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DIRECT PLANT REGENERATION FROM *Pysalis peruviana* L. EXPLANTS

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The aim of the work was to establish the effective culture medium for the regeneration of *Physalis peruviana* for further micropropagation and obtaining of adult plants from regenerants *in vitro* conditions. After conducting series of experiments, effective culture media for the regeneration of *Ph. peruviana* was established. The most effective media for shoot regeneration from leaf explants were MS_{30} supplemented with 1mg/l Kin + 3 mg/l BAP; MS_{30} + 2 mg/l Kin + 1 mg/l BAP (33.33% of regeneration on both media). Good results were obtained on the media MS_{30} supplemented with 1 mg/l Kin and 2 mg/l BAP (28.57% explants regenerated) and MS_{30} supplemented with 2 mg/l Kin and 3 mg/l BAP (26.31% of regeneration). Root induction from stem and leaf explants were obtained on medium MS_{30} with NAA (0.2 mg/l; 0.5 mg/l), IAA (0.2 mg/l; 0.5 mg/l). Root induction frequency on these media was 100%. The obtained regenerants were separated from the explants and were transferred on the medium MS_{30} with 1 mg/l of BAP for elongation, and then on a medium MS_{30} or MS_{30} with 0.2 mg/l NAA for subsequent rooting. After one month of cultivation on mediums MS_{30} or MS_{30} with 0.2 mg/l NAA were successfully received adult plants.

Key words: *Physalis*, regeneration.

Due to the medicinal and horticultural values *Physalis peruviana* is widely cultivated in tropical and subtropical countries. *Physalis* finds its application in medicine due to rich biochemical composition (the main components are 15-desacetylphysabubenolide and betuline). *Physalis* species have antitumor effect and used to treat inflammations.

Ph. peruviana is highly productive plant. From one plant it is possible to collect about 300 fruits.

In Ukraine, *Ph. peruviana* is grown only in private collections. It is not grown on an industrial scale, therefore, in the case of obtaining transgenic *Physalis* plants, it is easier to prevent the possible leakage of unauthorized transgenes.

According to mentioned above information, *Physalis* is a promising plant for the production of recombinant proteins for pharmaceutical use.

A sensational article about editing of *Ph. pruinosa* genome was recently published [1]. *Physalis* can be a good model object for studying the functioning of heterologous genes in its tissues and organs.

At present, there are many works devoted to the callus formation and regeneration of *Physalis*. Basically, researchers who obtained regenerants had the main goal of using them as a source of secondary metabolites and other valuable substances, therefore the largest number of works devoted to the study of *Physalis* has a biochemical direction.

The study of regenerative ability was undertaken by a group headed by Rao. They obtained regenerants for *Ph. pubescence*. Initially, they received a callus tissue from the leaves and internodes. Then, regenerants were received on the medium MS_{30} + 2 mg/l BAP + 0.5 mg/l NAA and on the medium MS_{30} + 2.5 mg/l BAP + 0.5 mg/l NAA, from the callus tissue [2].

Ramar K. and Ayyadurai V. chose *Physalis maxima* as an object of research. They managed to obtain regenerants for this specie from the leaf segments on the media: MS_{30} + 1 mg/l BAP + 0.5 mg/l NAA; MS_{30} + 2 mg/l BAP + 1 mg/l NAA + 1 mg/l Kin and regenerants from nodal segments on mediums: MS_{30} + 2 mg/l BAP + 1.5 mg/l NAA + 0.5 mg/l

GA_3; MS_{30} + 3 mg/l BAP + 1.5 mg/l; NAA + 1.5 mg/l GA_3 [3].

Sandhya H., and Srinath R. received regenerants from nodal segments of *Physalis minima* on media: MS_{30} + 2 mg/l 2.4 — D + 2 mg/l NAA; MS_{30} + 2 mg/l 2.4 — D + 1 mg/l Kin [4].

Ramar K. with colleagues received a positive regeneration result for *Ph. peruviana* on media: MS_{30} + 1.5mg/l BAP + 0,5 mg/l GA3 + 0.5 mg/l 2.4 — D; MS_{30} + 2 mg/l BAP + 1mg/l GA3 + 1 mg/l 2.4 — D (for nodal and internodal segments); and on media MS_{30} + 2.5 mg/l BAP + 1 mg/l GA_3 + 0.5 mg/l 2.4 — D; MS_{30} + 3 mg/l BAP + 1 mg/l GA_3 + 1 mg/l 2.4 — D (for leaf explants) [5].

Bergier K. and colleagues received *Ph. ixocorpa* regenerants from the hairy root's culture on MS_{30} + 5 μM Kin + 1 μM BAP [6].

Kumar O.A. and colleagues received regenerants of *Ph. angulata* from meristems on medium MS_{30} + 1 mg/l BAP + 0.5 mg/l IAA + 0.25 mg/l GA_3 [7].

Swartwood K. and Van Eck obtained *Ph. pruinosa* regenerants from hypocotyls on medium MS_{30} + 2 mg/l ZEA [8].

Assad-Garcia N. received regenerants from the cotyledons of 12-day's seedlings of *Ph. ixocorpa* cv. Rendidora on medium MS_{30} + 1 μM NAA + 12.5 μM BAP [9].

Singh P. and colleagues received regenerants from the nodal segments of *Ph. peruviana* on medium MS_{30} + 2.5 mg/l BAP + 0.05 mg/l IBA [10].

Several scientific groups worked with *Ph. minima*. Regenerants of this species were obtained from apical meristems of 15-day — old seedlings and nodal segments [11]; from the callus on the medium MS_{30} + 1 mg/l BAP + 1 mg/l Kin + 3.5 mg/l GA_3 [12]; from nodal segments on medium MS_{30} + 2 mg/l BAP + 0.25 mg/l IAA [13]; from callus on medium MS_{30} + 3.5 mg/l BAP + 0.4 mg/l Kin [14]; from the apical meristems of seedlings and nodal segments on medium MS_{30} + 1 mg/l BAP [15].

Although a sufficient quantity of works dedicated to the regeneration of *Ph. peruviana*, an effective protocol has not yet been developed for obtaining a large number of regenerants from *Physalis* leaf explants.

Our objective was to establish effective culture medium for the regeneration of *Ph. peruviana* for further obtaining of adult plants from regenerants *in vitro* conditions.

Materials and Methods

The objects of the research were *Ph. peruviana* plants.

Seeds of *Physalis* germinated on the sterile nutrient agar medium Murashige and Skoog (MS_{30}) [16] with 30 g/l sucrose (22–26 °C, 14-hour light period, illumination — 3000–4500 lx).

For regeneration we used the internodes' segments, segments of leaflets, petioles of leaflets and leaflets with petioles (without separation) 1 cm long, derived from 1-month-old plants of *Ph. peruviana*. The explants were cultivated horizontally for 1 month on MS_{30} medium, containing 30 g/l sucrose (pH 5.7–5.9) with the addition of 6-benzylaminopurine (BA), 1-naphthylacetic acid (NAA), kinetine (Kin) in different concentrations.

Obtained shortened shoots were separated and transferred for 2 weeks on MS_{30} medium with 1 mg/l of BAP for elongation, and then on a medium MS_{30} or $MS_{30}$with 1 mg/l NAA for subsequent rooting.

Data collection and Statistical analysis

For each experiment, 15 explants were used. Data was analyzed using the general procedure of Statistica software package, Version 12. When the P indicated significant treatment effects (5, 1 or 0,1%) based on the Spearman's rank order correlation (R), the Least Significant Difference test ($P \leq 0{,}05$; $P \leq 0{,}01$; $P \leq 0{,}001$) was used as a method to determine which treatments were significantly different from others treatments. The significance levels (P) of averages differences or relations of the mean values were determined by tables for small samples.

We carried out Spearman analysis. In order to confirm the validity of the results, the comparison of the group values was carried out. The comparison with control wasn't carried out, as for the control group we have value "0".

The control group were plants, parts of which were placed on the medium without the addition of stimulants. experimental groups — plants placed on medium with growth stimulants. No changes were observed in the control group (therefore, in the control group 0).

The Spearman's rank correlation coefficient is used to identify and assess the closeness of the connection between two ranks of compared quantitative indicators.

The correlation coefficient can take values from −1 to 1, and with R = 1 there is

a strictly direct connection, and with R = −1 there is a inverse connection. If the correlation coefficient is zero, then the relationship between the values is practically absent. The closer the correlation coefficient to one, the stronger is the connection between the measured values.

When using the rank correlation coefficient, conditionally assess the closeness of the connection between the signs, considering the values of the coefficient equal to 0.3 or less — indicators of weak closeness of the connection; values of more than 0.4, but less than 0.7 are indicators of moderate closeness of connection, and values of 0.7 or more are indicators of high closeness of connection.

In our work we compared the connection of effect of growth regulators (which was expressed in the appearance of different quantity of regenerants per one explant) with used concentrations & combinations of growth regulators.

Results and Discussion

After cultivation explants on MS_{30} medium with different concentrations of BAP and Kin were obtained explants (Fig. 1, 2).

Highest levels of regeneration were obtained, while cultivating the leaf explants on media MS_{30} with 2 mg/l Kin + 3 mg/l BAP and MS_{30} with 2 mg/l Kin + 1 mg/l BAP (Fig. 1). Quite good levels of regeneration were received on media MS_{30} with 1 mg/l Kin + 2 mg/l BAP and MS_{30} 2 mg/l Kin + 3 mg/l BAP. Low levels of regeneration were received on medium MS_{30} with 1 mg/l Kin + 1 mg/l BAP; 1 mg/l Kin + 2 mg/l BAP; 2 mg/l BAP; 2 mg/l BAP + 2 mg/l Kin; 3 mg/l BAP; 4 mg/l BAP + 1 mg/l Kin; 4 mg/l BAP + 2 mg/l Kin. The explants cultured on MS_{30} medium without addition of growth regulators, didn't regenerate (Table 1, 2; Fig. 2). Also, there weren't obtained regeneration on medium with addition only cytokinins (MS_{30} + 1–2 mg/l Kin). Absence of regeneration was also on medium with addition only low or high amounts of auxins (MS_{30} + 1 mg/l BAP, MS_{30}+ 4 mg/l BAP) (Table 1).

According to our result, the most effective media for shoot regeneration were MS_{30} + 1 mg/l Kin + 3 mg/l BAP and MS_{30} + 2 mg/l Kin + 1 mg/l BAP (33,33% of regeneration on both mediums) (Fig. 2, 3). Also, quite good results were obtained on the media MS_{30} + 1 mg/l Kin + 2 mg/l BAP (28.57% explants regenerated) and MS_{30} + 2 mg/l Kin + 3 mg/l BAP (26.31% of regeneration) (Fig. 2, 3).

Also several research groups obtained positive results for regeneration of species of *Physalis* genus. In majority of works were used growth regulators BAP and Kin with addition of 3-rd growth regulator [3, 5, 7, 12]. We decide to simplify the methodic of regeneration and use only 2 growth regulators: BAP (concentration 0–4 mg/l) with Kin (0–2 mg/l). According previous works, highest frequency of regeneration was obtained on mediums, which contain BAP (concentration 1–3 mg/l), Kin 1 mg/l [3, 6, 12]. In our results, highest frequency of regeneration was obtained while using the same concentrations of BAP and Kin. It was claimed, that mean quantity of regenerated plants was about 11–13 pieces per one explants which is matches our results (for several variants of mediums, mean quantity of regenerants per one explant was 11–12 pc.).

For root induction was used MS_{30} medium with NAA (0.2 mg/l; 0.5 mg/l), IAA (0.2 mg/l; 0.5 mg/l). No significant difference was found between the media used for root induction (Fig. 4).

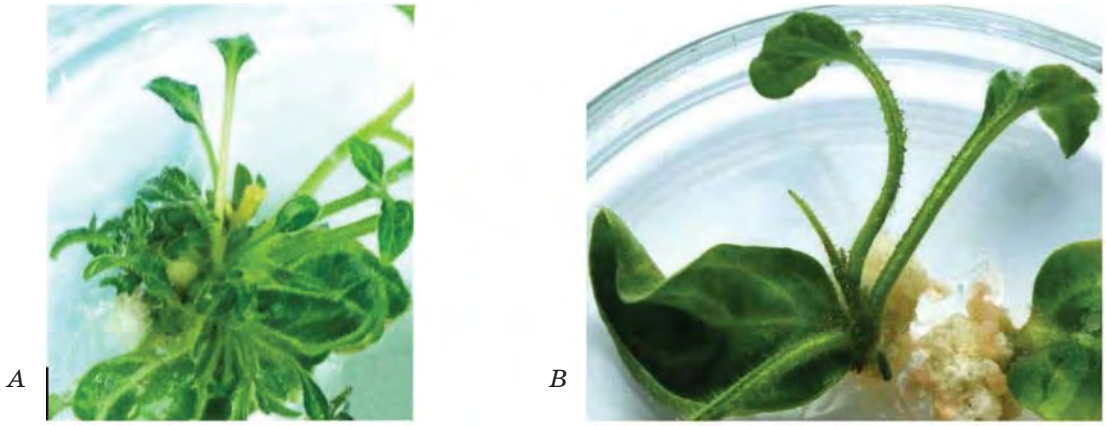

Fig. 1. **Shoot induction from leaf explants in MS_{30} medium with plant growth regulators (*A* — 2 mg/l Kin + 3 mg/l BAP; *B* — 0 mg/l Kin + 3 mg/l BAP) after 1 month of cultivation**

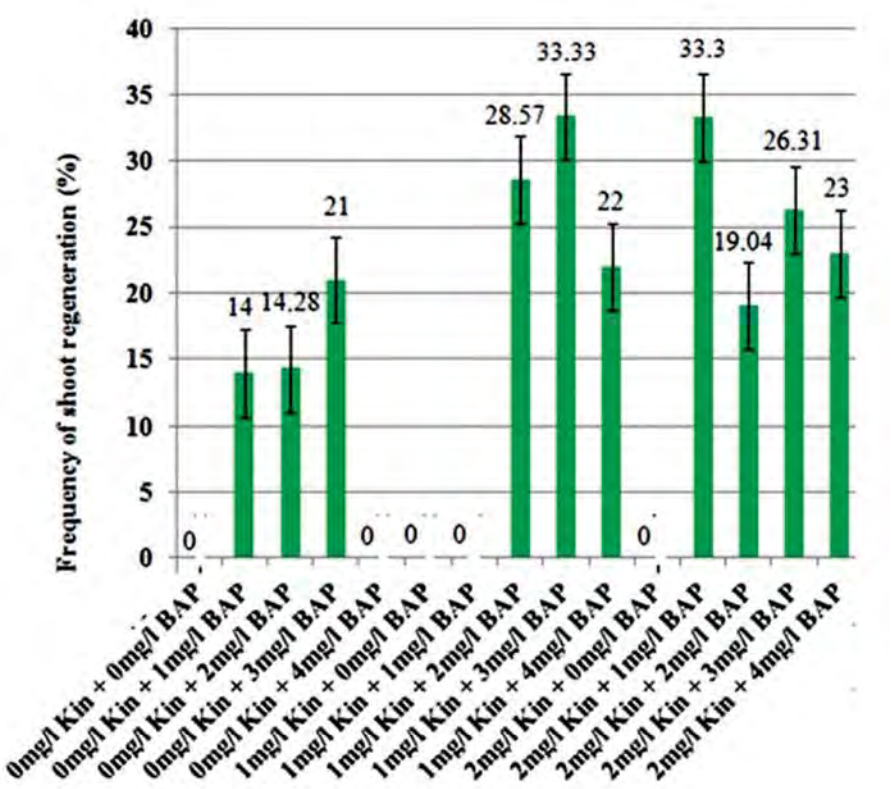

Fig. 2. **Effect of growth regulators on frequency of shoot regeneration from leaf segments of *Physalis peruviana* in MS_{30} medium after 1 month of cultivation**
Data are the mean ±SE (standard error); $n = 15$

Table 1. **Effect of growth regulators on shoot induction of *Physalis peruviana* from leaf explants on MS_{30} medium**

	0 mg/l BAP	1 mg/l BAP	2 mg/l BAP	3 mg/l BAP	4 mg/l BAP
0 mg/l Kin	–	–	*	*	–
1 mg/l Kin	–	*	**	***	*
2 mg/l Kin	–	***	*	**	*

Where – no regeneration;
* — frequency of regeneration from14% to 20%;
** — frequency of regeneration from 20% to 30%;
*** —frequency of regeneration more than 30%.

Table 2. **Effect of growth regulators on quantity of regenerated shoots of *Physalis peruviana* from leaf explants on MS_{30} medium**

	0 mg/l BAP	1 mg/l BAP	2 mg/l BAP	3 mg/l BAP	4 mg/l BAP
0 mg/l Kin	–	–	6 ± 1.15	6 ± 1.15	–
1 mg/l Kin	–	10 ± 0.84	11.8 ± 0.882	12.5 ± 0.72	11.2 ± 0.805
2 mg/l Kin	–	12.3 ± 0.76	11.6 ± 0.93	12 ± 0.85	9 ± 0.654

Quantity of shoots (M±SE).

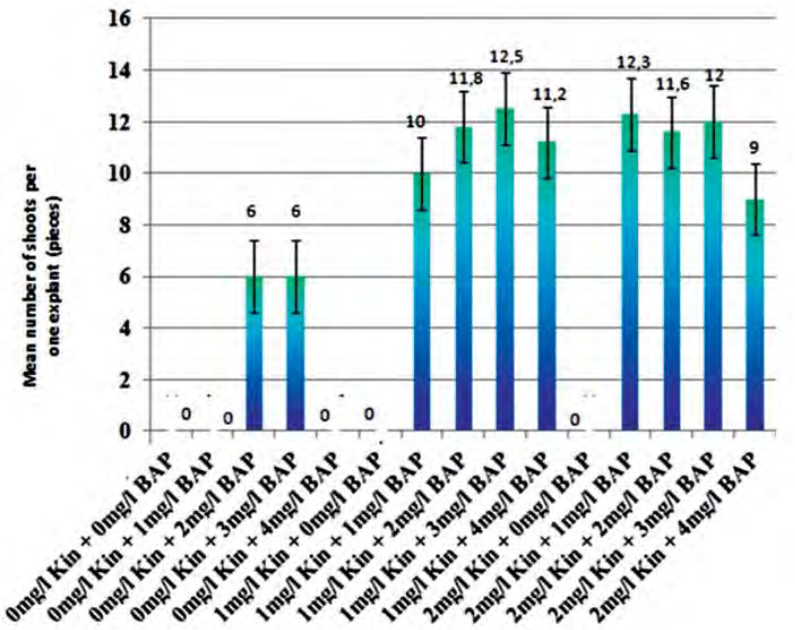

Fig. 3. **Effect of growth regulators on mean number of shoot of *Physalis peruviana* in MS_{30} medium after 1 month of cultivation**

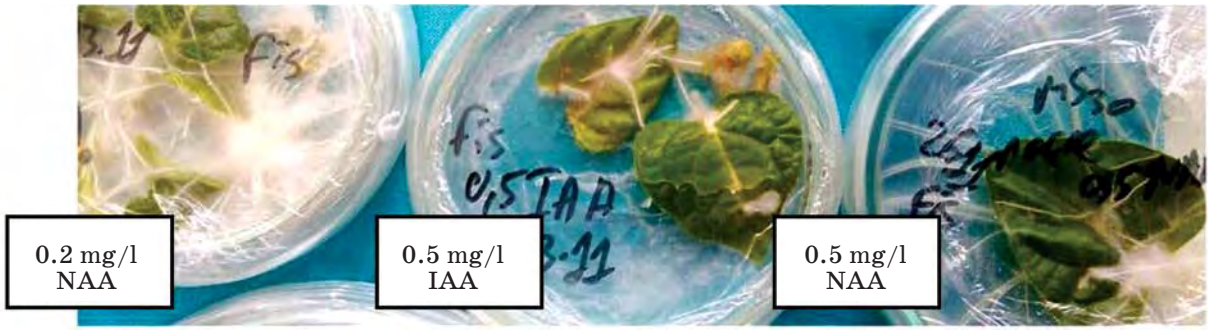

Fig. 4. **Root induction from leaf explants in MS_{30} medium supplemented with various plant growth regulators after one month of cultivation**

Obtained shortened shoots were separated and transferred for 2 weeks on MS_{30} medium with 1 mg/l of BAP for elongation, and then on a medium MS_{30} or MS_{30} with 0,2 mg/l NAA for subsequent rooting (Fig. 5).

Statistical analysis

After summarizing obtained results, statistical analysis was conducted for data. The results of regeneration (number of shoots per explants) were significantly different from those which were obtained without treatment with growth regulators and when comparing the effect of different growth regulators.

Null hypothesis is rejected, with significant ($P \leq 0,05$); highly significant ($P \leq 0,01$) and extremely significant ($P \leq 0,001$) levels of averages differences.

Fig. 5. **Rooted young regenerants on MS_{30} medium**

According Spearman's rank correlation, there were direct moderate closeness of connection between used concentration of growth regulators and quantity of regenerants per 1 explant (in most cases). Also were found inverse connection between used concentration of growth regulators and quantity of regenerants per 1 explant, when the concentration of BAP growth regulator was 4 mg/l. Thus, it can be argued that the concentration of 4 mg/l BAP regulator had a depressing effect on regeneration.

Thus, effective culture media for the regeneration of *Physalis peruviana* were established. The most effective media for shoot regeneration from leaf explants were MS_{30} + 1 mg/l Kin + 3 mg/l BAP and MS_{30} + 2 mg/l Kin + 1 mg/l BAP (33.33% of regeneration on both media). Good results were obtained on the media MS_{30} + 1 mg/l Kin + 2 mg/l BAP (28.57% explants regenerated) and MS_{30} + 2 mg/l Kin + 3 mg/l BAP (26,31% of regeneration). Excellent results for root induction from stem and leaf explants were obtained on medium MS_{30} with NAA (0.2 mg/l; 0.5 mg/l), IAA (0.2 mg/l; 0.5 mg/l). Root induction frequency on these media was 100%. The obtained regenerants were grown on the medium MS_{30} with 1 mg/l of BAP for elongation, and then on a medium MS_{30} or MS_{30} with 0.2 mg/l NAA for subsequent rooting. After one month of cultivation on mediums MS_{30} or MS_{30} with 0.2 mg/l NAA were successfully received adult plants.

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ПРЯМА РЕГЕНЕРАЦІЯ РОСЛИН *Physalis peruviana* L. З ЕКСПЛАНТІВ

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Метою роботи було виявлення ефективного культурального середовища для регенерації *Physalis peruviana* з метою його подальшого розмноження і отримання дорослих рослин з регенерантів за умов *in vitro*. Після проведення серії експериментів було підібрано ефективні живильні середовища для регенерації *P. peruviana*. Найбільш ефективними середовищами для регенерації пагонів з листових експлантів були $МС_{30,}$ доповнене 1 мг/л Кін та 3 мг/л БА і $МС_{30,}$ доповнене 2 мг/л Кін та 1 мг/л БА (33,33% регенерації на обох середовищах). Хороші результати було отримано на середовищах $МС_{30}$ з додаванням 1 мг/л Кін та 2 мг/л БА (28,57% експлантатів регенерували) і $МС_{30}$ з 2 мг/л Кін та 3 мг/л БА (ефективність регенерації — 26,31%). Індукцію коренів на стеблових і листових експлантатах було отримано на середовищі $МС_{30}$ з додаванням НОК (0,2 мг/л; 0,5 мг/л), ІОК (0,2 мг/л; 0,5 мг/л). Частота коренеутворення на цих середовищах становила 100%. Одержані регенеранти відокремлювали від експлантів і переносили на середовище $МС_{30}$ або $МС_{30}$ з 0,2 мг/л НОК для подальшого вкорінювання. Після одного місяця культивування на середовищах $МС_{30}$ або $МС_{30}$ з 0,2 мг/л НОК було отримано дорослі рослини.

Ключові слова: *Physalis*, регенерація.

ПРЯМАЯ РЕГЕНЕРАЦИЯ РАСТЕНИЙ *Physalis peruviana* L. ИЗ ЭКСПЛАНТОВ

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Целью работы было выявление эффективной культуральной среды для регенерации *Physalis peruviana* с целью его дальнейшего размножения и получения взрослых растений из регенерантов в условиях *in vitro*. После проведения серии экспериментов были подобраны эффективные питательные среды для регенерации *P. peruviana*. Наиболее эффективными средами для регенерации побегов из листовых эксплантов были $МС_{30}$, дополненное 1 мг/л Кин и 3 мг/л БА, и $МС_{30}$, дополненное 2 мг/л Кин и 1 мг/л БА (33,33% регенерации на обеих средах). Хорошие результаты были получены на средах $МС_{30}$ с добавлением 1 мг/л Кин и 2 мг/л БА (28,57% эксплантатов регенерировали) и $МС_{30}$ с 2 мг/л Кин и 3 мг/л БА (эффективность регенерации составила 26,31%). Индукция корней на стеблевых и листовых эксплантатах получена на среде $МС_{30}$ с добавлением НУК (0,2 мг/л; 0,5 мг/л), ИУК (0,2 мг/л; 0,5 мг/л). Частота корнеобразования на этих средах составила 100%. Полученные регенеранты отделяли от эксплантов и переносили на среду $МС_{30}$ или $МС_{30}$ с 0,2 мг/л НУК для последующего укоренения. После одного месяца культивирования на средах $МС_{30}$ или $МС_{30}$ с 0,2 мг/л НУК были получены взрослые растения.

Ключевые слова: *Physalis*, регенерация.

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