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Using plant extracts for the micropropagation of buckwheat



Abstract

Background. Various plant hormones are used (cytokinins, auxins) to increase the regeneration efficiency and the net reproduction rate of buckwheat *in vitro*. However, the growth and development rates of plantlets have been noted to be low under these conditions. For this reason, search for the plant extracts that are able to stimulate the regenerative ability of plants is a promising direction of biotechnological research.

Materials and methods. Aseptic single-node cuttings of common buckwheat plantlets (varieties Dikul and Izumrud) were grown on MS nutrient media with plant extracts from *Fagopyrum esculentum* and *Reynoutria japonica* (0.1, 0.5, and 1%) for 21 days. The following morphobiological parameters of the plantlets were evaluated: plant height, the number of internodes, the number of leaves, leaf length, and the number and length of roots.

Results. Dealcoholized aqueous solutions of the extracts from *F. esculentum* and *R. japonica* in the studied concentrations (0.1-1%) significantly stimulated the growth and development of the buckwheat plantlets increasing their net reproduction rate (4.00-6.00) and rhizogenesis. The media with the plant extracts in concentrations of 0.1-0.5% were observed to produce the strongest positive effect. As the result, the morphobiological characteristics of the plantlets and the success rate of the micropropagation were the highest.

Keywords: *Fagopyrum esculentum* Moench, plant extracts, morphobiological parameters, *in vitro*

Использование фитоэкстрактов для микроклонального размножения гречихи

Резюме

Актуальность. Для повышения эффективности регенерации и коэффициента размножения гречихи *in vitro* используют различные фитогормоны (цитокинины, ауксины). Однако при этом отмечаются невысокие темпы роста и развития регенерантов. Поэтому поиск фитоэкстрактов, способных стимулировать регенерационную способность растений, является перспективным направлением биотехнологических исследований.

Материалы и методы. На питательных средах МС с экстрактами растений *Fagopyrum esculentum* и *Reynoutria japonica* (0,1; 0,5 и 1%) в течение 21-х суток культивировали асептические одноузловые черенки регенерантов гречихи посевной сортов Диколь и Изумруд. Оценку морфобиологических показателей микроклонов проводили по следующим показателям: высота растения, число междоузлий, число листьев, длина листовой пластинки, количество и длина корней.

Результаты. Добавление dealcoholизированных водных растворов экстрактов *F. esculentum* и *R. japonica* в питательную среду в диапазоне исследуемых концентраций 0,1-1% существенно стимулировало рост и развитие регенерантов гречихи, повышая коэффициент размножения (4,00-6,00) и ризогенез. Наибольший положительный эффект наблюдался на средах 0,1-0,5%-м содержанием фитоэкстрактов, где обнаружены максимальные значения морфобиологических характеристик и выхода черенков для микроклонального размножения.

Ключевые слова: *Fagopyrum esculentum* Moench, фитоэкстракты, морфобиологические показатели, *in vitro*

Introduction

Currently, various methods of plant micropropagation *in vitro* are gaining in recognition and popularity. An important step in the micropropagation is the selection of an optimal nutrient medium, which will enable the highest possible yield of experimental material for cultivation. This is especially relevant for cross-pollinated and rare plant species. Additionally, this method allows researchers to study the biological effect of plant extracts under laboratory conditions reducing the cost of field experiments.

Common buckwheat *Fagopyrum esculentum* Moench, 1794 is a species of herbaceous plants from the genus *Fagopyrum* of the family *Polygonaceae*. Preserving and multiplying valuable genotypes with the use of micropropagation methods is of considerable importance for this cross-pollinated crop [1]. According to A. Tomasiak et al. [2], buckwheat is sensitive to growing conditions *in vitro*, which is widely used for studying the regeneration of sprouts, the induction of callus formation, organogenesis, somatic embryogenesis, and the synthesis of phenolic compounds [3-5].

The methods of buckwheat tissue culture are believed to be well developed in fundamental and applied aspects [6]. Different variants of the Murashige and Scoog nutrient medium [7] supplemented with plant hormones, vitamins, and minerals [8-14] are employed for the biotechnological propagation of buckwheat. All the developed methods for the micropropagation of buckwheat allowed researchers to prevent callus induction in explants and obtain clones that were genetically identical to their parents. However, if regeneration is stimulated with a high concentration of hormones, which cause cell dedifferentiation and prolonged cultivation, the occurrence frequency of genetic modifications rises [6]. For this reason, search for the plant extracts that are able to stimulate the regenerative ability of plant organisms is a promising direction of biotechnological research because the effect on their genetic apparatus is eliminated in this case.

There are limited data on the use of plant extracts as components of nutrient media for plant micropropagation *in vitro*. To increase the efficiency of this process, I.M. Fardzinova suggested to use an infusion of magnolia vine [15] and tropical chestnut [16], extracts from aloe [17], rose root [18], and Siberian ginseng [19]. Seaweed concentrate «Kelpak» (0.25%) was added to a culture medium for potato and improved the quality of plantlets [20]. I.Yu. Solokhina [21] recommends extracts from common oat, buckwheat, and Jerusalem artichoke for the micropropagation of cucumber.

There is a hypothesis that phenolic compounds, e.g. phenolcarboxylic acids, increase the efficiency of plant propagation at the stages of proliferation, root formation, and adaptation *in vitro* [22]. The potential sources of phenolic compounds, including flavonoids, are plants

from the buckwheat family *F. esculentum* and *Reynoutria japonica* Houtt. For example, the above-ground parts of buckwheat plants belonging to varieties Pri 7 and Izumrud were determined to contain rutin in a concentration of 12.5-21.7 mg/g of the dry matter [23]. A study on the flavonoid composition of extracts from *R. japonica* leaves identified eleven flavonoid compounds; the total amount of flavonoids was 3.66% on average, the rutin content was 1.28% and the content of quercetin was 0.53% [24]. There is some evidence that a solution of *R. japonica* extract has a stimulating effect on the germination rate and seed vigor of *Triticum aestivum* L., *Hordeum vulgare* L. and *Glycine max* (L.) Merr. [25], *Tagetes erecta* [24]. *F. esculentum* extracts demonstrate growth-stimulating activity during the germination of buckwheat seeds [26]. Thus, bioactive compounds contained in plant extracts, including flavonoids from the representatives of the buckwheat family, can produce growth-stimulating effect on test-tube buckwheat plantlets.

Our research goal was to study the effect of extracts from plants of the family *Polygonaceae* on the growth and development of buckwheat plantlets *in vitro*.

Materials and methods

Sterilizing the box, laboratory glassware, and instruments, preparing and autoclaving the nutrient media were performed according to generally accepted protocols [27].

Matured seeds of two common buckwheat varieties were used as starting explants. Variety Dikul was created in FSBSI "Federal Scientific Center of Legumes and Grain Crops" and variety Izumrud was bred in FSBSI "Federal Scientific Center of Agricultural Biotechnology of the Far East named after A.K. Chaiki". Previously reported methods [28] were employed to sterilize the plant material, introduce it into the *in vitro* culture, and obtain the needed quantity of plantlets for the experiment.

Preparing aqueous-alcoholic extracts from F. esculentum and R. japonica

Plant leaves were dried by the method of air drying in shade to achieve a moisture content of 12% and then ground to 1 mm fractions using a laboratory LHM mill. The extraction was performed employing a reflux reaction apparatus with C₂H₅OH 70% (t 90°C) and vacuum filtration; the obtained extracts were transferred into a volumetric flask. Before use, the necessary amount of the extracts was dealcoholized by evaporation until the smell of alcohol disappeared and reconstituted to the initial volume with distilled water.

Studying the growth-stimulating activity of the plant extracts for the micropropagation of buckwheat in vitro

The MS culture media were supplemented with the dealcoholized extracts from *F. esculentum* and *R. japonica* in a concentration of 1 ml, 5 ml, and 10 ml per 1 l (Table 1).

Table 1. Variants of the content of the extracts from *F. esculentum* and *R. japonica* in the MS nutrient medium
Таблица 1. Варианты содержания экстрактов *F. esculentum* и *R. japonica* в питательной среде МС

Component of the nutrient medium	Content of the extract in the nutrient medium, ml/L			
	Control	0.1%	0.5%	1%
MS1. Extract from <i>F. esculentum</i>	-	1	5	10
MS2. Extract from <i>R. japonica</i>	-	1	5	10

Statistical analysis

Microsoft Excel 2010 programmes were used for data input, processing of the original data, and statistical analysis. Software Statistica 6 was used to perform single-factor dispersion analysis. The results are expressed as means $\pm$ standard deviation.

The nutrient medium without extracts served as the control variant. The morphobiological parameters of the plantlets (plant height, the number of internodes, the number of leaves, leaf length, and the number and length of roots) were determined on the 21st day of cultivation. The length of roots was measured using a web cam on a stand and software IC Measure 1.0. The experiment was conducted with three repetitions.

Results and discussion

As the result of the conducted research, it was discovered that the nutrient media with the extracts had a stimu-

lating effect on the development of the buckwheat plantlets already at the first stages of cultivation *in vitro*. The fast growth of microshoots and the appearance of leaves were observed (fig. 1).

The analysis of the obtained data showed that a significant positive effect was achieved on the MS1 media with the extract from *F. esculentum* on the 21st day of cultivation. The morphobiological parameters of the plantlets were considerably higher in all experimental variants compared to the control (table 2).

The number of internodes is an important parameter determining the efficiency of micropropagation through the use of microcuttings. The media with the extracts increased their number up to 5.67-6.00 and the net reproduction coefficient was 4.67-5.67 exceeding the control by more than two times. The highest number of leaves and leaf length were noted in the variants with the use of the extracts.

The key moment in micropropagation is rhizogenesis *in vitro*. A well-developed root system produces a beneficial effect on the growth of test-tube plantlet. The root formation was improved in the variants with the use of the extracts, which facilitated an increase in the regenerative ability of the plantlets. Variety Izumrud had a higher number of roots than the control (by 4.5-5.5 times on average, 3.00-3.67 pcs.); the maximal observed root length was 35.12-89.55 mm (table 2, fig. 2). Variety Dikul formed a lower number of shorter roots (2.00-3.33 pcs. on average, 14.88-74.95 mm).

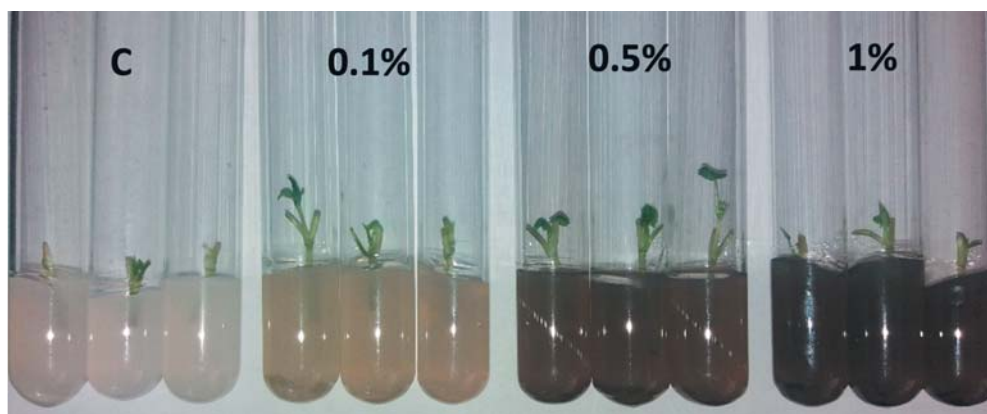


Fig. 1. Buckwheat plantlets of variety Izumrud on the nutrient media with the extract from *F. esculentum* (MS1) on the 4th day of cultivation
Рис.1. Микроклоны гречихи сорта Изумруд на питательных средах с экстрактом *F. esculentum* (MC1) на 4-е сутки культивирования

Table 2. Effect of the nutrient medium with the extract from *F. esculentum* (MS1) on the growth and development of buckwheat *in vitro* (on the 21st day of cultivation)
Таблица 2. Влияние питательной среды с экстрактом *F. esculentum* (MC1) на рост и развитие гречихи *in vitro* (21-е сутки культивирования)

Variant	Plant height, cm	Number of internodes, pcs.	Number of leaves, pcs.	Leaf length, mm	Number of roots, pcs.	Root length, mm	Net reproduction rate
variety Izumrud							
Control	7.46 $\pm$ 2.32	4.00 $\pm$ 0.58	6.33 $\pm$ 0.57	8.67 $\pm$ 3.21	0.66 $\pm$ 0.60	12.31 $\pm$ 12.00	2.33
0.1%	14.37 $\pm$ 4.13*	5.67 $\pm$ 1.15	7.33 $\pm$ 2.08	12.00 $\pm$ 3.61*	3.00 $\pm$ 2.00*	89.55 $\pm$ 45.50*	5.33
0.5%	14.20 $\pm$ 3.60*	5.67 $\pm$ 0.58	7.67 $\pm$ 1.53	12.67 $\pm$ 3.51*	3.33 $\pm$ 2.00*	86.05 $\pm$ 33.66*	5.67
1%	8.67 $\pm$ 3.55	4.67 $\pm$ 1.15	6.33 $\pm$ 1.15	10.67 $\pm$ 0.58	3.67 $\pm$ 0.58*	35.12 $\pm$ 17.85*	4.67
variety Dikul							
Control	2.30 $\pm$ 1.13	3.33 $\pm$ 2.08	5.00 $\pm$ 3.6	6.33 $\pm$ 4.73	0 $\pm$ 0	0 $\pm$ 0	2.66
0.1%	11.03 $\pm$ 2.22*	6.00 $\pm$ 2.00*	8.00 $\pm$ 1.00*	13.67 $\pm$ 1.53*	2.00 $\pm$ 1.0*	59.97 $\pm$ 16.01*	5.67
0.5%	13.90 $\pm$ 4.01*	6.00 $\pm$ 2.65*	9.00 $\pm$ 4.58*	14.33 $\pm$ 2.08*	3.33 $\pm$ 2.08*	74.95 $\pm$ 49.67*	5.00
1%	4.33 $\pm$ 0.45*	5.67 $\pm$ 0.57*	8.67 $\pm$ 1.53*	8.67 $\pm$ 2.52	2.00 $\pm$ 1.73*	14.88 $\pm$ 13.89*	5.67

Note. Differences are relevant at * $p < 0.05$ compared to the control.

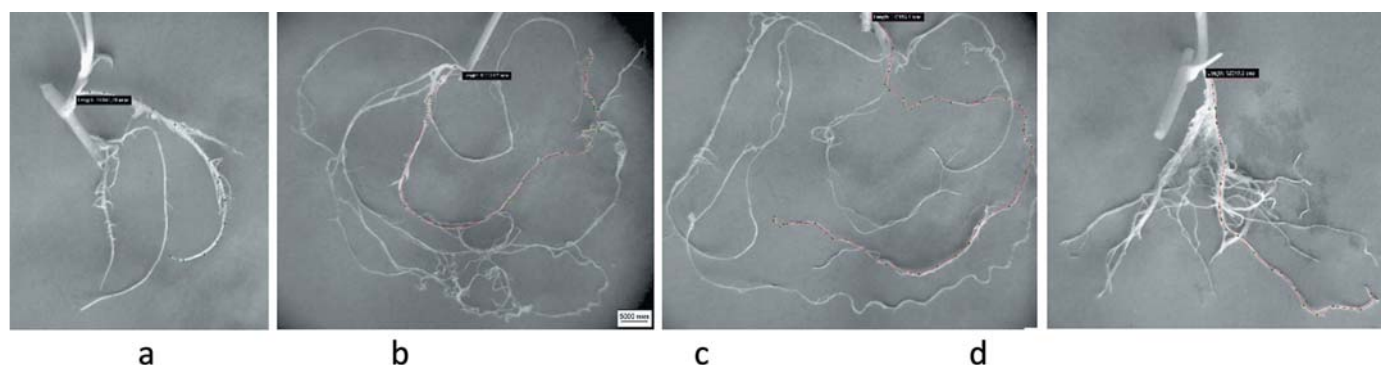


Fig. 2. Root development in the buckwheat plantlets of variety Izumrud on the MS nutrient media containing the extract from *F. esculentum* (a – control, b – 0.1%, c – 0.5%, d – 1%)

Рис. 2. Корнеобразование регенерантов гречихи сорта Изумруд на питательных средах МС1, содержащих экстракты *F. esculentum* (a – контроль; b – 0,1%; c – 0,5%; d – 1%)

The strongest positive effect was observed on the media supplemented with the *F. Esculentum* extract in a concentration of 0.1-0.5%; the regeneration of plants was the most intensive in these variants. The morphobiological parameter values were as follows: plant height – 11.03-14.37 cm, the number of internodes – 5.67-6.00 pcs., the number of leaves – 7.33-9.00 pcs., leaf length – 12.00-14.33 mm, the number of roots – 2.00-3.33 pcs., root length – 59.97-89.55 mm, and the net reproduction rate – 5.00-5.67.

Our study on the regenerative ability of the buckwheat plantlets growing on the nutrient media with the extract from *R. japonica* (MS2) showed that the plant height was by 2.3-7.5 times higher in the experimental variants than in the control (11.27-17.27 cm on average) (table 3). The exception was the variant with variety Izumrud and the nutrient media with the extract in a concentration of 1%. No significant differences in the morphobiological parameters from the

control group were detected in this case, probably, due to the fact that the high concentration of the active ingredient had a slight inhibitory effect on the growth of this variety.

The number of internodes (4.67-7.33 pcs.) and the net reproduction rate increased; the latter was 4.00-6.33 exceeding the control by 1.7-2.7 times. The extract did not affect the number of leaves significantly but the leaf length increased to 9.00-13.67 mm.

The medium supplemented with the extract considerably stimulated the rhizogenesis in the plantlets (table 3, fig. 3). Thus, the number and length of roots were higher (2.33-4.33 pcs. and 30.23-110.33 mm, respectively) on average than the control (0.00-0.65 pcs. and 0.00-12.31 mm, respectively).

The buckwheat plantlets developed better on the nutrient media with concentrations of the *R. japonica* extract within 0.1-0.5 %. The observed morphological parameter

Table 3. Effect of the nutrient medium with the extract from *R. japonica* (MS2) on the growth and development of buckwheat in vitro (on the 21st day of cultivation)
Таблица 3. Влияние питательной среды с экстрактом *R. japonica* (МС2) на рост и развитие гречихи in vitro (21-е сутки культивирования)

Variant	Plant height, cm	Number of internodes, pcs.	Number of leaves, pcs.	Leaf length, mm	Number of roots, pcs.	Root length, mm	Net reproduction rate
variety Izumrud							
Control	7.46±2.32	4.00±0.58	6.33±0.57	8.67±3.21	0.65±0.60	12.31±12.00	2.33
0.1%	17.27±1.79*	7.00±1.00*	7.33±0.58	13.33±0.58*	3.67±0.58*	110.33±42.14*	6.33
0.5%	17.23±1.94*	7.33±1.15*	8.33±3.06	13.67±3.21*	4.33±2.89*	92.65±35.54*	6.00
1%	6.70±4.46	4.67±0.58	7.00±1.00	12.00±2.65	3.67±1.53*	30.23±23.41*	4.00
variety Dikul							
Control	2.30±1.13	3.33±2.08	5.00±3.6	6.33±4.73	0±0	0±0	2.67
0.1%	15.77±0.61*	5.67±0.58*	7.33±3.21	12.33±2.08*	3.33±0.58*	74.70±32.43*	4.66
0.5%	11.67±6.98*	5.67±0.58*	6.67±1.53	12.00±1.00*	3.33±0.58*	31.37±10.53*	5.33
1%	11.27±5.39*	5.33±1.15*	8.00±2.00	9.00±3.46*	2.33±1.15*	38.19±14.73*	5.33

Note. Differences are relevant at **p* < 0.05 compared to the control.

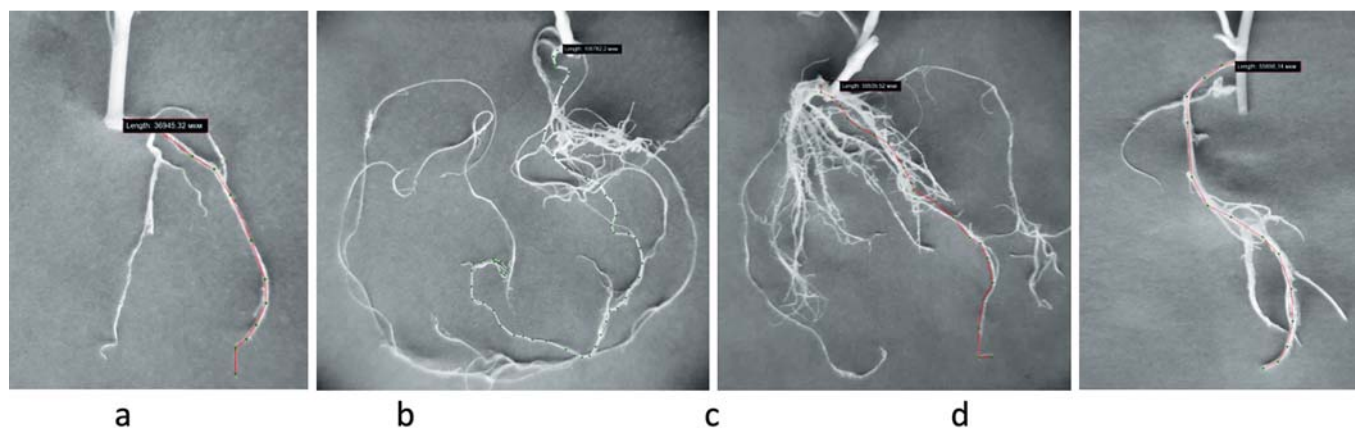


Fig. 3. Root development in the buckwheat plantlets of variety Izumrud on the MS nutrient media containing the extract from *R. japonica* (a – control, b – 0.1%, c – 0.5%, d – 1%)
Рис. 3. Корнеобразование регенерантов гречи сорта Изумруд на питательных средах МС, содержащих экстракты рейннурии японской (a – контроль; b – 0,1%; c – 0,5%; d – 1%)

values were the highest: plant height – 11.67-17.27 cm, the number of internodes – 5.67-7.33 pcs., leaf length – 12.00-13.67 mm, the number of roots – 3.33-4.33 pcs., root length – 74.7-110.33 mm, and the net reproduction rate – 5.33-6.33.

Extracts from common buckwheat have been reported to produce a growth-stimulating effect by some authors. It was discovered that bioflavonoids from plant extracts were able to activate the growth of roots and shoots in potato under *in vitro* conditions on ½ B5 nutrient medium [29] and Gamborg's medium [30]. A considerable growth rate of roots was noted in plantlets on a MS medium supplemented with this extract. According to B.A. Kapusina et al. [31],

the addition of extracts from buckwheat and common oat to a culture medium might stimulate the growth and development of potato plantlets and decrease fungal infection.

Conclusions

1. The extracts from plants of the family *Polygonaceae* (*F. esculentum* and *R. japonica*) stimulated the growth and development of the buckwheat plantlets *in vitro* considerably increasing the net reproduction rate (up to 4.67-6.33) and facilitating the root development.

2. The increase in the concentration of the extract to 1% in the nutrient medium did not produce a significantly stronger effect.

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