EFFECT OF GROWTH STIMULANTS ON ROOTING AND GROWTH OF SWEET AND SOUR CHERRY CLONAL ROOTSTOCKS SOFTWOOD CUTTINGS

VLIV STIMULÁTORŮ NA ZAKOŘEŇOVANI VYBRANÝCH PODNOŽÍ TREŠNÍ A VIŠNÍ

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ABSTRACT
This study investigates methods to improve the rooting efficiency of sweet and sour cherry clonal rootstocks. Propagation with softwood cuttings is one of the effective ways of speeding up the production of high-quality planting material. We evaluated the use of different types of biostimulants for rooting rate, quality, quantity of roots, and growth of softwood cuttings. The study involved propagation of the five most popular rootstocks in Ukraine: Krymsk® 5, Krymsk® 6, Krymsk® 7, Gisela 5, and Colt. Results indicated that softwood cuttings, particularly for Krymsk-type rootstocks, offer a cost-effective propagation method. Rooting efficiency varied from 53.6±5.1% to 65.1±4.4%, and stimulant Zircon at a concentration of 0.5 ml/L was the least effective. Among other stimulants, IBA at 50 mg/L (control) and Zircon at 0.1 ml/L showed the best results. Rootstock Krymsk® 7 had the highest percentage of rooting (90.7±1.3%) and the longest roots (10.3±0.2 cm), while Gisela 5 had the lowest values of the indices (41.5±1.5% rooting and 3.9±0.4 roots per plant). The control (Krymsk® 5) variant showed the highest shoot growth (12.1±0.8 cm). The results show that the rootstock genotype has a decisive influence on the process of rooting and plant growth, rather than the stimulants.

Keywords: sweet and sour cherry rootstocks, growth stimulants, cuttings, rooting

ABSTRAKT
Tato studie zkoumá metody zlepšení účinnosti zakořeňování klonálních podnoží pro višně a třešně. Množení zelených řízků je jedním z účinných způsobů, jak urychlit produkci kvalitního sadebního materiálu. Hodnotili jsme použití různých typů biostimulátorů pro rychlost zakořeňování, kvalitu, množství kořenů a růst řízků. Studie zahrnovala množení pěti široce využívaných podnoží na Ukrajině: Krymsk® 5, Krymsk® 6, Krymsk® 7, Gisela 5 a Colt. Výsledky ukázaly, že řízky pro podnože typu Krymsk nabízejí finančně efektivní metodu

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množení. Účinnost zakořenění se pohybovala od 53,6 ± 5,1 % do 65,1 ± 4,4 % a stimulant Zirkon v koncentraci 0,5 ml/L byl nejméně účinný. Mezi ostatními stimulanty vykazovaly nejlepší výsledky IBA v dávce 50 mg/L (kontrola) a Zirkon v dávce 0,1 ml/L. Podnož Krymsk® 7 měla nejvyšší procento zakořenění (90,7 ± 1,3 %) a nejdelší kořeny (10,3 ± 0,2 cm), zatímco Gisela 5 měla nejnižší hodnoty indexů (41,5 ± 1,5 % u zakořenění a 3,9 ± 0,4 v počtu kořenů na rostlinu). Kontrolní varianta (Krymsk® 5) vykazovala nejvyšší růst výhonků (12,1 ± 0,8 cm). Výsledky ukazují, že na proces zakořeňování a růst rostlin má rozhodující vliv spíše genotyp podnože než růstové stimulanty.

Klíčová slova: třešňové a višňové podnože, růstové stimulanty, vegetativní řízky, zakořeňování

INTRODUCTION

The modern intensive orchards of sweet and sour cherry should be characterized by low growth, sufficient winter and cold hardiness, precocity, ensuring high yields, and longevity. Since sweet cherry (*Prunus avium* L.) is the most vigorous among stone fruit crops, it is important to restrict its growth through the selection of dwarfing rootstocks. The role of the rootstock is crucial when choosing the orchard design. Currently, rootstocks such as Krymsk® 5, Krymsk® 6, and Krymsk® 7 are characterized by good winter hardiness, providing moderate growth force for the trees, and have a high multiplication rate (Maas, 2014; Kishchak and Kishchak, 2016). They have also shown positive results in orchards with various scion-rootstock combinations. Rootstocks Colt and Gisela 5 have also demonstrated good results in orchards in Ukraine. Despite their high demands on soil quality and climatic conditions, the positive aspect is their good compatibility in scion-rootstock combinations (Kishchak and Kishchak, 2005). Therefore, the propagation of these rootstocks is a relevant objective.

Two main methods are usually used for the propagation of clonal rootstocks: propagation in aseptic conditions *in vitro* and propagation with softwood cuttings. However, microclonal propagation requires a well-equipped laboratory and facilities with special conditions, as well as expensive chemicals. In contrast, propagation with softwood cuttings is a relatively inexpensive and economically advantageous method that allows growing planting material in a short period and preserves its genetic identity (Kishchak, 2013). The automation of all processes, including mineral fertilizer feeding, could simplify the labor-intensity of propagation. Softwood cuttings are planted in controlled greenhouse conditions, so their cultivation doesn't depend on the weather. To achieve good results with this type of propagation, it is necessary to maintain appropriate soil and air moisture, air temperature, and lighting. Adhering to basic cultivation requirements allows obtaining clonal rootstocks with a vigorous root system, ensuring the endurance, longevity, and high productivity of orchards.

The primary task in such type propagation is the rooting of cuttings. The efficiency of this stage is enhanced through the use of rooting stimulants. Such auxins as indole-3-butyric acid (IBA), indoleacetic acid (IAA), and 1-naphthaleneacetic acid (NAA), as well as various commercial preparations based on auxins, are employed. Despite their positive role in growing planting material, they also have their disadvantages. The main problem with the use of these substances is their toxicity, leading to environmental pollution and health issues in living organisms (Le *et al*., 2020). Currently, biologically active substances known to promote rooting

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in small concentrations are successfully applied in the process of rhizogenesis. Therefore, there is a need to search for alternative stimulants that will effectively induce rooting and be environmentally safe.

Biostimulant Zircon® is derived from purple coneflower and is composed of a mixture of hydroxycinnamic acids at 0.1 g/L concentrations, mainly caffeic and its derivatives – chicoric and chlorogenic acids. Its positive impact on the rooting of *Morus nigra* L., alder, and other woody plants has been noted (Gorelov, 2010; Karabulut and Saraçoğlu, 2020). The use of Kornevin-Grandis®, containing low concentrations of IBA in combination with amino acids and B-group vitamins, provided rooting for approximately 90% of cuttings of *Alnus incana*, *A. subcordata*, and *Buxus sempervirens* (Gorelov, 2010; Prokopchyk *et al*., 2018). A similar effect is observed with the natural plant growth regulator Rybav-extra® (0.00152 g/L L-alanine + 0.00196 g/L L-glutamic acid). It is a product of the metabolism of mycorrhizal fungi isolated from the root of ginseng. In addition to enhancing rooting, it stimulates the synthesis of phytohormones and growth processes, and may increase resistance to temperature stress (Gruznova *et al*., 2018).

Therefore, the objective of our study was to investigate the impact of growth regulators on the rooting of sweet and sour cherry clonal rootstocks, identify the most effective concentrations of the bio stimulants, and optimize the protocols for softwood cuttings propagation.

MATERIALS AND METHODS

The study was conducted from 2016 to 2019 at the Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine. The cuttings were cultivated under controlled conditions, using commonly accepted agronomic, physiological, and statistical methods for data collection and processing.

Plant material

The objects of the study were clonal rootstocks for sweet and sour cherry – Colt (*P. avium* F 299/2 × *P. pseudocerasus* Lind), Gisela 5 (*P. cerasus* × *P. canescens*), Krymsk® 7 (*Cerasus lannesiana* Carr), Krymsk® 5 (*Cerasus fruticose* Pall. × *Cerasus lannesiana* Carr) as a control, and Krymsk® 6 (*P. cerasus* × (*P. cerasus* × *P. Maackii*)).

For propagation were used softwood cuttings (12 cm) at the stage when the shoot growth had not yet ceased, but the basal and medial parts of it had already partially lignified, while the apical part was still herbaceous.

Root growth stimulation

Effect of various root growth stimulants at different concentrations was studied: Kornevin 1 g/L, Zircon 0.1 ml/L and Zircon 0.5 ml/L, Rybav-extra 0.1 ml/L and Rybav-extra 0.5 ml/L, IBA (control) 50 mg/L.

The basal part of cuttings (2–3 cm) was dipped in the stimulant solution for 16 hours. After treatment, the cuttings were rinsed with running tap water and planted in a peat substrate (DomoFlor Mix-4) with added perlite at a ratio of 3:1. The experiment was made in three repeats, with 15 cuttings of each rootstock in each repetition.

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The care for the cuttings during the rooting process followed standard practices (Polikarpova, 1981). The softwood cuttings were planted in plastic trays and placed in an unheated greenhouse equipped with an automatic intermittent misting system. During the rooting of the cuttings, the temperature in the greenhouse was maintained between 18 and 30°C, while the humidity level was kept between 80 and 90%.

The cuttings were removed from the substrate in late autumn (October–November). The percentage of cuttings rooting, number and length of roots, and growth of cuttings were measured.

Statistical analysis of the research data was carried out using Minitab 19 software (Minitab LLC, 2019). The data were subjected to a two-way analysis of variance after the Kolmogorov–Smirnov normality test. In order to determine significant differences between the means, Tukey's post-hoc test at $p \leq 0.05$ was used.

RESULTS AND DISCUSSION

The studied vegetative rootstocks have a high potential for propagation with softwood cuttings, while the percentage of rooting and biometric parameters depending on the rootstock, growth regulator and its concentration (Table 1).

Table 1. *Rooting and main biometric parameters of sweet and sour cherry rootstock cuttings mean for the factor "Stimulant"*

Tabulka 1. *Zakořenění a hlavní biometrické parametry podnožových řízků pro višně a třešně, průměr pro faktor „Stimulant "*

Stimulant	Rooting of the rootstocks (%)	Number of roots (pcs)	Average root length (cm)	Length of shoot growth (cm)
Zircon 0.1 ml/L	63.8±4.2 a	6.8±0.6 c	8.1±0.4 a	9.2±0.8 ab
Zircon 0.5 ml/L	53.6±5.1 c	8.7±0.8 b	8.7±0.4 a	8.6±0.8 ab
Rybav-extra 0.1 ml/L	62.3±4.7 ab	7.2±0.7 c	8.6±0.3 a	8.5±0.7 b
Rybav-extra 0.5 ml/L	65.1±4.4 a	7.1±0.6 c	8.6±0.4 a	8.4±0.6 b
Cornevin 1 g/L	57.3±4.3 bc	8.5±0.7 b	8.6±0.3 a	6.7±0.7 c
IBA 50 mg/L (control)	61.7±4.2 ab	10.0±0.6 a	8.6±0.4 a	9.3±0.8 a

Data presented as mean ± SE. Different letters within each column indicate significant difference between the mean values according to Tukey's test at p ≤ 0.05.

Rooting is the main criterion when propagating plants by softwood cuttings, because it determines the further growth and development of the plant itself. In our experiment, we observed a range of rooting from 53.6±5.1 to 65.1±4.4%. However, after statistical data processing, we can say that almost all growth regulators variants had the same positive effect on this index, except for Zircon at 0.5 ml/L concentration. It is obvious that the effectiveness of the stimulator also depends on the biological characteristics of the plant. The effectiveness of Zircon in concentrations of 1 and 1.5 ml/L exceeded the effectiveness of other stimulants, including Kornevin, when propagating thuja by green cuttings (Ivashchenko and Kozachenko, 2013). The effect of Zircon on root formation is related to the inhibition of the natural breakdown

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of auxin in the plant, minimizing oxidative damage, which ensures the maintenance of cellular homeostasis and the protection of vital cellular components from destruction (Khawula *et al*., 2024). Kornevin stimulant, applied in the form of a powder, is the most effective in restoring green plum cuttings of the group of clonal rootstocks Pumiselect, Marianna 2624, Myrobalan clonal rootstocks (Okhudzhanov, 2020). Zircon in 0.1 ml/L concentration demonstrated the highest efficiency for Gisela 5 rootstock with 47.3%±0.5 rooting of cuttings. This preparation showed the best effect for rooting *Alnus subcordata* and *Thuja plicata* (Gorelov, 2010; Ivashchenko and Kozachenko, 2013).

Control variant – IBA (indole-3-butyric acid at 50 mg/L), was also observed to have a positive effect on rooting. Its efficacy in concentrations of 10–70 mg/L were described when growth regulators were used (Kniga, 2000; Islamov *et al*., 2023). However, statistical analysis of the data did not show a difference between the control and other variants in our experiment, except for the Zircon (0.5 ml/L) variant, which decreased the rooting by 13.1% compared to the control.

Not only the percentage of rooted plants, but also the number of roots, their length and plant growth are important indices of the effective clonal rootstock propagation. Therefore, the influence of growth regulators on these parameters was additionally evaluated. When calculating the number of roots, treatment with a solution of indole-3-butyric acid at 50 mg/L concentration had the greatest effect. When using this stimulant, the average number of roots per plant was 10.0±0.6 pcs. The positive effect of this auxin was also noted on apple rootstocks and universal rose rootstocks (Stashenko, 2005; Pelekhata, 2015). A slightly lower result of 8.5±0.7 and 8.7±0.8 roots per plant was recorded for Kornevin (1 g/L) and Zircon (0.5 ml/L) variants, respectively. Other preparations had equally lower effect on the ability to form roots.

The length of the roots varied from 8.1±0.4 to 8.7±0.4 cm depending on the active substance of the stimulator, but according to the statistical analysis of the data, no significant differences were observed between the growth regulators. Similar results were obtained when measuring the length of shoot growth. The exception was Kornevin (1 g/L), with the smallest values – 6.7±0.7 cm.

In addition to the effect of the active substance of the stimulant on the parameters of the rootstock propagation, the effect of the rootstock itself was also important (Table 2).

Table 2. *Rooting and main biometric parameters of sweet and sour cherry rootstock cuttings mean for the factor "Rootstock"*

Tabulka 2. *Zakořenění a hlavní biometrické parametry podnožových řízků pro višně a třešně, průměr pro faktor "Podnož"*

Rootstock	Rooting of the rootstocks (%)	Number of roots (pcs)	Average root length (cm)	Length of shoot growth (cm)
Krymsk® 6	49.1±1.5 c	8.6±0.5 b	9.3±0.2 b	9.7±0.2 b
Krymsk® 7	90.7±1.3 a	8.4±0.4 b	10.3±0.2 a	9.6±0.2 b
Gisela 5	41.5±1.5 d	3.9±0.4 d	7.1±0.3 c	5.2±0.2 c
Colt	49.7±2.4 c	7.5±0.3 c	6.8±0.1 c	5.5±0.3 c
Krymsk® 5 (control)	72.2±1.7 b	11.8±0.3 a	9.2±0.2 b	12.1±0.8 a

Data presented as mean ± SE. Different letters within each column indicate significant difference between the mean values according to Tukey's test at p ≤ 0.05.

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The highest percentage of rooting was observed in Krymsk® 7 rootstock – 90.7±1.3%, the rooting of this rootstock exceeded Krymsk® 5 control variant, which was 72.2±1.7%. Krymsk® 6 and Colt rootstocks did not differ significantly and amounted to of 49.1±1.5% and 49.7±2.4%, respectively. The worst rooting was observed in Gisela 5 rootstock - 41.5±1.5%. However, under *in vitro* conditions, it is still possible to achieve 100% rooting of this rootstock (Kumar *et al*., 2020). The potential for hard-to-propagate genotypes is considered to be 30%, while a material yield of 50% can be considered successful. Propagation of any crop is considered economically efficient when 60% of the material can be obtained, while an indicator of 75% is highly efficient propagation (Kracíková, 1997). It is known that the rooting ability of vegetatively propagated cuttings of the *Prunus* genus is a complex and variable process that to some extent depends on the genotype (Scaltsoyiannes *et al*., 2009). Such dependence in the root-forming ability was also observed in our trial.

The largest number of roots was noted in Krymsk® 5 control variant (11.8±0.3 pcs per plant), which may indicate a strong root system capable of providing active growth of the above-ground part of the plant. In general, the rootstocks of the Krymsk group showed the same result. On the other hand, Gisela 5 showed the lowest number of roots (3.9±0.4 pcs). However, in other studies this indicator reached 11 roots per plant (Gulen *et al*., 2004).

Krymsk® 7 rootstock exhibits the longest roots among the studied variants, reaching an average length of 10.3±0.2 cm. This can contribute to more efficient water and nutrient absorption, especially important in closed soil conditions where the amount of available substrate is limited. Although the Colt rootstock was significantly inferior in this parameter – 6.8±0.1 cm. However, the rootstocks in our experiment are superior in this respect to *Prunus serotine* (Pijut and Espinosa, 2004).

Krymsk® 5 (control) rootstock demonstrated the highest shoot growth, 12.1±0.8 cm. This may indicate the vigorous growth of the plant and its potentially high productivity (Kishchak and Kishchak, 2016). On the other hand, for Gisela 5 and Colt rootstocks, the growth of the shoots turned out to be lower – 5.2±0.2 and 5.5±0.3 cm, respectively.

CONCLUSIONS

The study evaluated the influence of different types of stimulants on the rooting and growth of softwood cuttings of stone fruit rootstocks. All studied clonal rootstocks can be propagated by softwood cuttings, although the level of rooting differs. Generally, rootstock genotype had a greater effect on all the studied parameters compared to the stimulants. For all indices, the lowest values were observed in Gisela 5 and Colt (to a lesser extent), and the highest values for the rootstocks of Krymsk series. Zircon at a concentration of 0.5 ml/L showed the lowest percentage of rooting among the studied stimulants (53.6%). The control variant treated with IBA (50 mg/L) significantly (from a statistical point of view) outperformed all other preparations in terms of the number of roots on the cuttings. The variant treated with Kornevin showed by far the worst result in terms of the growth of the above-ground part of the cuttings.

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