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TECHNOLOGICAL ASPECTS OF IN VITRO PROPAGATION OF ORGANIC STRAWBERRIES

M. SARSHAYEVA[1], A. TASHKENBAYEVA[1*], A. BILIBAYEVA[1], ZH. IRSALIYEVA[1], and A.M. USTEMIROVA[2]

[1]LLP - Kazakh Research Institute of Fruit and Vegetable Growing, Almaty, Kazakhstan
[2]LLP – Kazakh Research Institute of Agriculture and Plant Growing, Almaty, Kazakhstan
*Corresponding author's email: etashkenbayeva@mail.ru
Email addresses of co-authors: moka-1993@mail.ru,
abilibayeva1@gmail.com, irsalieva1996@mail.ru, zhanel-aigul@mail.ru

SUMMARY

The article showcased experimental data on key stages of the propagation technology of four varieties of an organic strawberry (*Fragaria x ananassa*) planting material in apical meristem culture, using molecular methods of virus identification and chimeras' absence, for the creation of central and reproductive mother plantations and accelerated introduction of the world collection's best varieties into production. The screening of newly introduced strawberry cultivars for five viruses by polymerase chain reaction (PCR), comprised the strawberry vein banding virus (SVBV), the strawberry crinkle virus (SCV), the strawberry mottle virus (SMV), the strawberry pallidosis-associated virus (SPaV), and the beet pseudo yellows virus (BPYV). The strawberry cultivars were free from any virus infection. At the introduction stage into tissue culture, sterilizing strawberry plants with active chlorine preparations ensued in sodium hypochlorite with different concentrations and exposures. Also, initiating environmentally safe growth regulators, amino acid proline, adenosine triphosphate (ATP), and vitamin C-ascorbic acid at a concentration of 10 mg/L into the nutrient medium helped activate regeneration after the introduction of apexes into tissue culture. Plants introduced into *in vitro* culture attained propagation at a required amount on MS medium containing 1% *Eleutherococcus* extract instead of cytokinin-6-BAP at the proliferation stage and 1% alcohol extract from the bark of Salix weeping willow instead of auxin-IMC at the rhizogenesis stage. The time of introduction into tissue culture and regeneration helped determine the ascorbic acid effect on the reduction of phenolic oxidation of apexes. The medium induced with amino acid proline and ATP increased regeneration by 10%–15%. The inclusion of Eleutherococca increased the multiplication ratio to 1:3 per one passage with the Salix extract, which increased rhizogenesis by 75%–80%. All the obtained primary strawberry plants incurred testing for chimeras' absence using molecular markers.

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Keywords: Organic strawberry (*Fragaria x ananassa*), clonal micropropagation, in vitro, ex vitro adaptation, polymerase chain reaction, strawberry viruses, chimera

Key findings: The task sought to study environmentally safe growth regulators of plant nature in the biotechnological recovery process and clonal micropropagation of garden strawberries with the release of organic seedlings tested for the absence of viruses and chimeras. Planting a central nursery with eco-strawberry seedlings targets the production of organic plants, with the subsequent establishment of industrial plantations and organic berries manufacture.

INTRODUCTION

An underdeveloped strawberry (*Fragaria x ananassa*) cultivation in Kazakhstan provided insufficient supply to the population of this valuable fruit throughout the year. Strawberry is one of the main berry crops with unlimited demand and has always been highly profitable in all regions of the republic. As an excellent dietary product, strawberries contain various compounds, including simple sugars and carbohydrates, fiber, vitamin C, and varied secondary metabolites. However, there is an acute shortage of planting material nowadays. Developing virus-free mother plants is necessary, which affected the commercial strawberry plantations. *In vitro* methods' extensive use has been ongoing for propagating valuable horticultural crops; especially berry crops (Elec *et al*., 2023; Osman *et al*., 2023; Pang *et al*., 2023).

In horticultural crops, the biotechnological techniques provide a high economic effect associated with early maturity, high reproduction rate, and enhanced yield with better quality. Various studies have reported that *in vitro* propagation is an effective way to obtain strawberry clones free from viral diseases (https://altyn-orda.kz/diplom-antonovka-vedushhij-sort-yablon/). However, the reproducibility results of the clonal propagation of different genotypes are low. The varietal response of genotypes is sturdier than in traditional breeding methods, and the physiological, hormonal, and physical factors have a significant impact, which needs accounting for successful breeding.

The strawberry propagation through tissue culture's development began as early as the 20th century (Boxus, 1974). To date, the *in vitro* microclonal propagation of garden strawberries is quite well-established, with continued use for a long time (Rattanpal *et al*., 2011; Ara *et al*., 2012; Jan *et al*., 2013; Mahmoud *et al*., 2017; Jhajhra *et al*., 2018; Yakovenko and Lapshin, 2018; Ionela *et al*., 2019). Despite this, continuous technology improvement is mandatory due to the varied collection of garden strawberries, wherein the genotypic characteristics still need identification when grown under *in vitro* conditions.

The microclonal propagation comprises several steps. These include the following: a) Transformation of nutrient media with various content of mineral composition, vitamins, and physiologically active substances specifically for culture; b) For selection and preparation of plant material, and by introducing tissues into tissue culture, an apical meristematic bud with two leaf primordia 0.1–0.2 mm in size, became initial explants of strawberries, which are sterilized and introduced into tissue culture under a binocular lens; c) For explant development, the culturing of explants is under the illumination of about 2,000–3,000 lux and a 16-h day at a temperature of 24 °C–25 °C; d) During the process of apical meristem cultivation and development of apexes, transplanting pursues the goals of renewing nutrient media, placing developing apexes in large culture vessels, reculturing secondary explants to maximize multiplication rate, and managing the regeneration process; e) A strawberry plant up to 20 mm in size with several leaves ensures better plant development for transplanting to a rooting medium. Growth regulators of auxin nature help stimulate root formation; and f) Adaptation of test tube plants during transfer from *in vitro* to *ex vitro*. Test tube microcloned strawberry plants with a developed root

system, a 1–3 cm length, and a rosette with several leaves entailed transfer to non-sterile conditions. Light, moisture-intensive, well-aerated materials (peat, sand, and perlite) served as a substrate (Dolgikh, 2020).

The primary *in vitro* culture determines the entire subsequent technological cycle of clonal micropropagation of plants. The rate of meristematic tissue initiation leading to the formation of whole plants depends on the rate of its discrimination. However, it always varied with different genotypes and was ascertained by the ability of explants to form axillary buds for cloning.

A necessary condition for working with isolated tissue culture is the observance of strict sterility, using a wide range of chemicals for surface sterilization of plant tissues. Sterilizing the explants consists of solution of compounds containing active chlorine (chloramine, calcium, sodium hypochlorite, mercury [II] chloride), bromine (bromine water), diacid, hydrogen peroxide, alcohol, and antibiotics. However, with the correct choice of a sterilizing agent, the degree of tissue infection should not exceed 1%–3%.

For strawberry large-scale production through a propagation system, the MS nutrient medium is suitable, as characterized by a high content of inorganic nitrogen that stimulates the processes of organogenesis (Murashige and Skoog, 1962). The ability of explants to regenerate depends on the mother plant's condition during isolation. For strawberries, the best period of *in vitro* introduction is the start of the active vegetation phase after dormancy and when the meristem can regenerate.

The morphogenetic potential of plants largely relies on growth regulators, primarily cytokinins. Strawberry micropropagation often uses the 6-BAP, which shows higher activity in maintaining tissue growth and inducing organogenesis. However, in producing strawberry eco-seedlings, Eleutherococci extract replaced the 6-BAP at a 1% concentration in the nutrient medium at the proliferation stage. Eleutherococcus is well-known to contain 0.26%–0.35% eleutherosides from a dry material, which promotes rapid regeneration of damaged tissues (Razumnikov, 2011).

Garden strawberries often form roots already on the propagation medium, bypassing the rooting stage. Semenas and Kukharchik (2000) recommended using a medium without phytohormones at the rooting stage. Some scientists believe short-term exposure to auxin on microshoots has a better stimulating effect (Suleimenova, 2016). Studies showed that in addition to increased hardiness, the root system of plants that have received supplemental willow bark extract develops better. The roots grow faster with a healthier and stronger root system, allowing for plants' better nutrients and water absorption. Early growth occurs as the root system quickens, leaves grow more actively, photosynthesis occurs more intensively, and the plant develops well. Growing fruits and berries leads to higher fruit weight by having larger and heavier fruits with a constant and better dry matter content on the Brix scale.

Under *in vitro* conditions, the spectral composition of light is one of bioproductivity's main factors greatly influencing growth, regeneration, and rhizogenesis processes. In this regard, the selective response of strawberry microshoots during microclonal propagation to irradiation with light of different spectral compositions is favorable for the growth and development of microshoots. Usually, fluorescent lamps benefit plant cultivation; however, LED irradiators are preferable (Bula *et al*., 1991). Using irradiators made of LEDs causes plants to develop faster, saving energy significantly compared with fluorescent lamps (Brown *et al*., 1995).

Adaptation to *ex vitro* conditions considers the main anatomical and physiological factors, i.e., poor development of conductive xylem vessels, absence of cuticular wax layer, and non-functioning stomatal apparatus. In reducing transpiration, sowing plants requires a sterile substrate under moist chamber conditions (95%–98% humidity). When developing the optimal conditions of *ex vitro* adaptation, its efficiency reaches 90%–100%.

Growing certified planting material using *in vitro* technology under production conditions is economically profitable. Producing organic fruit and berry products needs the

initial quality of planting material. Strawberry old cultivars fall below the agro-industrial complex requirements because the last zoning of strawberry cultivars happened 25 years ago in Southeast Kazakhstan. Therefore, the new strawberry cultivars of global importance need introduction and multiplication using highly effective methods of propagation.

Despite the great advantage of clonal micropropagation and its widespread use for strawberries, the technology of clonal micropropagation with molecular genetic evaluation of the genome to exclude chimeras in the production system of environmentally friendly planting material is yet to succeed. Having worked out all vital stages of the technology of clonal micropropagation of strawberries on modified nutrient media and checked the stability of its genome by molecular markers to exclude chimeras, it became possible to multiply principal plants up to the third reproduction and obtain the necessary amount of virus-free planting material for the planting of primary and reproductive nursery stock to obtain eco-seedlings and further use for year-round production of eco-harvest.

MATERIALS AND METHODS

Plant material

The objects of research were strawberry varieties, Malwina (Germany), Sabrina and Black Prince (Italy), and Red Gauntlet (Scotland), which transpired in 2021–2023 at the laboratory of the Biotechnology of Horticultural Crops KazF & VRI. Purebred, high-yielding, and outwardly healthy rosettes of garden strawberries incurred selection visually. Cutting horns with meristems from the base of leaves occurred for outwardly asymptomatic bushes. The best time for introducing apexes into tissue culture was March-May, with the meristems extracted under a binocular lens. For strawberry propagation, the size of optimal explants was 0.1–0.2 mm.

Enhancing the explant yield and multiplication rate necessitated sterilizing the initial plant tissue of the selected strawberry cultivars by the following steps:1) using first a soap containing triclosan (five times) and treating with 0.1% solution of sodium hypochlorite (5') and $MnO_4$5’; 2) next, washing three times with sterile water for 15 min each; 3) proceeding to in-vitro shoot regeneration used meristem culture, where the apical meristems’ isolation from sterilized buds continued under a binocular lens and inoculated into tissue culture on a nutrient medium containing macro and micro salts (Murashige and Skoog, 1962).

Introducing apexes into tissue culture, adding ascorbic acid (10 mg/L) and adenosine triphosphate (ATP) to the nutrient medium helped remove phenolic oxidation. The explants’ culturing on this medium at 20 °C–25 °C and 16 h of light ensued until active regeneration and formation of microshoots for transplanting to fresh nutrient medium every two weeks. The prime composition of the nutrient medium included macro and micro salts according to Murashige and Skoog, double amount of iron chelate, vitamins, i.e., ascorbic acid (1 mg/L), thiamine (10 mg/L), pyridoxine (5.5 mg/L), nicotinic acid (4.5 mg/L), para-aminobenzoic k (5 mg/L), and meso inositol (75 mg/L), and carbohydrates, such as, sucrose (30 g/L).

Culturing the explants had no growth regulators at the regeneration stage, with the nutrient medium containing ATP and proline amino acid (1 mg/L) each at the proliferation stage. The nutrient medium’s supplementation with 1% Eleutherococcus extract included eleutherosides, resins, essential oils, lipids, pectins, flavonoids, alkaloids, and polysaccharides that promote tissue regeneration. After inoculating, the transfer of explants in culture vessels to a ‘light’ room continued. This room contained an air conditioner to maintain an optimal cultivation temperature and lamps for illumination with different spectral compositions (red - regeneration and proliferation stage; blue - rhizogenesis). Before planting the microcloned plants for induction of rhizogenesis in vitro, a short-term treatment of the plants’ basal part used 1% extract of weeping willow bark.

Acclimatization of meristem regenerated plants

After rooting micro rosettes from in vitro conditions, their transplanting into ex vitro conditions for adaptation proceeded. Before planting, removing plants from the test tube with tweezers ensured the root system's thorough washing with tap water from the remaining nutrient medium. Its placement in a weak potassium permanganate (KMnO4) solution for a few seconds followed. Then the plants continued transplanting in the soil pre-moistened and treated with KMnO4 solution. Adaptation on the soil mixture had a soil: peat: sand ratio of 1:1:1. Planting one plant with a well-developed root and aboveground system in each container progressed. Keeping the substrate moist had the container covered with a food film in one layer or a glass. Development of plants took place in the adaptation greenhouse of the laboratory until they reached standard sizes at a temperature of 22 °C–24 °C with a 2–3 thousand lux illumination, relative humidity of 60%–70%, and a 16-h daylight. As the plants grew and new leaves appeared, a small ventilation hole in the food film became necessary. After transferring test tube plants into the substrate, treating them with a weak solution of KMnO4 for a week continued. After 3–4 weeks, the fully opened strengthened plants remained longer in the adaptation greenhouse before transplanting them into the base nursery.

Virus infection control was by PCR, and the presence of chimeric plants detection was by RAPD. Four strawberry cultivars identification proceeded to study the genetic profile and detect five viruses, namely, Strawberry vein banding virus (SVBV), Strawberry crinkle virus (SCV), Strawberry mottle virus (SMV), Strawberry pallidosis-associated virus (SPaV), and Beet pseudo yellows virus (BPYV) (Seliger, 1990).

Extraction of total RNA from strawberry leaves continued by the STAV method. Preparing a reaction mixture of 2 µL of RNA, 0.5 µL of 40 mMOligodt, and 0.5 µL of 40 mMNeq R enabled set-up reverse transcription. The mixture received incubation for 10 min at 72 °C. Next, adding 4µl of 5x Buffer RT, 0.5µl of dNTP, and 0.5µl of RevertAidTranscriptase to 15µl of the reaction mixture ensued. The cDNA synthesis happened for 60 min at 45 °C. The PCR entailed preparing a reaction mixture of 2 µl DNA, 2.5 µlTaqBuffer (NewEnglandBiolabs), 0.5 µl dNTP, 0.5 µl primers, and 0.5 µlTaq polymerase (NewEnglandBiolabs).

Employing RAPD analyzed DNA isolated from in vitro explants to detect possible deviations in the genetic stability of micropropagated plants (Degani *et al.*, 1998). The PCR-RAPD method depended on the amplification of DNA fragments using a randomly selected short random primer that amplifies multiple discrete regions of genomic DNA under appropriate PCR conditions. Following it was the separation of amplified fragments based on their size using gel electrophoresis, with the samples identified by comparing the DNA bands of the resulting patterns. For this analysis, isolating genomic DNA from leaves used STAB. Using 15 random primers help identify strawberry somaclonal variants: A-03, A-15, AA-01, AA-19, AF-06, AL-04, D-16, E-12, G-01, G-10, H-14, I-04, O-05, T-04, and J-19. The PCR program consisted of the following steps: denaturation at 92 °C for 3 min, 45 cycles of denaturation at 92 °C for 30 s, annealing at 35 °C for 1 min, elongation at 72 °C for 2 min, and final elongation for 10 min at 72 °C. The resulting PCR amplicons' separation continued in 2% TBE agarose gel (Figure 4). The results' visual analysis used the GelDocImagingSystem gel-documentation equipment, BioRad. The original basic strawberry plants, grown to standard size under indoor conditions, proceeded for planting in the spring of 2023 in a regular nursery to produce organic plants and products.

RESULTS AND DISCUSSION

Strawberries (*Fragaria x ananassa*) are usually by stolon propagation; however, this method does not guarantee better plant quality due to the risk of spreading diseases (Rojas *et al.*, 2013). Thus, in vitro, meristem culture is ideal for obtaining healthy and homogeneous plants (García-Gonzáles *et al*., 2010; Moradi *et al*., 2011; Quiroz *et al*., 2017). However, to

achieve high multiplication rates and better plant quality, it is a must to optimize the propagation technology to consider the characteristics of the genotypes, thereby minimizing the loss of material due to oxidation and contamination of tissues and a low response to in vitro growth and development conditions (Ko *et al*., 2009; Elec *et al*., 2023; Osman *et al*., 2023).

In the presented study, meristems treated with 0.1% sodium hypochlorite (NaClO) solution for 5 min achieved higher viability (60%) and eliminated contamination by 90%. Similar results came from the disinfection of strawberry meristems (cultivars Oso Grande and Toro) with 0.5% NaClO for 10–15 min recorded a grafting rate of 75% and less than 15% contamination (Munir *et al.*, 2015). It is worth noting that adding antioxidants is crucial at the stage of tissue culture induction and regeneration in vitro, as it reduces the phenolic oxidation of explants (Bhatia and Ashwath, 2008; Quiroz *et al*., 2017).

As a result of this research on the technological features of planting material propagation to obtain virus-free, environmentally friendly (organic) strawberry seedlings, plant extracts of Eleutherococcus, willow bark, the amino acid proline adenosine triphosphate (ATP), and vitamin C-ascorbic acid introduction into the nutrient medium replaced growth regulators of chemical nature (cytokinins and auxins). It was also apparent that introducing ascorbic acid (10 mg/L) in the nutrient medium's composition eliminated phenolic oxidation of apexes at the stage of inserting into tissue culture and regeneration. In strawberry cultivars, incorporating proline amino acid and ATP as part of the nutrient medium increased regeneration by 10%–15%. As shown in Table 1, the maximum rosette growth manifested in strawberry cultivars Sabrina and Black Prince. Plant growth and development are ultimately outcomes of light energy captured during photosynthesis. ATP is a universal cellular energy cofactor that fuels all vital processes, including gene expression and metabolism (De-Col *et al.*, 2017). Eleutherococcus extract doubled the multiplication rate as part of the nutrient medium (Table 1). The multiplication ratio of strawberry cultivars Sabrina and Black Prince was the maximum at 1:12–21 micro rosettes per explant compared with Malwina and Red Gauntlet cultivars' 1:8–10. The developmental stages of garden strawberries through in vitro tissue culture appear in Figure 1.

The biological activity of Eleutherococcus links with compounds called eleutherosides. The plant contains pectin substances, essential oil, coumarin derivatives, flavonoids, vegetable wax, resins, gum, phenolcarboxylic acids, vitamins, and trace elements, which universally contribute to activating regeneration processes in microplants in tissue culture. At the rhizogenesis stage, the macro-salt concentration reduced twofold, while the sucrose concentration decreased to 20 g/l. The strawberry microplants treatment with weeping willow bark extract enhanced the quality of the root system (Figure 2). Figure 2 shows Malwina and Red Gauntlet strawberry varieties rooted in the nutrient medium with the weeping willow (Salix) extract treatment compared with the Black Prince variety, rooting on a medium with an added IBA (control).

Table 1. Effect of proline amino acid, ATP, and eleutherococcus on regeneration and proliferation activity of strawberry cultivars in vitro.

Varieties of garden strawberries	Rosette length after two months of cultivation, cm		Reproduction rate by 6th passage, pcs./1 explant	
	Murashige-Skoog, control	Murashige-Skoog + proline, ATP	Murashige-Skoog, control	Murashige-Skoog + eleutherococcus solution
Malvina	0,1-0,4	0,2-0,5	1:5	1:10
Red Gontlit	0,1-0,2	0,2-0,3	1:4	1:8
Sabrina	0,7-1,4	1,0-2,0	1:10	1:21
Black Prince	0,5-1,0	0,8-1,2	1:8	1:12

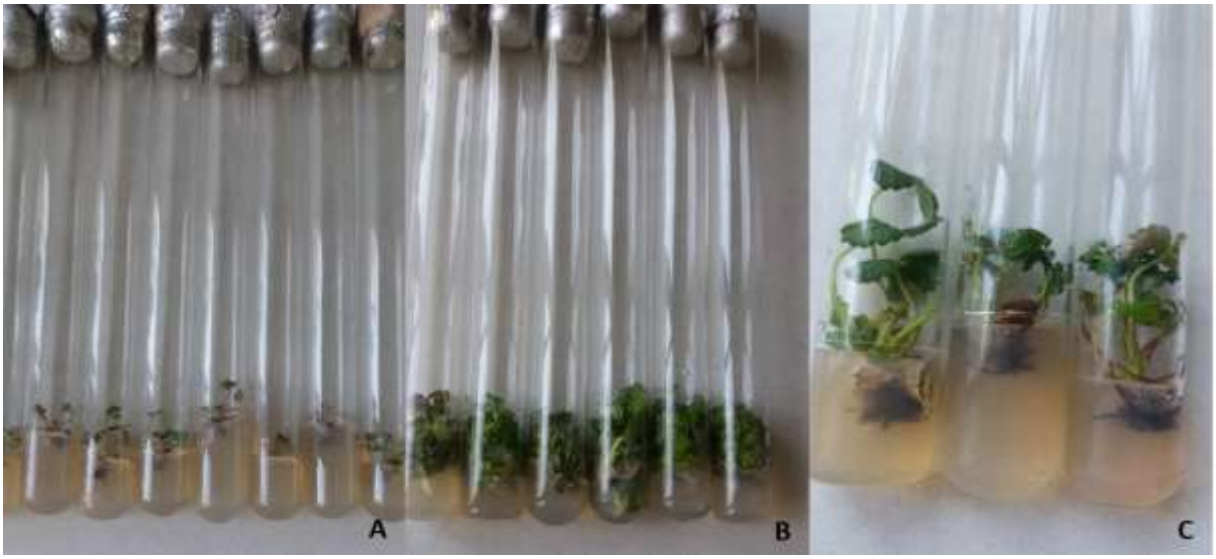

Figure 1. Stages of strawberry in tissue culture: A- regeneration; B- proliferation; C- rhizogenesis.

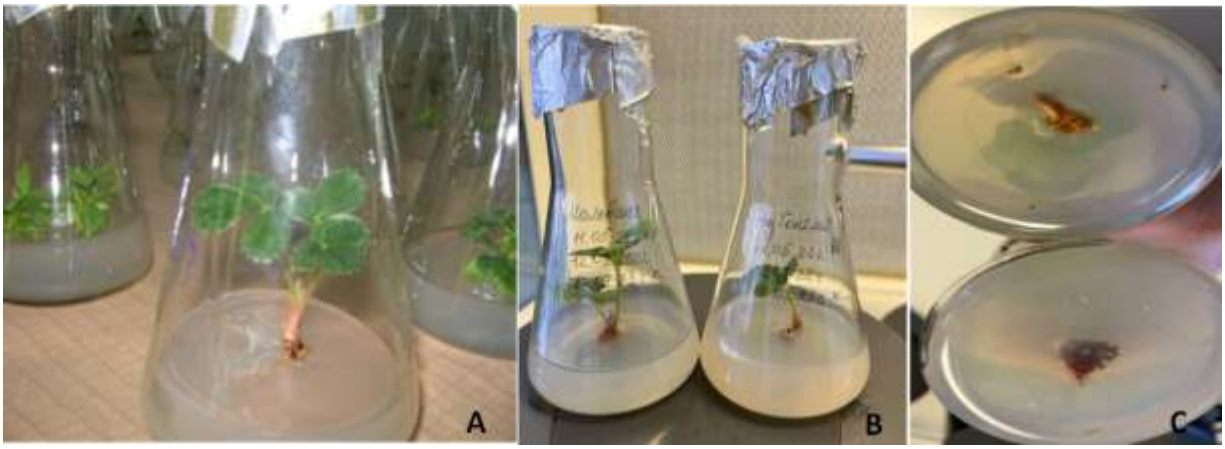

Figure 2. Rooting of microcloned plants at the rhizogenesis stage.

In general, modifying a nutrient medium to produce organic strawberry seedlings for all stages of clonal micropropagation, namely, tissue culture induction, regeneration, proliferation, and rhizogenesis flourished (Table 2). It is also well known that any micropropagation system's success connects with the effective transfer of seedlings from vessels for tissue cultivation in environmental conditions and adaptation during acclimatization (Hazarika, 2003). The ex vitro conditions, such as greenhouse and field settings, have characteristics of lower relative humidity and higher light levels than in vitro conditions. Therefore, a better container culture substrate with high moisture holding capacity, porosity, and drainage is essential for the proper growth and development of in vitro-derived seedlings (Chugh *et al*., 2009; Kaviani and Negahdar, 2017; Adibi-Baladeh and Kaviani, 2021; Sahari-Moghadam *et al*., 2022). In addition, the efficiency of acclimatization and new introduction gain impacts from environmental conditions, i.e., light intensity, humidity, and air temperature (Jeon *et al*., 2005).

Table 2. Effect of variety on in vitro regeneration processes.

A. auxin - indole-3- butyric acid, B,C. Auxin is an extract of the bark of weeping willow (*Salix*).Cultivars	Number of introduced explants	Regeneration rate (%)	Proliferation, reproduction coefficient	Rhizogenesis (%)
Malwina	105	95	1:10	90
Sabrina	90	85	1:7	80
Black Prince	100	95	1:8	90
Red Gauntlet	95	90	1:5	85

Figure 3. Adaptation of basal plants of garden strawberry during the in vitro - ex vitro transition period.
A- in vitro - ex vitro adaptation , **B-** container culture of strawberry plants

The high rooting rate during the adaptation period (90%) of the obtained microplants showed a soaring level of the developed technological aspects. It was evident that microplants treated with 1% alcohol extract of weeping willow (Salix) bark provided much rooting and high adaptability during acclimatization (90%–95%) compared with plants grown on medium with added IBA. The mechanism of action was that willow bark contains two essential compounds: salicin and indole acetic acid. Indole acetic acid (IAA) is a plant hormone that stimulates root growth. Salicylic acid has a stimulating effect on cuttings and in microclonal propagation of plants. It blocks the stress hormone caused by the damage the plant seeks to heal immediately after introduction into tissue culture.

It was also noticeable that the willow tincture may be an alternative for well-known and much more expensive preparations. It was available in greenhouses and hothouses before the appearance of artificial preparations that accelerated the process of root emergence. Likewise, it can also cause a protective reaction in nearby plants by converting salicylic acid into a volatile chemical form (https://dzen.ru/a/Xh7U6yvrSQCtpaxY). As studies have shown, the soil composition (soil: peat: sand ratio of 1:1:1) greatly influenced the rooting of plants in non-sterile conditions. For strawberry cultivars, the percentage of adapted prime plants under greenhouse conditions showed an average of 70% (Figure 3).

For accelerated microclonal propagation of organic strawberry cultivars, the following resolved chief tasks included: a) Identifying valuable strawberry cultivars; b) Selecting strawberry cultivars for introduction into tissue culture with the improvement of the technological scheme of microclonal propagation specifically for each variety at the stages of sterilization of explants, tissue culture introduction, primary regeneration of

Table 3. Identification of virus infection of strawberry varieties.

Virus name	Strawberry varieties			
	Malvina	Sabrina	Red Gotlit	Black Prince
Strawberry vein banding virus (SVBV)	-	-	-	-
Strawberry crinkle virus (SCV)	-	-	-	-
Strawberry mottle virus (SMV)	-	-	-	-
Strawberry pallidosis-associated virus (SPaV)	-	-	-	-
Beet pseudo yellows virus (BPYV).	-	-	-	-

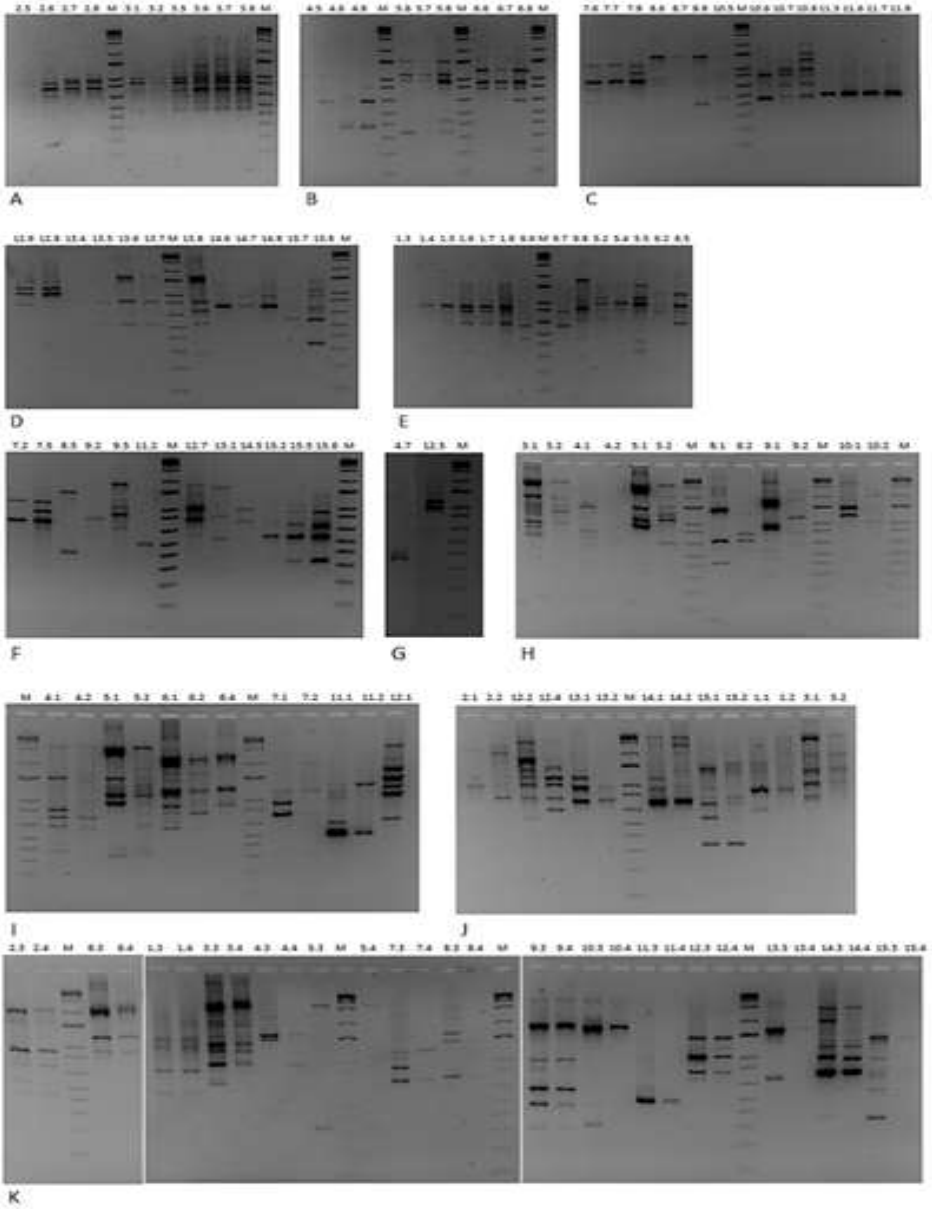

Figure 4. Results of RAPD amplification with markers.

A-samples 5-8 with marker №. 1; 1-8 with marker №. 8. B-samples 5, 6, and 8 with marker №. 4; 6-8 with marker №. 5; 6-8 with marker №. 6. C- samples 6-8 with markers №. 7 and №. 8; 5-8 with markers №. 10 and №. 11. D-samples 6 and 8 with marker №. 12; 4-8 with marker №. 13; 6-8 with marker №. 14; samples 7 and 8 with marker №. 15. E- samples 3-8 with marker № 1; 6-8 with marker №. 9; samples 2, 4, and 5 with marker №. 5; 2 and 5 with marker №. 6. F- samples 2 and 5 with markers №. 7 and 9; 2nd sample with markers №. 11 and 13; samples 2, 5, and 6 with marker №. 15. G-1 and 2 samples with 4-7 and 11 markers; 4th sample with 6 markers. H- 1 and 2 samples with 3-5 and 8-10 markers. I-1 and 2 samples with 1-3 and 12-15 markers. J - 7th sample with marker № 4; 5th sample with marker №. 12. K- samples 3 and 4 with markers 1-15. M-1 kb Ladder.

Figure 5. Basic and baseline brood stock strawberry: A-basic, B-baseline.

apexes, and proliferation and rhizogenesis with genotype preservation; c) A sufficient amount of material propagated in the tissue culture, improving the adaptation period - during in vitro - ex vitro plant transition; d) in the greenhouse, microcloned plants grown to standard sizes of the regular category and replicated to basic rosettes; e) Control of virus infection by OT-PCR and the absence of chimeras by RAPD for establishing standard and reproductive mother plantings; and f) Seedlings planted for the establishment of prime and major mother plants.

The control of virus infection by OT-PCR of microcloned strawberry plants showed no virus infections. Identification of virus infection ran on four strawberry cultivars (Table 3) to study the genetic profile and detection of five viruses: SVBV, SCV, SMV, SPaV, and BPYV.

RAPD analysis used 15 primers to genotype microcloned plants of four strawberry cultivars (Figure 4) for the absence of chimeric plants. Figure 4 shows the results of RAPD amplification with markers that indicate a reliable chimera absence. Most of the 15 primers used in the RAPD analysis successfully generated assessable bands for both conventionally propagated and meristem-derived plants; however, the pattern of RAPD bands differed slightly among the tested cultivars. As expected, the banding patterns observed in meristem-derived plants were similar to those observed in conventionally propagated plants for all four cultivars. In 2023, primary and basal mother plants of strawberry varieties continued for planting in the spring on 0.02 ha (Figure 5).

CONCLUSIONS

In strawberries (*Fragaria x ananassa*), the clonal micropropagation technology for obtaining eco-seedlings was better. The nutrient medium modifications at all stages of the technological scheme comprised the introduction of growth regulators of a plant origin into the nutrient medium composition. The propagated strawberry planting material occurred free from virus infections, namely, Strawberry vein banding virus (SVBV), Strawberry crinkle virus (SCV), Strawberry mottle virus (SMV), Strawberry pallidosis-associated virus (SPaV), and Beet pseudo yellows virus (BPYV). The healthy planting material was free from chimeras and served as plant base in nurseries.

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REFERENCES

Adibi-Baladeh D, Kaviani B (2021). Micropropagation of medlar (*Mespilus germanica* L.), a Mediterranean fruit tree. *Int. J. Fruit Sci*. 21(1): 242-254.

Altyn-orda Home Page. Available online: https://altyn-orda.kz/diplom-antonovka-vedushhij-sort-yablon/. (accessed on January 14, 2023).

Ara MT, Karim R, Karim MR, Ahmad Sh, Islam R, Hossain M (2012). Effects of different hormones on in vitro regeneration of strawberry (*Fragaria x ananassa* Duch.). *Int. J. Biosci.* 2(10): 1.

Bhatia P, Ashwath N (2008). Improving the quality of in vitro cultured shoots of tomato (*Lycopersicon esculentum* Mill. cv. Red coat). CQ University. Journal contribution. https://hdl.handle.net/10018/17259.

Boxus PH (1974). The production of strawberry plants by in vitro micro-propagation. *J. Hortic. Sci.* 49(3): 209-210.

Brown CS, Schuerger AC, Sager JC (1995). Growth and photomorphogenesis of pepper plants under red light-emitting diodes with supplemental blue or far-red lighting. *J. Am. Soc. Hortic. Sci.* 120(5): 808-813.

Bula RJ, Morrow RC, Tibbitts TW, Barta DJ, Ignatius RW, Martin TS (1991). Light-emitting diodes as a radiation source for plants. *HortSci.* 26(2): 203-205.

Chugh S, Guha S, Rao IU (2009). Micropropagation of orchids: A review on the potential of different explants. *Scien. Hortic.* 122(4): 507-520.

De-Col V, Fuchs P, Nietzel T, Elsässer M, Voon CP, Candeo A, Schwarzländer M (2017). ATP sensing in living plant cells reveals tissue gradients and stress dynamics of energy physiology. *Elife* 6: e26770.

Degani C, Rowland LJ, Levi A, Hortyński JA, Galletta GJ (1998). DNA fingerprinting of strawberry (*Fragaria* × *ananassa*) cultivars using randomly amplified polymorphic DNA (RAPD) markers. *Euphytica* 102: 247-253.

Dolgikh S (2020). Technology of production of virus-free planting material of fruit crops and grapes. Recommendation, Almanac, pp. 90.

Elec VH, Cadorna CAE, Rey JD (2023). In silico mining and validation of simple sequence repeat (SSR) markers derived from the Fragaria x ananassa genome assembly. *SABRAO J. Breed. Genet*. 55(5): 1573-1586. http://doi.org/10.54910/sabrao2023.55.5.11.

García-Gonzáles R, Quiroz K, Carrasco B, Caligari P (2010). Plant tissue culture: Current status, opportunities and challenges. *Int. J. Agric. Nat. Resour*. 37(3): 5-30.

Hazarika BN (2003). Acclimatization of tissue cultured plants. *Curr. Sci*. 85: 1704-1712.

Ionela R, Aurel P, Valentina I, Dorel H (2019). Micropropagation of strawberry cv. Magic. Analele Universitatii din Craiova. Seria Biologie, Horticultura, Tehnologia Prelucrarii Produselor Agricole, Ingineria Mediului, 24.

Jan A, Bhat KM, Bhat SJA, Mir MA, Bhat MA, Imtiyaz A, Rather JA (2013). Surface sterilization method for reducing microbial contamination of field grown strawberry explants intended for in vitro culture. *Afr. J. Biotechnol*. 12(39): 5749-5753.

Jeon MW, Ali MB, Hahn EJ, Paek KY (2005). Effects of photon flux density on the morphology, photosynthesis and growth of a CAM orchid, *Doritaenopsis* during post-micropropagation acclimatization. *Plant Growth Regul*. 45: 139-147.

Jhajhra S, Dashora LK, Singh J, Bhatnagar P, Kumar A, Arya CK (2018). In-vitro propagation of strawberry (*Fragaria* × *ananassa* Duch.). *Int. J. Curr. Microbiol. Appl. Sci*. 7(10): 3030-3035.

Kaviani B, Negahdar N (2017). Propagation, micropropagation and cryopreservation of *Buxus hyrcana* Pojark., an endangered ornamental shrub. *South Afr. J. Bot*. 111: 326-335.

Ko C, Al-Abdulkarim AM, Al-Jowid SM, Al-Baiz A (2009). An effective disinfection protocol for plant regeneration from shoot tip cultures of strawberry. *Afr. J. Biotechnol.* 8(11): 2611-2615.

Mahmoud KB, Najar A, Jedid E, Jemai N, Jemmali A (2017). Tissue culture techniques for clonal propagation, viral sanitation and germplasm improvement in strawberry (*Fragaria* ×

ananassa Duch.). *J. New Sci.,* 47: 2564-2576.

Moradi K, Otroshy M, Azimi MR (2011). Micropropagation of strawberry by multiple shoots regeneration tissue cultures. *J. Agric. Technol*. 7(6): 1755-1763.

Munir M, Iqbal S, Baloch JUD, Khakwani AA (2015). In vitro explant sterilization and bud initiation studies of four strawberry cultivars. *J. Appl. Hortic*. 17(3): 192-198.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plant*. 15(3): 473-497.

Osman A, Sitohy M, Mohsen FS, Abbas E (2023). Effectiveness of 7s globulin against Botrytis cinerea causing gray mold in strawberry. *SABRAO J. Breed. Genet*. 55(5): 1690-1702. http://doi.org/10.54910/sabrao2023.55.5.21

Pang WQ, Tan ST, Mad'Atari MF, Yoong ICK, Subramaniam S (2023). Establishment of an efficient micropropagation protocol for Cameron Highlands White Strawberry (*Fragaria x ananassa*) using light emitting diodes (LEDs) system. *South Afr. J. Bot*. 157: 189-200.

PlantoSys Home Page. Available online: https://www.plantosys.com/ru/zdorovye-kultury/ingredienty/ekstrakt-kory-ivy. (accessed on 2023).

Quiroz KA, Berríos M, Carrasco B, Retamales JB, Caligari PD, García-Gonzáles R (2017). Meristem culture and subsequent micropropagation of Chilean strawberry (*Fragaria chiloensis* [L.] Duch.). *Biol. Res*. 50.

Rattanpal HS, Gill MIS, Sangwan AK (2011). Micropropagation of strawberry through meristem culture. *Acta Hortic*. 890: 149-153.

Razumnikov N (2011) Content of eleutherosides and trace elements in rhizomes and roots of Eleutherococcus pricklypus. Niva Povolzhya. *Indust. Biotechnol*. 90-94.

Rojas P, Almada RD, Sandoval C, Keller KE, Martin RR, Caligari PDS (2013). Occurrence of aphid-borne viruses in southernmost South American populations of *Fragaria chiloensis* ssp. chiloensis. *Plant Pathol*. 62(2): 428-435.

Sahari-Moghadam A, Kaviani B, Torkashvand MA, Abdossi V, Eslami A (2022). Micropropagation of English yew, an ornamental-medicinal tree. *J. Ornam. Plants* 12(2): 91-99.

Seliger HH (1990). "PCR protocols — A guide to methods and applications." Trends in Biotechnology 8: 335-335.

Semenas SE, Kukharchik NV (2000). Clonal micropropagation technique of garden strawberry cultivars. *Plodovodstvo (Belarus)*.

Suleimenova S (2016). Microclonal propagation of fruit crops. Wschodnioeuropean Czasopismo Naukove, No. 11, Part2, pp. 47-54.

Yakovenko V, Lapshin V (2018). Strawberry varieties for ecological study in the North Caucasus zone. Scientific Proceedings of the North Caucasus Federal Scientific Center of Horticulture, Viticulture, Winemaking, T. 14, pp. 147-154.

Zen Home Page. Available online: https://dzen.ru/a/Xh7U6yvrSQCtpaxY (accessed on January 16, 2020).