occurs with an increase in the content of chloroplast pigments in the leaves. Also, in the opinion of Borowiak and Korszun [2012], the intensity of photosynthesis may be determined by the leaf area of the plants. In the experiment, all preparations containing synthetic auxins increased the leaf area and the content of chloroplast pigments, which suggested more significant growth activity of cuttings, confirmed by better growth parameters obtained in the study. Based on the conducted experiment and the opinions of other researchers [Sims and Gamon 2002, Steele et al. 2008], it can be concluded that determining the content of chloroplast pigments in leaves can provide us with valuable information about the physiological state of the tested plants.

Research was also conducted on selecting appropriate substrates for rooting shoot cuttings to ensure optimal air-water relations [Owen 2007]. Many authors [Sardoei 2016, Rajkumar et al. 2017, Kapczyńska et al. 2020] confirmed the improvement in rooting of woody plant shoot cuttings when perlite was used as

a homogeneous substrate or as one of its components. The results of our experiment are different. A higher percentage of rooting of shoot cuttings of 'Pi-ku 1' rootstock was obtained when peat mixed with sand was used as a substrate. Other authors [Štefančič and Stampar 2005, Exadaktylou 2009, Mezey and Leško 2014] used different substrates for rooting shoot cuttings of cherry rootstocks. The results of their experiments and this study confirm that the composition of the substrate does not have a significant effect on the rooting of cuttings of *Prunus* rootstock. Moreover, the substrates tested in this experiment had an equally favorable effect on the number and sum of root lengths.

## CONCLUSIONS

The use of synthetic auxins significantly improved the percentage of rooting of 'Pi-ku 1' cuttings. Taking the two applied biostimulants into account, only Goteo increased the rooting percentage of the cuttings compared to the control. Although the cuttings of the rootstock under study were rooted without additional treatment with auxins, using the auxins improved the results by several percent. Also, the number of roots of the cuttings increased by three times for four auxin treatments, on average, and their length increased by two-times, depending on the year. Such a strong effect of auxin treatments concerning the intensity of development of the above-ground parts of the cuttings has not been observed. However, all parameters improved significantly. The effect of the applied biostimulants on the growth of cuttings turned out to be insignificant. A higher percentage of rooting of the cuttings was obtained in the peat and sand substrate. Also, in one year of the research, the number and sum of root lengths were higher for that substrate. An inverse relationship was found between the number of shoots and their sum depending on the type of substrate used. The synthetic auxins increased the chlorophyll content in the leaves of the cuttings. The most effective treatment was the use of two auxins in the form of an alcohol solution at a concentration of 1%. The Goteo biostimulant also increased the percentage of rooted cuttings.

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## REFERENCES

- Aysanov, T.S., Romanenko, E.S., Selivanova, M.V., Esaulko, N.A., Mironova, E.A., German, M.S. (2019). Improving the technology of obtaining clonal root stocks of stone fruit crops. *Earth Environ. Sci.* 315(2), 022017. <https://doi.org/10.1088/1755-1315/315/2/022017>
- Bassi, G., Fajt, N., Biško, A., Donik Purgaj, B., Draicchio, P., Folini, L., Gusmeroli, F., Steinbauer, L. (2016). Vegetative and productive performances of 'Kordia' and 'Regina' sweet cherry cultivars grafted on four clonal rootstocks in the Alpe Adria region. *Acta Hort.* 1139, 159–166. <https://doi.org/10.17660/ActaHortic.2016.1139.28>
- Bondarenko, P. (2019). Physiological basics of sweet cherry productivity depending on rootstocks, interstems and plant density. *Open Agric.* 4(1), 267–274. <https://doi.org/10.1515/opag-2019-0025>
- Borowiak, K., Korszun, S. (2012). Investigations of photosynthetic activity parameters in relation to berries yield of selected grapevine cultivars. *Nauka Przym. Technol.* 6(1), 8.
- Bourrain, L., Charlot, G.I. (2014). In vitro micrografting of cherry (*Prunus avium* L. 'Regina') onto 'Piku® 1' rootstock [*P. avium* × (*P. canescens* × *P. tomentosa*)]. *J. Hort. Sci. Biotech.* 89(1), 47–52. <https://doi.org/10.1080/14620316.2014.11513047>
- Calvo, P., Nelson, L., Kloepper, J.W. (2014). Agricultural uses of plant biostimulants. *Plant Soil* 383, 3–41. <https://doi.org/10.1007/s11104-014-2131-8>
- Cmelik, Z., Druzic, J., Duralija, B., Bencic, D. (2004). Influence of clonal rootstocks on growth and cropping of 'Lapins' sweet cherry. *Acta Hort.* 658, 125–128. <https://doi.org/10.17660/ActaHortic.2004.658.16>
- Dessy, S., Radice, S., Andorno, A., Ontivero, M. (2004). Ferdor-Julior, Myriam-Yumir and Julien GF 655-2 rootstocks: Propagation by cuttings with growth regulators and bottom heat. *Acta Hort.* 658, 629–635. <https://doi.org/10.17660/ActaHortic.2004.658.95>
- Dinkova, H., Stoyanova, T., Dragoyski, K., Minkov, P., Bozhanska, T., Kutinkova, H. (2006). Possibilities of improving methods of vegetative propagation for currants. *Latv. J. Agron.* 9, 16–19.
- Drabudko, N.N., Samus, V.A., Leles, S.V. (2016). [Propagation of cherry clonal rootstocks by green cuttings]. *Plodovodstvo* 28, 131–137. In Russian.
- Drobek, M., Frąc, M., Cybulska, J. (2019). Plant biostimulants: importance of the quality and yield of horticultural crops and the improvement of plant tolerance to abiotic stress – a review. *Agronomy* 9(6), 335. <https://doi.org/10.3390/agronomy9060335>

- Eremin, G.V., Podorozhnyi, V.N., Eremina, O.V. (2017). Use of genetic diversity of the genus *Prunus* L. in selection of clonal rootstocks for stone fruit crops and features of their reproduction. *Proc. Latvian Acad. Sci.* 71(3), 173–177. <https://doi.org/10.1515/prolas-2017-0029>
- Exadaktylou, E., Thomidis, T., Grout, B., Zakynthinos, G., Tsipouridis, C. (2009). Methods to improve the rooting of hardwood cuttings of the 'Gisela 5' cherry rootstock. *HortSci.* 19(2), 254–259. <https://doi.org/10.21273/HORTSCI.19.2.254>
- Franken-Bembenek, S. (2010). [GiSelAs, PIKUs and new Giessen Clone: Results from European and North American cherry rootstock trials]. *Erwerbs-Obstbau*, 52, 17–25. In German. <https://doi.org/10.1007/s10341-010-0100-9>
- Gergoff Grozeff, G.E., Romero, Á.M. De Los, Aubone Videla, M. (2018). Nitric oxide in combination with indole-3-butyric acid improves root growth in 'Ferdor Julior' hardwood cuttings (*Prunus insistitia* (L.) × *Prunus domestica* (L.)). *J. Hortic. Sci. Biotech.* 93(2), 175–184. <https://doi.org/10.1080/14620316.2017.1358592>
- Gyeviki, M., Bujdosó, G., Hrotko, K. (2008). Results of cherry rootstock evaluations in Hungary. *Inter. J. Hortic. Sci.* 14(4), 11–14. <http://dx.doi.org/10.31421/IJHS/14/4/1522>
- Hiscox, J.D., Israelstam, G.F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.* 57(12), 1332–1334. <https://doi.org/10.1139/b79-163>
- Hrotkó, K., Magyar, L., Gyeviki, M. (2009a). Effect of rootstocks on vigor and productivity in high density cherry orchards. *Acta Hort.* 825, 245–250. <https://doi.org/10.17660/ActaHortic.2009.825.38>
- Hrotkó, K., Magyar, L., Hoffmann, S., Gyeviki, M. (2009b). Rootstock evaluation in intensive sweet cherry (*Prunus avium* L.) orchard. *Inter. J. Hortic. Science.* 15(3), 7–12.
- Kapczyńska, A., Kowalska, I., Prokopiuk, B., Pawłowska, B. (2020). Rooting media and biostimulator goteo treatment effect the adventitious root formation of pennisetum 'Vertigo' cuttings and the quality of the final product. *Agriculture* 10(11), 570. <https://doi.org/10.3390/agriculture10110570>
- Kaviani, B., Negahdar, N., Hashemabadi, D. (2016). Improvement of micropropagation and proliferation of *Robinia pseudoacacia* L. using plant growth regulators and extracts of brown seaweed *Ascophyllum nodosum*. *J. Crop Prod.* 6(21), 61–79. <https://doi.org/10.18869/acadpub.jcpp.6.21.61>
- Kumar, A., Sharma, R., Thakur, M. (2020). In vitro rooting and hardening of clonal cherry rootstock Gisela 5 (*Prunus cerasus* × *Prunus canescens*). *Indian J. Agric. Sci.* 90(5), 1032–1035. <https://doi.org/10.56093/ijas.v90i5.104389>
- Loconsole, D., Cristiano, G., De Lucia, B. (2022). Improving aerial and root quality traits of two landscaping shrubs stem cuttings by applying a commercial brown seaweed extract. *Horticulturae* 8(9), 806. <https://doi.org/10.3390/horticulturae8090806>
- Malinowska, A., Urbaniak, M., Świerczyński, S. (2018). [Foliar treatment with selected preparations and their effect on the rooting of stem cuttings of two varieties of gymnosperm species]. *Nauka Przyn. Technol.* 12(3), 261–272. In Polish. <http://dx.doi.org/10.17306/J.NPT.00242>
- Markovski, A., Popovska, M., Gjamovski, V. (2015). Investigation of the possibility for production of some stone fruit rootstocks by rooting cuttings. *Acta Agric. Serb.* 20(39), 75–83. <https://doi.org/10.5937/AAS-er1539075M>
- Mezey, J., Leško, I. (2014). Callus and root-system formation in cherry rootstock GiSelA 5. *Acta Hort. Regiot.* 17(1), 5–7. <https://doi.org/10.2478/ahr-2014-0002>
- Monder, M.J. (2019). Rooting and growth of root cuttings of two old rose cultivars 'Harison's Yellow' and 'Poppius' treated with IBA and biostimulants. *Acta Agrobot.* 72(2), 1774. <https://doi.org/10.5586/aa.1774>
- Murawska, B., Gabrowska, M., Spychaj-Fabisiak, E., Wszelaczyńska, E., Chmielewski, J. (2017). Production and environmental aspects of the application of biostimulators Asahi SL, Kelpak SL and stimulator Tytanit with limited doses of nitrogen. *Envir. Protec. Natural Res.* 28(4), 10–15. <https://doi.org/10.1515/oszn-2017-0024>
- Myndra, V., Chernets, A., Kozhoharenko, V., Kuku, G., Kalashian, Yu. (2010). [Improvement of rootstocks of cherry trees – a way of intensification of culture]. Conference "Horticulture, Viticultura și vinificație, Silvicultură și gardin publice, Protecția plantelor", 24(1), 31–34. In Belarusian.
- Nasri, F., Fadakar, A., Saba, M.K., Yousefi, B. (2015). Study of indole butyric acid (IBA) effects on cutting rooting improving some of wild genotypes of damask roses (*Rosa damascena* Mill.). *J. Agric. Sci.* 60(3), 263–275. <https://doi.org/10.2298/JAS1503263N>
- Nečas, T., Krška, B. (2013). Propagation of different stone fruit rootstocks using softwood and hardwood cuttings. *Acta Hort.* 985, 127–138. <https://doi.org/10.17660/ActaHortic.2013.985.16>
- Otiende, M.A., Nyabundi, J.O., Ngamau, K., Opala, P. (2017). Effects of cutting position of rose rootstock cultivars on rooting and its relationship with mineral nutrient

- content and endogenous carbohydrates. *Sci. Hortic.* 225, 204–212. <https://doi.org/10.1016/j.scienta.2017.07.009>
- Owen J.S., Maynard, B.K. (2007). Environmental effects on stem-cutting propagation: a brief review. *Com. Proc. Inter. Plant Propag. Soc.* 57, 558–565.
- Pacholczak, A., Nowakowska, K. (2017). Effect of the biopreparation Goteo on rooting of hydrangea stem cuttings (*Hydrangea paniculata* Siebold 'L imelight' and Vanille freise® 'Renhy'). *Propag. Orn. Plants* 17(4), 126–133.
- Pacholczak, A., Nowakowska, K. (2020). The effect of biostimulators and indole-3-butyric acid on rooting of stem cuttings of two ground cover roses. *Acta Agrobot.* 73(1), 7314. <https://doi.org/10.5586/aa.7314>
- Pacholczak, A., Nowakowska, K., Mika, N., Borkowska, M. (2016). The effect of the biostimulator Goteo on the rooting of ninebark stem cuttings. *Folia Hort.* 28(2), 109–116. <https://doi.org/10.1515/fhort-2016-0013>
- Pacholczak, A., Szydło, W., Jacygrad, E., Federowicz, M. (2012). Effect of auxins and the biostimulator Algamino-plant on rhizogenesis in stem cuttings of two dogwood cultivars (*Cornus alba* 'Aurea' and 'Elegantissima'). *Acta Sci. Pol. Hortorum Cultus* 11(2), 93–103.
- Pacholczak, A., Szydło, W., Petelewicz, P., Szulczyk, K. (2013). The effect of Algamino-plant on rhizogenesis in stem cuttings of *Physocarpus opulifolius* 'Dart's Gold' and 'Red Baron'. *Acta Sci. Pol. Hortorum Cultus.* 12(3), 105–116.
- Rajkumar, Gora, J.S., Kumar, R., Singh, A., Kumar, A., Gajender (2017). Effect of different growing media on the rooting of pomegranate (*Punica granatum* L.) cv. 'Phule arakta' cuttings. *J. Appl. Nat. Sci.* 9(2), 715–719. <https://doi.org/10.31018/jans.v9i2.1263>
- Raju, N.L., Prasad, M.N.V. (2010). Influence of growth hormones on adventitious root formation in semi-hardwood cuttings of *Celasturus paniculatus* Willd. a contribution for rapid multiplication and conservation management. *Agrof. Syst.* 79, 249–252. <https://doi.org/10.1007/s10457-009-9251-9>
- Rouphael, Y., Spichal, L., Panzarova, K., Casa, R., Colla, G. (2018). High-throughput plant phenotyping for developing novel biostimulants: from lab to field or from field to lab? *Front. Plant Sci.* 9, 1197. <https://doi.org/10.3389/fpls.2018.01197>
- Sansavini, S., Lugli, S. (2009). New rootstocks for intensive sweet cherry plantations. *Acta Hort.* 1020, 411–434.
- Sardoei, A.S. (2016). Effect of different media of cuttings on rooting of guava (*Psidium guajava* L.). *Euro. J. Exp. Bio.* 4(2), 88–92.
- Sarropoulou, V., Dimassi-Theriou, K., Therios, I. (2015). Effects of exogenous indole-3-butyric acid and myo-inositol on in vitro root-ing, vegetative growth and biochemical changes in leaves and roots in the sweet cherry rootstock M×M 14 using shoot tip ex-plants. *Theor. Exp. Plant Physiol.* 27, 191–201. <https://doi.org/10.1007/s40626-015-0044-4>
- Sedlak, J., Paprstein, F., Erbenova, M. (2008). In vitro propagation of PHL dwarfing sweet cherry rootstocks. *Acta Hort.* 795, 395–400. <https://doi.org/10.17660/ActaHortic.2008.795.59>
- Sharma, R., Kumar, A. (2019). Influence of indole butyric acid on propagability of clonal rootstock of *Prunus* species through cuttings and stooling. *J. Pharmac. Phytochem.* 8(6), 2483–2487.
- Sims, D.A., Gamon, J.A. (2002). Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sens. Environ.* 81(2–3), 337–354. [https://doi.org/10.1016/S0034-4257\(02\)00010-X](https://doi.org/10.1016/S0034-4257(02)00010-X)
- Skůpa, P., Opatrný, Z., Petrášek, J. (2014). Auxin biology: Applications and the mechanisms behind. In: Applied plant cell biology, plant cell monographs, Nick P. Opatrný Z. (eds.). Berlin, Heidelberg, Springer-Verlag, pp. 69–102. [http://dx.doi.org/10.1007/978-3-642-41787-0\\_3](http://dx.doi.org/10.1007/978-3-642-41787-0_3)
- Spornberger, A., Hajagos, A., Modl, P., Vegvari, G. (2015). [Impact of rootstocks on growth, yield and fruit quality of sweet cherries (*Prunus avium* L.) cv. 'Regina' and 'Kordia' in a replanted cherry orchard in Eastern Austria]. *Erwerbs-Obstbau* 57(2), 63–69. In German. <https://doi.org/10.1007/s10341-015-0232-z>
- Steele, M., Gitelson, A.A., Rundquist, D. (2008). Nondestructive estimation of leaf chlorophyll content in grapes. *Am. J. Enol. Vitic.* 59(3), 299–305.
- Świerczyński, S. (2023). Assessment of the effect of treating 'GiSela 5' softwood cuttings with biostimulants and synthetic auxin on their root formation and some of their physiological parameters. *Plants* 12(3), 658. <https://doi.org/10.3390/plants12030658>
- Štefančič, M., Stampar, F., Osterc, G. (2005). Influence of IAA and IBA on root development and quality of *Prunus* 'GiSela 5' leafy cuttings. *Hort. Sci.* 40(7), 2052–2055. <https://doi.org/10.21273/HORTSCI.40.7.2052>
- Štefančič, M., Stampar, F., Osterc, G. (2006). Influence of endogenous IAA levels and exogenous IBA on rooting and quality of leafy cuttings of *Prunus* 'GiSela 5'. *J. Hort. Sci. Biotech.* 81(3), 508–512. <https://doi.org/10.1080/14620316.2006.11512095>
- Štefančič, M., Vodnik, D., Štampar, F., Osterc, G. (2007). The effect of a fogging system on the physiological status and rooting capacity of leafy cuttings of woody species. *Trees* 21, 491–496. <https://doi.org/10.1007/s00468-006-0121-z>

- Szabó, V., Magyar, L., Hrotkó, K. (2016). Effect of leaf spray treatments on rooting and quality of *Prunus mahaleb* (L.) cuttings. *Acta Sci. Pol. Hortorum Cultus* 15(1), 77–87.
- Traversari, S., Cacini, S., Nesi, B. (2022). Seaweed extracts as substitutes of synthetic hormones for rooting promotion in rose cuttings. *Horticulturae* 8(7), 561. <https://doi.org/10.3390/horticulturae8070561>
- Trobec, M., Stampar, F., Veberic, R., Osterc, G. (2005). Fluctuations of different endogenous phenolic compounds and cinnamic acid in the first days of the rooting process of cherry rootstock 'GiSelA 5' leafy cuttings. *J. Plant Phys.* 162(5), 589–597. <https://doi.org/10.1016/j.jplph.2004.10.009>
- Usenik, V., Štampar, F., Fajt, N. (2008). Sweet cherry rootstock testing in Slovenia. *Acta Hort.* 795, 273–276. <https://doi.org/10.17660/ActaHortic.2008.795.37>
- Usenik, V., Štampar, F., Šturm, K., Fajt, N. (2005). Rootstocks affect leaf mineral composition and fruit quality of 'Lapins' sweet cherry. *Acta Hort.*, 667, 247–252. <https://doi.org/10.17660/ActaHortic.2005.667.36>
- Wang, Y., Fu, F., Li, J., Wang, G., Wu, M., Zhan, J., Chen, X., Mao, Z. (2016). Effects of seaweed fertilizer on the growth of *Malus hupehensis* Rehd. Seedlings, soil enzyme activities and fungal communities under re-plant condition. *Eur. J. Soil Biol.* 75, 1–7. <https://doi.org/10.1016/j.ejsobi.2016.04.003>