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Features of micro clone reproduction of some currant representatives of the genus *Ribes spp.*: review

N. V. Ryago[1]✉
[1] **Russian Research Institute of Fruit Crop Breeding, Zhilina, Russia**
✉*E-mail: ryago@orel.vniispk.ru*

Abstract. **Purpose.** The systematization of information on the micro clonal reproduction of representatives of the *Ribes* spp. genus, including the rooting of micro shoots and their adaptation in *ex vitro* conditions. **Methods.** A comparative analysis was used to review foreign and domestic literature on the cultivation of berry crops using the method of microclonal propagation. **Results.** It is possible to increase the reproduction coefficient of healthy pure-cultivar *Ribes* spp. planting material in conditions of micro clone reproduction, taking into account genotypic features. Obtaining viable explants when introduced into *in vitro* culture is achieved, first of all, by choosing a high-quality source material, the phase of plant development during the isolation of explants and the selection of nutrient media. In addition, the positive introduction is influenced by the effectiveness and toxicity of the sterilizing agent, the fight against phenolic oxidation of explants and the medium, the range of growth regulators during the periods of the introduction, micropropagation and rhizogenesis. The greatest production losses occur at the stage of acclimatization of micro-shoots in non-sterile conditions, which is associated with the weak work of enzymes for carbon fixation in the required volumes and the unformed water regime due to the difference in the microclimate in the test tube and open ground conditions. Various technologies of adaptation of the representatives of this genus to external conditions are presented, ranging from the selection of a substrate in greenhouses to the development of special climate chambers with optimal conditions. Despite the contradictory results, the idea of the success of the use of certain preparations for *Ribes* spp. is expanding, the quality of the planting material is improving, however, the methodology for obtaining pure-variety planting material taking into account the varietal characteristics, has not been fully developed, and the issues of adaptation at the *ex vitro* stage have not been fully resolved. **Scientific novelty.** The review of the reports is presented taking into account new domestic and foreign scientific developments of the stages of microclonal reproduction of berry crops, depending on the species and genotypes, as well as the issue of acclimatization in *ex vitro* conditions is discussed.
Keywords: *in vitro*, the term of introduction into culture, nutrient media, regulators of plant growth, rooting, *ex vitro* adaptation.

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Особенности микроклонального размножения некоторых представителей смородины рода *Ribes* spp.: обзор

Н. В. Ряго[1]✉
[1] **Всероссийский научно-исследовательский институт селекции плодовых культур, Жилина, Россия**
✉*E-mail: ryago@orel.vniispk.ru*

Аннотация. **Цель** – систематизация информации по вопросам микроклонального размножения представителей рода *Ribes* spp., в том числе укоренение микропобегов и их адаптация в условиях *ex vi-*

tro. **Методы.** Использовали сравнительный анализ при обзоре зарубежной и отечественной литературы по выращиванию ягодных культур методом микроклонального размножения. **Результаты.** Увеличить коэффициент размножения оздоровленного чистосортного посадочного материала представителей рода *Ribes* spp. возможно в условиях микроклонального размножения с учетом генотипических особенностей. Получение жизнеспособных эксплантов при введении в культуру *in vitro* достигается прежде всего выбором качественного исходного материала, фазы развития растения в период изоляции эксплантов и подбора питательных сред. Кроме того, на положительный исход введения влияет эффективность и токсичность стерилизующего агента, борьба с фенольным окислением эксплантов и среды, ассортимент регуляторов роста в периоды введения, микроразмножения и ризогенеза. Наибольшие производственные потери происходят на этапе акклиматизации микропобегов в нестерильных условиях, что связывают со слабой работой ферментов для фиксации углерода в необходимых объемах и несформировавшимся водным режимом из-за разницы микроклимата в пробирке и условий открытого грунта. Представлены разные технологии адаптации представителей данного рода к внешним условиям, начиная от подбора субстрата в теплицах до разработки специальных климатических камер с оптимальными условиями. Несмотря на противоречивые результаты, расширяется представление об успешности применения определенных препаратов для рода *Ribes* spp., повышается качество посадочного материала, однако полностью не отработана методика получения чистосортного посадочного материала с учетом сортовых особенностей, и полностью не решены вопросы адаптации на этапе *ex vitro*. **Научная новизна.** Представлен обзор литературы с учетом новых отечественных и зарубежных научных разработок этапов микроклонального размножения ягодных культур в зависимости от видовой принадлежности и генотипа, а также вопрос акклиматизации в условиях *ex vitro.*

Ключевые слова: культура in *vitro*, срок введения в культуру, питательные среды, регуляторы роста растений, укоренение, адаптация *ex vitro*.

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Introduction

Since 2013, the volume of the yield of fruits and berries has grown 2.3 times in Russia and in 2022 reached a peak of more than 1.5 million tons of products. In parallel, during this period, over 140 thousand hectares of perennial plantings of fruit and berry crops were laid, of which 66 % were intensive orchards [1].

Berry production requires high costs and includes the costs of planting, tending care before and during fruiting, organization of harvesting and sale of products. To increase the profitability of berry crop cultivation, it is necessary to modernize technologies [2].

Most pathogens, pests and diseases spread mainly with planting material. It is known that the quality of seedlings highly affects the future state of plantations and their productivity [3].

It is impossible to obtain healthy planting material propagating berry crops by traditional methods. *In vitro* culture technology alone or together with other methods of healing (thermo-, chemotherapy) contributes to achieving high elimination of viruses, bacterial and fungal diseases and obtaining more regenerating plants [4].

The plants obtained by this method are characterized by increased frost resistance, enhanced formation of basal shoots, branch buds, high yield (1.5–4 times more), and the seedlings themselves have a higher rooting ability and development of the root system [3; 5].

Currant is a popular and valuable berry crop due to high concentrations of biologically active substances, antioxidants, vitamins, sugars and mineral components. It can be propagated by traditional methods – softwood and hardwood cuttings, layering, bush division and grafting. But these methods cannot provide a high rate of expansion of currant plantings. The use of *in vitro* culture technology is more promising, with the help of which it is possible to obtain a genetically homogeneous and healthy material [6].

Microclonal reproduction is studied taking into account the biological characteristics of the cultivar, so the issue of improving the technique of *in vitro* culture remains relevant, in particular for currants.

The purpose of this study was to generalize the literature data on the technology of microclonal reproduction of *Ribes* spp. representatives.

Methods

The main research method was a comparative analysis of the scientific publications of domestic and foreign experts mainly over the last 20 years on the use of growth regulators in the *in vitro* culture, selection of conditions for introduction, micropropagation and adaptation of berry crops *in vivo.*

Results

Cultivation of plants *in vitro* is currently a popular method of plant reproduction, since it is possible to solve a number of tasks:

– improving the quality of planting material: the increase of genetic homogeneity and cropping power;

– getting healthy plants from viral, bacterial, fungal diseases and pests through the use of meristem culture *in vitro*;

– a large number of the resulting planting material with shorter production terms;

– the possibility of year-round production of plants and their release by a certain date;

– long-term deposition of mericlones without contact with the external environment;

– exchange of plant material with other organizations without the risk of infecting it with quarantine facilities [7].

In breeding, the method of microclonal reproduction makes it easier to create new forms and varieties: the time for releasing valuable genotypes is reduced, the genotypes of parental forms are preserved and the duration of the breeding process is shortened. To date, both in Russia and abroad, the list of species and varieties, to which the method of culture of isolated tissues is being applied, is gradually expanding [7].

According to the degree of involvement in breeding, V. S. Shevelukha and co-authors divided the *in vitro* tissue culture method into two groups:

1.Technologies that supplement breeding: *in vitro* fertilization (overcoming progamous incompatibility); cultivation of ovules and immature hybrid embryos (overcoming postgamous incompatibility); cultivation of anthers and microspores with subsequent production of haploids; cryopreservation of isolated cells, tissues and organs; microclonal reproduction of remote hybrids.

2. Production of new forms and varieties, separate from traditional breeding methods: cell selection using callus cells; somatic hybridization (fusion of isolated protoplasts and production of non-generic hybrids); application of genetic engineering technology [8].

To obtain and cultivate remote hybrids, the following *in vitro* culture methods are used: *in vitro* fertilization, embryoculture, microclonal reproduction of valuable hybrids, haploid production *in vitro*, cryopreservation [8].

The process of microclonal reproduction usually consists of several stages:

1. Preliminary stage: disinfection of the initial plant material taken in the optimal phase of development for introduction into culture.

2. Introduction to *in vitro* culture: organization of favorable conditions for explant cultivation on nutrient medium.

3. Proper microclonal reproduction: stimulating the development of lateral buds by removing apical dominance; micro propagation by cuttings of shoots that have retained apical dominance.

4. Extension of micro-shoots and rhizogenesis.

5. Transfer of test tube plants to ex vitro conditions, adaptation to non-sterile conditions and transplanting into the greenhouse [9; 10].

The effect of the introduction timing and size of explants on the development of micro-shoots. The result of the introduction of *Ribes* spp. representatives into the culture *in vitro* depends mainly on the quality of the source material and on the phase of plant development during the isolation of explants. Annual shoots are suitable for introduction. Usually, both dormant buds taken at the end of winter / early spring and young tops of shoots in the active growth phase (May – June) are used [4; 11–13]. If adult plants serve as the starting material, necrosis of explant tissues and phenolic oxidation may increase, which, according to the authors' assumptions, is associated with the physiological age of the original plant. However, in experiments with golden currant, there are no significant differences in the reproduction coefficient of materials taken from juvenile and adult sources [14].

The size of the explant affects the rate of microreproduction. Larger explants reduce the time of obtaining a full-fledged plant; however, this option is only suitable for taking plant material from a virus-free plantation [12].

According to the reports of M. J. S. Cárdenas (2016), the size of the explant affects its survival and small sizes (2–3 mm) are not optimal for high survival of meristems on the medium. The use of explants with a height of 6–10 mm contributes to the preservation of above 50 % of meristems *in vitro*, and this has been confirmed by other authors [13; 15]. C. G. Manole and co-authors took Ribes rubrum explants with a size of 3–5 mm, which allowed them to obtain viable microplants and study the relationship between the height of sprouts and the concentration of sucrose in the nutrient medium [16].

There is a difference in the growth rates of plants from the meristems of apical and axillary buds [12] and this is associated with inhibition of the growth of axillary buds due to the dominant influence of the leading shoot [17].

Phenolic oxidation and ways to fight it. When buds and micro-shoots are injured during *in vitro* culture and transplants, phenolic compounds are oxidized due to the release of toxic substances. Phenolic oxidation visually looks like darkening of the tissue and nutrient medium and physiologically it interferes with the normal course of growth processes. In this regard, G. P. Atroschenko [18] recommends pretreatment of currant explants with a solution of a mixture of antioxidants (ascorbic acid, EDTA), as well as treatment with these substances before separating conglomerates and transferring them to a new medium of antioxidants. Buds can also be immersed for 30 minutes in an antioxidant mixture of citric and ascorbic acids (0.1 g/l of each acid) [13]. For black currant, S. A. Matushkin recommends pre-washing explants in ascorbic acid

(20.0 mg/l) and transplanting them to a nutrient medium with its content. This procedure will not only reduce the negative effect of phenols on growth, but will also increase the regenerative capacity by 20.0–-44.4 % [19]. In experiments with golden currant, glutathione (200 mg/l) had a positive effect). This antioxidant contributed to an increase in the reproduction coefficient of 'Valentine' from 3.9 to 6.8 pcs/explant. The authors noted the manifestation of varietal specificity of the reaction to glutathione [14].

Disinfection of the initial plant material and selection of a sterilizing agent. Disinfection of the starting material is an important step before introduction into culture, since pathogenic microflora – bacteria, fungi and their spores remain on the surface of plant tissues. Before sterilizing explants, they are washed with running water for 30–45 minutes [12]. Also, washing is carried out with the addition of detergents. M. J. S. Cárdenas [13] recommends keeping buds before sterilization in a mixture of fungicidal-bactericidal solution (captan 2.7 g/l + streptomycin 0.5 g/l + benomyl 1.8 g/l) for 12 hours in a shaker to disinfect the explants completely.

The sterilizing agent should neutralize the microflora without damage to plant tissues [9]. The sterilization time is regulated depending on the concentration, type of sterilizing agent and type of the explant. Plants grown in the field are recommended to be sterilized twice with a 24-hour interval. According to the degree of disinfecting effect, sterilizers can be conditionally divided into groups: with strong, medium and weak disinfecting effect.

The group with a strong disinfecting effect includes compounds containing mercury: sulema (mercury chloride (II)), diacid, mercury nitric acid and silver nitric acid. The group with an average disinfecting effect includes chlorine-containing compounds: sodium and potassium hypochlorites, chloramine and chloride lime. Hydrogen peroxide and potassium permanganate have oxidizing properties and belong to the group with a weak disinfecting effect [20].

Various compounds are used as a sterilizer for currants. For *Ribes* spp. the protocol "Micropropagation of *Rubus* and *Ribes* spp." recommends rinsing the buds in 70% alcohol for 1 min and then in 10–12 % calcium hypochlorite solution for 10–12 minutes. If the tops of annual softwood shoots are treated, alcohol treatment should be avoided and the tips of the shoots should be washed in a solution of calcium hypochlorite of the same concentration, but within 5 minutes [12]. M. J. S. Cárdenas [13] used a combination of 70 % ethanol (5 seconds) and 1% sodium hypochlorite solution (10 minutes) for disinfection of three *Ribes* representatives. Contamination of explants varied from 16 to 44 % depending on the type of plant. Chlorine–based disinfectants – 0.3 % solution of HMI UNI S and 1 % solution of HMI Tabidez-56 proved to be optimal for washing buds, and alternate treatment with them made it possible to achieve 100 % pure culture. However, the explants did not develop for a long time after that, and in some cases died [21]. In experiments with golden currant, 70 % ethanol (3–5 seconds) and 0.1% solution of sulema (mercury (II) chloride) (20 minutes) were used for buds sterilization followed by 4-fold washing with sterile distilled water [14]. An increase in the exposure of 0.1 % sulema from 1 minute to 7 minutes in combination with treatments with 70 % ethanol and 0.2 % benlate led to a decrease in the number of infected explants (from 50 % in 'Fertodi Piros' to 10 % in 'Nenaglyadnaya') and an increase in the number of viable explants [4]. Mercury (II) chloride at a concentration of 0.15 % with the addition of a wetting agent (Tween-20) (immersion for 1 min.) reduced the level of contamination of the medium to an average of 25.7 %, while no darkening of explants or the medium caused by the oxidation of phenolic substances was observed [15]. The application of 0.1 % merthiolate solution (3 minutes) for explant treatment reduced the proportion of contamination to 13.2–64 % [22]. Hydrogen peroxide is used as a sterilizer for fruit crops in various concentrations from 3 to 30 % [4; 23; 24]. Silver nitrate (0.1 %) is used as a sterilizer not only for disinfection of buds, but also seeds [20; 25].

Experience in the use of nutrient media on *Ribes* plants and their modification at the stage of introduction and micro reproduction. The composition of the culture medium significantly affects the success of microclonal reproduction. Murashige – Skoog (MS) medium is more often used for the culture of currant cells, while changing the salt composition depending on the stage of cultivation: introduction, reproduction and rooting of micro-shoots [12; 26]. There are also works using McCown medium for woody plants (WPM), characterized by a lower content of macronutrients [27]. However, it turned out to be unsuitable for 'Detvan', 'Vitan' and 'Rote Hellandische' at the stage of micro-propagation of the tops of shoots. The plants had symptoms of yellowing or chlorosis. 'Rotte Holländische' had a higher multiplication coefficient (2.1 pcs/exp) on MS medium with a BAP content of 1.0 mg/l and 2.0 mg/l than on WPM medium with the same BAP concentrations [15]. In addition to Murashige – Skoog and McCown media, it is possible to use macro- and microsols for the introduction of red currant explants according to the Anderson, Lee and de Fossard media [4; 28]. Most of the valuable fruit and berry crops studied in the work (honeysuckle, aronia, raspberry, blackberry, currant, gooseberry, actinidia, blueberry) showed better results of the reproduction coefficient on the nutrient medium QL compared with MS [29; 30]. The positive effect of the QL nutrient medium was noted in the experiments with gooseberries: the reproduction coefficient increased by 1.3–1.8 times compared to the Murashige – Skoog medium [31]. In

addition to the above-mentioned culture media, DKW (Driver – Kuniyuki Walnut medium) was tested for Ribes horasanicum, however, it was less effective than Murashige – Skoog [32].

Vitamins included in the composition of the medium are important for the full development of plants, since vitamins perform various key roles in metabolism (participation in carbohydrate metabolism; as part of energy carriers and activators of phosphoric, carbohydrate and fat metabolism; as part of transaminases; as a component of redox reactions). The following vitamin concentrations are used more often for fruit plants: B1 (thiamine), PP (nicotinic acid), B6 (pyridoxine), H (biotin) in concentrations of 0.1–05 mg/l, C (ascorbic acid) – 1.0–3.0 mg/l, mesoinosite – 10–100 mg/l; glycine content varies from 1.0 to 8.0 mg/l [33]. In the studies of N. V. Kukharchik, the following quantitative content of vitamins for red currant is found: B1 – 0.1 and 10 mg/l, B6 – 0.5 and 10 mg/l, PP – 0.5 and 5 mg/l, ascorbic acid – 10 mg/l, mesoinosite – 100 mg/l. Glycine content − 2.0 mg/l [4]. For black currant at the rooting stage, mineral and vitamin complex "Complivit" (2.0 mg/l) was added to the medium to enhance growth [22].

Culture tubes are incubated for 3–4 weeks at a temperature of 22–24 °C with a photoperiod of 16/8 hours [12].

Various growth regulators which are growth and development activators (cytokinins, auxins, gibberellins, etc.) are introduced into the media. Their qualitative and quantitative ratios depend on the objectives of the study and micro-reproduction stage: for proliferation at the stages of reproduction, for the induction of rhizogenesis at the stages of micro-shoots rooting or for the induction of callus formation [33]. Also, the choice of a growth regulator is influenced by the genotype, the rate of reproduction of shoots, the height of shoots and the frequency of genetic variations. Higher concentrations of cytokinin usually cause the proliferation of shoots, but developed shoots have a smaller size and may have signs of hyperhydration [34]. There is also a possibility to detect changes in plant morphology, slowdown of the process of laying and development of axillary meristems, as well as reducing the ability of shoots to rhizogenesis is possible. Due to these undesirable phenomena, the use of the lowest concentrations of cytokinin, which can provide the maximum available rate of micropropagation, or the alternation of culture media with high and low growth regulator content will make it possible to avoid the toxic effects of cytokinins because of their constant presence in the nutrient medium. [35].

Benzylaminopurine (benzyladenine, 6- BAP, BAP) is the most commonly used inducer of shoot formation. The range of BAP concentrations in studies can reach 20 microns [14]. To introduce red currant meristems into the culture *in vitro*, the authors recommend a combination of benzylaminopurine 2.0 mg/l, indolyl-3-butyric acid (IBA) 0.5 mg/l and gibberellic acid (GA) 0.1 mg/l [12]. For red currant at the stage of introducing, the BAP content in the medium is recommended to be kept at 0.2 mg/l, which is significantly lower than for black currant − 0.5 mg/l [4].

At the stage of micro-reproduction proper, there are more references in the literature to the use of benzylaminopurine, and its concentrations vary from 0.4 to 1.0 mg/l.

E. Dziedzic and J. Jagła [12] after 3–4 weeks transferred micro-shoots to MS medium enriched with 1.0 mg/l BAP and 0.1 mg/l IBA. Another combination with benzylaminopurine was also successful: MS medium was supplemented with 1.0 mg/l BAP, 100 mg/l inositol and 2.0 mg/l glycine at pH 5.8 [15].

In the study of M. J. S. Cárdenas [13], a different species reaction to the composition of the medium *in vitro* was noted. The most suitable conditions for the vegetative development of red currant explants were created by a nutrient medium according to Murashige – Skoog supplemented with 0.1 mg/l gibberellic acid (GA), 0.5 mg/l BAP, 0.05 mg/l or 0.1 mg/l 1-naphthaleneacetic acid (NAA). For black currant and gooseberry, on the contrary, such a composition of growth regulators was not suitable: for black currant, MS medium with 0.1 mg/ l gibberellic acid (GA) with or without growth regulators did not have a strong effect on the morphogenesis of the explant, and for gooseberry, the presence of only one hormone, gibberellic acid, stimulated the laying of additional buds [13]. Thus, the choice of the type and combination of growth regulators will depend on the genotype selected for micro-propagation.

In the experiments with red currant, MS medium with a mineral composition of 1/2 concentration and a BAP content of 0.4 mg/l, IBA of 0.02 mg/l and GA of 0.2 mg/l allowed to obtain more high shoots with green leaves and a high degree of density [11]. For the reproduction of black currant, the optimal concentration of BAP cytokinin was 0.5–1.0 mg/l (reproduction coefficient 3.0–12.6 pcs/exp) [19]. At the same time, it is not necessary to exclude the influence of the genotype: experiments confirm that reproduction depends not only on the concentration of cytokinins in the medium, but also on the individual reaction of the cultivar. The authors suggest that this is due to the genetic control of the metabolism of various auxins and cytokinins in plant tissue [15].

In addition to BAP, the growth regulator 'Cytodef' shows its effectiveness among cytokinins. An active ingredient of this growth regulator is N-(1,2,4-triazol-4-yl)-N'-phenylureas. Under stress, 'Cytodef' increases resistance to lack of moisture, provides stable synthesis of chlorophyll and stable operation of the photosynthetic apparatus at low temperatures, accelerates the accumulation of sugars [36]. Thus, the cultivars 'Shedraya' and 'Jonkheer Van Tets' on MS nutrient medium with the content of 'Cytodef' at concentrations of 0.5 and 1.0 mg/l acquired an increase in the number and

total length of shoots by 1.5–2.0 times, in comparison with BAP at the same concentrations [37].

Thidiazuron (TDZ) and forchlorfenuron (CPPU) are also derivatives of diphenylurea. In experiments with cultivars of gooseberry Russkiy and Severnyy Kapitan and honeysuckle Berel, the effects of these drugs and BAP were compared. The survival rate was higher in the presence of TDZ and CPPU (94.5–100 % vs. 85 % in gooseberries and 22 % in honeysuckle with BAP). Larger plants were formed on medium with the addition of BAP (up to 11.3 mm in gooseberries and 30 mm in honeysuckle). According to the reproduction coefficient, forchlorfenuron was more effective on gooseberries (up to 10.2 pcs/exp), while BAP (6.3 pcs/exp) was more effective on honeysuckle [38].

At the stage of blackcurrant proliferation, a high reproduction coefficient was noted – 4.7–7.6 pcs/exp. when kinetin 2.5 mg/l was added to the medium [19].

Gibberellic acid (GA_3) can have a positive effect on the cultivation of shoots: it promotes elongation and development of shoots and reduces the formation of callus [11]. In particular, in experiments with golden currant, its stimulating effect on the length of shoots was noted (they increased by 2 times) with the simultaneous presence of 5 microns of BAP and GA3 in the medium. This approach is also applied to black currants and blackberries, as the authors note [14]. For red currant, GA was used at a concentration of 0.1–0.2 mg/l [11–13].

When signs of chlorosis are detected, micro-shoots are transferred to a medium with doubled content of iron chelate [12].

The total period of red currant cultivation in the form of actively growing culture at the stage of micropropagation proper is 7–9 passages (about 9 months), after which the shoots either stop multiplying or turn brown-yellow and die [15]. A decrease in the reproduction rate of *Ribes uva-crispa* L. after the 4th passage on MS medium with 1.0 microns of BAP was shown, and for a number of black currant varieties, on the contrary, the level of BAP 0.5–2.0 mg/l was satisfactory for the induction of shoots and further reproduction. When propagating *in vitro*, red currant turned out to be a difficult crop, for which only the short-term maintenance of varieties is possible, but not the sustainable development on an industrial scale [15].

To lengthen the shoots of black currant, it is recommended to reduce the concentration of BAP in the medium to 0.2 mg/l or add adenine sulfate (50 mg/l) or GA (0.3 and 0.4 mg/l) to BAP (1.0 mg/l). It is possible to cultivate regenerants without hormones, as well as with putting 5.0 mg/l kinetin into the medium [19].

Rhizogenesis and selection of growth regulators for *Ribes* spp. At the rooting stage, the amounts of salts and carbohydrates are usually reduced, cytokinins are excluded and auxins are added [35]. The development of the root system *in vitro* is determined primarily by the genotype: the predisposition of shoots to rooting. The composition of the nutrient medium has a great influence on rooting, i. e. micro- and macroelements at all stages of micro-reproduction, the source and concentration of carbohydrates, the consistency of the medium, the proportion in the medium and the combination of phytohormones and the direction of their action on the explant, the degree of development of the regenerant itself, etc. However, auxins have a leading influence on the rooting process and without them this process is practically impossible [10].

For hard-to-root genotypes of red currant, a two-stage procedure is used to increase the rate of appearance and development of roots. Before rooting, the shoots are transferred first to a medium with a high content of auxin until the roots appear (about 10–11 days), and then transplanted to a medium without phytohormones to lengthen the roots [12]. Other researchers transfer the shoots to MS medium with a small concentration of BAP (up to 1.0–2.0 microns) and cultivate them for 7–10 days. By virtue of this method, the number of explants suitable for rooting increases by up to 80 % and the negative impact of the accumulation of these growth regulators on rhizogenesis decreases [14].

E. Dziedzic and J. Jagła [12] use Murashige – Skoog medium containing IBA of 1.0 mg/l (2.0 mg/l for hard-to-root genotypes) as the mineral basis of the medium at the stage of rhizogenesis. The concentration of IBA of 1 mg/l contributes to the rooting of up to 70 % of black currant plants during adaptation in autumn (in September). By the end of the growing season, the shoots have a developed root system, 5–7 leaves and reach a height of 15–20 cm [21]. In experiments with golden currant at the rooting stage, researchers used ½ of the MS mineral base supplemented with IBA auxin with a concentration of 2.0 microns, since with a higher concentration (5.0 microns and 10.0 microns), active callus formation is noted, preventing the development of roots. The exception was the Valentine variety, in which the number of shoots with roots increased with a high content of auxin [14]. In the work of C. G. Manole and co-authors [11], the medium for rooting red currant contained 1/5 of the MS macronutrients with the addition of BAP 0.01 mg/l. The shoots were first grown in the darkness for 5 days, then transferred to the light for a period of 3 weeks, later the process of root formation became less intense. The most suitable medium for rooting black currant shoots was Quorin, Lepoivre without the addition of hormones with half content of macronutrients [19].

The use of another auxin, naphthaleneacetic acid (NAA), in the experiments with golden currant was ineffective, since even in low concentrations it caused callusogenesis in the basal part of micro-shoots, and the formation of single roots was recorded only in one cultivar ‘Valentina’. Such undesirable effects when using NAA were noted earlier in experiments with goose-

berries [14]. In the experiments of M. J. S. Cárdenas [13], the combined content of 0.1 mg/l NAA and 0.5 mg/l BAP in the medium had an inhibitory effect on the laying of shoots without a positive effect on their length, which contradicts the work of other scientists who showed that the decrease in BAP from 1.0 mg/l to 0.5 and 0.25 mg/l led to an increase in growth and the number of shoots.

Some researchers note the positive effect of indolyl-3-acetic acid (IAA) on the process of root formation [6; 39; 40]. IAA is the most common and basic natural auxin; according to the degree of compliance with the auxin receptor, it prevails over other compounds. The addition of this auxin to the nutrient medium in comparison with IBA and NAA had a stimulating effect on golden currant shoots greater in strength and quality [14]. However, in experiments with the rooting of black currant micro shoots, the growth and development of roots on media containing IAA was worse than on media containing IBA: the roots acquired an unnatural thickness, lateral roots were not formed and callus growth, reducing the quality of planting material, was observed [10].

Physiological prerequisites for the difficulties of adapting mericlones to non-sterile conditions and working out how to achieve them. One of the most difficult processes is the adaptation of regenerating plants to *ex vitro* conditions. It is at this stage that large losses of plants occur during microclonal reproduction on an industrial scale [41]. Cessation of growth, leaf loss and death are observed in plants, when transplanted into non-sterile conditions. These phenomena are caused, first of all, by the weak control of transpiration by micro-plants themselves and the heterotrophic way of nutrition [42].

For better survival of plants in new conditions, it is necessary to prepare them to overcome stress. First of all, water stress negatively affects regenerants, leading to dehydration of tissues and damage to membranes [43].

Carbohydrates are added to the nutrient medium at all stages of micro-reproduction, since plants *in vitro* are only heterotrophic. Chlorophyll of plants *in vitro* is not fully capable of photosynthesis due to the low content of carbon dioxide in the vessels. The ability to photoautotropy *in vitro* will vary depending on the type of vessel coating, light intensity, sucrose content in the medium. After transplantation into *ex vitro* conditions, plants do not have time to adapt to photoautotropy due to carbon-fixing enzymes that do not work at full strength [44; 45].

The leaves of plants from test tubes are able to absorb CO_2 4–5 times less in comparison with already adapted plants, which is not enough for the needs of the respiration process in photosynthesis products [46; 47]. In this regard, before transferring plants to non-sterile conditions, many scientists recommend to grow test tube plants for 2 weeks at a light level of 10000 lux [48].

The adaptation technology includes the selection of the substrate and optimal conditions: illumination, photoperiod, air and substrate humidity and temperature regime. Some authors recommend adhering to the following parameters: gentle illumination, active growth temperature (20 °C), high relative humidity – about 95–99 % before the formation of a new leaflet in non-sterile conditions and good aeration [49].

The essence of adaptation is to maintain high humidity in the ground part of the plant and to reduce it systematically. Plants are more often adapted in greenhouse conditions, where high humidity is created. The humidity can be achieved by using artificial fog installations, a wet awning, an individual plastic coating and a "smog" system. Artificial fog allows monitoring the humidity level of the air, but it leads to a decrease in the nutrient content in plants, overwetting of the substrate and creating conditions for the development of fungal diseases [50]. A. B. Burgutin [51] proposed a method in which it is possible to exclude the use of a fog-forming installation during the adaptation of grapes. To do this, it is necessary to remove the plugs from those test tubes in which the shoots occupied the entire height of the container, and leave the plants open for 1.5–2 weeks. After these weeks, the plants should be transplanted together with agar into the soil with the stem deepening into the substrate.

A wet awning in comparison with fog accelerates the adaptation of plants and stimulates their growth more. However, when using it, it is difficult to control the temperature and the required humidity at a particular stage [52].

At the stage of adaptation, plastic food containers with a volume of 0.5 liters with a lid are used. Half of their volume is filled with soil composition, a couple of plants are planted and hermetically covered with a lid. For a period of 3–4 weeks, containers are placed under lamps with a capacity of 4000–5000 lux without the need for watering and additional care. After the time has elapsed, holes with an area of 0.5–1.0 cm^2 are made in the lid to reduce humidity, and after a week the lids are completely removed. When using this adaptation technique on cherry and plum plants, the yield was 70–90 % [53].

An alternative option is the use of hydroponic installations that contribute to the growth of the height of shoots, the number of leaves, the number and length of roots by the end of the acclimatization period in non–sterile conditions [14].

In most works, adaptation occurs in the soil substrate. Mixtures of 2–4 components (soil, sand, peat, zeolite, vermiculite, humus, etc.), ionite substrates (Cion, Bione, etc.) are most often used as a substrate [49].

Researchers recommend transferring rooted red currant shoots previously washed in water from agar separately to the mixture of soil and perlite 2 : 1 [12]. The high survival rate of currants is also noted on substrates: coconut (96 %), peat + sand (94 %), and turf (90 %) [54]. When evaluating the results of adaptation of regenerating *R. Meyeri* plants to *ex vitro* conditions, a mixture of peat and sand (3 : 1) contributed to the survival rate of 60 % of plants; a mixture of peat, soil and sand (2 : 2 : 1) contributed to the survival rate of 70 % plants [42]. Black currant seedlings showed high survival rate (90–95 %) on peat and peat substrates with the addition of perlite in a ratio of 3 : 1 [55].

The phytosanitary condition of the substrate is very important in the adaptation of plants *ex vitro*. For example, for grapes, the sterilization of sand with a solution of potassium permanganate had a significant impact on the survival of plants. Treatments of the soil substrate with hot steam, 0.1 % solution of fungicides (Benlat, Euparen), antimicrobials with the addition of another fungicide (Terrazol) have proven effectiveness. However, there is an opinion that there is no significant difference between the use of a sterilized and non-sterilized substrate. For example, for the development of strawberries obtained *in vitro*, the best option was a non-sterile mixture of forest soil and sand (3 : 1) [56].

The ideal temperature for growing many types of plants, is +23…+25 °C. At the initial stage of adaptation, exceeding and lowering of this factor will negatively affect the further development of the plant. A systematic decrease in temperature to +15…+20 °C and an increase in the humidity of the substrate transfer the micro-plants to a state of prolonged dormancy with a low probability of getting out of it without the selection of special conditions [57]. The temperature in the range of +40…45 °C and above when planting plants in combination with high humidity in the adaptation room leads to a violation of the cellular structure of regenerants.

Illumination is one of the important factors influencing the full development of the photosynthetic apparatus of plants *in vitro*. In the culture rooms of biotechnology laboratories, the lighting intensity is usually maintained at 3000–3500 lux and the photoperiod is 16 hours a day / 8 hours a night. In the first weeks after planting plants in the ground, it is recommended to keep the light at the same level. On grape plants, the illumination in the climate chamber of 3000 lux at a temperature of +25…+27 °C showed 100 % efficiency and contributed to the development of the root system [58]. The lack of lighting suppressed the growth and development of plants, which led to plant death in 2–3 weeks [59].

It is recommended to cover the planting of red currants with a transparent plastic film and maintain a constant temperature of 20 ± 1 °C with a photoperiod of 16/8 hours and a light intensity of 300 mmol·m^{-2}c^{-1}. After 5–6 days, the authors advise starting the hardening of plants in a climatic box or greenhouse. In studies, they reduced humidity by removing the lid for an hour, gradually increased the acclimatization time to ex vitro conditions for 4 weeks. After 4 weeks, the plants were transferred to the greenhouse, and in the next 4 weeks, the plants were ready to move to field conditions [12].

Blackcurrant plants were kept in conditions of high humidity under the film for longer than two weeks, at a temperature of 22–26 °C, and only then they were gradually accustomed to a decrease in air humidity [55]. The increased humidity of the air did not cause an increase in the intensity of transpiration, which protected the plants against wilting.

Now, special installations (climate chambers) are being developed to adapt test tube plants to non-sterile environmental conditions. Climate chambers are capable to keep the degree of aeration of the soil substrate, humidity and air temperature in a given mode. The main task of the chamber is to mitigate the stress load of plants and ensure a smooth transition to cultivation in new conditions. At the Magarach Institute of the Russian Academy of Sciences (Yalta), the KBWF Binder 240 climate chamber is used. This chamber allows simulating cultivation modes for different stages of the technology of clonal micro-propagation of grapes and helps accelerate adaptation processes while maintaining relatively high viability of grape plants [60]. Prior to that, an adaptation mode was developed and tested there, which allows for partial lignification of grape shoots, which further increases the viability of plants during the adaptation and after the adaptation period [61].

Currently, there is a large range of chambers for the adaptation of plants *in vitro*, but they are all foreign-made and quite expensive. In this regard, some domestic research institutes are developing similar chambers and modes for cultivated plants, which is more cost-effective to produce planting material.

Discussion and Conclusion

Due to the increase in demand for domestic products and state support for the development of their own farms, the number of studies aimed at modernizing existing technologies for growing plants is increasing. The researchers include more and more new growth regulators, sterilizers in technological maps, and improve ways to adapt plants to non-sterile conditions in order to reduce plant losses. For currants in the early 2000s, there was little work on *in vitro* culture. Now there are much more of such work: there are protocols for microclonal reproduction, various recommendations for a greater yield of developed plants. Despite sometimes contradictory results, the idea of the success of using certain drugs for currants is expanding. Thanks to further tests, the quality of planting material will increase, which should have a positive effect on the productivity of berry crops and the profitability of production.

References

1. Shokurova E. Proizvodstvo plodovo-yagodnoy produktsii v Rossii prevysilo 1,5 mln tonn [Production of fruit and berry products exceeded 1.5 million tons in Russia] [e-resource]. URL: https://www.agroinvestor.ru/markets/news/39833-proizvodstvo-plodovo-yagodnoy-produktsii-v-rossii-prevysilo-1-5-mln-t (date of reference: 30.03.2023). (In Russian.)
2. Khromenko V. V., Vorob'yev V. F. Tekhnologicheskiye zatraty i ekonomicheskaya effektivnost' vyrashchivaniya yagodnykh kul'tur [Technological costs and economic efficiency of growing berry crops] // Horticulture and viticulture. 2013. No. 2. Pp. 44–48. (In Russian.)
3. Pronina I. N., Matushkina O. V. Ekonomicheskiye aspekty ispol'zovaniya klonal'nogo mikrorazmnozheniya v sisteme proizvodstva posadochnogo materiala plodovykh i yagodnykh kul'tur [Economic aspects of using clonal micropropagation in the system of production of planting material of fruit and berry crops] // Pomiculture and small fruits culture in Russia. 2011. Vol. 26. Pp. 82–88. (In Russian.)
4. Kukharchik N. V., Kastritskaya M. S., Semenas S. E., Kolbanova E. V., Krasinskaya T. A., Volosevich N. N., Solovey O. V., Zmushko A. A., Bozhiday T. N., Rundya A. P., Malinovskaya A.M. Razmnozheniye plodovykh i yagodnykh rasteniy v kul'ture *in vitro* [Reproduction of fruit and berry plants in culture *in vitro*]. Minsk: Belaruskaya navuka, 2016. 208 p. (In Russian.)
5. Orazbayeva G. K., Khasanova V. T., Isakov A. R., Shvidchenko V. K. Klonal'noye razmnozheniye rasteniy chërnoy smorodiny (*Ribes nigrum* L.) *in vitro* [Clonal propagation of black currant plants (*Ribes nigrum* L.) *in vitro*] // Herald of Science of S. Seifullin Kazakh Agrotechnical University. 2012. No. 1 (72). Pp. 115–124. (In Russian.)
6. Ishmuratova M. M., Golovina L. A. Razmnozheniye sortov smorodiny chernoy (*Ribes nigrum* L.) bashkirskoy selektsii v kul'ture *in vitro* [*In vitro* propagation of blackcurrant (*Ribes nigrum* L.) cultivars of bashkir breeding] // Bulletin of Udmurt University. Series Biology. Earth Sciences. 2017. Vol. 27. No. 4. Pp. 455–-461. (In Russian.)
7. Suleymanova S. J. Mikroklonal'noye razmnozheniye plodovykh kul'tur (obzor) [Microclonal propagation of fruit crops (review)] // East European Scientific Journal. 2016. No. 11. Pp. 47–54. (In Russian)
8. Shevelukha V. S., Kalashnikova E. A., Voronin E. S., Kovalev V. M., Kovalev A. A., Kochiyeva E. Z., Novikov N. N., Provorov, N. A., Prokof'yev M. I., Pronina N. B., Sventitskiy I. O., Tikhonov I. V., Tikhonovich I. A. Sel'skokhozyaystvennaya biotekhnologiya [Agricultural biotechnology] // Under the editorship of V. S. Shevelukha. Moscow: Vysshaya shkola, 1998. 416 p. (In Russian)
9. Programma i metodika selektsii plodovykh, yagodnykh i orekhoplodnykh kul'tur [Program and methodology for breeding fruit, berry and nut crops] // Under the general editor ship of E. N. Sedov. Oryol: Russian research institute of fruit crop breeding, 1995. 502 p. (In Russian.)
10. Geneticheskiye osnovy selektsii: v 4 t. T. 3. Biotekhnologiya v selektsii rasteniy. Kletochnaya inzheneriya [Genetic foundations of breeding: in 4 vol. Vol. 3. Biotechnology in plant breeding. Cell engineering] / Under the editorship of A. V. Kil'chevskiy, L. V. Khotyleva. Minsk: Belaruskaya navuka, 2012. 489 p. (In Russian.)
11. Manole C. G., Balan V., Mencinicopschi I. C., Golea D., Rodino S., Butu, A. The influence of growth regulators concentrations on *in vitro* micropropagation of *Ribes rubrum* species // Scientific Bulletin, Series F, Biotechnologies. 2012. Vol. 16. Pp. 26–29.
12. Dziedzic E., Jagła J. Micropropagation of *Rubus* and *Ribes* spp. // Protocols for Micropropagation of Selected Economically-Important Horticultural Plants. 2013. Pp. 149–160. DOI: 10.1007/978-1-62703-074-8_11.
13. Cárdenas M. J. S. Adaptación de protocolos de establecimiento *in vitro* de *Ribes rubrum* L., *Ribes nigrum* L. y *Ribes uva-crispa* L.: doctoral dissertation, Universidad Austral de Chile. 2016. 41 p. (In Spanish.)
14. Erst A. A., Vechernina N. A. Razmnozheniye *Ribes aureum* (sem. *Glossulariaceae*) v kul'ture *in vitro* [Propagation of *Rubes aureum* (family of *Grossulariaceae*) in *in vitro* culture] // Biotechnology. 2010. No. 5. Pp. 37–44. (In Russian.)
15. Sedlák J., Paprštein F. *In vitro* establishment and proliferation of red currant cultivars // Hort. Sci. Prague. 2012. Vol. 39. No. 1. Pp. 21–25. DOI: 10.17221/40/2011-HORTSCI.
16. Manole C., Bălan V., Tudora C., Butu M., Fidler G., Rodino S., Golea D., Butu A. Influence of sucrose concentration on *in vitro* multiplication of *Ribes rubrum* species // Banat's Journal of Biotechnology. 2011. Vol. 2. No. 4. Pp. 73–75.
17. Vechernina N. A., Plaksina T. V., Tikhomirova L. I. Biotekhnologicheskiye metody sokhraneniya i izmeneniya genomov sadovykh rasteniy [Biotechnological methods for preserving and changing the genomes of garden plants] // Programma rabot selektsentra nauchno-issledovatel''skogo instituta sadovodstva Sibiri imeni M. A. Lisavenko do 2030 goda. Novosibirsk: Nauchno-issledovatel'skiy institut sadovodstva Sibiri imeni M. A. Lisavenko Sibirskogo otdeleniya Rossel'khozakademii NII. 2011. Vol. 3. Pp. 51–56. (In Russian.)
18. Sposob klonal'nogo mikrorazmnozheniya smorodiny i antioksidant dlya klonal'nogo mikrorazmnozheniya smorodiny [Method of clonal micromultiplication of currant and antioxidant for currant clonal micromultiplica-

tion] / G. P. Atroshchenko Patent 20080060 Russian Federation No. 94007611/13; application: 1.03.1994, date of publication: 27.05.1997. 7 p. (In Russian)

19. Matushkin S. A. Optimizatsiya elementov tekhnologii razmnozheniya smorodiny chernoy i kryzhovnika *in vitro*: avtoref. dis. … kand. s.-kh. nauk [Optimization of the elements of the technology of reproduction of black currant and gooseberry *in vitro*: abstract of the dissertation ... candidate of agricultural sciences]. Michurinsk: Michurinsk State Agrarian University, 2020. 24 p. (In Russian.)

20. Kutas E. N., Garaninova M. V. Vliyaniye sterilizuyushchikh soyedineniy na vykhod zhiznesposobnykh eksplantov rododendronov (*Rhododendron* L.) pri vvedenii v kul'turu *in vitro* [Influence of sterilizing compounds on the yield of viable explants rhododendron (*Rhododendron* L.)] // Proceedings of the National Academy of Sciences of Belarus. 2015. No. 2. Pp. 13–17. (In Russian.)

21. Kovalenko G. Biotekhnologicheskiye priyemy razmnozheniya yagodnykh kul'tur [Biotechnological methods of berry crop propagation] // Horticultură, Viticultură şi vinificaţie, Silvicultură şi grădini publice, Protecţia plantelor. 2010. Vol. 24. Pp. 197–199. (In Russian.)

22. Skovorodnikov D. N. Klonal'noye mikrorazmnozheniye v uskorenii selektsionnogo protsessa smorodiny chernoy [Clonal micropropagation in accelerating the breeding process of black currant] // Belgorod State University Scientific Bulletin. Series Natural Sciences. 2012. Vol. 21. No. 21-1 (140). Pp. 58–61. (In Russian.)

23. Shakhov V. V., Fedotova I. E., Tashmatova L. V., Matsneva O. V., Khromova T. M. Sravnitel'nyy analiz sterilizatorov na osnove periodizatsii ikh ispol'zovaniya [Comparative analysis of sterilizers on the basis of periodization of their use] // The Bulletin of KrasGAU. 2019. No 12 (153). Pp. 38–42. DOI: 10.36718/1819-4036-2019-12-38-42. (In Russian.)

24. Kambarova A., Karipbayeva R. K., Bakhtaulova A. S. Poluchenie asepticheski chistoy kul'tury smorodiny Meyera dlya vvedeniya v kul'turu *in vitro* [Obtaining an aseptically pure 'Meyer' currant culture for *in vitro* culture] // Selektsiya i sortorazvedeniye sadovykh kul'tur. 2020. Vol. 7. No. 1-2. Pp. 80–82. DOI: 10.36718/1819-4036-2019-12-38-42. (In Russian.)

25. Sakhvon E. V., Kudryashova O. A., Vaynovskaya I. F., Volotovich A. A. Sravnitel'nyy analiz sposobov asepticheskogo vvedeniya v kul'turu *in vitro* rasteniy *Melittis sarmatica* Klok. [Comparative analysis of administration aseptic in culture *in vitro* plants *Melittis sarmatica* Klok.] // Bulletin of Polessky State University. Series in Natural sciences. 2016. No. 1. Pp. 22–27. (In Russian)

26. Murashige T., Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures // Physiologia plantarum. 1962. Vol. 15 (3). Pp. 473–497. DOI: 10.1111/J.1399-3054.1962.TB08052.X.

27. Lloyd G., McCown B. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. 1980. Vol. 30. Pp. 421–427.

28. Anderson W. C. A revised tissue culture medium for shoot multiplication of rhododendron // Journal of the American Society for Horticultural Science. 1984. Vol. 109. Pp. 343–347. DOI: 10.21273/JASHS.109.3.343.

29. Quorin M., Lepoivre P. Improved media for *in vitro* culture of *Prunus* sp. // Acta Horticulturae. 1977. Vol. 78 (4). Pp. 437–442. DOI: 10.17660/ActaHortic.1977.78.54.

30. Molkanova O. I., Koroleva O. V., Stakheyeva T. S., Krakhmaleva I. L., Meleshchuk E. A. Sovershenstvovaniye tekhnologii klonal'nogo mikrorazmnozheniya tsennykh plodovykh i yagodnykh kul'tur dlya proizvodstvennykh usloviy [Improvement of clonal micropropagation technology of valuable fruit and berry crops varieties for commercial conditions] // Achievements of Science and Technology of AIC. 2018. Vol. 32 (9). Pp. 66–69. DOI: 10.24411/0235-2451-2018-10915. (In Russian.)

31. Krakhmaleva I. L., Molkanova O. I. Osobennosti razmnozheniya sortov kryzhovnika v kul'ture *in vitro* [Peculiarities of *in vitro* propagation of gooseberry varieties] // Pomiculture and small fruits culture in Russia. 2020. Vol. 62. Pp. 105–114. DOI: 10.31676/2073-4948-2020-62-105-114. (In Russian.)

32. Daroudi H., Akbarniya M., Hosseini S. M., Hajiyan Shahri M. Micropropagation of *Ribes khorasanicum* species by tissue culture // Iranian Journal of Rangelands and Forests Plant Breeding and Genetic Research. 2015. Vol. 23. No. 1. Pp. 65–76. DOI: 10.22092/IJRFPBGR.2015.101539. (In Persian.)

33. Dzhigadlo E. N., Dzhigadlo M. I., Golyshkina L. V. Metodicheskiye rekomendatsii po ispol'zovaniyu biotekhnologicheskikh metodov v rabote s plodovymi, yagodnymi i dekorativnymi kul'turami [Guidelines for the use of biotechnological methods in the work with fruit, berry and ornamental crops]. Oryol: Russian research institute of fruit crop breeding, 2005. 51 p. (In Russian)

34. Bad'yakov I. Georgiev V., Georgieva M., Dincheva I., Vrancheva R., Ivanov I., Georgiev D., Hristova D., Kondakova V., Pavlov A. Bioreactor technology for *in vitro* berry plant cultivation // In: Plant Cell and Tissue Differentiation and Secondary Metabolites: Fundamentals and Applications / K. G. Ramawat, H. M. Ekiert, S. Goyal (eds.). Cham: Springer, 2021. Pp. 383–431. DOI: 10.1007/978-3-030-30185-9_18.

35. Timofeeva O. A., Nevmerzhitskaya Yu. Yu. Klonal'noye mikrorazmnozheniye rasteniy: uchebno-metodicheskoye posobiye [Clonal micropropagation of plants: Educational and methodical manual]. Kazan: Kazan Federal University, 2012. 56 p. (In Russian.)
36. Tsitodef-100 [Cytodef-100] [Electronic resource] // Agrosintez [website]. URL: https://agrosintez.ru/catalog/czitodef-100-vrp (date of reference: 3.03.2022). (In Russian.)
37. Makarov S. S., Chudetskiy A. I., Kuznetsova I. B. Vliyaniye tsitokininov na protsess pobegoobrazovaniya rasteniy krasnoy smorodiny na etape "sobstvenno mikrorazmnozheniye" [The effect of cytokinins on the process of shoot formation of red currant plants at the stage of "true micropropagation"] // Izvestia Orenburg State Agrarian University. 2020. No. 3 (83). Pp. 101–103. DOI: 10.37670/2073-0853-2020-83-3-101-103. (In Russian.)
38. Milekhina N. V., Skovorodnikov D. N. Vliyaniye proizvodnykh difenilmocheviny na vvedeniye v kul'turu *in vitro* yagodnykh rasteniy [Influence of diphenyl-urea derivatives on small fruit introduction in culture *in vitro*] // Bulletin of the Bryansk State Agricultural Academy. 2017. No. 4 (62). Pp. 30–34. (In Russian.)
39. Matsneva O. V., Tashmatova L. V. Klonal'noye mikrorazmnozheniye zemlyaniki – perspektivnyy metod sovremennogo pitomnikovodstva (obzor) [Clonal micro-propagation of strawberries is a promising method of modern nursery practice (review)] // Contemporary Horticulture. 2019. No. 4. Pp. 113–119. DOI: 10.24411/2312-6701-2019-10411. (In Russian.)
40. Sidorevich M. S. Razrabotka etapa mikroklonal'nogo razmnozheniya v introduktsii smorodiny krovavo-krasnoy [Development of the stage of microclonal propagation in the introduction of blood-red currant] // Ustoychivoye razvitiye: regional'nyye aspekty: sbornik materialov XI Mezhdunarodnoy nauchno-prakticheskoy konferentsii molodykh uchenykh. Brest, 2019. Pp. 429–431. (In Russian.)
41. Kim K. W., Byun M. S., Kang M. S. Effect of ABA and agar in preventing vertitication of carnation plantlets cultured *in vitro* // Journal of the Korean Society for Horticultural Science. 1988. Vol. 29. No. 3. Pp. 208–215.
42. Karipbayeva R. K., Kanayev A. T., Ismailova M. E., Khani A. B. Adaptatsiya regenerantov smorodiny Mey-Yera (*Ribes Meyeri*) k usloviyam *ex vitro* [Adaptation of Meyer currant regenerants (*Ribes Meyeri*) to *ex vitro* conditions] // Ustoychivoye razvitiye territoriy: teoriya i praktika: materialy mezhdunarodnoy nauchno-prakticheskoy konferentsii. Sibay, 2020. Pp. 159–161. (In Russian.)
43. Demenko V. I., Lebedev V. G. Adaptatsiya rasteniy, poluchennykh *in vitro*, k nesteril'nym usloviyam [Adaptation of plants obtained *in vitro* to non-sterile conditions] // Izvestiya of Timiryazev Agricultural Academy. 2011. No 1. Pp. 60–70. (In Russian.)
44. Ivanova-Khanina L. V. Adaptatsiya rasteniy-regenerantov ezheviki k usloviyam *ex vitro* [Adaptation of blackberry plants-regenerators to *ex vitro* conditions] // Scientific Notes of V. I. Vernadsky Crimean Federal University. Biology. Chemistry. 2019. Vol. 5 (71). No. 1. Pp. 30–39. (In Russian.)
45. Crane J., Hughes H. Medium overlays for improved hardening of micropropagated potatoes // Horticultural Science. 1990. Vol. 25 (7). Pp. 794–795. DOI: 10.21273/HORTSCI.25.7.794.
46. Giang D. T. T., Tanaka M. Photoautotrophic micropropagation of *Epidendrum (Orchidaceae)* using disposable, gas permeable film vessel // Propagation of Ornamental Plants. 2004. Vol. 4 (2). Pp. 41–47.
47. Xiao Y., Kozai T. Comparison of micropropagation costs by photoautotrophic and photomixotrophic systems // Agricell Report. 2004. Vol. 4. Pp. 5–7.
48. Gront B. W. W. Photosynthesis of regenerated plantles *in vitro* and the stresses of transplantins // Acta Horticulturae. 1988. Vol. 230. Pp. 129–135.
49. Pavlova I. A., Gavrilenko I. V., Matyash Yu. S., Gavrilenko A. V., Shanin D. A., Likhovskoy V. V., Goncharenko V. A. Faktory effektivnoy adaptatsii rasteniy vinograda *in vitro* k usloviyam *ex vitro* [Original research factors of effective adaptation of grape plants *in vitro* to *ex vitro* conditions] // Magarach. Viticulture and winemaking. 2021. Vol. 23. No. 3. Pp. 226–232. DOI: 10.35547/IM.2021.30.22.003. (In Russian.)
50. Polyakov S. A., Rastorguyev S. L., Verzilin A. V. Adaptatsiya rasteniy-regenerantov zemlyaniki k nesteril'nym usloviyam [Adaptation of strawberry regenerated plants to non-sterile conditions] // Povysheniye effektivnosti sadovodstva v sovremennykh usloviyakh: materialy vserossiyskoy nauchno-prakticheskoy konferentsii. Michurinsk, 2003. Vol. 2. Pp. 335–339. (In Russian.)
51. Burgutin A. B. Mikroklonal'noye razmnozheniye rasteniy vinograda. Perevod probirochnykh rasteniy v pochvennuyu kul'turu [Micropropagation of grapes. Converting Test Tube Plants to Soil Culture] // Biologiya kul'tiviruyemykh kletok i biotekhnologiya: tezisy dokladov mezhdunarodnoy konferentsii. Novosibirsk, 1988. P. 12. (In Russian.)
52. Zhang B., Stoltz L. P. Acclimatization systems for *Euphorbia fulgens* microcuttings // HortScience. 1989. Vol. 24 (6). Pp. 1025–1026.
53. Kornatskiy S. A. Naiboleye znachimyye elementy promyshlennoy tekhnologii klonal'nogo mikrorazmnozheniya drevesnykh kul'tur [The most significant elements of the industrial technology of clonal micropropagation of tree crops] // Promyshlennoye proizvodstvo ozdorovlennogo posadochnogo materiala plodovykh, ya-

godnykh i tsvetochno-dekorativnykh kul'tur: materialy mezhdunarodnoy nauchno-prakticheskoy konferentsii. Moscow, 2001. Pp. 103–104. (In Russian.)

54. Makarov S. S., Kuznetsova I. B., Chudetskiy A. I. Izucheniye protsessa adaptatsii krasnoy smorodiny *in vitro* k nesteril'nym usloviyam *in vivo* [Studies on the adaptation process of red currant *in vitro* to non-sterile conditions *in vivo*] // Izvestia Orenburg State Agrarian University. 2020. No. 5 (85). Pp. 104–107. DOI: 10.37670/2073-0853-2020-85-5-104-107. (In Russian.)

55. Kovaleva I. S., Matsneva A. E., Khanbabayeva O. E., Mazayeva A. S. Vvedeniye v kul'turu *in vitro* i klonal'noye mikrorazmnozheniye perspektivnogo seyantsa smorodiny chernoy (*Rubes nigrum* L.) [The introduction to culture *in vitro* and clonal micropropagation of perspective seedling of black currant (*Ribes nigrum* L.)] // The Bulletin of KrasGAU. 2019. No. 12 (153). Pp. 43–48. DOI: 10.36718/1819-4036-2019-12-43-48. (In Russian.)

56. Muratova S. A., Trunov I. A., Melekhov I. D. Osnovnyye faktory, opredelyayushchiye uspekh perevoda mikrorasteniy v nesteril'nyye usloviya [Main factors determining the success of the transfer of microplants to non-sterile conditions] // Nauka i obrazovaniye. 2020. Vol. 3. No. 4. Pp. 298. (In Russian.)

57. Trunov I. A., Khoroshkova Yu. V. Optimizatsiya usloviy rosta mikrorasteniy sadovykh kul'tur na etape adaptatsii [Optimization of growing conditions of microplants of horticultural crops on the adaptation stage] // Bulletin of Michurinsk State Agrarian University. 2020. No. 1. Pp. 90–97. (In Russian.)

58. Byadovskiy I. A. Optimizatsiya usloviy rosta kostochkovykh kul'tur posle mikrorazmnozheniya: avtoref. dis. … kand. s.-kh. nauk [Optimization of growth conditions for stone fruit crops after micropropagation: abstract of the dissertation … candidate of agricultural sciences]. Moscow: Federal Horticultural Center for Breeding, Agrotechnology and Nursery, 2007. 25 p. (In Russian.)

59. Pierik R. L. M., Steegmans H. H. M., Sprenkels P. A. Micropropagation of lilac (*Syringa vulgaris* L.) // Biotechnology in agriculture and forestry. 1992. Vol. 20. Pp. 407–426.

60. Pavlova I. A., Klimenko V. P. Modelirovaniye klimaticheskikh usloviy dlya adaptatsii rasteniy vinograda *in vitro* k usloviyam *in vivo* [Modeling of the climatic conditions for the adaptation of grape plants *in vitro* to conditions *in vivo*] // Scientific works of SKFNTsSVV. 2019. Vol. 25. Pp. 164–168. DOI: 10.30679/2587-9847-2019-25-164-168. (In Russian.)

61. Klimenko V. P., Pavlova I. A. Sozdaniye posadochnogo materiala vinograda vysokikh biologicheskikh kategoriy kachestva na osnove ispol'zovaniya sovremennykh agrobiotekhnologiy [Production of grapevine planting material of high biological quality using modern agribiotechnologies] // Magarach. Viticulture and winemaking. 2018. No. 4. Pp. 34–36. (In Russian.)

Author's information:

Nelli V. Ryago[1], junior researcher, ORCID 0000-0003-2871-6126, AuthorID 1110256; *+7 996 164-79-91*, *ryago@orel.vniispk.ru*

[1] Russian Research Institute of Fruit Crop Breeding (VNIISPK), Zhilina, Russia