DOI 10.31489/2022BMG4/140-147

UDC 576.3

M.K. Tynykulov*

L.N. Gumilyov Eurasian National University, Astana, Kazakhstan
**Corresponding author: tynykulov@list.ru*

Peculiarity of currant and perpetual repair cell culture

Perpetual raspberry is a group of raspberry varieties distinguished by their ability to bear fruit on two-year and one-year stem shoots. Black currant (Ríbes nígrum) is a deciduous shrub, family Gooseberry (Grossulariaceae), related to currants (Ribes). The article presents methods of cell selection of perpetual raspberries and currants. Crop varieties resistant to extreme natural factors, obtaining high harvest, and two harvests per year were considered. The chemical composition of the used nutrient medium, the content of micro- and macroelements, and organic substances were taken into account. The qualitative composition of callus shoots extracted from plant cells was determined. A strong relationship between the frequency of callusogenesis in black currant and the balance of phytohormones in the nutrient medium has been shown. Progress in the field of gene and cell biotechnology is directly related to the development of the basics of the cultivation of plant cells and tissues under in vitro conditions. In addition, it is of particular interest to choose a suitable nutrient medium, to establish the mode of cultivation in order to determine the species and volume of explants, the totipotency of representatives of different taxonomic groups of plants. At present, this unique ability of somatic cells is found in various cultures, most of which are annual and practically vegetative propagating plants. These are vegetable crops and ornamental plants, the list of which grows annually. The scientific novelty of the work is the possibility of obtaining somatic hybrids and transgenic plants of repair raspberry and black currant using biotechnology methods. Therefore, the study of theoretical foundations and development of applied aspects of cells and tissues of perennial plants, in particular fruit and berry and agricultural crops, their accelerated reproduction, obtaining somatic hybrids and transgenic plants are considered relevant.

Keywords: currant, raspberry, biotechnology, in vitro, perpetual, totipotency, breeding, cells, callus, phytohormones.

Introduction

There are more than 150 species of currants worldwide and in Kazakhstan — 11 species of currants. Currants are an excellent source of flavonoids, vitamin C, proanthocyanidins, and anthocyanins. It consists of 15-195 fatty acids such as alpha- and gamma-linolenic acid, anthocyanidins, stearidonic acid, and flavonoids. It also has an antioxidant effect [1].

Perpetual raspberries are popular among amateur gardeners in Kazakhstan. Such varieties effectively use favorable environmental factors due to the annual cycle of crop formation and peculiarities of growing technology. Its peculiarity is that in a season it is possible to harvest two crops [2].

It is of theoretical interest to determine the degree of totipotency of somatic cells of black currant and raspberry. In addition, it is interesting to study the fundamentals of callus formation, somatic cell morphogenesis, and morphogenesis of black currant and raspberry somatic cells by selecting culture media to develop applied aspects of micropropagation of these cultures.

Due to the popularity of these crops, scientific programs to produce new varieties exist in many breeding centers, where scientists are working on resistance to diseases and high-quality fruits, in particular the high content of biologically active substances — antioxidants. In the last 5 years, about 30 new varieties of strawberries and 20 raspberries were included in the state register of breeding achievements. Unfortunately, classical breeding, in which the evaluation of new objects is carried out by external features (phenotype), is time-consuming and expensive. For example, it takes up to 15 years to produce a new raspberry variety [3].

Biotechnological methods are widely used primarily in breeding for resistance of fruit crops to pathogens, where genetically distant species must be used to attract their resistant genes, as well as to study their biology and conduct artificial infestation to collect a collection of pathogens in the culture of in vitro.

In domestic breeding, there is no purposeful work on the creation of raspberry perpetual varieties. I.V. Michurin’s perpetual raspberry is known for reason that it gives at least some har vest in the fall if there are optimal conditions. A number of perpetual varieties have been created abroad that bear fruit mainly on annual shoots. The best-known of them are September, Heritage, Lulin, Redwing, Zeva, and Ottom. These varieties require a frost-free period of 150-160 days and an active temperature above 3000 °C for full

ripening of harvest on one-year shoots. These foreign varieties manage to give only 15-30 % of their potential harvest before the autumn frosts come. In terms of one bush, it is not more than 300 g of berries. For this reason, foreign perpetual raspberry varieties for many regions of our country are not of interest [4].

However, new biotechnological methods do not stand still. The development of molecular biology methods made it possible to introduce into the practice of breeders such a method as the evaluation of plant material using chains of molecular DNA markers associated with genes encoding valuable traits. They are able to use for quick test hundreds or thousands of plants and identify whether their genome contains the genes for the desired traits. With this approach, the breeding process is able to be significantly accelerated and made cheaper because of molecular markers: conducting an assessment of breeding material at an early stage (e.g., on shoots), thereby significantly reducing the interval between generations in breeding selection; complex, costly and phenotypic (e.g., sensitivity to environmental conditions: pests, diseases, drought, etc.) long testing and assessment of traits; viral diseases of fruit crops are chronic and systemic: once damaged, a crop remains incurable for life. As a rule, all its organs become infected. The use of such plants as pestles during vegetative reproduction contributes to the mass spreading of diseases [5].

Experimental

The object of research is black currant (*Ribes nigrum* L.) and perpetual raspberries (*Rubus idaeus* L.). Representatives of the *Ribes* species usually have 2p = 16 chromosomes, and their cultivated varieties are diploids [6].

The main method of black currant propagation is cuttings, but this method does not allow obtaining a large amount of planting material. To solve this problem, the in vitro microtonal method is promising, which allows to significantly increase the reproduction coefficient using the method of cell and shoot cultivation.

The perpetual variety of the perpetual raspberry Hercules and the variety of the American black currant Lia were selected as the studied samples.

These explants from the samples underwent stepwise sterilization-in soapy water washing with a magnetic mixer for 10 minutes, rinsing 3 times with distilled water, rinsing with potassium permanganate for 5 minutes, rinsing again with distilled water, keeping whiteness 70/30 for 20 minutes, treating with 70 % ethanol for 1 minute and rinsing with distilled water for 5 minutes. Then, under aseptic conditions, explants 3-5 mm in size were removed from them in a laminar box, cleaned from the surface film and cut crusts, and grown in test tubes of 15 mm in diameter for the induction of callusogenesis. The callus tissue was divided into 10-15 segments and transplanted into 6-cm diameter jars for induction of morphogenesis after triplicate transplantation into a new culture medium. Up to 300-400 explants were planted in each series of experiments. A modified nutrient medium was used to cultivate explants for the purpose of inducing callus formation and morphogenesis of callus tissues based on the composition proposed by T. Murasi and F. Skoog. Phytohormones 2,4-D and kinetin were used as hormonal additives. The pH of the whole nutrient medium was 5.6-5.8.

Calluses were obtained in MS induction media (Tab. 1). At the end of the passage (30 ± 5 days), the morphological characteristics of the callus were produced and measured by color, consistency, and degree of tissue necrotization.

Table 1

The composition of the Murashige and Skoog nutrient medium (MS)

Composition	Doze
Micronutrients, mg / L	
NH_4NO_3	1650
$KN0_3$	1900
$MgS0_47H_20$	370
KH_2P0_4	170
$CaCL_2$. $2H_20$	440
$FeS0_4$. $7H_20$	27.85
Na_2EDTA. $2H_20$	37.25
Micronutrients, mg / L	
Na_2Mo0_4. $2H_20$	0.25
H3BO3	6.20
$MnS0_4.4H_20$	22.30

$ZnS0_4$. $4H_20$	8.30
$CuS0_4$. $5H_20$	0.025
$CoCI_2$. $6H_20$	0.025
KI	0.83
Organic compounds	
Pyridoxine, mg/L	0.50
Ascorbic acid, mg / L	1.00
Nicotinic acid, mg / L	0.50
Thiamine HCl, mg/L	0.1
Sucrose, g/L	30.00
Agar, g/L	7.00

The calluses were then placed in an appropriate, fresh nutrient medium and incubated in a 16-hour photoperiod and in diffuse light (1-2 thousand lux) at 22-24/14-16 °C (day/night). Part of the callus from each passage was transferred to morphogenic media MS6 and MS7 (Tab. 2).

Table 2

Composition of the modified MS nutrient medium used for the induction of callusogenesis and morphogenesis, mg/L

Composition	MS 1	MS 2	MS 3	MS 4	MS 5	MS 6	MS 7
2,4-D	1.00	2.00 ^	4.00	8.00	10.00	-	-
Kinetin	0.05	0.10	0.20	0.40	0.50	-	-
Folicacid	0.50	0.50	0.50	0.50	0.50	-	-
Glycine	1.00	1.00	1.00	1.00	1.00	-	-
Adenine	1.00	1.00	1.00	1.00	1.00	-	-
IMA	-	-	-	-	-	-	-
ICA	-	-	-	-	-	0.05	0.10
Article-6	-	-	-	-	-	0.20	0.20
Zeatin	-	-	-	-	-	0.40	0.40
Mesoinosite	100.0	100.0	100.0	100.0	100.0	-	-
Casein hydrolysate	100.0	100.0	100.0	100.0	100.0	-	-

Note: MS 1, MS2, MS3, MS4, MS5 — nutrient media used in the induction of callusogenesis; MS6, MS7 — nutrient media used in the induction of morphogenesis; 2,4-D — 2,4-dichlorophenoxyacetic acid; IMA- indolemic acid; ICA- indoleacetic acid; 6-BAP — 6-benzoaminopurine

The formation of morphogenic structures was observed, and the number of obtained regenerants was counted, they were characterized and developmental abnormalities were noted. The release of regenerants was observed as the number of plants consisting of 10 or 100 callus and planted in a reducing medium.

Results and Discussion

The medium used in in vitro plant shoot culture work as a medium for culturing T. Murasi plant cells and shoots. In this environment, the process of morphogenesis in vitro can often be successfully regulated by adjusting the concentration and changing the types of phytohormones. The productivity of callusogenesis and morphogenesis depends on the ratio of phytohormones and their input into the nutrient medium.

In vitro, 2,4-D and kinetin are the most common stimulants for obtaining and maintaining callus culture. Therefore, to create optimal conditions, maximum output of callus and morphogenic structures, we set up a series of experiments to study various concentrations and effects of two main ratios of phytohormone-2,4-D and kinetin.

Explants from fragments, apical and meristems of leaves of perpetual raspberry and black currant were grown in a modified MS medium with different concentrations of 2,4-D and kinetin. These nutrient media are MS1, MS2, MS3, MS4, and MS5 (Tab. 1).

The callus usually grows in the dark because light causes morphogenesis and greenishness of the callus. In this regard, callus cultivation was carried out in a bright room with weak diffuse light.

The study of the features of callus formation of different somatic cells of black currant is characterized by indirect and apical bud explants with high activity of callus genesis induction and callus growth, which are permanent holders of genetic information of growth and development. The newly formed callus had a

yellowish color and relatively loose consistency. On day 30 of in vitro cultivation, the implants contained 2 and 4 mg/L of 2,4-D and 0.1 and 0.2 mg/L of kinetin.

The process of formation of calluses and bones of currant leaves from the explants obtained from the leaves has its own peculiarities. They are characterized by the induction of callusogenesis and the intensity of growth of callus tissue when grown in a relatively high nutrient medium containing phytohormone — 8 mg/L 2,4-D and 0.4 mg/L kinetin (nutrient medium MS 4). Explants taken from the stem were observed with slow callusogenesis of the leaf compared to its own explants (Tab. 3).

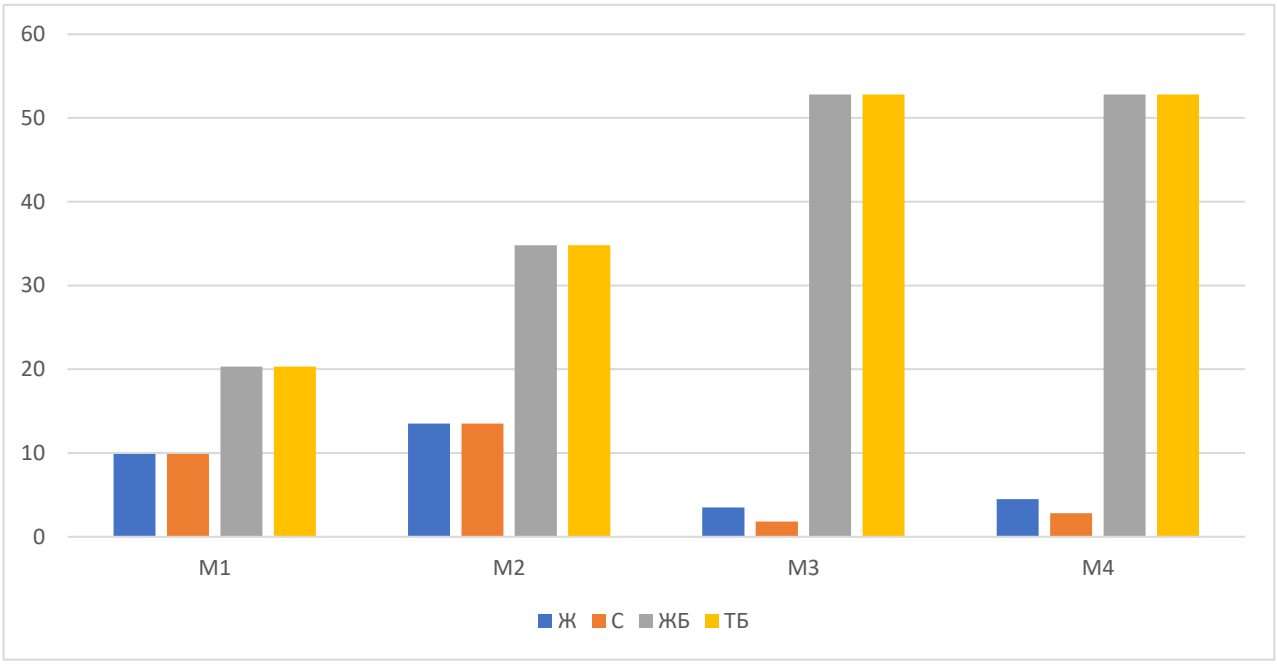
Table 3

Effect of phytohormone concentration on the intensity of callusogenesis inside black currant explant type

Nutrient medium	Fruitful variety of currant Lia			
	Explant type			
	L	S	IB	TB
Size of calluses on the 30th day, mm				
MS1	0.8±0.2	0.9±0.3	1.4±0.2	2.1±0.3
MS2	1.4±0.3	1.3±0.2	2.3±0.3	4.1+0.2
MS3	2.0+0.4	1.6±0.3	3.8+0.2	3.3±0.4
MS4	2.5±0.3	2.2±0.3	2.9±0.3	2.5±0.3
MS5	1.5±0.2	1.3±0.2	1.2±0.4	0.9±0.2
L-leaf; S-stem; IB-indirect bud; TB-top bud				

During observations of black currants, it was shown that the frequency of callusogenesis strongly depends on the balance of phytohormones in the nutrient medium (Fig. 1).

At high rates of callusogenesis frequency, explants of indirect and top buds differed when they were grown in MS3 medium, which was 52.8 % in the cultivar Lia fertile. MS4 proved to be the most suitable nutrient medium for leaf explants and stem frequency. At lower concentrations of phytohormones (MS1 and MS2 medium), the frequency of callusogenesis of leaves and stems is within 9.9 and 13.5 %, and that of indirect and apical buds is 20.3 and 34.8 %. Increasing the concentration of phytohormone did not increase the rate of callusogenesis in all explants studied. At the same time, the frequency of callusogenesis decreased to 20.4-21.6 % depending on the black currant variety and explant species (Tab. 4).



Ж — leaves, С — stem, ЖБ — lateral buds, ТБ — top buds

Figure 1. Influence of phytohormone concentration and explant type on the callusogenesis of the currant Lia variety, %

Table 4

Effect of phytohormone concentration and explant type on callusogenesis duration in black currant varieties (days)

Nutrient medium	Harvest variety of currant Lia			
	Explant type			
MS 1	L	S	IB	TB
MS2	119±2	134±3	103±2	100±2
MS3	113±3	109±3	95±3	89±3
MS4	100±3	106±3	103±2	99+2
MS5	96+2	107±2	89±3	82±3

Callus cultures with different morphogenesis abilities also differ in morphology, allowing the morphogenic parts of the callus to be visually identified for further subculture.

It is known that the differentiation of a plant cell can be observed not only in the light microscope but also in the fine structure of the cell and changes in its organelles. In the in vitro culture, this is associated with the processes of organogenesis, histogenesis, and somatic embryogenesis.

As a result of numerous experiments on the study of callus genesis and callus shoot growth, a large variety of calluses with different morphological characteristics (consistency, color, morphogenetic potential) was obtained. The study of morphological features of calluses induced from black currant and perpetual raspberry organs revealed that there is no clear difference between the selected cultures in the type of formed calluses. However, the calluses were morphologically heterogeneous: loose, hard, easily decomposed, and granularly dense. Therefore, the calluses formed by explants of perpetual raspberry and black currant can be grouped according to their morphology, coloring, and morphogenetic ability (Tab. 5).

Table 5

Description of currant and perpetual raspberry callus

Types of callus	Morphological structure	Color	Type of morphogenesis
1	loose callus	colorless	no morphogenetics
2	large bumps	light yellow	homogeneous
3	there are fine-grained sprouts on the callus	dark yellow to brown	embryogenic
4	the surface of the callus has a thin filament	white	rhizogenic

The 1st type of callus is a solid oval, transparent, colorless glandular mass, with no visible external tumors. This species, capable of continuous growth when transplanted, has accumulated considerable biomass. However, this species has no morphogenetic potential.

The 2nd type of callus has a dense consistency, consisting of large roll-shaped bulges, bright yellow. When grown in the light, the callus tissue is green. Growing in a morphogenic medium is accompanied by the formation of buds and the growth of primary leaves. According to the type of morphogenesis, these calluses are homogeneous.

The 3rd type of callus consists of small structures, which approach each other with their bases and resemble a lightning bolt. When slowed down in the nutrient medium, they easily decompose, giving rise to globules and further somatic embryoids. This embryogenic callus tissue is an ideal model system to study the process of somatic embryogenesis in the culture of perpetual raspberry and black currant. The 4th type of callus is characterized by the appearance of many thin filamentous structures on the tissue surface. During further cultivation, some of these structures form roots.

Summarizing the results of this series of experiments, the obtained callus tissues of perpetual raspberry and black currant have different types of morphogenesis: homogenesis, rhizogenesis, and embryogenesis. These experimentally obtained types of callus tissues are valuable as a model to study the physiological mechanisms of cell differentiation in vitro.

To study the possibility of preserving their morphological characteristics and morphogenetic potential by callus type, fragments of different callus types were transferred to a new medium containing the same components of the nutrient medium that contributed to the formation of these calluses.

Experiments on the origin of somatic cells of black currant and perpetual raspberry have established that the studied objects can induce callusogenesis in vitro with proper selection of phytohormones, especially their ratio, as well as cultivation regimes. The intensity of callus formation and growth of callus structures is influenced by the origin of the explant and the concentration of phytohormones in the nutrient medium.

In the process of callusogenesis, somatic cells are dedifferentiated. As a result, different cell types are formed, which causes heterogeneity of callus tissue. The degree of heterogeneity depends on the genetic characteristics of the plants from which the explants are obtained. When different phytohormones and cultivation conditions are changed, an increase in cell diversity in the callus mass is possible. However, in our experiment, we obtained calluses from different explants only in media with relatively low concentrations of 2,4-D phytohormones and kinetins. Calluses and regenerants were cultured under the same conditions. Therefore, the difference between black currant and perpetual raspberry in the influence of other factors, the rate and duration of callus formation, is purely genetic in nature. Our studies have once again confirmed that high concentrations of 2,4-D and kinetin are necessary for cell and tissue growth.

According to the results of the experiments, it can be concluded that the intensity of callusogenesis depends on plant genotype, but often depends on the type of selected explant. Callusogenesis can be induced in all organs of black currant and perpetual raspberry.

Conclusions

The results of obtaining growth from currant culture varieties and micropropagation were as follows: 57.8 % of pure growth was obtained from currant culture varieties, and bud proliferation was performed from all varieties of pure currant culture.

The development of shortened roots of currant varieties was stimulated by 28.6 %. The development of shoots on shoots of Russian currant culture varieties was 23.4 %.

About 80 % of shoots were obtained from local currant crops. Thus, it has been proved that the stage of obtaining pure shoots by microclonal propagation of plants is the most difficult process. As a result of our research, the method of obtaining pure growth and its propagation has been improved. It is the end of month and the weekend.

References

1 Ikuta K. Anti-viral and anti-bacterial activities of an extract of blackcurrant (*Ribes nigrum* L.) / K. Ikuta, K. Hashimoto, H. Kaneko, S. Mori, K. Ohashi, T. Suzutani // Microbiol Immunol. — 2012. — Vol. 56 (12). — P. 805–809. https://doi.org/10.1111/j.1348-0421.2012.00510.x

2 Bishayee A. Anthocyanin — rich black currant extract suppresses the growth of human hepatocellular carcinoma cells / A. Bishayee, E. Haznagy-Radnai, T. Mbimba, P. Sipos, P. Morazzoni, A.S. Darvesh, D. Bhatia, J. Hohmann // Nat Prod Commun. — 2010. — Vol. 5 (10). — P. 1613–1618.

3 Оразбаева Г.К. Клональное размножение растений черной смородины (*Ribes nigrum* L.) *in vitro* / Г.К. Оразбаева, В.Т. Хасанов, А.Р. Искаков, В.К. Швидченко // Вестн. науки КазАТУ им. С. Сейфуллина. — 2012. — № 1. — С. 115–124.

4 Казаков И.В. Малина ремонтантная / И.В. Казаков, С.Н. Евдокименко. — М.: Всерос. селекц.-технол. ин-т садоводства и питомниководства, 2006. — 288 с.

5 Жбанова Е.В. Плоды малины *Rubus idaeus* L. как источник функциональных ингредиентов (обзор) / Е.В. Жбанова // Техника и технология пищевых производств. — 2018. — Т. 48, № 1. — С. 5–14. https://doi.org/10.21603/2074-9414-2018-1-5-14

6 Акимов М.Ю. Пищевая ценность плодов перспективных сортов земляники / М.Ю. Акимов, Е.В. Жбанова, В.Н. Макаров, И.Б. Перова, Л.В. Шевякова, О.А. Вржесинская, Н.А. Бекетова, О.В. Кошелева, М.Н. Богачук, Е.В. Рылина, И.В. Лукьянчук, А.М. Миронов // Вопросы питания. — 2019. — Т. 88, № 2. — С. 64–72. https://doi.org/10.24411/0042-8833-2019-10019

М.К. Тыныкулов

Қарақат және ремонтантты таңқурай жасушалары дақылдарының ерекшеліктері

Ремонтантты таңқурай — екіжылдық және біржылдық сабақ өркендерінде жеміс беру қабілетімен ерекшеленетін таңқурай сорттарының тобы. Қара қарақат (*Ríbes nígrum*) — түспежапырақты бұталы,

қарлығандар тұқымдасына (*Grossulariaceae*), қарақат (*Ribes*) туыстасына жатады. Ауыл шаруашылығы дақылдарының экстремалды табиғи факторларға төзімді, жоғары өнім және жылына екі өнім алатын сорттары қарастырылған. Қолданылатын қоректік ортаның химиялық құрамы, микро- және макроэлементтердің, органикалық заттардың мөлшері есепке алынды. Өсімдіктердің жасушаларынан алынған каллус өсінділерінің сапалы құрамы анықталған. Қара қарақат каллусогенезінің жиілігі қоректік ортаның фитогормондарының балансына қатты байланысы көрсетілді. Гендік және жасушалық биотехнология саласындағы прогресс *in vitro* жағдайында өсімдік жасушалары мен ұлпаларын өсіру негіздерінің дамуымен тікелей байланысты. Сонымен қатар, қолайлы қоректік ортаны таңдау, эксплантты түрі мен көлемін, өсімдіктердің әртүрлі таксономиялық топтарының өкілдерінің тотипотенттілігін анықтау мақсатында өсу режимін белгілеу ерекше қызығушылық тудырады. Қазіргі уақытта соматикалық жасушалардың бұл ерекше қабілеті әртүрлі дақылдарда кездеседі, олардың көпшілігі біржылдық және іс жүзінде вегетативті көбейетін өсімдіктер. Бұл көкөніс дақылдары мен сәндік өсімдіктер, олардың тізімі жыл сайын толықтырылып отырады. Жұмыстың ғылыми жаңалығы — биотехнология әдістерінің көмегімен ремонтантты таңқурай мен қара қарақаттың соматикалық будандары мен трансгенді өсімдіктерін алу мүмкіндігі. Сондықтан көпжылдық өсімдіктердің жасушалары мен ұлпаларының, атап айтқанда жеміс-жидек және ауылшаруашылық дақылдарының теориялық негіздерін зерттеу және қолданбалы аспектілерін дамыту, олардың тез көбеюі, соматикалық будандар мен трансгенді өсімдіктерді алу өте өзекті болып саналады.

Кілт сөздер: қарақат, таңқурай, биотехнология**,** in vitro, ремонтанттылық, тотипотенттілік, селекция**,** жасауша, каллус, фитогормондар.

М.К. Тыныкулов

Особенности культуры клеток смородины и ремонтантной малины

Ремонтантная малина — группа сортов малины, отличающихся способностью плодоносить на двухлетних и однолетних стеблевых побегах. Черная смородина (*Ríbes nígrum*) — листопадный кустарник, семейство крыжовниковые (*Grossulariaceae*), относится к родству смородины (Ribes). В статье представлены методы клеточной селекции ремонтантной малины и смородины. Рассмотрены сорта сельскохозяйственных культур, устойчивые к экстремальным природным факторам, получающие высокие урожаи и два урожая в год. Учитывались химический состав использованной питательной среды, содержание микро- и макроэлементов, органических веществ. Определен качественный состав побегов каллуса, извлеченных из клеток растений. Показана сильная связь частоты каллусогенеза черной смородины с балансом фитогормонов питательной среды. Прогресс в области генной и клеточной биотехнологии напрямую связан с разработкой основ культивирования клеток и тканей растений в условиях *in vitro*. Кроме того, особый интерес представляет выбор подходящей питательной среды, установление режима выращивания с целью определения вида и объема экспланта, тотипотентности представителей различных таксономических групп растений. В настоящее время эта уникальная способность соматических клеток обнаруживается в различных культурах, большую часть которых составляют однолетние и практически вегетативные растения размножения. Это овощные культуры и декоративные растения, список которых ежегодно пополняется. Научной новизной работы являетя возможность получения соматических гибридов и трансгенных растений ремонантной малины и черной смородины с помощью методов биотехнологии. Поэтому изучение теоретических основ и разработка прикладных аспектов клеток и тканей многолетних растений, в частности, плодово-ягодных и сельскохозяйственных культур, их ускоренное размножение, получение соматических гибридов и трансгенных растений считаются весьма актуальными.

Ключевые слова: смородина, малина, биотехнология, *in vitro*, ремонтантность, тотипотентность, селекция, клетки, каллус, фитогормоны.

References

1 Ikuta, K., Hashimoto, K., Kaneko, H., Mori, S., Ohashi, K., & Suzutani, T. (2012). Anti-viral and anti-bacterial activities of an extract of blackcurrant (*Ribes nigrum* L.). *Microbiol Immunol., 56 (12)*; 805–809. https://doi.org/10.1111/j.1348-0421.2012.00510.x

2 Bishayee, A., Haznagy-Radnai, E., Mbimba, T., Sipos, P., Morazzoni, P., Darvesh, A. S., Bhatia, D., & Hohmann, J. (2010). Anthocyanin — rich black currant extract suppresses the growth of human hepatocellular carcinoma cells. *Nat Prod Commun., 5 (10)*; 1613–1618.

3 Orazbaeva, G.K., Khasanov, V.T., Iskakov, A.R., & Shvidchenko, V.K. (2012). Klonalnoe razmnozhenie rastenii chernoi smorodiny (*Ribes nigrum* L.) *in vitro* [Clonal reproduction of blackcurrant plants (*Ribes nigrum* L.) *in vitro*]. *Vestnik nauki Kazakhskogo agrotekhnicheskogo universitets imeni S. Seifullina — Bulletin of Science of the S. Seifullin Kazakh Agro Technical University, 1*; 115–124 [in Russian].

4 Kazakov, I.V., & Evdokimenko, S.N. (2006). *Malina remontantnaia [Raspberry repair]*. Moscow: All-Russian Selctive-technological Institute of gardening and nursery farming [in Russian].

5 Zhbanova, E.V. (2018). Plody maliny *Rubus idaeus* L. kak istochnik funktsionalnykh ingredientov (obzor) [*Rubus idaeus* L. raspberry fruits as a source of functional ingredients (review)]. *Tekhnika i tekhnologiia pishchevykh proizvodstv — Technique and food production technology, 48 (1)*; 5–14. https://doi.org/10.21603/2074-9414-2018-1-5-14 [in Russian].

6 Akimov, M.U., Zhbanova, E.V., Makarov, V.N., Perova, I.B., Shevyakova, L.V., Vrzhesinskaya, O.A., Beketova, N.A., Kosheleva, O.V., Bogachuk, M.N., Rylina, E.V., Lukyanchuk, I.V., & Mironov, A.M. (2019). Pishchevaia tsennost plodov perpektivnykh sortov zemlianki [Nutritional value of fruits in promising raspberry varieties]. *Voprosy pitaniia — Problems of Nutrition, 88 (2)*; 64–72. https://doi.org/10.24411/0042-8833-2019-10019 [in Russian].