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Plant growth regulators on the micropropagtion of *Actinidia* cultivars

Reguladores de crescimento vegetal na micropropagação de cultivares de *Actinidia*

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ABSTRACT

Actinidia Lindl., commonly known as kiwifruit, is a valuable berry crop. The area of commercial kiwifruit plantations is increasing; the global production of kiwifruit is about 0.62% of the total production of major fruit crops. The use of biotechnological methods, which can significantly accelerate the propagation of quality planting materials, is considered to be relevant for the propagation of this crop. In this study, we optimized the culture medium composition at the micropropagation stage for the effective cultivation of promising cultivars of *A. arguta*, *A. kolomikta*, and *A. polygama*. We investigated the features of *Actinidia* morphogenesis depending on the genotype, the concentration of 6-Benzylaminopurine (0.5, 0.8, and 1.0 mg L^{-1}), and plant growth regulators (6-Benzylaminopurine, meta-topolin, and 2-isopentenyladenine at a concentration of 0.5 mg L^{-1}) in the media Quoirin and Lepoivre. *Actinidia arguta* (multiplication rate of 8.0) and *A. polygama* (6.8) developed faster at the micropropagation stage compared to *A. kolomikta* (4.9). The studied *Actinidia* representatives were cultured most effectively on Quoirin and Lepoivre media supplemented with 0.5 mg L^{-1} meta-topolin, compared to the media containing 0.5 mg L^{-1} 6-Benzylaminopurine and 0.5 mg L^{-1} 2-isopentenyladenine. The use of meta-topolin in the medium contributed to the increase in various morphometric traits, such as the height of microshoots (up to 28% depending on the species), their number (up to 52%), and their multiplication rate (up to 42%). We also recorded a high morphogenic capacity of the investigated species.

Index terms: *A. arguta*; *A. kolomikta*; *A. polygama*; cytokinin; regeneration.

RESUMO

A *Actinidia* Lindl., vulgarmente conhecida como kiwi, é uma frutífera valiosa. A área de plantio comercial de kiwis está a aumentando; a produção global de kiwis é de cerca de 0,62% da produção total das principais culturas frutícolas. A utilização de métodos biotecnológicos, que podem acelerar significativamente a produção de mudas de qualidade, é considerada relevante para a propagação desta cultura. Neste estudo, optimizámos a composição do meio de cultura na fase de micropropagação para o cultivo eficaz de cultivares promissoras de A. *arguta*, A. *kolomikta* e A. *polygama*. Investigámos as características da morfogénese de *Actinidia* em função do genótipo, da concentração de 6-Benzilaminopurina (0,5, 0,8 e 1,0 mg L^{-1}) e dos reguladores de crescimento de plantas (6-Benzilaminopurina, meta-topolina e 2-isopenteniladenina a uma concentração de 0,5 mg L^{-1}) nos meios Quoirin e Lepoivre. *Actinidia arguta* (taxa de multiplicação de 8,0) e *A. polygama* (6,8) desenvolveram-se mais rapidamente na fase de micropropagação em comparação com *A. kolomikta* (4,9). Os representantes de *Actinidia* estudados foram cultivados mais eficazes nos meios Quoirin e Lepoivre suplementados com 0,5 mg L^{-1} de meta-topolina, em comparação com os meios contendo 0,5 mg L^{-1} de 6-benzilaminopurina e 0,5 mg L^{-1} de 2-isopenteniladenina. A utilização de meta-topolina no meio contribuiu para o aumento de vários traços morfométricos, tais como a altura dos micro rebentos (até 28%, dependendo da espécie), o seu número (até 52%) e a sua taxa de multiplicação (até 42%). Foi registrado, também, uma elevada capacidade morfogénica das espécies investigadas.

Termos para indexação: *A. arguta*; *A. kolomikta*; *A. polygama*; citocinina; regeneração.

INTRODUCTION

The genus *Actinidia* Lindl. includes about 70 species cultivated around the world (Ferguson; Huang, 2007; POWO, 2023). Most *Actinidia* species have edible fruits widely used in various branches of the food industry, especially in canning (Ma et al., 2019; Kozak et al., 2020) and in the wine industry (Zakharenko, 2010; Park et al., 2013). *Actinidia* flowers and fruits are promising raw materials for the cosmetic industry (Chen et al., 2010; Kim et al., 2023). Different parts of the plants (leaves, vines, roots, and especially fruits) contain many types of bioactive compounds (triterpenoids, polyphenols, vitamin C, carbohydrates, amino acids, and minerals), which have laxative, anti-diabetic, antioxidant, anti-inflammatory, and

other health-promoting effects (Abe et al., 2010; Chang et al., 2010; Ciacci et al., 2014; Wojdylo et al., 2017; Li et al., 2018; Panishcheva; Motyleva; Kozak, 2021). Therefore, these plants are used in traditional medicine and also as pharmaceutical raw materials in folk medicine (He et al., 2019; Ma et al., 2021).

The area of commercial kiwifruit plantations is increasing; the world production of kiwifruit is about 0.62% of the total production for major fruit crops (Rey et al. 2020). The most common commercially cultivated *Actinidia* spp. (including *Actinidia chinensis* Planch., *Actinidia deliciosa* (A. Chev.) C.F. Liang & A.R. Ferguson) are grown in subtropical regions. However, there are only three frost-resistant species that can be cultivated in places with cold winters, which include *A. arguta* (Siebold et Zucc.) Planch. et Miq., *A. kolomikta* (Rupr. Et Maxim.) Maxim., and *A. polygama* (Sieb. et Zucc.) Maxim. (Chat, 1995). According to Avery (1991), *A. arguta* and *A. polygama* can withstand temperatures up to –31 °C without injury, while *A. kolomikta* has greater resistance to winter frosts (up to –40 °C). These species are cultivated in Russia.

The most representative collection of *Actinidia* in Russia of about 200 samples is available in the Federal Horticultural Center for Breeding, Agrotechnology, and Nursery (Kozak et al., 2020). Some valuable and unique genotypes are represented by a small quantity.

The vegetative propagation of unpopular cultivars is difficult because of the lack of the required number of donor plants. Therefore, innovative biotechnological methods need to be applied for mass propagation of high-quality planting material. The first micropropagation protocol of *Actinidia* (*A. chinensis*) was developed by Harada (1975), and it was further improved by Standardi (1980). Most studies on *Actinidia* were based on the *in vitro* culture of *A. deliciosa* (Mitrofanova; Mitrofanova, 2000; Nasib; Ali; Khan, 2008; Thakur et al., 2022;).

Some studies, however, have also investigated the micropropagation of other species, such as *A. arguta* (Hameg; Gallego; Barreal, 2017; Hameg et al., 2020), *A. kolomikta* (Kovac, 1993), *A. polygama* (Takahashi et al., 2004), and *A. melanandra* (Debenham; Seelye; Mullan, 2016). These researchers investigated the effect of macronutrients, plant growth regulators, and genetic characteristics on the *in vitro* shoot regeneration of these species (Hameg; Gallego; Barreal, 2017; Malaeva; Molkanova, 2021; Molkanova; Krakhmaleva; Kozak, 2022).

In this study, we optimized the content of the growth medium at the multiplication stage for the effective culture of the promising cultivars of *A. arguta*, *A. kolomikta*, and *A. polygama*. One objective of this study was to select the most optimal cytokinin and its concentration for better growth and development of plantlets of the studied *Actinidia* cultivars and species.

MATERIAL AND METHODS

All experiments were conducted in the Laboratory of Plant Biotechnology at Tsitsin Main Botanical Garden of the Russian Academy of Sciences (MBG RAS), Moscow, Russia. The generally accepted methods of plant biotechnology (Butenko, 1964) developed in the Laboratory (Molkanova et al., 2018) were used in this study.

Plant material

Cultivars of three *Actinidia* species with valuable features and in demand for mass production were selected for this study. All plant materials for *in vitro* initiation were obtained from the donor plants of the collection of the Federal Horticultural Center for Breeding, Agrotechnology, and Nursery located in the village of Mikhnevo, Moscow Region, Russia. Seven cultivars were selected from the *in vitro* gene bank of the Laboratory of Plant Biotechnology at MBG RAS. These cultivars were developed earlier while starting the institutional research project (male *A. arguta* cultivar, 'Rebristaya', 'Komandir'. 'Zemlyanichnaya', 'Izobilnaya', male *A. polygama* cultivar, and 'Beta'). Eight cultivars were introduced into the *in vitro* culture as part of the implementation of the grant ('Solnechnyj', 'Zolotaya Kosa', 'Taezhnyy Dar', male *A. kolomikta* cultivar, 'Moma', 'Pamyati Kolbasinoy', 'Osennyaya', and 'Perchik'). In total, 15 promising cultivars of three *Actinidia* species were used in this study (Table 1).

Surface sterilization

The explants (apical and lateral buds) were first washed under running tap water for 15 min, then submerged in 2% fungicide solution for 10 min and 70% ethanol for 1–2 min. The explants were rinsed for 5–7 min in 5–7% calcium hypochlorite solution, and 1–2 drops of Tween 20 were added. After adding each reagent, the explants were washed 4–5 times in sterile distilled water (Molkanova; Krakhmaleva; Kozak, 2022).

Table 1: The main characteristics of the studied *Actinidia* cultivars.

Species	Cultivar	Sex	Fruit color	Fruit mass (g)	Fruit taste
A. arguta	Male form	male	-	-	-
	'Solnechnyj'	male	-	-	-
	'Zolotaya Kosa'	female	yellowish green, green	8.0	sweet, with apple flavor
	'Rebristaya'	female	green	7.6	sweet and sour, with pineapple flavor
	'Taezhnyy Dar'	female	green	7.6	sweet and sour, with strong pineapple flavor
A. kolomikta	Male form	male	-	-	-
	'Komandir'	male	-	-	-
	'Zemlyanichnaya'	female	yellowish green	2.8	sweet, with strawberry flavor
	'Izobilnaya'	female	yellowish green, with light longitudinal stripes	3.6	sweet and sour, with strong pineapple flavor
	'Moma'	female	yellowish green	2.7	sweet, with pineapple flavor
	'Pamyati Kolbasinoy'	female	olive green, with light longitudinal stripes	4.3	light sweet and sour, with pineapple flavor
A. polygama	Male form	male	-	-	-
	'Beta'	female	orange, to dark orange	3.7	like bell pepper, with pepper aroma
	'Osennyaya'	female	dark orange	4.5	like bell pepper, with pepper aroma
	'Perchik'	female	orange	2.7	like bell pepper, with pepper aroma

Culture initiation

The initiation medium QL (Quoirin and Lepoivre, 1977), solidified with 6.6 g L^{-1} agar (Roeper, Germany), containing 30 g L^{-1} sucrose and 0.3 mg L^{-1} 6-Benzylaminopurine (6-BAP) (Sigma, USA), was used for shoot induction (Molkanova; Krakhmaleva; Kozak, 2022). The medium was adjusted to pH 5.8 with potassium hydroxide and sterilized in an autoclave (WAC-60; Daihan Scientific, South Korea) using pressurized saturated steam (P = 101 kPa) at 121 °C for 20 min.

Sterilized explants were grown in test tubes on the initiation medium. Explants were planted on the medium in a laminar flow cabinet, following the rules for working with sterile material. The explants were cultured at 23 ±2 °C and a 16-h photoperiod under cool-white light fluorescent lamps (light intensity 3,000–3,500 lux).

The duration of the initiation stage was 14 days. Shoots obtained after initiation were propagated and transferred to the multiplication medium. Later explants were subcultured every 30–35 days, before which, the multiplication rate (expressed as the product of the number of microshoots and the number of nodes per explant) was calculated.

Shoot multiplication

Explants (~1.0–1.5 cm long) with two nodes raised *in vitro* were transferred into 250 mL glass jars containing 50 mL of the QL medium supplemented with 6.6 g L^{-1} agar (Roeper, Germany), 30 g L^{-1} sucrose, and various cytokinins. Two experiments were conducted. The first experiment was designed to determine the optimal level of 6-BAP; 0.5, 0.8, and 1.0 mg L^{-1} 6-BAP were tested. In the second experiment, the effect of different cytokinins (6-BAP, meta-topolin (mT) (Duchefa, Netherlands), and 2-isopentenyladenine (2-iP) (Sigma, USA)) on plant morphogenesis was investigated at the concentration that was found to be optimal in the first experiment (0.5 mg L^{-1}). The explants for the second experiment were isolated from plants obtained as a result of the first experiment. The medium supplemented with 0.5 mg L^{-1} 6-BAP was used as a control. The pH of all media was adjusted to 5.8 before

the experiment, and then, the media were sterilized in an autoclave (WAC-60) using pressurized saturated steam (P = 101 kPa) at 121 °C for 20 min.

During this stage, the explants were cultured at 23 ±2 °C and a 16-h photoperiod under cool-white light fluorescent lamps (light intensity of 3,000–3,500 lux).

All experiments were repeated in triplicate; for each treatment, 10 explants were used for each variant. After 30–35 days of culture, the data on the number of microshoots per explant, the length of the microshoots, the frequency of spontaneous rhizogenesis, and the multiplication rate (expressed as the product of the number of microshoots and the number of nodes per explant) were calculated.

Statistical analysis

All data were expressed as the mean ±SD. The data were analyzed using PAST 2.17c (PAleontological STatistics), SPSS Statistics 23, and Microsoft Office Excel 2010. The differences between the mean values of multiple groups were determined by Duncan's multiple-range test. All differences were considered to be statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Culture initiation

During the *in vitro* initiation of the explants, the main and axillary shoots developed after 14 days. After 45 days, some genotypes showed induction of the development of adventitious buds and shoots. The multiplication rate of the representatives of the genus *Actinidia* changed significantly during *in vitro* culture. However, all studied species showed a general pattern of change, in which during the first and second subcultures the multiplication rate increased and reached the highest values by the third to fifth subcultures, and then, it decreased from the fourth to sixth subcultures. *Actinidia arguta* showed the highest multiplication rate in the third subculture, *A. kolomikta* showed the highest multiplication rate in the fifth subculture, and *A. polygama* showed the highest multiplication rate in the fourth subculture.

A similar pattern was found in other cultures. The micropropagation of lavender and essential oils rose during nine subcultures showed a similar increase in the multiplication index by the third to fourth passage; further subcultures were characterized by a decrease in this parameter (Yegorova et al., 2019; Yegorova et al., 2021).

Shoot multiplication

While optimizing the clonal micropropagation technique, the biological traits and details of the *in vitro* regeneration of each taxon must be considered. They depend on several factors, which include the genotype, epigenetic traits of explant cells, the physiological state of intact plants, the composition of the medium, and culture conditions (Hameg; Gallego; Barreal, 2017; Mitrofanova, 2018; Molkanova et al., 2018).

Some studies showed that the determination of the morphogenetic potential of most taxa mostly depends on the genetic traits of the plants (Mitrofanova, 2018; Molkanova et al., 2018; Marino; Bertazza, 1990). The regeneration capacity of the explants of different *Actinidia* species varied significantly; *A. arguta* and *A. polygama* developed faster than *A. kolomikta* at the micropropagation stage (Figure 1), which correlated with their growth dynamics during introduction. These findings matched the results of our previous study (Molkanova et al., 2014). However, in this study, we found that the traits of the genus affected the multiplication rate of the representatives of the genus *Actinidia* more than the traits of the cultivars (62.7% vs. 28.4%).

The genetic traits of cultivars are not only indicators of the ability of explants to micropropagate, but they also control their ability to micropropagate. Thus, the variation in the limits of the regeneration capacity of explants and the number of formed micro-plantlets were determined genotypically (Figures 2 and 3).

The multiplication rate of *A. kolomikta* cultivars varied from 4.6 to 5.5 and was lower than the multiplication rate of the cultivars of *A. arguta* and *A. polygama*. The cultivars of *A. arguta* and *A. polygama* had similar multiplication rates (6.3–8.4 and from 6.0–7.7, respectively). *Actinidia arguta* cv. 'Zolotaya Kosa' had the highest multiplication rate (10.1).

Plant growth regulators are essential components of the medium for clonal micropropagation. Cytokinins are important plant growth substances that can regulate morphogenesis in plant tissue and organ cultures. They affect various plant growth processes. When added to the medium, these compounds promote cell division, induce the differentiation of shoots, and remove apical dominance. The type of cytokinin used and its concentration significantly affect shoot formation and the quality of plantlets. The synthetic cytokinin 6-BAP is most commonly used in micropropagation. Other naturally occurring cytokinins, including mT and 2-ip, also have high physiological activity and are effective for the micropropagation of some plants; however, they are mostly used for research purposes (Van Staden; Zazimalova; George, 2008). A commonly used and effective cytokinin at the micropropagation stage for some

Actinidia representatives is 6-BAP, used at concentrations of 0.5–2.0 mg L^{-1} (Marino; Bertazza, 1990; Akbaş et al., 2007). It can be added to the medium along with auxins, gibberellic acid, or other plant growth regulators (Hameg; Gallego; Barreal, 2017). The differences in the response of different species and cultivars of *Actinidia* to growth regulators can help determine the relevance of the studies to optimize the micropropagation procedure and better understand the effect of various plant growth regulators, especially cytokinins, on shoot induction and development.

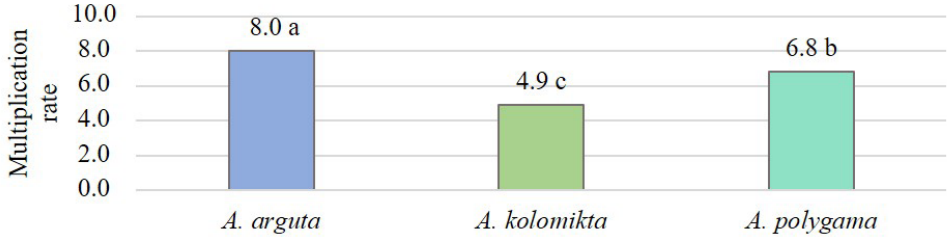

Figure 1: Effect of the genotype on the multiplication rate of the representatives of the genus *Actinidia* grown in the QL medium with 0.5 mg L^{-1} 6-BAP. The mean values followed by the same letters in the column indicate that the differences were not significant, as determined by Duncan's test ($p \leq 0.05$).

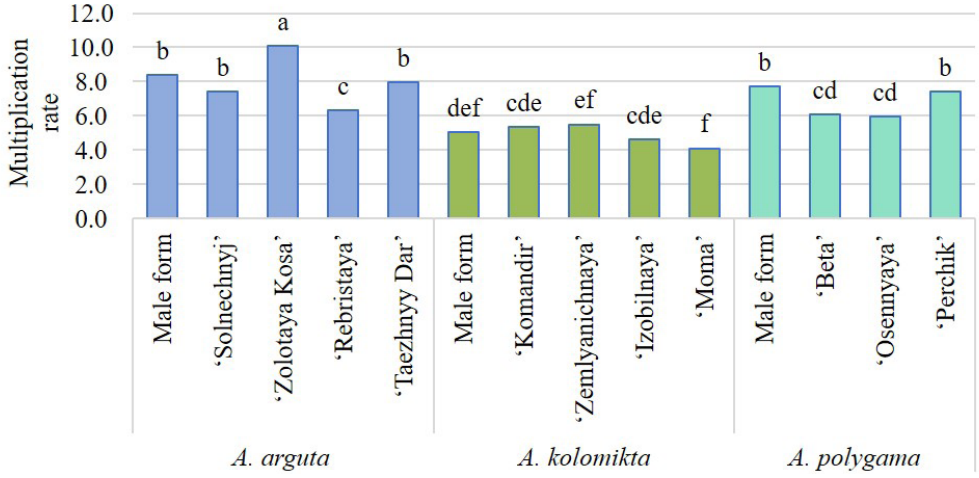

Figure 2: The multiplication rate of the representatives of the genus *Actinidia* in the QL medium with 0.5 mg L^{-1} 6-BAP is presented. The mean values followed by the same letters in the column indicate that the differences were not significant, as determined by Duncan's test ($p \leq 0.05$).

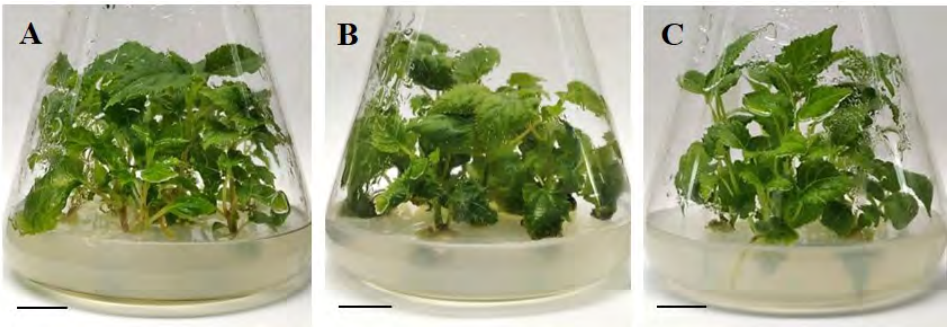

Figure 3: The representatives of the genus *Actinidia* with high morphogenetic potential are shown. A – *A. arguta* 'Zolotaya Kosa', B – *A.kolomikta* 'Komandir', and C – *A. polygama* male cultivar. Scale bars correspond to 1 cm.

The concentration of 6-BAP in the medium showed a lower effect on the changes in the multiplication rate of the representatives of *Actinidia* than the cultivar traits of the studied species (2.6 and 9.2% vs. 69.2–85.4%). Thus, no significant influence of the hormone composition of the medium and interaction of the studied factors were found on the multiplication rate of *A. arguta*.

Our results showed that increasing the concentration of 6-BAP increased the number of microshoots for most of the studied species, whereas the multiplication rates changed for only *A. kolomikta* and *A. polygama* (Table 2).

When the concentration of 6-BAP was increased from 0.5 to 1.0 mg L^{-1} in the medium, the number of microshoots and multiplication rate increased slightly (by 1.1 times). Therefore, we suggest using 0.5 mg L^{-1} 6-BAP while growing the *Actinidia* representatives to reduce cytokinin accumulation in the tissues of explants and increase their commercial efficiency.

Some researchers have reported that 6-BAP often negatively affects plant development due to the formation of stable compounds (6-benzylaminopurine-9-glucosides). The accumulation of these compounds inhibits the development of tissues (Yablonskaya; Knishkajte; Romanova, 2014). *Actinidia* spp. may develop microshoots with hyperhydricity when cultured on a medium containing this plant growth regulator (Marino; Bertazza, 1990; Saeiahagh et al., 2019).

An analog of 6-BAP, known as mT, can affect the *in vitro* shoot multiplication of *A. deliciosa* (Prado; Herrera, 2005), *Actinidia chinensis* var. *chinensis* (Saeiahagh et al., 2019), and other plant species, including *Maytenus emarginata* (Willd.) Ding Hou (Shekhawat et al., 2020), *Pogostemon cablin* (Blanco) Benth. (Lalthafamkimi et al., 2020), *Allamanda cathartica* L. (Khanam et al., 2020), *Ribes grossularia* L. (Kucharska et al., 2020), *Scaevola taccada* (Gaertn.) Roxb. (Shekhawat et al., 2021), and *Opuntia stricta* Haw. (de Souza et al., 2019).

Malaeva (2020) showed that 2-ip (3.0–5.0 mg L^{-1}) positively affects the multiplication rate of some representatives of *Actinidia*.

While culturing *Actinidia* representatives on media containing different cytokinins (6-BAP, mT, and 2-ip), a significant change in the multiplication rate was found. *Actinidia kolomikta* (77.2%) and *A. arguta* (64.1%) had the greatest effect of growth regulators on the morphogenetic capacity of the studied plants. The effects of various plant growth regulators on the morphometric traits of promising cultivars of the genus *Actinidia* are presented in Table 3.

The growth of *A. arguta* and *A. kolomikta* on media supplemented with mT and 2-ip promoted the increase in the height of the microshoot (*A. arguta*: 2.3 cm and 2.3 cm vs. 1.7 cm (6-BAP); *A. kolomikta*: 2.5 cm and 1.9 cm vs. 1.7 cm). The cultivars of *A. polygama* showed no significant differences in the height of the microshoots grown on these media (2.6 cm (mT) and 2.7 cm (2-ip) vs. 2.4 cm (6-BAP)). Similar effects of mT on the height of micro-plantlets were reported by other researchers who studied various horticultural species (Moyo et al., 2018; Kucharska et al., 2020; Nowakowska et al., 2020). Other studies found that 2-ip might have different effects on the length of the microshoot, but for some tree crops, 2-ip can positively affect shoot growth compared to 6-BAP (Debnath; Kenneth, 2001; Faisal et al., 2006; Singh, 2020; Wulandari et al., 2021; Haida et al., 2022).

Table 2: The effect of different concentrations of 6-BAP on the morphometric traits of the representatives of the genus *Actinidia*.

Species	6-BAP (mg L^{-1})	Length of shoot (cm)	Number of shoots per explant (unit)	Multiplication rate
A. arguta	0.5 (control)	1.7 a*	1.9 b	8.0 a
	0.8	1.6 a	2.0 ab	8.0 a
	1.0	1.6 a	2.2 a	8.2 a
A. kolomikta	0.5 (control)	1.6 a	1.4 b	4.9 b
	0.8	1.6 a	1.5 a	5.1 b
	1.0	1.6 a	1.6 a	5.4 a
A. polygama	0.5 (control)	2.5 a	1.7 b	6.8 b
	0.8	2.6 a	1.9 a	7.1 a
	1.0	2.5 a	1.8 a	7.2 a

*Means of each species, followed by the same letters in the column, do not differ by Duncan's test ($p \leq 0.05$).

Table 3: A list of the morphometric traits of promising *Actinidia* cultivars used for evaluating the effects of different plant growth regulators at 0.5 mg L^{-1}.

Species	Cultivar	Plant growth regulator	Length of shoots (cm)	Number of shoots per explant (unite)
A. arguta	Male form	6-BAP (control)	1.7±0.4 b*	1.8±0.6 b
		mT	2.2±0.5 a	2.1±0.7 a
		2-ip	2.4±0.4 a	1.1±0.3 c
	'Solnechnyj'	6-BAP (control)	1.4±0.4 c	1.7±0.5 b
		mT	2.3±0.6 a	2.3±0.8 a
		2-ip	1.7±0.3 b	1.2±0.4 c
	'Zolotaya Kosa'	6-BAP (control)	1.5±0.4 b	2.1±0.5 b
		mT	2.4±0.6 a	2.7±0.6 a
		2-ip	2.4±0.6 a	1.1±0.3 c
	'Rebristaya'	6-BAP (control)	1.7±0.4 b	1.5±0.5 b
		mT	1.8±0.5 b	2.2±0.6 a
		2-ip	2.5±0.6 a	1.1±0.3 c
	'Taezhnyy Dar'	6-BAP (control)	2.0±0.5 c	1.8±0.4 b
		mT	3.0±0.7 a	2.2±0.4 a
		2-ip	2.7±0.7 b	1.2±0.4 c
A. kolomikta	Male form	6-BAP (control)	1.6±0.7 a	1.6±0.5 b
		mT	1.4±0.4 a	2.3±0.5 a
		2-ip	1.4±0.5 a	1.2±0.4 b
	'Komandir'	6-BAP (control)	1.1±0.4 b	1.9±0.6 b
		mT	1.4±0.3 a	2.3±0.6 a
		2-ip	1.5±0.4 a	1.1±0.3 c
	'Zemlyanichnaya'	6-BAP (control)	1.5±0.5 b	1.6±0.6 b
		mT	1.8±0.2 a	2.0±0.0 a
		2-ip	1.8±0.5 a	1.2±0.4 c
	'Izobilnaya'	6-BAP (control)	2.0±0.5 b	1.0±0.2 b
		mT	2.6±0.7 a	2.0±0.5 a
		2-ip	1.6±0.5 c	1.0±0.0 b
	'Moma'	6-BAP (control)	1.8±0.5 c	1.1±0.3 b
		mT	3.0±0.6 a	2.3±0.8 a
		2-ip	2.4±1.0 b	1.0±0.0 b
	'Pamyati Kolbasinoy'	6-BAP (control)	2.1±0.8 a	1.4±0.5 b
		mT	1.7±0.4 b	1.9±0.6 a
		2-ip	2.4±0.6 a	1.1±0.3 b

Continue...

Table 3: Continuation.

Species	Cultivar	Plant growth regulator	Length of shoots (cm)	Number of shoots per explant (unite)
A. polygama	Male form	6-BAP (control)	1.9±0.5 a	1.9±0.6 ab
		mT	2.0±0.5 a	2.1±0.5 a
		2-ip	2.0±0.6 a	1.8±0.6 b
	'Beta'	6-BAP (control)	2.6±0.7 a	1.4±0.5 b
		mT	3.0±0.6 a	2.0±0.7 a
		2-ip	3.3±1.0 a	1.3±0.1 b
	'Osennyaya'	6-BAP (control)	3.1±0.8 b	1.5±0.5 a
		mT	3.5±1.1 a	1.5±0.5 a
		2-ip	3.8±0.9 a	1.2±0.4 b
	'Perchik'	6-BAP (control)	1.9±0.6 a	2.1±0.4 a
		mT	1.8±0.5 a	1.9±0.4 b
		2-ip	1.9±0.7 a	1.6±0.6 c

*Values of each cultivar are mean ±SD, followed by the same letters in the column do not differ by Duncan's test ($p \leq 0.05$).

The studied plant growth regulators could be arranged according to their effect on the ability to promote shoot formation in the following order: mT > 6-BAP > 2-ip. Some cultivars had no significant differences in the number of microshoots formed during cultivation on media containing 6-BAP and mT. Also, 0.5 mg L^{-1} 2-ip did not significantly affect the formation of new microshoots for the cultivars of *A. arguta* and *A. kolomikta*. When 2-ip was used, the number of microshoots ranged from 1.0 ±0.0 to 1.2 ±0.6 units per explant.

The cultivation of samples on the medium containing mT resulted in a significant increase in the multiplication rate of *A. arguta* (by 1.3 and 1.7 times) and *A. kolomikta* (by 1.5 and 1.7 times) compared to cultivation on media containing 6-BAP and 2-ip. *Actinidia polygama* also showed an increase in the multiplication rate when grown on the medium with mT; however, no significant differences were recorded when the plants were grown on the medium with 6-BAP (Figures 4 and 5).

Our results showed that the variability limits of the regeneration capacity of explants and the number of formed plantlets depend not only on the genotype but also on the composition of the medium.

Rhizogenesis is an important stage of the micropropagation development technique. It may occur spontaneously and can also be induced. Spontaneous rhizogenesis is interpreted by some researchers as the "hormonal autonomy" of plant cells under *in vitro* conditions (Gamburg; Leonova; Rekoslavskaya, 1974). When plants under *in vitro* conditions are unable to synthesize hormones necessary for root formation, auxin needs to be added to the medium to induce root formation. High concentrations of cytokinins (generally used at the micropropagation stage) may inhibit or delay root formation and prevent root growth even in the presence of auxins. Therefore, one or more subcultures in a cytokinin-free medium may be required for the efficient rooting of micro-plantlets (Van Staden; Zazimalova; George, 2008). Thus, spontaneous root formation is a cheaper and less labor-intensive clonal micropropagation technique, as there is no need for a separate rooting stage and no additional costs of applying chemical reagents. These inexpensive options can contribute to the efficiency of micropropagation and the popularization of the *in vitro* propagation protocol for mass production (FAO, 2004).

Spontaneous root formation at different frequencies was noticed during the culture of *Actinidia* representatives on different media (Figure 6). Thus, the studied *Actinidia* species represent crops that can be easily rooted without the use of auxins.

The studied species can be arranged according to the frequency of spontaneous root formation in the following order: *A. arguta* (52.8%) < *A. kolomikta* (68.0%) < *A. polygama* (95.6%). This allowed us to conduct micropropagation and rooting in one subculture period, which increased the efficiency of *in vitro* cultivation.

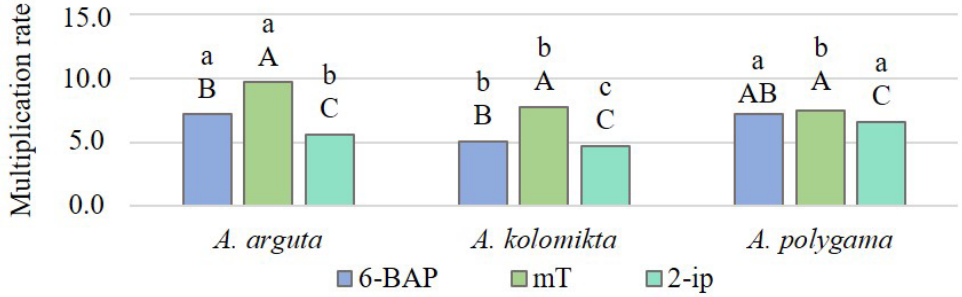

Figure 4: The change in the multiplication rate of the representatives of the genus *Actinidia* grown on media with different plant growth regulators with a concentration of 5 mg L^{-1}. Different capital letters indicate significant differences between the tested growth regulators for each species, as determined by Duncan's test ($p \leq 0.05$); different lowercase letters indicate significant differences between species grown on the same medium, as determined by Duncan's test ($p \leq 0.05$).

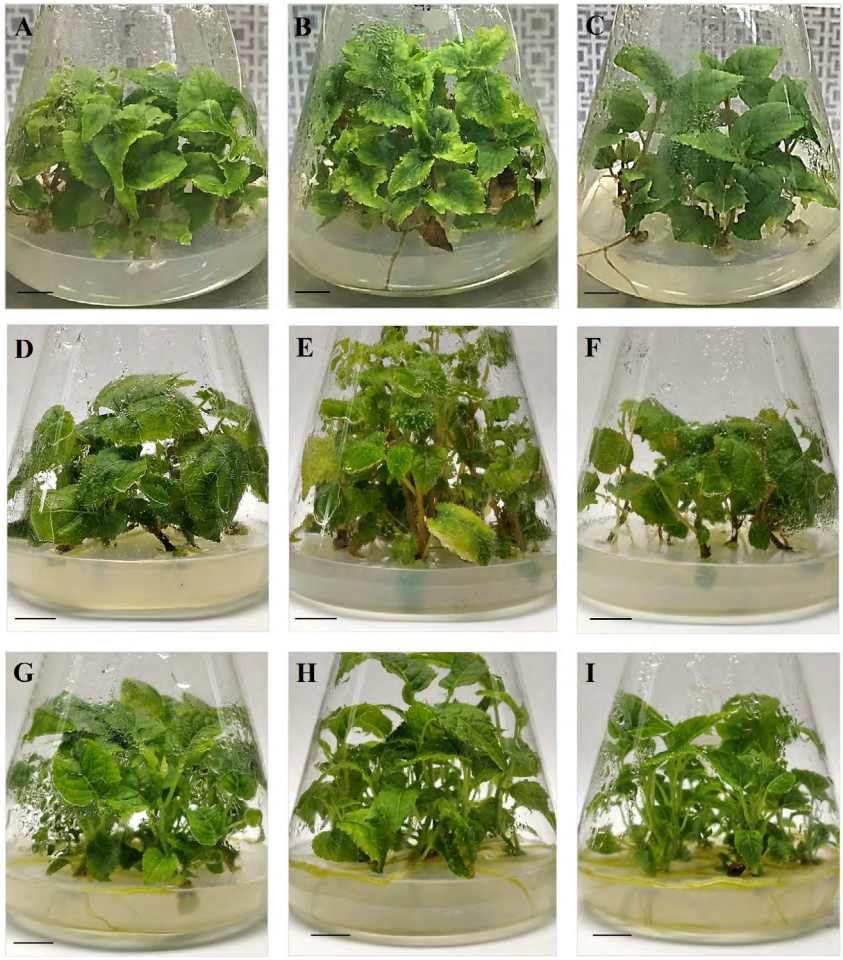

Figure 5: Microshoot development in the representatives of the genus *Actinidia* on media supplemented with different cytokinins at a concentration of 0.5 mg L^{-1}; *A. arguta* cv. 'Taezhnyy Dar' was grown on the medium with 6–BAP (A), mT (B), and 2-ip (C); *A. kolomikta* cv. 'Moma' was grown on the medium with 6–BAP (D), mT (E), and 2-ip (F); male cultivar of *A. polygama* was grown on the medium with 6–BAP (G), mT (H), and 2-ip (I). Scale bars correspond to 1 cm.

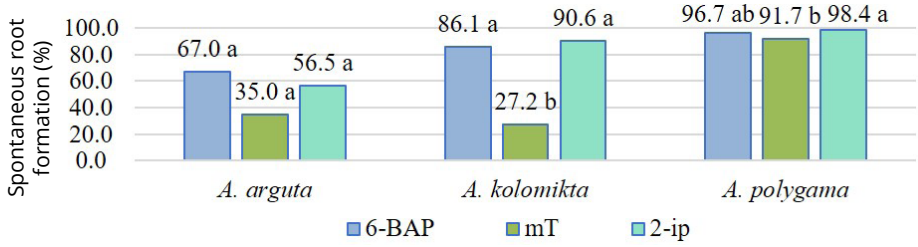

Figure 6: The frequency of spontaneous root formation of three *Actinidia* species when using different plant growth regulators at a concentration of 0.5 mg L^{-1}. The mean values of each species followed by the same letters in the column indicate that the differences are not significant, as determined by Duncan's test ($p \leq 0.05$).

In this study, we developed effective techniques of clonal micropropagation for promising *Actinidia* cultivars. These techniques might facilitate the propagation and *in vitro* long-time preservation of this crop.

CONCLUSIONS

In this study, we found that the regeneration capacity of the studied *Actinidia* cultivars depended on the genotype, growth regulators, and their concentrations in the medium. The efficiency of explant culturing on the QL medium supplemented with 0.5 mg L^{-1} mT contributed to the increase in the height and number of microshoots, and also the multiplication rate. We also found that mT induced adventitious shoot formation at the explant base and the induction of bud development on microshoots.

AUTHOR CONTRIBUTION

Conceptual idea: Mitrofanova, I.V.; Molkanova, O.I.; Methodology design: Molkanova, O.I.; Mitrofanova, I.V.; Data collection: Krakhmaleva, I.L.; Orlova N.D.; Data analysis and interpretation: Krakhmaleva, I.L.; Koroleva O.V.; Orlova N.D.; and Writing and editing: Krakhmaleva, I.L.; Koroleva O.V.; Molkanova, O.I.; Mitrofanova, I.V.

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REFERENCES

ABE, D. et al. A fraction of unripe kiwi fruit extract regulates adipocyte differentiation and function in 3T3-L1 cells. Biofactors, 36:52–59, 2010.

AKBAŞ, F. A. et al. Micropropagation of kiwifruit (*Actinidia deliciosa*). International journal of agriculture & biology, 9(3):489-493, 2007.

AVERY, J. *Actinidia*. Annual Report Northern Nut Growers Association, 82:175–179, 1991.

BUTENKO, R. G. Isolated tissue culture and physiology of plant morphogenesis. Moscow, Russia: Science, 1964, 272 p.

CHANG, C. C. et al. Kiwifruit improves bowel function in patients with irritable bowel syndrome with constipation. Asia Pacific Journal of Clinical Nutrition, 19(4):451–457, 2010.

CHAT, J. Cold hardiness within the genus *Actinidia*. HortScience, 30(2):329–332, 1995.

CHEN, X. et al. Characterisation of an (S)-linalool synthase from kiwifruit (*Actinidia arguta*) that catalyses the first committed step in the production of floral lilac compounds. Functional Plant Biology, 37(3):232–243, 2010.

CIACCI, C. et al. The kiwi fruit peptide kissper displays anti-inflammatory and anti-oxidant effects in *in-vitro* and *ex-vivo* human intestinal models. Clinical & Experimental Immunology, 175(3):476–484, 2014.

DE SOUZA, L. M. et al. Use of meta-Topolin, an unconventional cytokinin in the *in vitro* multiplication of *Opuntia stricta* Haw. Biotecnologia Vegetal, 19:85–97, 2019.

DEBENHAM, M. C.; SEELYE, J. F.; MULLAN, A. C. An *in vitro* repository for clonal kiwifruit. Acta Horticulturae, 1113:93–98, 2016.

DEBNATH, S. C.; KENNETH B. M. An efficient *in vitro* shoot propagation of cranberry (*Vaccinium macrocarpon* ait.) by axillary bud proliferation. In Vitro Cellular & Developmental Biology – Plant, 37:243–249, 2001.

FAISAL, M. et al. *In vitro* rapid regeneration of plantlets from nodal explants of *Mucuna pruriens*: a valuable medicinal plant. Annals of Applied Biology 148:1–6, 2006.

FAO. Low cost options for tissue culture technology in developing countries: proceedings of a technical meeting organized by the joint FAO/IAEA division of nuclear techniques in food and agriculture and held in Vienna 26–30 August 2002. Austria: IAEA, 106 p., 2004.

FERGUSON, A.R.; HUANG, H. Genetic Resources of Kiwifruit: Domestication and Breeding. *In* JANICK J. Horticultural Reviews. Volume 33. Hoboken. New Jersey. USA: John Wiley and Sons. Inc, p.1–121, 2007.

GAMBURG K.Z.; LEONOVA L.A.; REKOSLAVSKAYA N.I. Hormonal autonomy of plant cells in isolated culture. *In*: GAMBURG K.Z. Growth and hormonal regulation of plant life. Irkutsk, Russia: Siberian Institute of Plant Physiology and Biochemistry, p.85–101, 1974.

HAIDA, Z. et al. Shoot Induction, Multiplication, Rooting and Acclimatization of Black Turmeric (*Curcuma caesia* Roxb.): An Important and Endangered Curcuma Species. Horticulturae, 8(8):740, 2022.

HAMEG, R. et al. Modeling and optimizing culture medium mineral composition for *in vitro* propagation of *Actinidia arguta*. Frontiers in Plant Science, 11:1–19, 2020.

HAMEG, R.; GALLEGO, P.; BARREAL, M. E. *In vitro* establishment and multiplication of hardy kiwi (*Actinidia arguta* 'Issai'). Acta Horticulturae, 1187:51–58, 2017.

HARADA, H. *In vitro* organ culture of *Actinidia chinensis* PI. as a technique for vegetative multiplication. Journal of Horticultural Science, 50(1):81–83, 1975.

HE, X. et al. *Actinidia chinensis* Planch.: A Review of Chemistry and Pharmacology. Frontiers in pharmacology, 10:1236, 2019.

KHANAM, M. N. et al. meta-Topolin induced *in vitro* regeneration and metabolic profiling in *Allamanda cathartica* L. Industrial Crops and Products, 145:111944, 2020.

KIM Y. J. et al. Effect of *A. polygama* APEE (*Actinidia polygama* ethanol extract) or APWE (*Actinidia polygama* water extract) on wrinkle formation in UVB-irradiated hairless mice. Journal of Cosmetic Dermatology, 22(1):311–319, 2023.

KOVAC, J. Micropropagation of *Actinidia kolomikta*. Plant cell, tissue and organ culture, 35:301–303, 1993.

KOZAK, N. V. et al. Rare berry crops: morphology; biochemistry; ecology. Moscow, Russia: FSBSI ARHIBAN, 2020, 72 p.

KUCHARSKA, D. et al. Application of meta-Topolin for improving micropropagation of gooseberry (*Ribes grossularia*). Scientia Horticulturae, 272:109529, 2020.

LALTHAFAMKIMI, L. et al. Direct organogenesis mediated improvised mass propagation of *Pogostemon cablin*: A natural reserve of pharmaceutical biomolecules. South African Journal of Botany, 140:375–384, 2020.

LI H. Y. et al. Phenolic Profiles; Antioxidant Capacities; and Inhibitory Effects on Digestive Enzymes of Different Kiwifruits. Molecules, 23(11):2957, 2018.

MA J. T. et al. Advances in Research on Chemical Constituents and Their Biological Activities of the Genus *Actinidia*. Natural Products and Bioprospecting, 11(6):573–609, 2021.

MA T. et al. Nutritional properties and biological activities of kiwifruit (*Actinidia*) and kiwifruit products under simulated gastrointestinal *in vitro* digestion. Food & Nutrition Research, 63:1674, 2019.

MALAEVA E. V.; MOLKANOVA O. I. Regeneration peculiarities of *in vitro* berry cultures. Acta Horticulturae, 1324:89–94, 2021.

MALAEVA, E.V. Theoretical and practical aspects of clonal propagation of small fruit cultures. The Problems of Botany of South Siberia and Mongolia, 19(2):19–23, 2020.

MARINO, G.; BERTAZZA, G. Micropropagation of *Actinidia deliciosa* cvs. "Hayward" and "Tomuri". Scientia Horticultura, 45(1–2):65–74, 1990.

MITROFANOVA, I. V. Fundamentals of *in vitro* genebank creation of species, cultivars and forms in ornamental, aromatic and fruit crops: A collective monograph. Simferopol, Russia: PH "ARIAL", 2018, 260p.

MITROFANOVA, I. V.; MITROFANOVA, O. V. Using broad genetic diversity and *in vitro* culture to enhance breeding of some subtropical fruit plants. Acta Horticulturae, 538:169–172, 2000.

MOLKANOVA, O. I. et al. Biological peculiarities of far eastern species of the genus *Actinidia* Lindl. Bulletin of Udmurt University. Series Biology. Earth Sciences, 1:42-48, 2014.

MOLKANOVA O.; KRAKHMALEVA I.; KOZAK N. Genetic resources and features of clonal micropropagation of Far Eastern species of *Actinidia*. BIO Web of Conferences, 43:1–9, 2022.

MOLKANOVA, O. I. et al. Improvement of clonal micropropagation technology of valuable fruit and berry crops varieties for commercial conditions. Achievements of Science and Technology of AIC, 32(9):66–69, 2018.

MOYO, M. et al. Deciphering the growth pattern and phytohormonal content in Saskatoon berry (*Amelanchier alnifolia*) in response to *in vitro* cytokinin application. New biotechnology, 42:85–94, 2018.

NASIB, A.; ALI, K.; KHAN, S. An optimized and improved method for the *in vitro* propagation of kiwifruit (*Actinidia deliciosa*) using coconut water. Pakistan Journal of Botany, 40(6):2355–2360, 2008.

NOWAKOWSKA, K. et al. The use of tissue cultures in the mass production of Heuchera 'Silver Scrolls'. Annals of Warsaw University of Life Sciences - SGGW. Horticulture and Landscape Architecture, 41:5–16, 2020.

PANISHCHEVA, D.; MOTYLEVA, S.; KOZAK; N. The comparison of biochemical composition of *Actinidia kolomikta* and *Actinidia polygama* fruits. Potravinarstvo Slovak Journal of Food Sciences, 15:723–731, 2021.

PARK, K. L. et al. Manufacturing and physicochemical properties of wine using hardy kiwi fruit (*Actinidia arguta*). Korean Journal of Microbiology and Biotechnology, 41(3):327–334, 2013.

PLANTS OF THE WORLD ONLINE. *Actinidia* Lindl. 2023. Available in: <https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:38999–1>. Access in: June 22, 2023.

PRADO M. J.; HERRERA M. T. Micropropagation of two selected male kiwifruit and analysis of genetic variation with AFLP Markers. HortScience, 40(3):740–746, 2005.

QUOIRIN, M.; LEPOIVRE, P. Improved medium for *in vitro* culture of Prunussp. Acta Horticulturae, 78:437–442, 1977.

REY, M. et al. Actinidia spp. kiwifruit. *In*: LITZ, R. E.; PLIEGO-ALFARO, F.; IGNACIO HORMAZA J. Biotechnology of fruit and nut crops. Wallingford, United Kingdom: CAB International, p. 1–18, 2020.

SAEIAHAGH, H. et al. Effect of cytokinins and sucrose concentration on the efficiency of micropropagation of 'Zes006' *Actinidia chinensis* var. *chinensis*; a red-fleshed kiwifruit cultivar. Plant Cell, Tissue and Organ Culture, 138:1–10, 2019.

SHEKHAWAT, J. K. et al. Synergism of m-Topolin with auxin and cytokinin enhanced micropropagation of *Maytenus emarginata*. *In Vitro* Cellular & Developmental Biology – Plant, 57:418–426, 2020.

SHEKHAWAT, M.S. et al. Meta-topolin and liquid medium enhanced *in vitro* regeneration in *Scaevola taccada* (Gaertn.) Roxb. *In Vitro* Cellular & Developmental Biology - Plant, 57:296–306, 2021.

SINGH, B. M. S. Effects of Cytokinin on *in vitro* Propagation of *Bauhinia variegata* L. European Journal of Biology and Biotechnology, 1(6):123, 2020.

STANDARDI, A. Micropropagazione dell' *Actinidia chinensis* Pl. mediante coltura *in vitro* di apici meristematici. Frutticoltura, 43(1):23–27, 1980.

TAKAHASHI, W. et al. Plant regeneration in *Actinidia polygama* Miq. by leaf; stem; and petiole culture with zeatin; and from stem-derived calli on low-sucrose medium. Journal of Forest Research, 9(1):85–88, 2004.

THAKUR, D. et al. Critical factors governing the efficient direct organogenesis in green-fleshed kiwifruit (*Actinidia deliciosa*) [A. Chev.] var. *deliciosa*. *In Vitro* Cellular & Developmental Biology-Plant, 58:1107–1116, 2022.

VAN STADEN, J.; ZAZIMALOVA, E.; GEORGE, E. F. Plant growth regulators II: cytokinins, their analogues and antagonists. *In*: GEORGE, E. F.; HALL, M. A.; KLERK, G.-J. D. Plant Propagation by Tissue Culture*:* Volume 1. The Background. Dordrecht, The Netherlands: Springe, p.205–226, 2008.

WOJDYLO, A. et al. Phytochemical compounds and biological effects of *Actinidia* fruits. Journal of Functional Foods, 30:194–202, 2017.

WULANDARI, D. et al. *In vitro* growth of Sempur (*Dillenia philippinensis* Rolfe) shoots in response to different types of plants growth regulators supplemented on MS medium. IOP Conference Series: Earth and Environmental Science, 762:012037, 2021.

YABLONSKAYA, M. I.; KNISHKAJTE, A. V.; ROMANOVA, E. V. Meta-topolin as alternative to benzyladenine in tissue culture. Innovative processes in the agro-industrial complex: collection of materials of the VI International scientific and practical conference of teachers, young scientists, graduate students and students, 88-89, 2014.

YEGOROVA, N. A. et al. Morphogenetic, physiological, and biochemical features of *Lavandula angustifolia* at long-term micropropagation *in vitro*. Russian Journal of Plant Physiology, 66:326–334, 2019.

YEGOROVA, N.; STAVTZEVA, I.; ZOLOTILOV, V. Micropropagation *in vitro* of essential oil rose hybrids obtained in embryoculture. BIO Web of Conferences, 38:00139, 2021.

ZAKHARENKO, E. M. Technology of fruit wines from fruit of *Actinidia* (*Actinidia kolomikta* and *Actinidia polygama*). Vestnik of Pacific State Economics University, 1(53):87–92, 2010.