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IN VITRO PROPAGATION OF PEAR (*PYRUS* L.)

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Background. The micropropagation methods are used for the conservation of *Pyrus* L. phytodiversity, as well as for the creation of collections of the threatened species and pear cultivars including genotypes necessary for breeding and genetic studies.

Materials and Methods. The two *Pyrus* species, *P. communis*, and *P. salicifolia* Pall. as well as four pear *P. communis* cultivars, 'Bere Desiatova', 'Umans'ka Juvileina', 'Kniahynia Olga', and 'Sofia Umans'ka', were chosen as the experimental plants. Young shoots of *Pyrus* species and cultivars with apical meristem from three-year-old plants were used as primary explants. The effectiveness of sterilization was determined by the rate of sterile and viable explants. Rooted *in vitro* test tube shoots with 3–4 roots 2–5 cm long were transplanted to adapt into nutrient disks Jiffy-7 and Ellepress. The statistical analysis of the results was performed according to Ronald Fisher (2017) and Peter Bruce with co-workers (Bruce *et al*., 2020) using Statistica ver.10 (StatSoft, Inc. STATISTICA version 10.0).

Results and Discussion. According to our results, mercury dichloride ($HgCl_2$) was the most effective of all tested sterilizers of *P. communis*. The *P. communis* explants sterilization technique was extremely difficult or unsuccessful for *P. salicifolia*, probably due to the dense pubescence on shoots and buds of this species. Therefore, we used the seeds as explants for micropropagation of *P. salicifolia* according to the standard protocol. In the best variant of Murashige–Skoog (MS) medium modification for *P. communis*, where MS-276 was modified by 2.0 mg/L 6-benzylaminopurine (6-BAP) and 0.01 mg/L indole-3-butyric acid (IBA), a value of 9.50 microclones per passage was obtained. However, the most active shoot formation of *P. salicifolia in vitro* was observed in the variant where the Driver and Kuniyuki (DKW) medium was modified by adding 2.5 mg/L

6-BAP. To induce rhizogenesis, the best medium for *P. communis* and *P. salicifolia* rhizogenesis was MS-302, modified by the addition of 0.5 mg/L α-naphthaleneacetic acid (NAA). In this variant, the first root in microclones developed in 8–10 days, and after a month, rooting reached 87.50% of *P. communis* and 98.54 % of *P. salicifolia*. The efficiencies of both *Pyrus* species adaptation were better with Ellepress peat plugs, than with Jiffy-7 peat pellets.

Conclusion. It is recommended to cultivate explants of *P. communis* on MS medium with the addition of 2.0 mg/L 6-BAP and 0.01 mg/L IBA, and *P. salicifolia* on DKW medium with the addition of 2.5 mg/L 6-BAP and 0.1 mg/L IBA. It is also recommended that MS medium with the addition of 0.5 mg/L NAA be used for rooting of the microclones of both *Pyrus* species, and Ellepress peat plugs for the adaptation of regenerated plants.

Keywords: *Pyrus communis* L., juvenile phase, seed reproduction, micropropagation, proliferation, *ex vitro* acclimatization

INTRODUCTION

The pear (*Pyrus communis* L.) is one of the species of the pome fruit group, traditionally classified into the genus *Pyrus* L. from the large and quite polymorphic family *Rosaceae* Juss. (Yamamoto & Terakami, 2016). However, controversies regarding this species, concerning its placement in intermediate (between family and genus) taxonomic categories continue to exist. Such taxonomic instability is due to several reasons, among which the incompleteness of the Rosaceae family system can be considered the most important. It is currently being revised by the flower and fruit structure as well as other morphological characteristics, in light of the latest results of molecular phylogenetic. Synonymy, which in some cases goes beyond the intra-generic confines, also complicates the Rosaceae family study. (Opalko *et al*., 2022).

The cultivated pears, namely the European common pear (*Pyrus communis*) cultivars, as well as the Oriental pear species, *Pyrus pyrifolia* (Burm.f.) Nakai, and *Pyrus bretschneideri* Rehder cultivars, are very important pomaceous fruits of temperate regions (*Pyrus*..., 2024; Simionca Mărcășan *et al*., 2023). The common pear (*Pyrus communis*) is the second most important pome fruit crop in Ukraine, second only to the apple tree in terms of share in the structure of industrial plantations. This is due to their high winter hardiness, and many biological and economic benefits. Pear fruits are consumed fresh and dried and are also used in the non-alcoholic, alcoholic beverages and other food products, such as juices, syrups, jellies, fruit candy, jams, extracts, fillings, kvass, etc. (Delpino-Rius *et al*., 2019; Opalko *et al*., 2022).

On the territory of Ukraine, the genus *Pyrus* is represented by 28 species, 25 of which are alien. The National Dendrological Park “Sofiyivka” has the largest collection of species of this genus in Ukraine, which includes 19 species, 7 of which are listed as rare and endangered (Opalko *et al*., 2022).

Pyrus seedlings, that have been grown from a seed, are characterized by a rather long (up to four to six years), although shorter by one to four years than *Malus*, juvenile stage of development, during which the plants grow, increase in size, but do not bear fruit. The generative stage of perennial woody plant development is triggered by the floral promoter, a gene whose expression can be modified to some extent by endogenous

and environmental signals such as plant age, endogenous and exogenous growth regulators, temperature, photoperiod, etc. (Chevreau *et al*., 2020; Kotoda, 2021; Pan *et al*., 2023; Wang & Ding, 2023).

The end of the juvenile period is defined by the achievement of the ability to flower, and the actual formation of fruits with seeds is the first indication that the plant has reached the generative phase. However, the end of the juvenile period and the beginning of flowering may not coincide in date. After the juvenile stage, seedlings do not necessarily flower. The formation of generative organs and subsequent flowering can be blocked by many factors, even when the plants reach the ability to flower. Scott Poethig refers to this stage of development of perennial woody plants as a transitional period or "*adult vegetative phase*". It is during "*adult vegetative phase*" that most methods of stimulating plants to flower can be successfully applied (Poethig & Fouracre, 2024).

Various methods of plant propagation by rooting cuttings in a fruit nursery are described in many sources, both classical (Hartmann & Kester, 2014) and modern (Ak *et al*., 2021; Habibi *et al*., 2022; Roberto & Colombo, 2020). However, in the practice of introducing alien woody plants, as well as in horticulture and landscape gardening, summer and autumn grafting with an eye (budding) and spring grafting with cuttings are mostly used (Adhikari *et al*., 2022; Hartmann & Kester, 2014), including for *Pyrus* (Machado *et. al*., 2017; Mudge *et. al*., 2009; Riya *et. al*., 2023), and even *in vitro* grafting (Hassanen, 2013).

In the fundamental review of the history of grafting, Ken Mudge and his colleagues describe quite sophisticated ancient grafting methods. Moreover, along with the relevant descriptions of the specifics of the grafting technique, there were ambiguous reports on the dependence of pear fruit quality on the rootstock. So, in the sixth century, Jia Sixie, who lived in China between 500 and 600 A.D., wrote not only about the importance of selecting a rootstock when creating an orchard as a decision that would affect the health of the tree and the yield over the life of both the tree and the orchard proper, but also pointed out the advantages of pear fruit quality from cultivars grafted onto ‘‘tang’’. In particular, Jia Sixie wrote that on the trees of pear cultivars grafted onto ‘’Tang’’ (modern researchers assume that it was *P. phaeocarpa* Rehder or *P. betulifolia* Bunge), the fruits were larger and tastier than those on the rootstock ’’Du’’ (we can assume that it was *P. calleryana* Decne. or *P. ussuriensis* Maxim. ex Rupr.). Back then, pear cultivars were grafted onto these rootstocks. Quince, now named *Cydonia oblonga* Mill., was known as early as the 17th century as a dwarf rootstock for pears. In particular, espalier pear cultivars grown for fresh consumption already back then were recommended to be grafted onto quince (Mudge *et al*., 2009).

The problems of introducing own-rooted vegetative propagation in industrial nurseries for pears, as well as other woody fruit plants, remain unresolved to this day, despite the advantages of such cultivars. However, the trend toward planting stock unification not only in the nurseries but also in the operation of commercial orchards encourages nurseries to propagate pear cultivars on propagated vegetative, clonal rootstocks that have already proven their advantages on apple trees. The transition of fruit tree planting stock production to the clonal rootstocks has led to the threat of the spread of viral (and not only viral) infections, epigenetic changes, and raised the problem of increasing the reproduction rates of rootstocks. These and other problems of vegetative propagation are proposed to be solved by apomixis (Feng *et al*., 2023) and micropropagation (Kaviani *et al*., 2023; Pandey *et al*., 2024).

Somatic embryogenesis and organogenesis as the basis of *in vitro* systems for propagation and preservation of many ornamental and fruit crops have already been used quite successfully, including cases with *Pyrus* spp. (Chevreau *et al*., 2020; Kotb *et al*., 2020). A group of researchers led by Barbara M. Reed has developed a highly successful technology for *Pyrus* spp. that includes the introduction, proliferation, *in vitro* rooting, and *ex vitro* acclimatization of the obtained plants (Reed *et al*., 2013). They found that the initiation of shoot formation and growth *in vitro* is most successfully achieved from buds that are in a state of forced dormancy. Such buds lend themselves well to sterilization and are characterized by significant morphogenic potential. Although micropropagation of pear species and cultivars is performed on some well-known media, the most commonly used are various modifications of the basic Murashige–Skoog (MS) medium (Murashige & Skoog, 1962). The above-mentioned studies reported that the proliferation and growth of pear shoots occurred best in a special medium developed by them for *Pyrus* with higher concentrations of calcium chloride, potassium phosphate, and magnesium sulfate than in the basic MS medium, and with the addition of 4.4 μM N^6-benzyladenine. Test-tube shoots of *Pyrus* spp. often do not root well, but a 5 s dip in 10 mM indole-3-butyric acid (IBA) or naphthalene acetic acid before transplanting into basal medium without growth regulators has proven effective for many genotypes. The micropropagation of old pear cultivars, as well as old cultivars of other perennial woody plants is highly challenging. Most authors, starting with Charles Darwin himself, who expressed the belief that the reproductive potential of juvenile animals is much higher than that of adult animals (Darwin, 1868), emphasize significant advantages in the regenerative potential of the animals and plants in their juvenile age (Chevreau *et al*., 2020; Hartmann & Kester, 2014; Isah, 2023; Krivmane *et al*., 2023).

When it is necessary to introduce *in vitro* explants from old cultivars-clones of perennial woody plants that have long since left the juvenile age (after several, even many years of fruiting), it is sometimes useful to grow grafted trees of these clone cultivars in a greenhouse as mother plants for explants cutting. Strong pruning, and bedimming the shoots of mother plants before cutting explants for their etiolation, etc. also helps. It has also been found that grafting onto one-year-old seedling rootstocks partially restores the juvenile state of the grafted cultivar and promotes *in vitro* engraftment of buds from cuttings grafted onto seedlings (Opalko & Ryabovol, 2000; Reed *et al*., 2013).

This study aims to improve micropropagation protocols for some species and cultivars of *Pyrus*. This will help to develop more efficient technologies in botanic collection conservation of specimens and their propagation for breeding purposes.

MATERIALS AND METHODS

Plant materials. In the study, two *Pyrus* species, *P. communis*, and *P. salicifolia* Pall. as well as four pear *P. communis* cultivars, ‘Bere Desiatova’, ‘Umans’ka Juvileina’, ’Kniahynia Olga’, and ’Sofia Umans’ka’, were chosen as the experimental plants. Young shoots of *Pyrus* species and cultivars with apical meristem from three-year-old plants were used as primary explants.

Explant sterilization. Sterilization of the starting material was performed according to the conventional method (Melnichuk *et al*., 2003). To increase the effectiveness of the main sterilization agent, a stepwise sterilization scheme was applied. Before treatment with the main sterilization agents, the excised shoots were washed thoroughly with

soapy water, then treated with 70% ethanol for 1 min, and then washed with distilled water, and only then by appropriate sterilization agents.

For pear tissue culture initiation, three sterilization agents were tested: sodium hypochlorite (NaOCl) in a concentration of 2.5%; mercury dichloride ($HgCl_2$) 0.1%; and silver nitrate ($AgNO_3$) in a concentration of 1.0% for 2.5, 5 and 10 min exposure. To improve the effectiveness of the sterilizing reagents, the emulsifying agent Tween 80 that adds better performance at lower usage was added to the solution of each of them (Kucher, 2012). After removing the sterilizer residues, the sterilized explants were placed on the modified MS nutrient medium (Murashige & Skoog, 1962). The rate of sterile and viable explants determined the effectiveness of sterilization.

Shoot regeneration, maintenance, and rooting. The capacity for proliferation and to morphogenesis of *P. communis* and *P. salicifolia* as well as four pear *P. communis* cultivars was examined on eleven variants of modified nutrient media according to Murashige–Skoog (MS), Driver and Kuniyuki (DKW), and Lloyd and McCown (WPM) (Driver & Kuniyuki, 1984; Lloyd & McCown, 1980; Murashige & Skoog, 1962). Cultures were grown at 24±2 °C under a 16-h photoperiod with an average of 80 to 90 % relative air humidity. To induce *in vitro* rhizogenesis, we used a basic MS medium with a halved content of mineral salts and without growth regulators, as well as some modified MS media with NAA, IBA, and indole-3-acetic acid (IAA) in different proportions.

Acclimatisation and hardening. Rooted *in vitro* test tube shoots with 3–4 roots of 2–5 cm in length were transplanted to adapt to non-sterile conditions *ex vitro* under natural autotrophic nutrition into Jiffy-7 peat pellets and Ellepress peat plugs based on sphagnum peat with the addition of a hydrogel. The plants planted in the nutrient disks were cultivated in the glass adaptation chamber, which was left closed for two days to maintain a consistently high air humidity. After two days, the chambers were gradually opened, thereby reducing the air humidity to 70–60 % and promoting the plants to adapt to conditions with lower air humidity. After 7–10 days, when the plants started to grow, the chambers were opened, and the plants gradually adapted to the ambient conditions.

Statistical analysis. The statistical analyses of the results were performed according to Ronald Fisher (2017) and Peter Bruce with co-worker guidelines (Bruce *et al*., 2020) using a two-way ANOVA and were conducted using Statistica ver.10 (StatSoft, Inc. STATISTICA version 10.0).

RESULTS AND DISCUSSION

Explant sterilization. According to our results, mercury dichloride ($HgCl_2$) was the most effective of all tested sterilizers of *P. communis* plant material. The contamination rate of explants sterilized in 0.1 % mercury dichloride for 5 min was the lowest, and the survival rate of explants introduced was the highest (**Fig. 1**). The phenomenon of direct organogenesis was observed in 75.7–86.9 % of explants sterilized with mercury dichloride. In the variants of sterilization with silver nitrate ($AgNO_3$), this rate was 24.9–32.2 %, and for sodium hypochlorite (NaOCl) – 24.6–45.7 %.

When mercury dichloride and silver nitrate were used, the sterilization efficiency rates were approximately at the same level, with a small 0.8–4.8 % advantage of mercury dichloride treatment. Increasing the exposure to sterilization in all variants increased the yield of sterile explants, but at the same time decreased the survival rate of explants introduced. Of all the sterilization options performed, sodium hypochlorite was the least effective. Under 5–10 minute exposures, 38.6–47.4 % of sterile explants were obtained,

and when the exposure period was reduced to 2.5 minutes, all explants were infected. When sterilized with sodium hypochlorite with a 10-minute exposure, the survival rate of the *P. communis* explants was half the variant with a 5-minute exposure. The explant sterilization techniques, which were used in *P. communis* micropropagation, turned out extremely difficult or unsuccessful *for P. salicifolia*. In the exposures that were successful for disinfection of other *Pyrus* species, all explants of *P. salicifolia* were infected, probably due to the dense pubescence on shoots and buds of this species. A wide range of fungi, yeast, bacteria, viruses, and other contaminants are difficult to remove from this pubescence. With an increased exposure to sterilization, all explants died from the toxic effects of the sterilizers. When using segments of semi-lignified shoots with a bud as explants, it was possible to obtain some sterile explants, but during subsequent cultivation, no developmental activation occurred. Therefore, we used the seeds as explants for micropropagation of *P. salicifolia*. The smooth surface of the seeds favors the removal of sources of infection before introduction to the culture medium. However, the seeds of *P. salicifolia*, like the rest of *Pyrus* and most fruit crops, are dormant after ripening and cannot germinate without stratification. Therefore, seeds sterilized according to the standard protocol with 0.1 % mercury dichloride for 5 min were introduced into test tubes with the basal medium (Murashige–Skoog inorganic medium). After such seed sterilization, 97 % of sterile seeds were obtained.

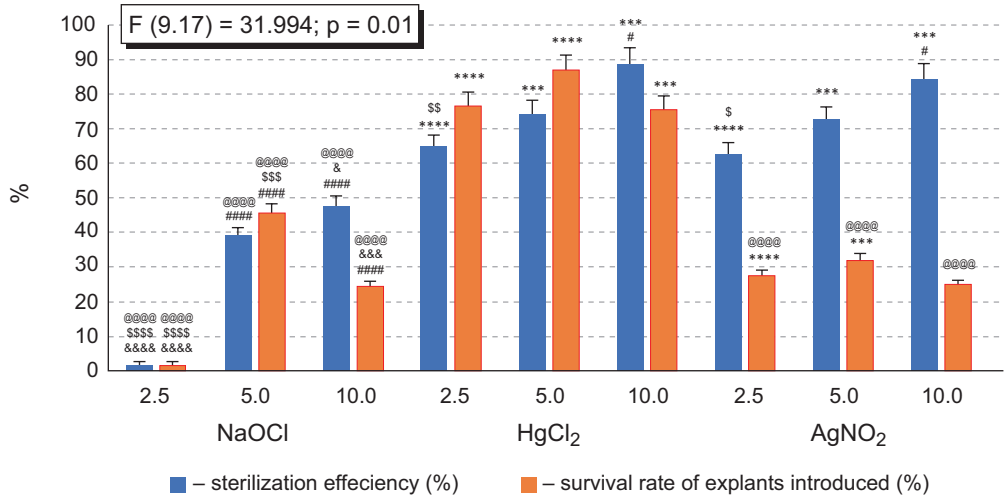


Fig. 1. *P. communis* explants sterilization efficiency (on average for the experiment)

Notes: * – p<0.05, ** – p<0.01, *** – p<0.001 – significant compared to the same NaOCl concentration; # – p<0.05, ## – p<0.01, ### – p<0.001 – significant compared to 2.5 min exposure to the selfsame sterilization agent; & – p<0.05, && – p<0.01, &&& – p<0.001 – significant compared to 5.0 min exposure to the selfsame sterilization agent $ – p<0.05, $$ – p<0.01, $$$ – p<0.001 – significant compared to 10.0 min exposure to the selfsame sterilization agent; @ – p<0.05, @@ – p<0.01, @@@ – p<0.001 – significant compared to the same $HgCl_2$ concentration

The sterilized seeds were placed in a cold store at +5 °C, that were stratified in test tubes. On the 99th day of stratification, the seeds began to germinate. Over the next three days, the number of germinated seeds reached 28 %, after which the germination of new seeds stopped. The test tubes with germinated seeds were transferred to the

culture room, where shoots and roots continued to grow, providing material for the next proliferation. After the appearance of 3–4 true leaves, the tops of the seedlings were separated from the root and transplanted to the nutrient medium for further propagation.

Shoot regeneration, maintenance, and rooting. The ways to improve the composition of the culture medium suitable for *in vitro* induction and activation of explant development, proliferation, stimulation of the morphogenesis of *P. communis* and *P. salicifolia*, as well as fruit cultivars of *P. communis* 'Bere Desiatova', 'Umans'ka Juvileina', 'Sofia Umans'ka' and 'Kniahynia Olga' depending on the concentration of phytohormones, and rhizogenesis of explants depending on the concentration of auxins were studied.

The classical proliferation protocol used in our studies with specimens of the genus *Pyrus* provided the possibility of microcloning in all variants of modification of the studied base media; however, successful microcloning with rates of more than 5 microclones per passage was characterized by species specificity. The proliferation of *P. communis* explants was the best with MS-276 medium, in which the basic Murashige–Skoog medium was modified by the addition of 2.0 mg/L 6-benzylaminopurine (6-BAP) and 0.01 mg/L IBA. The average multiplication rate of *P. communis* on this medium was 9.5. That is, an average of about 10 microclones were obtained from each planted explant during one passage (**Fig. 2**).

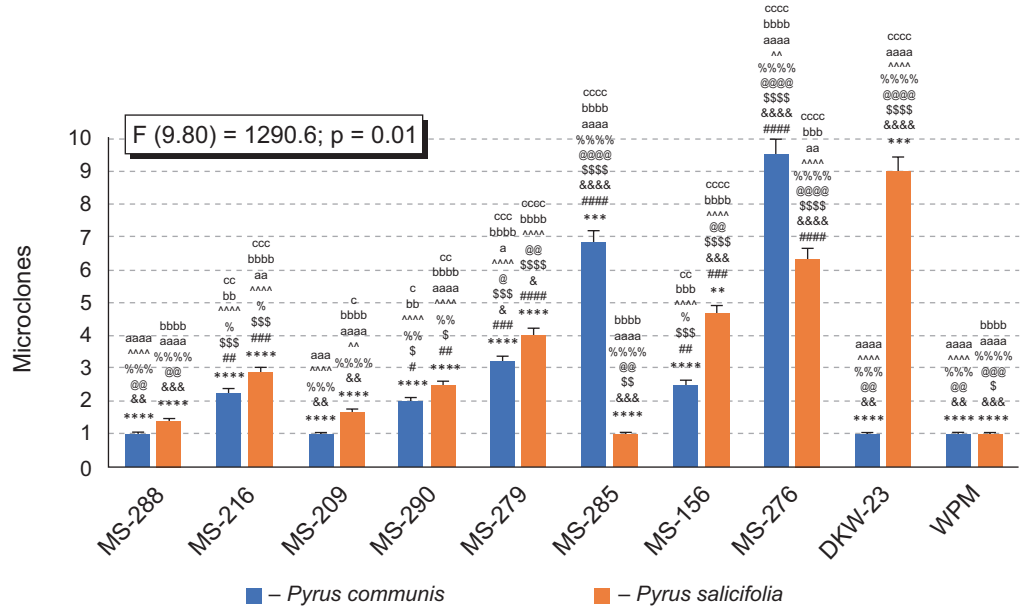
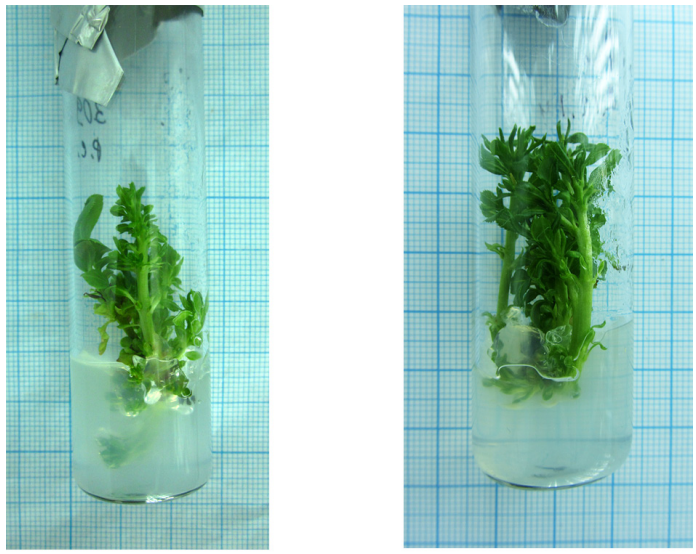


Fig. 2. Efficiency of *P. communis* and *P. salicifolia* proliferation *in vitro*

Notes: * – p<0.05, ** – p<0.01, *** – p<0.001 – significant compared to MS-276; # – p<0.05, ## – p<0.01, ### – p<0.001 – significant compared to з MS-288; & – p<0.05, && – p<0.01, &&& – p<0.001 – significant compared to з MS-216; $ – p<0.05, $$ – p<0.01, $$$ – p<0.001 – significant compared to MS-209; @ – p<0.05, @@ – p<0.01, @@@ – p<0.001 – significant compared to MS-290; % – p<0.05, %% – p<0.01, %%% – p<0.001 – significant compared to MS-279; ^ – p<0.05, ^^ – p<0.01, ^^^ – p<0.001 – significant compared to MS-285; a – p<0.05, aa – p<0.01, aaa – p<0.001 – significant compared to MS-156; b – p<0.05, bb – p<0.01, bbb – p<0.001 – significant compared to DKW-23; c – p<0.05, cc – p<0.01, ccc – p<0.001 – significant compared to WPM

The rates of more than 5 microclones of *P. communis* per passage were achieved in the variants MS-285 (with a rate of 6.85 microclones per passage), where 1.0 mg/L 6-BAP was added to the basic MS medium and MS-156 (5.25 microclones per passage), in which the basic MS medium was modified by adding 2.0 mg/L 6-BAP. Instead, in other variants of MS media modification and in all variants of DKW and WPM media modification, the multiplication rates were significantly lower. In the best variant of MS medium modification for *P. communis* (MS-276), the highest rate of all MS media was also obtained for the propagation of *P. salicifolia* (**Fig. 3**) with a value of 6.33 microclones per passage.



Pyrus communis *Pyrus salicifolia*

Fig. 3. Proliferation of *P. communis* and *P. salicifolia* with MS-276 medium

However, the most active shoot formation of *P. salicifolia in vitro* was observed in the variant where the DKW medium was modified by adding 2.5 mg/L 6-BAP.

The capacity for morphogenesis of *P. communis* cultivars was studied on six variants of culture media, previously selected experimentally, which provided a multiplication factor of more than two. Shoots with apical meristem began to develop within 10–15 days when cultivated on these media. The explants were transferred to the fresh culture media once a month. The multiplication rate after the first passage was zero, and during the second passage, proliferation of adventitious buds was observed. During subsequent passages, explants formed conglomerates of buds and shoots. Conglomerates of buds and shoots in the first passages were not divided into separate micro-clones, but transferred in large parts to accelerate reproduction. Evaluation of the effectiveness of the media and calculation of the multiplication rates were carried out after the fourth passage (see **Table**).

When explants were cultivated on MS-251 medium, the multiplication rate of all cultivars was in the range of 1.19–4.65 microclones per passage. In the variant with MS-203, an increase in the multiplication rate to 3.70–5.86 was observed. On MS-227 medium with the addition of 1.0 mg/L 6-BAP and 0.08 mg/L 2,4-D, the explants of ‘Bere

Desiatova' cultivar had a rather high multiplication rate of 8.68, and on MS-213 medium the addition of 1.5 mg/L 6-BAP contributed to an increase in the multiplication rate of 'Kniahynia Olga' cultivar to 7.91 and 'Umans'ka Juvileina' cultivar to 7.86. When explants of 'Sofia Umans'ka' were cultivated on MS-238 medium with the addition of 2.0 mg/L 6-BAP and 0.01 mg/L IAA, the multiplication rate was 8.50. The composition of the MS-226 medium reduced the yield of microclones in all studied cultivars to 3.93–4.71 microclones per passage.

The efficiency of *P. communis* cultivars proliferation on modified MS media (microclones average number per passage)

Cultivar	Nutrient medium					
	MS-251	MS-203	MS-227	MS-213	MS-238	MS-226
'Bere Desiatova'	4.65	5.86	8.68	8.53	7.42	4.46
'Kniahynia Olga'	1.19	3.70	6.85	7.91	5.77	4.33
'Umans'ka Juvileina'	1.22	4.45	6.33	7.86	6.79	3.93
'Sofia Umans'ka'	2.13	4.83	7.69	7.75	8.50	4.71
LSD_{05}	0.34	0.39	0.44	0.50	0.47	0.29

The 18-24-day period of explant cultivation in the media, selected as a result of the experiment, ensured active growth of both central and additional adventitious shoots. Within the next 25–35 days, 2 to 8 shoots were formed. The explants were transplanted after 35–50 days. For this purpose, micro-shoots 3–6 cm long were separated from the mother explants and cut into parts about 2–3 cm long. Thus, it was found that for the optimal morphogenesis processes of the explants with 'Bere Desiatova' cultivar, the most effective was the medium with the addition of 1.0 mg/L 6-BAP and 0.08 mg/L 2,4-D; for the cultivars 'Kniahynia Olga' and 'Umans'ka Juvileina' – the medium with the addition of 1.5 mg/L 6-BAP, and for the propagation of the cultivar 'Sofia Umans'ka', it was necessary to add 2.0 mg/L 6-BAP and 0.01 mg/L IAA to the medium.

Growth and development of *Pyrus* explants *in vitro* under different light conditions. The formation of the largest number of shoots was observed in the variant with an illumination intensity of 1500 lux; in particular, explants of *P. communis* developed 5.7 shoots on average, and *P. salicifolia* developed 3.3 shoots (**Fig. 4**).

In the variants with 1000 lux and 2000 lux illumination, 4.6 and 4.4 shoots were formed in *P. communis* explants, respectively. The smallest number (2.9 shoots) was formed by explants cultivated at a light intensity of 2500 lux. At the lowest light intensity of 500 lux, explants of *P. communis* formed an average of 3.2 shoots.

P. salicifolia explants cultivated at an illumination intensity of 1500 lux formed the largest number of shoots (3.3) from one explant. The explants of this species formed an equal number of shoots (2.3) at 500 lux and 1000 lux, while cultivated at an illumination intensity of 3000 lux, they formed the smallest number of shoots – 1.6.

The highest rates of apical growth of shoots in *P. communis* explants were obtained at light intensities of 2500 lux (2.6 cm) and 2000 lux (2.1 cm). In other variants, the apical growth of explants of this species did not exceed 1.2–1.6 cm (**Fig. 5**).

In *P. salicifolia*, the highest rate of shoot apical growth was obtained at illumination intensities of 2000 lux (2.9 cm), 1500 lux (2.8 cm), and 1000 lux (2.5 cm). In the other illumination variants, the length of shoot growth was within 1.6–2.1 cm.

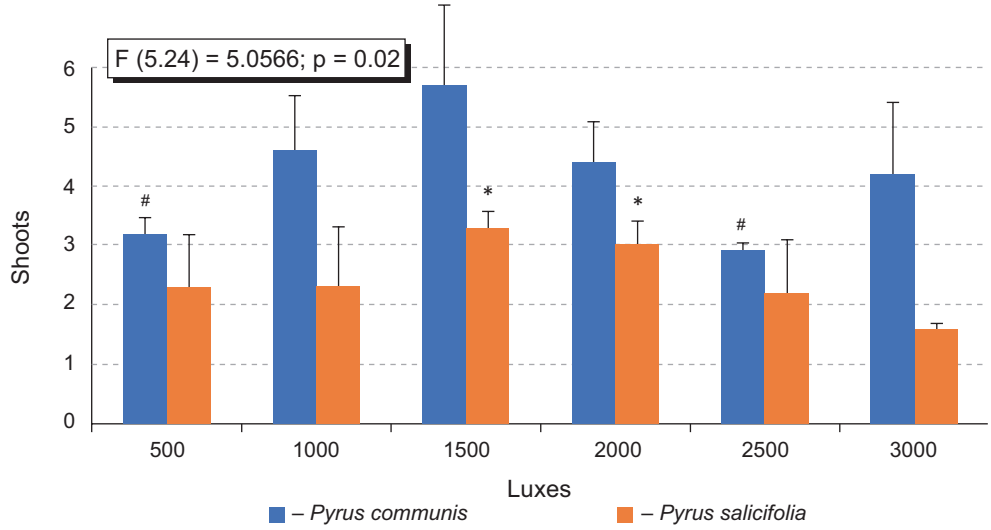


Fig. 4. The average number of *P. communis* and *P. salicifolia* shoots formed *in vitro* under different illumination intensities

Notes: * – p<0.05, ** – p<0.01, *** – p<0.001 – significant compared to 3000 lux illumination *P. salicifolia* explants; # – p<0.05, ## – p<0.01, ### – p<0.001 – significant compared to 1500 lux *P. communis* explants illumination

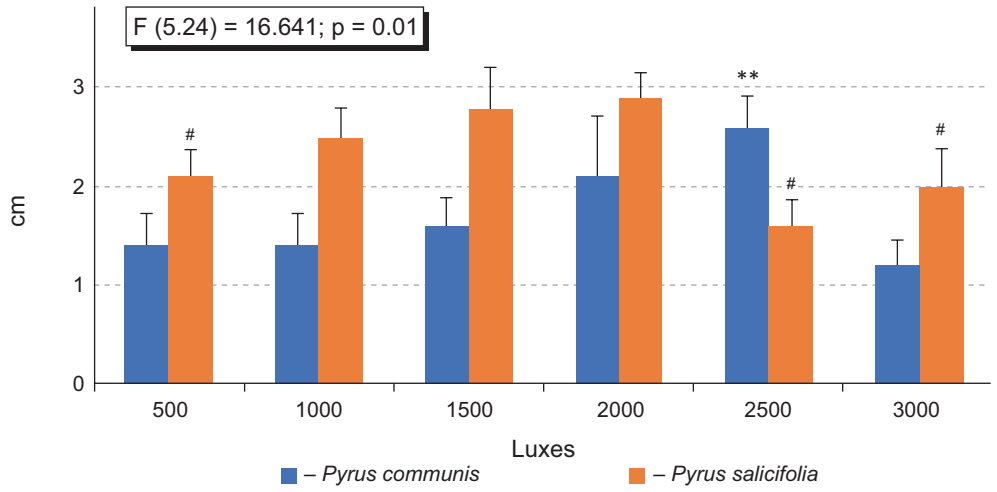


Fig. 5. The average apical growth of *P. communis* and *P. salicifolia* shoots formed *in vitro* under different illumination intensities

Notes: * – p<0.05, ** – p<0.01, *** – p<0.001 – significant compared to 3000 lux illumination *P. salicifolia* explants; # – p<0.05, ## – p<0.01, ### – p<0.001 – significant compared to 1500 lux *P. communis* explants illumination

According to the results of the multiplication ratio (9.1 and 9.2 microclones), the variants with illumination intensities of 1500 lux and 2000 lux were the best for the growth and development of *P. communis* explants (**Fig. 6**).

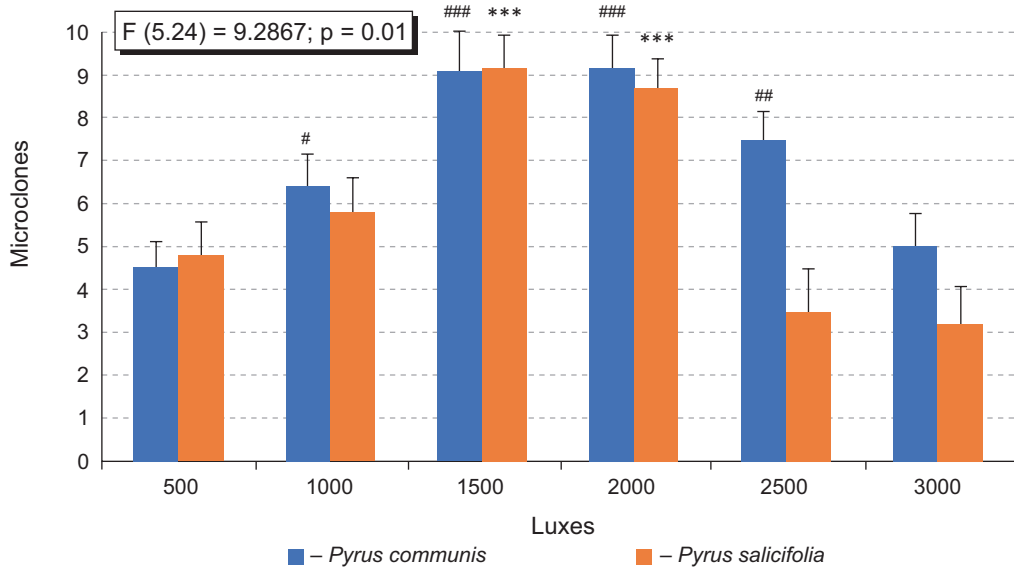


Fig. 6. The average number of *P. communis* and *P. salicifolia* microclones obtained *in vitro* under different illumination intensities

Notes: * – p<0.05, ** – p<0.01, *** – p<0.001 – significant compared to 3000 lux illumination *P. salicifolia* explants; # – p<0.05, ## – p<0.01, ### – p<0.001 – significant compared to 1500 lux *P. communis* explants illumination

Even though in the variant with 2500 lux illumination, the number of shoots was the lowest in the experiment (2.9 shoots), the length of the formed shoots was the highest (2.6 shoots), resuting in a fairly high multiplication factor of 7.5 microclones. The lowest multiplication coefficient was in the variants with the lowest (500 lux) and the highest (3000 lux) light intensity – 4.5 and 5.0 microclones, respectively.

For *P. salicifolia* with the multiplication rate (9.2 and 8.7), the variants with illumination of 1500 lux and 2000 lux were also beneficial for the growth and development of explants. Under 1000 and 500 lux illumination, this value was 5.8 and 4.8, respectively. And it was the lowest for the variants with the highest illumination intensity of 2500 lux (3.5) and 3000 lux (3.2).

Thus, the optimal light intensity for the growth and development of explants of *P. communis* and *P. salicifolia* was in the range of 1500–2500 lux, which provided the highest reproduction rate.

Rhizogenesis. To induce rhizogenesis, the basic MS medium was modified with auxin acids in different amounts. The best medium for *P. communis* and *P. salicifolia* rhizogenesis was MS-302, modified by the addition of 0.5 mg/L α-naphthaleneacetic acid (NAA). In this variant, the microclones developed the first roots within 8–10 days. On this medium, after a month, rooting reached 87.50 % for *P. communis* and 98.54 % for *P. salicifolia* (**Fig. 7**).

Fairly high rooting rates (71.43 % and 88.89 %) of these species were also obtained with MS-301 medium, modified with 0.5 mg/L of IBA. Reducing the amount of IBA to

0.25 mg/L in the nutrient medium reduced the rooting rate of *P. communis* by 4.98 % and *P. salicifolia* by 23.51 %. The better rooting of *P. salicifolia* microshoots (66.67 %) was obtained with 0.5 mg/L of IAA (MS-237). In *P. communis*, 44.44 % of rooted plants were obtained on this modification. However, on all the above-mentioned media, test tube plants suitable for transplanting for adaptation to non-sterile *ex vitro* conditions were obtained.

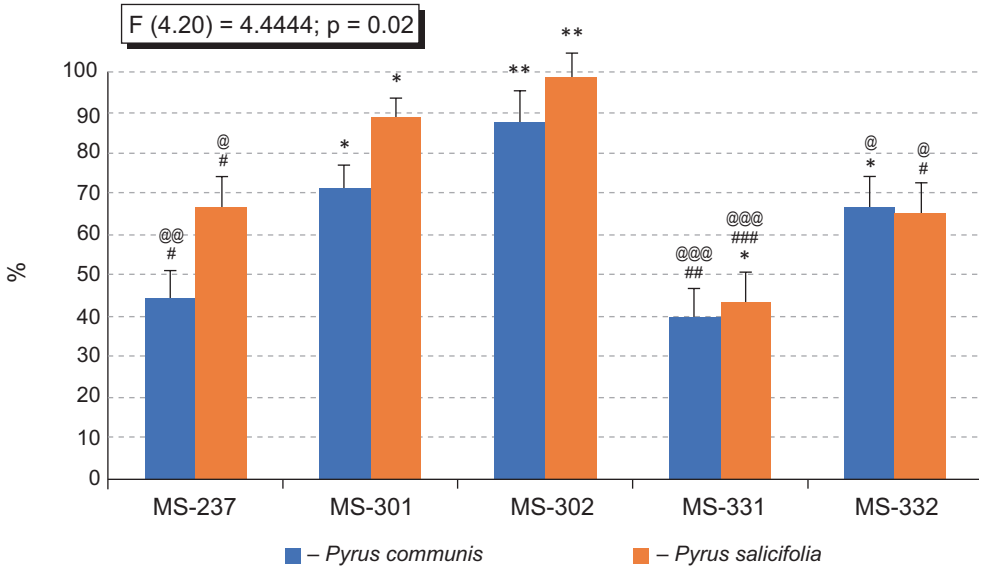


Fig. 7. The average rate of *P. communis* and *P. salicifolia* rooting, %
Notes: * – p<0.05, ** – p<0.01, *** – p<0.001 – significant compared to MS-237; # – p<0.05, ## – p<0.01, ### – p<0.001 – significant compared to MS-301; @ – p<0.05, @@ – p<0.01, @@@ – p<0.001 – significant compared to MS-302

With an increased IAA content up to 1 mg/L, the intensity of rhizogenesis in both species was the lowest (39.62 % and 43.47 %). In addition, when cultivated on MS-331 nutrient medium, along with root formation, the growth of a loose callus mass in the lower part of the shoot was observed.

The *P. communis* cultivars 'Kniahynia Olga' and 'Sofia Umans'ka' were rooted relatively more slowly than the studied natural *Pyrus* species on almost all the studied variants of the modified nutrient media. Only with the medium MS-237, the rooting efficiency of cultivar 'Sofia Umans'ka' exceeded the results of *P. salicifolia* by 2.56 % and *P. communis* by 24.79 %. At the same time, rooting in 'Kniahynia Olga' on this medium was the lowest in the experiment – 18.67 % (**Fig. 8**).

The average rooting rates of both cultivars with the medium MS-302 were about 60 %, and for 'Kniahynia Olga' this medium was the best. With the media MS-301, and MS-332, the rooting efficiencies of 'Kniahynia Olga' were 45.45 % and 41.67 %, and of 'Sofia Umans'ka' the results were 38.93 % and 34.71 %. On MS-331 medium with an increased IAA content up to 1.0 mg/L about 30 % of microshots of both cultivars were rooted. At the same time, the growth of loose callus mass in the lower part of the shoot was observed on both rooted and non-rooted shoots.

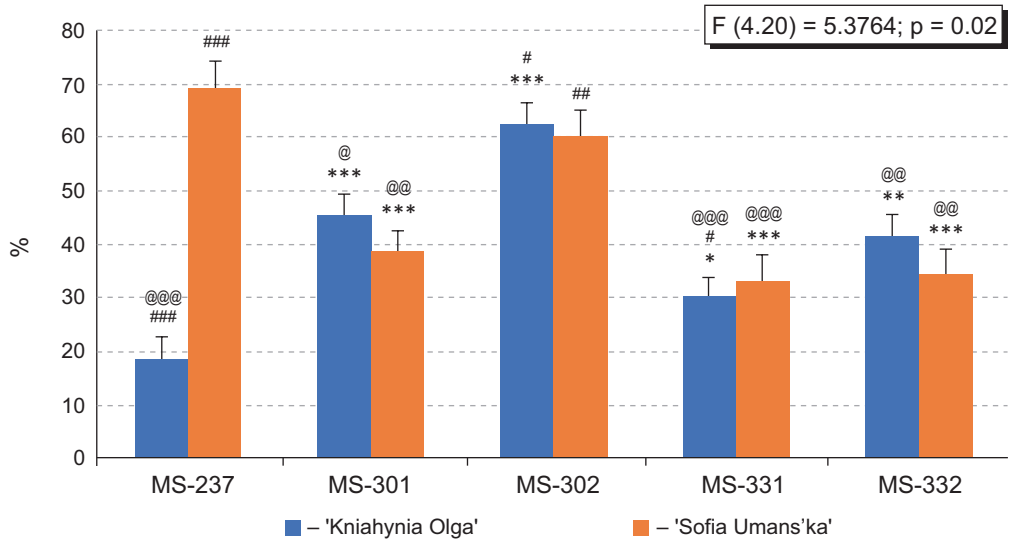


Fig. 8. The average rate of *P. communis* cultivars rooting, %
Notes: * – p<0.05, ** – p<0.01, *** – p<0.001 – significant compared to MS-237; # - p<0.05, ## – p<0.01, ### – p<0.001 – significant compared to MS-301; @ – p<0.05, @@ – p<0.01, @@@ – p<0.001 – significant compared to MS-302

The adaptation of rooted tube plants to *ex vitro* conditions. The obtained *in vitro* rooted test tube plants of the studied *Pyrus* species with 3–4 roots of 2–5 cm in length were transplanted for adaptation to non-sterile conditions *ex vitro* under the conditions of natural autotrophic nutrition with substrates of light sphagnum peat with the addition of hydrogel and were placed in a glass adaptation chamber of our construction to ensure appropriate air humidity. The root hairs of test tube plants, grown in artificial conditions of easy availability of nutrients and high air humidity, grow worse on nutrient media and the roots sluggishly absorb water. Therefore, test tube plants require gradual acclimatization to *ex vitro* conditions, including water deficiency. After 7–10 days, when the acclimatized plants began to grow, they were hardened. After 30–50 days, the pellets/plugs were planted in special containers.

The efficiencies of adaptation of rooted test-tube plants of the studied *Pyrus* species to *ex vitro* autotrophic nutrition were studied on two substrates of light sphagnum peat: Jiffy-7 peat pellets and Ellepress peat plugs (**Fig. 9**).

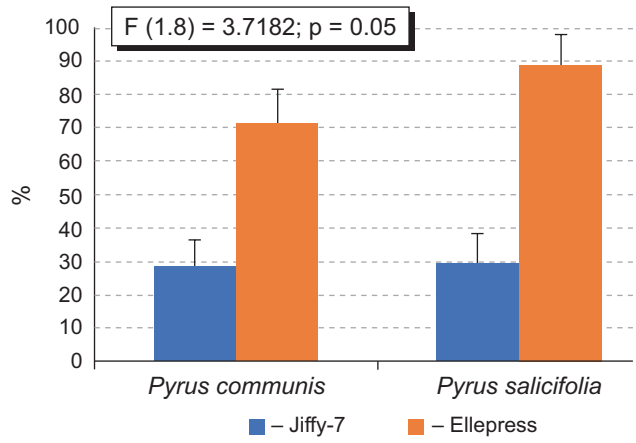


Fig. 9. The adaptation of *P. communis* and *P. salicifolia* rooted test-tube plants to non-sterile conditions *ex vitro* with Jiffy-7 peat pellets and Ellepress peat plugs

The efficiencies of both studied *Pyrus* species adaptation were better with Ellepress peat plugs, than Jiffy-7 peat pellets. In the test-tube plants, the success rate with Ellepress peat plugs reached 71.43 % for *P. communis* and 88.89 % for *P. salicifolia*, while with Jiffy-7 peat pellets these values were only 28.57% and 29.41%, respectively.

The test-tube plants of both studied *Pyrus* species growing with Ellepress peat plugs developed the absorbent root hairs within 3–4 days, and after 35–40 days the adapted regenerated plants (**Fig. 10**) were suitable for transplanting for further growing.

Pyrus communis *Pyrus salicifolia*

Fig. 10. The adapted regenerated plants of *P. communis* and *P. salicifolia* with Ellepress peat plugs

The regenerated plants adapted to *ex vitro* non-sterile conditions were transplanted into containers for growing into a special arched solar-heated greenhouse made of cellular polycarbonate. The term of growing was determined by the condition of the container plant and the suitability of seasonal conditions for stress-free transplanting of container plants into the open ground of the nursery. The survival rate of clones in the open field using this adaptation technology reached more than 90 %.

CONCLUSION

As a result of the search for the possibilities to improve micropropagation protocols for some *Pyrus* species and pear cultivars, the techniques of explants selection, preparing and sterilizing were optimized for the development and implementation of more efficient technologies for the conservation of plant botanical collections and their propagation for breeding purposes. The technologies of explant proliferation and morphogenesis, rooting technologies of the obtained microclones, and adaptation of rooted test-tube plants to non-sterile *ex vitro* conditions have been improved. The proliferation of *P. communis* explants was the most successful on the basic Murashige–Skoog medium modified by the addition of 2.0 mg/L 6-BAP and 0.01 mg/L IBA (the multiplication rate – 9.50±0.18). The most active shoot formation of *P. salicifolia in vitro* was observed in the variant where the DKW medium was modified by adding 2.5 mg/L 6-BAP, and 0.1 mg/L IBA (the multiplication rate – 9.11±0.14). The best for the rhizogenesis of *P. communis* and *P. salicifolia* was the nutrient medium according to the basic recipe of Murashige–Skoog modified by the addition of 0.5 mg/L NAA (rooting was 87.50 % and 98.54 %, respectively). The efficiencies of both studied *Pyrus* species adaptation were better with Ellepress peat plugs than with Jiffy-7 peat pellets. For the test-tube plants, the success rate with Ellepress peat plugs reached 71.43 % for *P. communis* and 88.89 % for *P. salicifolia*, while with Jiffy-7 peat pellets these values were only 28.57 % and 29.41 %,

respectively. It is recommended to cultivate explants of *P. communis* are on Murashige–Skoog medium with the addition of 2.0 mg/L 6-BAP and 0.01 mg/L IBA, and *P. salicifolia* on Driver and Kuniyuki medium with the addition of 2.5 mg/L 6-BAP and 0.1 mg/L IBA. For rooting of microclones of both *Pyrus* species, it is recommended to use Murashige–Skoog medium with the addition of 0.5 mg/L NAA.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Animal Rights: this article does not include animal studies.

Human Rights: this article does not contain any studies with human subjects performed by any of the authors.

AUTHOR CONTRIBUTIONS

Conceptualization, [A.O.; N.K.]; methodology, [A.O.; N.M.K.; O.O.]; validation, [A.O.; N.K.; V.Z.]; formal analysis, [A.O.; N.K.; V.Z.]; investigation, [N.K.; V.Z.]; resources, [V.H.]; data curation, [V.H.; N.K.]; writing – original draft preparation, [A.O.; N.K.; O.O.]; writing – review and editing, [A.O.; O.O.]; visualization, [N.K.; V.Z.] supervision, [N.K.]; project administration, [V.H.]; funding acquisition, [V.H.].

All authors have read and agreed to the published version of the manuscript.

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РОЗМНОЖЕННЯ ГРУШІ (*PYRUS* L.) *IN VITRO*

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Вступ. Методи мікророзмноження використовуються для збереження фіторізноманіття *Pyrus* spp., а також для створення колекцій рідкісних і зникаючих видів і сортів *Pyrus* spp. та генотипів, необхідних для селекційно-генетичних досліджень.

Матеріали та методи. За експериментальні рослини були обрані представники двох видів роду *Pyrus* L., *P. communis* і *P. salicifolia* Pall., та чотири сорти груші *P. communis*: "Бере Десятова", "Уманська ювілейна", "Княгиня Ольга" і "Софія Уманська". За первинні експланти використовували молоді пагони з апікальною меристемою від трирічних рослин видів і сортів *Pyrus*. Ефективність стерилізації визначали за відсотком стерильних і життєздатних експлантів. Укорінені в пробірках *in vitro* пагони пересаджували для адаптації в живильні диски Jiffy-7 та Ellepress. Статистичний аналіз отриманих результатів проводили за Рональдом Фішером (Fisher, 2017) з використанням Microsoft Office Excel 2010.

Результати. Дихлорид ртуті ($HgCl_2$) виявився найефективнішим із усіх досліджених стерилізаторів експлантів *P. communis*. Техніка стерилізації експлантів *P. communis* виявилася надзвичайно складною або неефективною для *P. salicifolia*, що, ймовірно, пов'язано з густим опушенням пагонів і бруньок цього виду. Тому для мікророзмноження *P. salicifolia* за експланти використовували стерилізоване за стандартним протоколом насіння. У найкращому для *P. communis* варіанті модифікації середовища Мурасіге–Скуга (МС) з додаванням 2,0 мг/л 6-бензиламінопурину (6-БАП) і 0,01 мг/л індоліл-3-масляної кислоти (ІМК) було отримано 9,50 мікроклонів на пасаж. Однак у *P. salicifolia* найактивніше пагоноутворення *in vitro* спостерігали у варіанті модифікації середовища Драйвера й Куніюкі (DKW) з додаванням 2,5 мг/л 6-БАП. Для індукування ризогенезу найкращим для *P. communis* і *P. salicifolia* було МС-302, модифіковане 0,5 мг/л α-нафтилоцтової кислоти (α-НОК). У цьому варіанті перші корені розвивалися через 8–10 діб, а через місяць укорінення досягло 87,5 % у *P. communis* і 98,54 % у *P. salicifolia*. Ефективність адаптації вкорінених пробіркових рослин обох видів *Pyrus* була краща у варіанті з живильними дисками Ellepress, аніж Jiffy-7.

Висновки. Експланти *P. communis* пропонується культивувати на середовищі Мурасіге–Скуга з додаванням 2,0 мг/л 6-БАП та 0,01 мг/л ІМК, а *P. salicifolia* – на середовищі Драйвера й Куніюкі з додаванням 2,5 мг/л 6-БАП і 0,1 мг/л ІМК. Для вкорінення мікроклонів обох видів *Pyrus* рекомендується використовувати середовище Мурасіге–Скуга з додаванням 0,5 мг/л α-НОК, а торф'яні живильні диски Ellepress – для адаптації регенерантів.

Ключові слова: *Pyrus communis* L., ювенільна фаза, насіннєве розмноження, мікророзмноження, проліферація, акліматизація *ex vitro*

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