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RESEARCH ARTICLE

The prognostication of the rooting ability of apple stem cuttings by indices of seasonal growth of shoots

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Abstract

The study involved seven species and cultivars of ornamental apple trees including *Malus × floribunda, M. halliana, M. niedzwetzkyana, M. × purpurea, M. × purpurea* 'Ola', *M. × purpurea* 'Royalty', and *M. × purpurea* 'Selkirk'. The average value of increment, average length, and duration of shoots' growth were determined to characterize the seasonal growth of *Malus* shoots. The percentages of rooted cuttings, callus formation without roots, and unviable cuttings were registered for each investigated taxon. Statistical analysis was performed in Microsoft Excel 2007 following Zaitsev (1990), and Atramentova & Utevskaya (2014).

The total duration of shoots growth of the studied species and varieties ranged from 72 (*M. × purpurea* 'Ola') to 118 days (*M. niedzwetzkyana*); annual shoots reached a length from 213.75 mm (*M. × purpurea*) to 448.75 mm (*M. niedzwetzkyana*); the average increment of shoots ranged from 3.90 mm (*M. × purpurea* 'Ola') to 14.70 mm (*M. floribunda*).

The rooting ability of *Malus* stem cuttings was limited to a reasonably short period of cutting procedure and depended on the application of biologically active substances, their concentrations, and complexes. The highest rooting rate (33.33 %) was observed in *M. × purpurea* 'Ola' after the treatment of its cuttings by 0.6 % indole-3-butyric acid (IBA) at the end of June. A slightly lower rooting rate (20.00 %) was observed in *M. halliana* cuttings treated by 0.4 % IBA and *M. × purpurea* 'Selkirk' cuttings treated by Podkorzen AB aqua at the end of June. Finally, 7.69 % of *M. × floribunda* cuttings were rooted with Podkorzen AB aqua at the beginning of July.

The prediction of the rooting ability of *Malus* stem cuttings was confirmed by a strong negative correlation between the percentage of rooted cuttings and the duration of shoots' growth (*r* = –0.88). Hence, the percentage of rooted cuttings increases with decreasing duration of shoots growth, depending on the genotype.

Keywords: *Malus*, ornamental apple, propagation, adventitious roots, duration of shoots' growth, correlation

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Introduction

Representatives of the genus *Malus* Mill. are prospective but uncommon in culture as ornamental plants. For their comprehensive introduction into production and landscaping, it is necessary to develop new technologies and adapt already known methods of their reproduction to local requirements.

In the natural conditions, apple plants mostly reproduce by seeds and only some species can form root suckers (Konopelko, 2020). At the same time, the genetic non-identity of seedlings caused by the rearrangement of chromosomes and individual genes in germ cells was observed during seed reproduction (Opalko & Opalko, 2019). Due to allogamy, successful sex reproduction of these plants can be ensured only by cross-pollination of at least 50–250 individuals of the same genotype, which is possible only in the case of sufficiently dense monospecific populations without admixture of other taxa of this genus (Opalko et al., 2004; Konopelko, 2020). That is why vegetative propagation (mainly by budding and grafting; rarely – by cuttings and microclonal reproduction) is preferred to preserve maternal traits of these plants in the conditions of introduction (Konopelko, 2020).

Propagation by stem cuttings is one of the methods of asexual propagation based on the natural ability of plants to regenerate and to produce adventitious roots from the shoot tissues (Ivanova, 1982; Bilyk, 1993; de Klerk et al., 1999; Hartmann et al., 2010). Cutting provides genetically homogeneous, anatomically and physiologically integral plants, the advantage of which is the ability to restore the aboveground system in case of damage or death (Polikarpova, 1990). Reproduction by cuttings allows avoiding rootstock suckering, crooks in the trunk, and graft incompatibility compared to traditional methods of budding and grafting (Hartmann et al., 2010).

The success of adventitious rooting depends on the physiological condition of the whole plant and its particular shoots, which change during the growing season (Bilyk, 1993). Besides this, it also depends on the environmental microclimatic conditions (such as humidity, air and substrate temperature, and light), the mechanical and chemical composition of the substrate, aeration, chemical composition of water. All these factors affect the cuttings simultaneously and jointly, determining the formation of meristematic cells in the basal part of the cuttings and photosynthetic activity of their leaves (Ermakov, 1981).

Malus belongs to the group of hardly rooting plants, and the low rooting rate of its cuttings is a biological problem (Ermakov, 1981). Investigations of the biology of mother plants, endogenous and exogenous factors of the shoots growth, and development of different rooting protocols allow segregating those genotypes that are potentially promising for propagation by cuttings (Polikarpova, 1990). Taking into account the high labor costs and general energy efficiency, it is necessary to develop the effective methods of prediction of the rooting ability, which would allow avoiding potentially ineffective cases (Opalko, 2003).

The attempts to explain the different rhizogenesis ability of plants depending on their life form, phylogeny or taxonomy were unsuccessful because among all these groups occur plants with different rooting rate (Bakhtaulova, 2020). The plants of the same genus and even closely related infraspecific taxa can demonstrate different success in formation of adventitious roots (Zhou et al., 1992).

The formation of root rudiments in dicotyledonous woody plants occurs in the stem's bark from the rays of the parenchyma. Many researchers pointed the correlation between the degrees of bark parenchymatization and rooting ability of stem cuttings (Bakhtaulova, 2020). In particular, Doud & Carlson (1977) observed a negative correlation between the percentage of cuttings' sclerification and the rooting success in *Malus* rootstocks (Amissah et al., 2008). Similarly, the correlation between the rupture in the sclerenchymatous ring and the rooting ability was also confirmed for *Quercus bicolor* Wild. and *Q. macrocarpa* Michx. cuttings (Amissah et al., 2008). The authors consider the sclerenchymatous ring as a physical barrier to cell expansion and proliferation, which is required to form root primordia. However, despite the similar sclerification, there was the higher rooting in the cuttings grown in greenhouse. During the study of the anatomical structure of *Malus sieversii* (Ledeb.) M. Roem., Bakhtaulova et al. (2015) found that the average percentage of medullar double-row rays (15.0 %) was close to the rooting rate of cuttings (12.0 %). However, application of semi-woody shoots and treat by 50 mg/l indole-3-butyric acid (IBA) allowed to increase the rooting rate in the same species to 46.7 % (Bakhtaulova, 2020). Hence, the shoot anatomy can serve as a basis for development of rooting protocols, but it is also necessary to consider other anatomical and physiological indicators ensuring rooting success.

There is a well-known method of evaluation of the quality of cuttings by starch content, which is determined by color reaction with iodine or Lugol's solution resulting in a dark blue color (Aleinikov & Mineev, 2015). The lignin content can be a criterion for the diagnosis of cuttings readiness; sufficiently developed xylem is stained with a phloroglucinol solution in a deep red color. For example, negative correlation between formation of adventitious roots and the lignification rate of shoots was found in the cuttings of different *Prunus* L. species (Polikarpova & Pilyugina, 1991).

The content of nutrients in annual shoots (cuttings) is considered as an objective assessment of the biological state of the plant, determining its ability for rooting (Besschetnova et al., 2017). High protein and nitrogen content, and low content of carbohydrates inhibit the growth of root primordia, while the excess of nitrogen provoke the cuttings rot (Polikarpova, 1990).

Seasonal growth of the shoots as a part of general plant development, is a genetically determined process reflected in its anatomical and physiological changes (Hansen, 1971; Schweingruber & Börner, 2018; Cubas, 2020). Recent molecular studies showed that plant rejuvenation in the way of manipulation on microRNA could improve the rooting rate (Yu et al., 2015; Aung et al., 2017; Ye et al., 2020). The ability to form adventitious roots decreases during the ontogenesis and is mediated by microRNA miR156, which supports juvenile traits by inhibiting the group of transcription factors SPL26 (Xu et al., 2017). Overexpression of miR156 increases the root regeneration ability (Aung et al., 2017). Although the detailed mechanism of this effect on adventitious rooting is still unclear, it has been found that the apple rootstocks under the influence of IBA have a higher expression of miR156.

Summarizing, the potential for adventitious rooting is a complex phenomenon related to anatomical, physiological and genetic parameters and affected by numerous exogenous factors.

Material and methods

Seven *Malus* genotypes (*M.* × *floribunda* Siebold ex Van Houtte, *M. halliana* Koehne, *M. niedzwetzkyana* Dieck ex Koehne, *M.* × *purpurea* (E. Barbier) Rehder, *M.* × *purpurea* 'Ola', *M.* × *purpurea* 'Royalty', and *M.* × *purpurea* 'Selkirk') grafted on the M9 rootstock, and growing in the V.V. Mitina introductory-experimental plot at the National Dendrological Park Sofiyivka of the NAS of Ukraine were used for this investigation. In the database Plants of the World Online (POWO, 2021) *M. niedzwetzkyana* is indicated as a synonym of *M. domestica* (Suckow) Borkh. However, in the World Flora Online (WFO, 2021) it is listed as an independent species. Taking into account such inconsistency, here we consider *M. niedzwetzkyana* independently following the World Flora Online.

Stem cuttings with two-three internodes, 10–15 cm long, were harvested from the shoots of current year in four periods: (1) at the beginning of June; (2) at the middle of June; (3) at the end of June; (4) at the beginning of July.

The ability to adventitious rooting was investigated in five experiment variants: (1) control; (2) the lower part of the cuttings was treated with 0.2 % w/w IBA in talc; (3) the lower part of the cuttings was treated with 0.4 % w/w IBA in talc; (4) the lower part of the cuttings was treated with 0.6 % w/w IBA in talc; (5) the lower part of the cuttings was treated with stimulator Podkorzen AB aqua, which contains cultures of live bacteria and humic components (0.2 % of 1-naphthylacetic acid, 0.1 % of IBA, and 0.1 % of ethanoic acid).

Certain quantitative traits (including the average increment of the shoot; the average length of the shoot, and duration of the increment period) were selected to characterize the seasonal growth and state of *Malus* shoots. The seasonal dynamics of shoots' growth was defined for each taxon by measuring ten annual shoots every three-four days with a ruler (with an accuracy of 1 mm) according to recommendations of Molchanov & Smirnov (1967). The daily increment was calculated and compared for different genotypes accordingly.

The following quantitative indices were used to characterize the success of adventitious rooting of stem cuttings: the percentage of rooted cuttings, the percentage of callus formation without roots, and the percentage of unviable cuttings. These indices were analyzed separately for each investigated taxon following described above five variants of treatment and four periods of the experiment. Hence, each sample consisted of 15–25 cuttings depending on the genotype and variant of the experiment. Cuttings of *M. niedzwetzkyana*, *M.* × *purpurea*, and *M.* × *purpurea* 'Royalty' did not produce roots in our experiment. Therefore, for the statistical analysis we took the maximum percentages of callusogenesis and the corresponding percentages of unviable cuttings of these taxa.

To determine the relationships between the indicators of seasonal shoot growth and the success of rooting of the stem cuttings, the paired correlation coefficients were calculated. A regression analysis was realized to establish a quantitative relationship between indicators characterized by a strong correlation. Statistical analysis was realized in Microsoft Excel 2007 software package following Zaitsev (1990) and Atramentova & Utevskaya (2014).

Results and discussion

The seasonal growth of shoots

The shoots started the growth at the beginning (*M.* × *floribunda*, *M.* × *purpurea*, and *M.* × *purpurea* 'Ola') or middle (*M. halliana*, *M. niedzwetzkyana*, *M.* × *purpurea* 'Royalty', and *M.* × *purpurea* 'Selkirk') of April. It lasted until the middle (*M.* × *purpurea* 'Ola' and *M.* × *purpurea* 'Selkirk') or end (*M. halliana* and *M. niedzwetzkyana*) of June, or was extended up to the beginning of July (*M.* × *floribunda*, *M.* × *purpurea*, and *M.* × *purpurea* 'Royalty'), depending on the genotype. The first flush of shoots growth lasted 68–92 days, with the maximum increment in the middle and end of May (3.26–6.24 mm) and the average length of newly formed annual shoots from 281.00 mm to 473.33 mm. Growth of shoots was observed for all investigated genotypes excepting *M.* × *purpurea* 'Ola'. It started mainly at the beginning of June (*M.* × *floribunda*, *M. halliana*, *M. niedzwetzkyana*, *M.* × *purpurea*, and *M.* × *purpurea* 'Selkirk'). Sometimes it began within the middle June (*M.* × *purpurea* 'Royalty'). Shoots growth ended at the beginning of July (*M.* × *floribunda*, *M. halliana*, *M.* × *purpurea*, and *M.* × *purpurea* 'Selkirk'), end of July (*M.* × *purpurea* 'Royalty'), or at the beginning of August (*M. niedzwetzkyana*). The second flush of shoots growth was characterized by 1.5 times higher average increments (2.97–9.50 mm), but 1.4 times shorter average length of shoots (127.50–323.20 mm). It ended two times faster compared to the first flush and lasted only 31–62 days.

The average duration of total shoot growth was 94.71 days (Table 1). The most extended growing period was observed for *M. niedzwetzkyana* (118 days), while the least extended – for *M.* × *purpurea* 'Ola' (72 days). The average growth value was 8.68 mm. The most significant average shoot growth was observed in *M. floribunda* (14.70 mm), while the smallest – in *M.* × *purpurea* 'Ola' (3.90 mm). Long-term annual shoots of the studied taxa on average reached 318.05 mm. The annual shoots of *M. niedzwetzkyana* were the longest (448.75 mm), and annual shoots of *M.* × *purpurea* – the shortest (213.75 mm).

The rooting ability of stem cuttings

The optimal time for cutting of ornamental apple trees was limited to a reasonably short period and coincided with the phase of attenuation or end of shoot growth at the first flush. According to our experiment, the end of June was the best time to take cuttings from *M. halliana*, *M.* × *purpurea* 'Ola', and *M.* × *purpurea* 'Selkirk'. For *M.* × *floribunda* it was the beginning of July. At this time, the shoots were most sensitive to stimulants' action. However, different genotypes reacted differently to the same biologically active components and to their different concentrations.

For *M. halliana*, the highest percentage (20.00 %) of rooted cuttings was recorded after treatment by 0.4 % IBA. The best rhizogenesis in *M.* × *purpurea* 'Ola' was achieved after application of 0.6 % IBA – as a result, 33.33 % of cuttings were rooted. The use of Podkorzen AB aqua resulted in 20.00 % rhizogenesis in *M.* × *purpurea* 'Selkirk' and

Table 1. Shoot growth characteristics of investigated *Malus* taxa.

Taxon	Growth start / end date	Growth duration, days	Daily increment of shoots, mm		The average length of shoots, mm
			min–max	M	
The first flush of shoots' growth					
M. × floribunda	05.04 / 03.07	90	0.55–11.67	5.20	473.33
M. halliana	13.04 / 26.06	75	1.11–10.56	5.03	363.33
M. niedzwetzkyana	11.04 / 23.06	74	1.25–12.13	6.24	462.50
M.×purpurea	06.04 / 06.07	92	0.00–8.84	3.26	300.00
M.×purpurea 'Ola'	06.04 / 16.06	72	0.88–11.68	3.90	281.00
M.×purpurea 'Royalty'	14.04 / 03.07	80	0.00–7.84	3.53	324.33
M.×purpurea 'Selkirk'	13.04 / 19.06	68	1.00–9.18	5.52	375.00
M ± m	n. a.	78.71 ± 3.41	n. a.	4.67 ± 0.42	368.50 ± 28.53
min–max	n. a.	68–92	n. a.	3.26–6.24	281–473.33
CV, %	n. a.	11.61	n. a.	6.45	20.49
The second flush of shoots' growth					
M. × floribunda	03.06 / 06.07	34	2.66–16.00	9.50	323.20
M. halliana	10.06 / 10.07	31	1.25–13.34	7.00	224.00
M. niedzwetzkyana	10.06 / 06.08	62	0.41–27.11	7.18	435.00
M.×purpurea	10.06 / 10.07	31	0.13–8.34	2.97	127.50
M.×purpurea 'Royalty'	10.06 / 27.07	48	0.57–10.36	5.00	240.00
M.×purpurea 'Selkirk'	06.06 / 10.07	35	0.63–13.00	6.93	242.50
M ± m	n. a.	40.17 ± 12.42	n. a.	6.43 ± 2.22	265.37 ± 42.43
min–max	n. a.	31–62	n. a.	2.97–9.50	127.50–435.00
CV, %	n. a.	30.91	n. a.	34.48	39.16
Total seasonal growth					
M. × floribunda	05.04 / 06.07	94	3.21–13.84	14.70	398.27
M. halliana	13.04 / 10.07	89	2.36–11.95	12.03	293.67
M. niedzwetzkyana	11.04 / 06.08	118	1.66–19.62	13.42	448.75
M.×purpurea	06.04 / 10.07	96	0.07–8.59	6.23	213.75
M.×purpurea 'Ola'	06.04 / 16.06	72	0.88–11.68	3.90	281.00
M.×purpurea 'Royalty'	14.04 / 27.07	105	0.29–9.10	4.27	282.17
M.×purpurea 'Selkirk'	13.04 /10.07	89	0.82–11.09	6.27	308.75
M ± m	n. a.	94.71 ± 14.33	n. a.	8.68 ± 4.55	318.05 ± 29.98
min–max	n. a.	72–118	n. a.	3.90–14.70	213.75–448.75
CV, %	n. a.	15.13	n. a.	52.43	24.94

Note. **M ± m** – the arithmetic mean and its error; **min** – minimum values; **max** – maximum values; **CV, %** – coefficient of variation; **n. a.** — not analyzed.

7.69 % rhizogenesis in M. × *floribunda*. Cuttings of M. *niedzwetzkyana*, M. × *purpurea*, and M. × *purpurea* 'Royalty' did not produce roots in our experiment (Table 2).

Correlation and regression analysis

Pairwise correlation coefficients were calculated between the percentage of rooted cuttings, percentage of the callus formation without roots, and the percentage of unviable

Table 2. The success of rooting of the stem cuttings of investigated *Malus* taxa.

Taxon	The best time to root stem cuttings	Phase of seasonal development of shoots	Experiment variant	Rooted stem cuttings,%	Callus formation, without roots, %	Unviable cuttings, %
M. × *floribunda*	beginning of July	attenuation of shoot growth	Podkorzen AB aqua	7.69 ± 0.31	23.08 ± 0.97	69.23 ± 3.11
M. halliana	end of June	attenuation of shoot growth of the first flush; maximum increment of shoots of the second flush	0.4 % IBA	20.00 ± 0.80	0.00	80.00 ± 3.20
M. niedzwetzkyana	beginning of July (callusogenesis)	end of shoot growth of the first flush; attenuation of shoot growth of the second flush	Podkorzen AB aqua	0.00	41.04 ± 1.64	58,96 ± 2.35
M. × *purpurea*	end of June (callusogenesis)	attenuation of shoot growth	0.6% IBA	0.00	22.22 ± 0.87	77.77 ± 3.03
M. × *purpurea* 'Ola'	end of June	end of shoot growth	0.6% IBA	33.33 ± 1.37	58.33 ± 2.32	8.33 ± 0.32
M. × *purpurea* 'Royalty'	end of June (callusogenesis)	attenuation of shoot growth of the first flush; maximum increment of shoots of the second flush	Podkorzen AB aqua	0.00	16.67 ± 0.63	83.33 ± 3.33
M. × *purpurea* 'Selkirk'	end of June	end of shoot growth of the first flush; maximum increment of shoots of the second flush	Podkorzen AB aqua	20.00 ± 0.78	70.00 ± 2.37	10.00 ± 0.39

cuttings on one hand and the average value of increments, the average length of shoots, and the duration of shoots' growth on other hand. This allowed determining the linkage between the rooting in the studied genotypes and the peculiarities of seasonal growth of their shoots. As a result, a strong negative correlation between the percentage of rooted cuttings and the duration of shoot growth in the studied genotypes was revealed (Table 3).

The regression analysis allowed to predict (calculate) the rooting success of the stem cuttings of investigated *Malus* genotypes according to a specific value of the growth duration of shoots (Fig. 1). As the duration of shoot growth decreases, the percentage of rooting will increase. However, since the percentage of rooted cuttings depends on a combination of endogenous and exogenous factors, the obtained value (y) should be considered only as a relative indicator of potential rooting ability (i.e., describing homogeneous group of plants under specific growing conditions). It should not be equated with the percentage of rooted cuttings.

Conclusions

The study of seasonal shoot growth can be considered a tool to predict the rooting ability of the stem cuttings in the genus *Malus*. It was confirmed by a strong negative correlation between the duration of shoot growth and the percentage of rooted cuttings, and the linear regression equation ($y = -1.055x + 108.93$) predicting that genotypes with a shorter duration of shoot growth will be characterized by the higher rooting ability. This prediction method can serve for choosing the best rooting *Malus* taxa and removing the inefficient variants.

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Table 3. Correlation between indicators of seasonal growth of shoots and rotting success of cuttings in investigated *Malus* taxa.

Indicators of seasonal growth of shoots	Correlation coefficients		
	Rooted cuttings	Callus formation	Unviable cuttings
The average increment	−0.27	−0.36	0.38
The average length of shoot	−0.21	0.13	−0.01
Duration of shoot growth	**−0.88**	−0.28	0.56

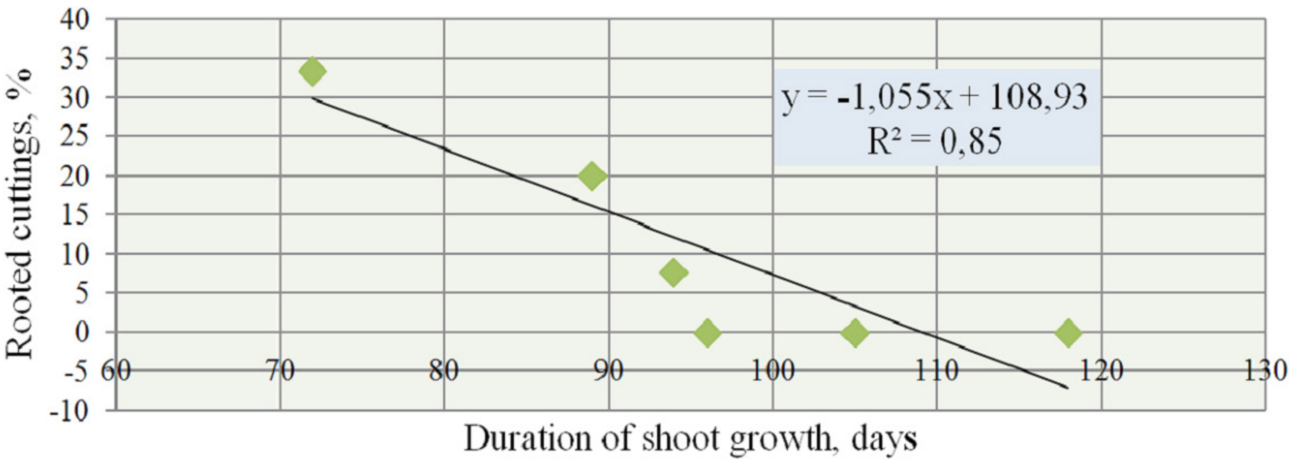

Figure 1. Relationship between rooted cuttings and duration of shoots growth in investigated *Malus* taxa.

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Прогнозування коренетвірної здатності стеблових живців яблуні за показниками сезонного росту пагонів

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До дослідження були залучені сім видів та сортів декоративної яблуні, включаючи *Malus × floribunda, M. halliana, M. niedzwetzkyana, M. × purpurea, M. × purpurea* 'Ola', *M. × purpurea* 'Royalty' та *M. × purpurea* 'Selkirk'. Для характеристики сезонного росту пагонів визначали середнє значення приросту, середню довжину і тривалість росту пагонів. Після живцювання визначали відсоток укорінених живців, відсоток живців з калюсом без коренів, а також відсоток нежиттєздатних живців окремо для кожного таксону. Статистичний аналіз проводили за методикою Зайцева (1990) та Атраментової і Утєвської (2014) за допомогою пакету програм Microsoft Excel 2007.

З’ясовано, що загальна тривалість росту пагонів досліджуваних видів та сортів становила від 72 (*M. × purpurea* ‘Ola’) до 118 діб (*M. niedzwetzkyana*); однорічні пагони після завершення росту були завдовжки від 213,75 мм (*M.× purpurea*) до 448,75 мм (*M. niedzwetzkyana*); а середній приріст пагонів складав від 3,90 мм (*M. × purpurea* ‘Ola’) до 14,70 мм (*M. floribunda*).

Коренетвірна здатність стеблових живців яблунь була обмежена досить короткими термінами проведення живцювання та визначалася застосуванням біологічно активних речовин, їх концентрацій та комплексів. Максимальний відсоток укорінення (33,33 %) був отриманий для *M. × purpurea* ‘Ola’ після обробки живців 0,6 % індоліл-3-масляною кислотою (ІМК) в третій декаді червня. Дещо нижчі показники вкорінення живців (20,00 %) спостерігали для *M. halliana* після застосування 0,4 % ІМК та для *M. × purpurea* ‘Selkirk’ після застосування Podkorzen AB aqua в третій декаді червня. Найменших показників вкорінення живців (7,69 %) було досягнуто для *M. floribunda* після використання Podkorzen AB aqua при живцюванні в першій декаді липня.

Перспектива прогнозування коренетвірної здатності стеблових живців підтверджується сильним зворотним кореляційним зв’язком між відсотком укорінених живців та тривалістю росту пагонів (r = –0,88). Відсоток укорінених живців збільшується зі зменшенням тривалості росту пагонів, залежно від генотипу.

Ключові слова: *Malus*, декоративна яблуня, розмноження, адвентивні корені, тривалість росту пагонів, кореляція