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IN VITRO PRODUCTION OF VIRUS-FREE CARNATION (*DIANTHUS CARIOPHYLLUS* L.) PLANTING MATERIAL

***O. KLIACHENKO*[1],**
doctor, Professor of the Department of Ecobiotechnology and Biodiversity,
https://orcid.org/0000-0002-4087-4082,
Klyachenko@ukr.net
***K. KUSHCHENKO*[1],**
master's degree Faculty of Plant Protection, Biotechnology and Ecology,
https://orcid.org/0009-0007-1134-6479
***I. SHIAKHTUN*[1],**
graduate student of the Department of Ecobiotechnology and Biodiversity,
https://orcid.org/0000-0003-4338-8474
***I. BEZPROZVANA*[2],**
Researcher of the Laboratory of Quality Indicators of Plant Varieties,
https://orcid.org/ 0000-0002-4240-7605
[1] National University of Life and Environmental Sciences of Ukrraine, Kyev, Ukraine
[2] Ukrainian Institute of Expertize of Plant Varieties, Kyev, Ukraine

Abstract. *The methods of culture of apical meristems and direct and indirect morphogenesis in vitro were used for production of virus-free planting material of carnation. A scheme for obtaining aseptic material has been developed, which consists of stepwise treatment of explants: Thimerosal - 2 min, 70% ethyl alcohol - 0.5 min and 0.08% $AgNO_3$ - 1 min, which reduces the level of contamination by fungal infection. Expounded the results of studies of callusogenesis and direct and indirect morphogenesis in the culture of in vitro explants of Dutch carnation, their dependence on the content of growth regulators in the nutrient medium. It was established that there were almost no significant differences in the course of callusogenesis processes within carnation varieties. At the same time, the frequency of callusogenesis was 100%. Under the conditions of indirect morphogenesis realization, it is necessary to take into account the age of callus tissues. The growth and intensive shoot formation of carnations was noted on the Murashige-Skoog nutrient medium supplemented with BAP at a concentration of 0.5 mg/l. The best medium for rooting was the MS medium with half the concentration of macro- and microsalts with the addition of 0.5 mg/l of NAA, which is recommended by us for rooting regenerating carnation plants of various varieties. Peat: perlite in a 1:1 ratio was used as a substrate for the adaptation of regenerating plants . Survival of carnation plants to conditions in vivo for the variety "Raffino Linde" was 90%, while for the variety "Tiya" - 83%, respectively.*

Key words: *carnation, callusogenesis, morphogenesis, shoot formation rhizogenesis.*

Introduction.

Carnation is a perennial plant, cultivated in almost all regions of Europe and is used in landscaping as an ornamental plant for planting in flower beds and alpine slides. In floriculture, carnations are used for cutting and sale. In greenhouses, remontant carnation, which is often called Dutch carnation, is grown for cutting. The main method of propagating Dutch carnation is meristem tissue culture, which makes it possible to obtain virus-free planting material in a timely manner (Pralhad, 2009; Khatun et al., 2018; Sreelekshmi and Syril, 2021).

In recent years, two trends can be clearly observed in research on the culture of tissues and cells of higher plants. The first is related to an in-depth study of the biology of cultured cells, examining features of their growth and differentiation (Gao et. al, 2017; Isah, 2019; Zhang et al., 2022). The second is more applied, which aims at a wider application of techniques and methods of tissue culture to solve important practical problems that arise in floriculture. One of such effective and cost-effective methods is the culture of apical meristems and microclonal propagation and improvement of plant material in *in vitro* culture (Klyachenko et. al.,2003; Kushnir and Sarnatska, 2005; Muszyńska and Hanus-Fajerska, 2017). This method makes it possible to obtain a large amount of plant material in a short period of time. In addition, during the propagation of plants in tissue culture, they are freed from pathogenic microorganisms and, in many cases, from viruses. In the process of micropropagation, there is no risk of re-infection of plants. The improvement of planting material improves the quality of products (Koldar, 2010; Sreelekshmi and Syril, 2021).

Despite the large number of experimental works devoted to morphogenesis and reproduction *in vitro*, the technology of microclonal reproduction has not been developed for many species of ornamental plants and in floriculture. The reason for this is, first of all, the lack of clear methods, their complexity, secondly, the use of scarce and expensive components of the nutrient medium and insufficient knowledge of the morphogenetic potential of plants and ways of managing them in tissue culture. Today, the issue of virus-free carnation planting material production, its reproduction and adaptation to conditions *in vivo* is still relevant. To satisfy the growing demand for carnations, the use of traditional approaches in the reproduction and selection of these plants is not enough.

The purpose of the work is to study the features of production of virus-free material of different varieties of Dutch carnation and its clone micropropagation in the conditions of *in vitro*.

Research materials and methods.

As explants, shoots with axillary and apical buds of two varieties of carnations, Dutch "Tiya" and "Raffino Linde" were used for introduction into the in vitro culture. The research methods generally accepted in biotechnology were used in the work (Melnichyk and Klyachenko, 2015). We conducted research in several directions: studying the ability of carnations to form callus, the intensity of shoot formation in conditions of *in vitro*, rooting of regenerating plants and their adaptation to conditions *in vivo*.

Callus tissue was obtained by cultivating stem explants. Apical meristems

1. Sterilant solutions, their concentration and treatment exposure

Sterilizing substances	Concentration, %	Exposure, min	
		1 option	2 option
Thimerosal	1	2	2
C_2H_5OH	70	1	0.5
$AgNO_3$	0.08	0.5	1

with leaf primordia 0.3-0.5 mm long were used to grow mother plants. When studying shoot formation, apical meristems and intermediate microstalks of carnations, 1-2 cm long with one pair of leaves, which contain axillary meristematic tissues, were used, as explants. With donor plants carnations were cut off the upper part of the stem, 1.5-2.0 cm long, thoroughly washed in a soapy solution, then washed with running water and rinsed twice with sterile distilled water.

For optimization of sterilization of plant material, sterilant solutions and step sterilization with different exposures were used. Thimerosal, 70% ethanol and 0.08% $AgNO_3$ were used as sterilants. Sterilization was carried out sequentially (table 1).

Explants were transferred to test tubes on a hormone-free Murashige-Skoog (MS1) nutritious medium (Murashige, Skoog, 1962) for adaptation. Tubes with explants were cultivated in a thermostat without lighting at a regulated temperature of 25±1°C. After a week, they were tested for microorganisms caused infection.

Sterilization efficiency (Es), in percentage, was calculated according to the formula:

$$Ec = \frac{Ke - Kvr.e.}{Ke} x100\%, \text{ where}$$

Ke - is the total number of explants.

Kvr.e - is the number of affected explants (Melnichyk and Klyachenko, 2015).

Carnation callus tissue was obtained from sterile leaf plates and fragments of microshoots (0.5-0.8 cm), by making additional incisions. Shoots were cut into segments of 2-4 mm carnation explants were cultured on a modified Murashige-Skoog medium (variant MSC1 with the addition of 0.2 mg/l BAP, 2 mg/l NAA, variant MSC2 with the addition of 0.5 mg/l BAP, 2 mg/l NAA and MSC3, supplemented with 1.0 mg/l BAP, 2 mg/l NAA).

For subcultivation, the mass of raw callus substance was 2.0±0.10 g, the duration of the passage was 28-30 days. The plant material was cultivated in penicillin vials in a TS-80 thermostat without lighting at a temperature of 25 ± 1 0C and humidity of 70−75%. The frequency of callus formation was determined as the percentage of explants that formed a callus from the total number of explants. The average monthly increase in raw callus mass was determined as the difference between the final and initial mass (Melnichuk and Klyachenko, 2015).

To induce indirect morphogenesis, callus tissues weighing 2.0±0.10 g were planted on MC medium with the addition of 0.1-3.0 mg/l BAP, 0.5-2.0 mg/l kinetin and 0.1- 1.0 mg/l 2,4-D. The plant material was cultivated at a temperature of 25±1 0C, illuminance of 2.0-3.0 klx, a 16-hour photoperiod, and humidity of 70-75%.

Direct morphogenesis was achived by cultivating apical meristems on

modified MSA nutrient medium supplemented with White's vitamins, BAP at a concentration of 0.5 mg/l. For the rooting of microshoots obtained in the culture of isolated apical meristems, they were transferred to the nutrient medium MCP with a half set of macro- and microsalts with the addition of 0.5-2.0 mg/l NAA.

Rooted carnation plants with well-developed leaf blades and petioles of dark green color were taken out of test tubes for adaptation . The plants were transferred to a substrate composed of perlite: peat (1:1), which was previously autoclaved at 2 atm according to Kushnir et all. (Kushnir and Sarnatska, 2005). After planting the regenerating plants in the substrate, they were covered with glass cylinders to prevent the plants from drying out, and after 7 days, the plants were fertilized with a solution of macro- and microsalts according to MS. After 2 weeks of cultivation, the glass cylinders were removed, the plants were kept for 7 days and transplanted into open ground conditions. Survival of plants in conditions of *in vivo* was recorded.

The experimental material obtained as a result of research was statistically processed using the MS Excel analysis package. The repeatability of experiments is 3-15 times, the number of samples used in the experiment is 10-30 pieces. Arithmetic mean values and their standard errors are given in the tables.

Results and discussion.

In the course of research, we conducted a number of experiments on obtaining an aseptic carnation culture. The carnations were stored in water, brought to the laboratory and thoroughly washed under running water to remove dirt from the stem surface. Difficulties associated with sterilization of explants have been noted in the literature (Radojevic et al., 2010; Qadri et al., 2018; Ram et al., 2019; Polivanova and Bedarev, 2022). We used gradual sterilization with several solutions of sterilants with different processing exposure. The first option – gradual treatment with Thimerosal - 2 min, 70% ethanol - 1 min and 0.08% AgNO3 - 0.5 min. The second option - gradual treatment with Thimerosal - 2 min, 70% ethanol - 0.5 min and 0.08% AgNO3 - 1 min followed by washing three times with sterile distilled water. The shoot tips and nodal segments were cut into small parts (1.0-1.5 cm) for explantation on a hormone-free MC medium. The results of the research showed that the effectiveness of sterilization depended on the option of exposure to treatment with sterilizing substances. The second option of sterilization turned out to be more effective, which made it possible to free the plant material from

2. Efficiency of sterilization of carnation explants under different schemes of aseptic processing

Variety	Number of explants	Number of infected explants on day 7, %		The number of infected explants on day 10, %	
		Version			
		1	2	1	2
"Tiya"	100	4	1	2	-
"Raffino Linde"	100	2	-	-	-
NIR_{05}		0.15	0.05	0.20	

exogenous and endogenous infection. Explants were screened for the presence or absence of infection on the third and seventh days of cultivation. The results are presented in table 2.

From the presented data, it can be seen that the second sterilization option was more effective, in which the sterilization efficiency was 99% for the "Tiya" variety on the 7th day, and 100% for the "Raffino Linde" variety, respectively.

After successful sterilization and production of aseptic carnation seedlings on the 10th day of cultivation, the explants were placed in biological test tubes on nutrient media supplemented with growth regulators to obtain callus and regenerating plants. On the first or second day of cultivation of sterile carnation explants, you can observe their increase in size.

Thus, after analyzing the results obtained by us, we can conclude that aseptic processing of explants is used the scheme of Thimerosal - 2 min, 70% ethanol - 0.5 min and 0.08% AgNO3 - 1 min is the most effective. In addition, the use of 0.08% AgNO3 reduces the level of fungal infection if the exposure time is lengthened.

According to research by domestic and foreign scientists, the intensity and efficiency of callusogenesis in plants cultivated in vitro depends on external factors, namely cultivation conditions, and above all, the presence of growth regulators in the nutrient medium (Gang et al., 2003; Khatun et al.,2018; Qadri, 2018). Growth regulators cause numerous changes in plant tissues and are the basis of structural transformation of cells during callusogenesis. The presence of growth regulators in the nutrient medium causes the formation of a callus mass with a high degree of proliferation, which makes it possible to obtain a large number of regenerating plants. It is known that the classic ratio of auxins to cytokinins (10:1) in the nutrient medium induces callusogenesis. In addition, a certain genotype has its own peculiarities and difficulties in the process of obtaining and cultivating callus tissues.

Callus tissue was obtained by culturing stem explants on a modified MC nutrient medium with the addition of growth regulators NAA and BAP in different concentrations and ratios. For callusogenesis, we used the following series of nutrient media presented in the table. 3.

3. Content of growth regulators in callusogenic media

Version	Growth regulators
1	0.2 mg/l BAP + 2 mg/l BAP
2	0.5 mg/l BAP + 2 mg/l BAP
3	1 mg/l BAP + 2 mg/l BAP

Penicillin vials with planted explants were placed in the dark in a thermostat with regulated temperature and humidity of 70% and cultivated for 12 days. Later, the explants were exposed to light at a temperature of +23 ± 1 0C and a 16-hour light period. On the 24th day, the formation of callus tissues was recorded, the results of which are presented in the table. 4.

From the presented data, it can be seen that the minimum mass of calli was formed on average on the nutrient medium of variant 1. In addition to the growth characteristics, we described the morphological characteristics of callus tissues, namely compactness and color, the results of which are presented in the table. 5.

Therefore, within carnation varieties, there were almost no significant dif-

4. Dependence of callusogenesis of carnation variety "Tiya" on the content of growth regulators in the nutrient medium

Variant	Composition of the nutrient medium	Number of explants	The number of callus-forming explants	
			%	mass of callus, mg
1	MS + 0.2 mg/l BAP + 2 mg/l BAP	40	100	350 ±17.5
2	MS + 0.5 mg/l BAP + 2 mg/l BAP	40	100	820 ±41.1
3	MS +1 mg/l BAP + 2 mg/l BAP	40	100	610 ±30.5

5. Qualitative characteristics of carnation callus tissues depending on the composition of the nutrient medium

Variant	Composition of the nutrient medium	Characteristics of carnation callus tissues	
		variety	
		"Tiya"	"Raffino Linde"
1	MS + 0.2 mg/l BAP + 2 mg/l BAP	light yellow, not dense	light yellow, not dense
2	MS + 0.5 mg/l BAP + 2 mg/l BAP	dense with green cells	dense with green cells
3	MS +1 mg/l BAP + 2 mg/l BAP	dense with green cells	dense with green cells

ferences in the course of callusogenesis processes, and its frequency was 100%.

One of the least studied aspects of differentiation is morphogenesis. At the same time, the complexity of researching the physiological, biochemical and molecular processes underlying morphogenesis lies in the fact that the differentiation of cells into organs occurs asynchronously. However, differentiating cells and differentiation centers are spatially separated. In the process of differentiation, morphological structures are formed in cultivated unorganized callus tissues, which lead to the formation of buds, roots, stems, flowers, whole plants in them (Murashige,1990; Jorapur et al., 2018). Indirect morphogenesis takes place in two stages, namely: the first stage is the process of formation of callus tissues on the explant; the second stage is the formation of morphological structures in callus tissues. At the same time, the formation of morphological structures is determined by a certain ratio of cytokines and auxins, which is considered classic 10:1. In the culture of callus tissues, somatic embryogenesis and organogenesis (stem, root, floral, leaf) an be distinguished (Zhu et al., 2018; Nikam et al., 2019; Zhang et al., 2022).

Research on the induction of organogenesis is caused primarily by the requests of breeders and geneticists who are interested in using tissue culture methods and genetic engineering to obtain new forms of the most interesting ornamental plants and flowers. The capacity for organogenesis decreases in tissues isolated by direction from apex to base stems. The tendency to organ-

6. Ability to organogenesis *in vitro* in carnation varieties

No	Variety	Intensity of organogenesis		
		Frequency of organogenesis, %	Number of shoots	Height of shoots, cm
1	"Tiya"	68.22	3.2 ± 0.36	6.15 ± 0.062
2	"Raffino Linde"	95.75	3.8 ± 0.13	8.28 ± 0.091

ogenesis also decreases with multiple transplants of callus tissues, and the ability to root is preserved for a longer time.

To induce indirect morphogenesis, callus tissues weighing 2.0±0.10 g were planted on MC medium with the addition of 0.1-3.0 mg/l BAP, 0.5-2.0 mg/l kinetin and 0.1- 1.0 mg/l 2,4-D. The plant material was cultivated at a temperature of 25 ± 10C, illuminance of 2.0-3.0 klx, a 16-hour photoperiod, and air humidity of 70-75%. In our studies, intensive organogenesis was observed for both varieties of carnations (table 6).

Thus, when obtaining indirect morphogenesis, it is necessary to take into account the fact that the regeneration ability of callus tissues of different carnation genotypes depends not only on the genotype of the plants, but also on the age category. With age, especially after the ninth passage, a decrease in regenerative capacity is observed. Perhaps the reason for this may be somaclonal variability, which occurs as a result of long-term cultivation of callus tissues.

It is generally known that one of the main conditions for the regeneration of plants with a meristem is the presence and optimal ratio of a growth regulator in the nutrient medium. Examples of organ formation in tissue and cell culture are extremely diverse in relation to certain types and concentrations of phytohormones (Muszyńska and Hanus-Fajerska, 2017; Jorapur et al., 2018; Khatun, 2018; Qadri, 2018). However, there is no general formula regarding the concentrations of phytohormones, as well as other physiologically active substances, which could be applied in all cases. An important role belongs to cytokinins, namely 6-BAP, kinetin and auxins, such as IAA, NAA, IBA. The types and level of exogenous phytohormones in the nutrient medium are currently one of the main factors that make it possible to direct the regulation of growth, development and morphogenesis processes in tissue culture (Koldar, 2010; Zhu et al., 2018).

Taking into account that the apical meristem contains endogenous auxins, primerely IAA, we have developed a nutrient medium for shoot formation from the apical meristems of carnations, which contains a substance from the group of cytokinins - 6-BAP. Cytokinins induce the development of axillary buds in apical culture, stimulate the growth of dormant organs, and also regulate the growth of somatic embryos and plant formation. as an explant, 0.3-0.5 mm long apical meristems of carnations of the "Tiya" and "Raffino Linde" varieties were used. The results of shoot formation are presented in the table. 7.

Carnation plants poduced from apical meristems are presented in fig. 1.

Thus, the addition of cytokines to the MC nutrient medium, and 6-BAP directly, enables the development of apical meristems and intensive carnation shoot formation.

7. Shoot formation from the apical meristems of carnations on the 7th week of cultivation

Variety	Number of explants	Number of shoots			The length of the shoots, cm
		Number of shoots, 1 shoots	The number of shoots is, 2 shoots	The number of shoots, 3 or more shoots	
"Tiya"	20	10	5	5	2.1 ± 0.09
"Raffino Linde"	20	3	5	12	2.8 ± 0.14

Fig. 1. Regenerative plants obtained from apical meristems of carnations of the "Tiya" variety

Study of the process of experimental morphogenesis in vitro at all levels of organization - from a single cell to the top of a shoot - led to the creation of microclonal reproduction of plants, which is put on a commercial basis in most countries. Clonal micropropagation is the use of the technique in vitro for quick asexual production of plants that are identical to the original. During the regeneration of microshoots, meristematic tissues that perform certain functions in the plant organism are reorganized, and at the same time their primary functions are restored.

One of the important problems with clonal micropropagation is a matter of concentration of growth regulators that add to the nutrient medium. Recently, there has been evidence that high concentrations of growth regulators negatively affect the morphogenetic features of the obtained plants, as well as the possibility of long-term micropropagation in culture in vitro (Nikam et al., 2019; Isah, 2019; Polivanova and Bedarev, 2022).

It is possible that in the process of cultivation of shoots, the gradual accumulation of growth regulators in the cultivated tissues exceeds the necessary physiological level and at the same time they become toxic. This leads to changes in the morphology of plants, suppression of the proliferation of axillary meristems, and a decrease in the ability to root. The use of media with a minimum concentration of cytokinins, which ensure a high rate of micropropagation, reduces their negative effect. Alternating cultivation cycles on media with low and high levels of growth regulators also prevents the negative effects of cytokinins (Das et al., 2012; Arif et al., 2014; Sreelekshmi and Syril, 2021).

Plants grown from the apical meristems of carnations, 5-6 weeks after the establishment of the experiment, were grafted and micro-cuttings 1-2 cm long with one internode were used. They were transplanted in such a way that the internode was on the surface of

8. Shoot formation of carnation variety from micro cuttings

Accounting time	Shoots 1-2.9 cm long	Shoots 3-4.9 cm long	Shoots 5 cm long or more	Total shoots
Variety " Tiya "				
The number of shoots after 4 weeks of cultivation	26 ± 1.31	10 ± 0.54	2 ± 0.12	38 ± 1.93
The number of shoots after 6 weeks of cultivation	51 ± 2.52	16 ± 0.81	4 ± 0.22	71 ± 3.55
Variety "Raffino Linde"				
The number of shoots after 4 weeks of cultivation	28 ± 1.40	18 ± 0.90	6 ± 0.30	52 ± 2.60
The number of shoots after 6 weeks of cultivation	64 ± 3.20	24 ± 1.20	8 ± 040	96 ± 4.80

the nutrient medium. Explants were cultivated at a temperature of +24-25 °C, a 16-hour photoperiod, and humidity of 70-80%. Samples were examined twice a week. Thirty explants were planted in total. On the 4th and 6th week of cultivation, shoot formation was recorded, the results of which are presented in the table. 8.

From the presented data, it can be seen that after 4 weeks of cultivation, the variety "Tiya" formed an average of 38 developed shoots, while the variety "Raffino Linde" - 71 shoots. After 6 weeks of cultivation, the "Tiya" variety produced an average of 52, while the "Raffino Linde" variety - 96 shoots, respectively.

Thus, our research clearly illustrates the advantages of microclonal propagation compared to conventional vegetative propagation, since an unlimited amount of planting material can be obtained from existing shoots by dividing newly formed shoots and transplanting them to a fresh nutrient medium.

Rooting of regenerative plants is the final and one of the most difficult stages in microclonal reproduction. Some authors (Ali et al., 2008; Melnychuk and, Klyachenko, 2015) recommend to use mineral medium diluted twice without growth regulators for rooting, others (Muszyńska and Hanus-Fajerska, 2017) to add auxins. We studied the effect of NAA auxin at a concentration of 0.5 mg/l to 2 mg/l in an MS medium, which contained half the concentration of mineral salts, on the process of root formation in the researched carnation varieties and rooting on a hormone-free nutrient medium.

Murashige-Skoog nutrient medium of various variants: MSR1 - half concentration of mineral salts without adding growth regulators; MCP2 - half concentration of mineral salts with the addition of 0.5 mg/l NAA; MCP3 - half the concentration of mineral salts with the addition of 2 mg/l NAA. It should be noted that root formation takes place at the base of the shoots. Analyzing the results of research on the rooting of regenerating plants, carnations are shown in the table. 9, it can be seen that MCP2 supplemented with 0.5 mg/l NAA is optimally effective for both the "Tiya" and "Raffino Linde" varieties. On this medium, 90% rooting was observed, while medium variants 1 and 3 gave, respectively, 20% and 50% rooting of shoots for the variety "Tiya". Regarding the variety "Raffino Linde" 95% rooting was observed on medium MCP1, while

9. The influence of the composition of the nutrient medium on the rooting of carnation shoots

Version	The composition of the environment	Number of shoots	Number of rooted shoots	
			units	%
Variety "Raffino Linde"				
1	½ MS	40	8	20
2	½ MC + 0.5 mg/l NAA	40	36	90
3	½ MS + 2.0 mg/l NAA	40	20	50
NIR_{05}		0.02	10.80	
Variety "Tiya"				
1	½ MS	40	10	25
2	½ MC + 0.5 mg/l NAA	40	38	95
3	½ MS + 2.0 mg/l NAA	40	18	45
NIR_{05}		1.10	2.75	

medium variants 1 and 3 gave 25% and 45% rooting of shoots, respectively.

Thus, it can be concluded that, according to the conducted research, the best for rooting was the MS medium with half the concentration of macro- and micro-salts and supplemented with 0.5 mg/l NAa, which can be recommended for rooting regenerating carnation plants of various varieties.

The final and one of the problematic stages of plant reproduction by the method of cultivation of isolated tissues is adaptation, which in plants obtained in vitro, quite complicated compared to plants in vivo. Plants obtained in conditions in vitro differ in anatomical features, namely, a thin cuticle, which contains little wax and wax-like substances; a small amount of mechanical tissues; leading bundles are weakly developed; organs that are necessary for photosynthesis (stoma) function in a limited way. Transplantation of intact plants in vitro in terms of in vivo leads to stress, because their organs, formed under high air humidity and low light intensity, cannot function adequately in new conditions. During the gradual decrease in air humidity, cuticles are formed in regenerating plants due to adaptation (Melnychuk et al., 2012).

We carried out the following manipulations during the adaptation of carnation regenerating plants. Test tubes with rooted plants were opened, plants with a well-developed root system were removed, washed from the remains of the nutrient medium, placed for 20 minutes in a 1% solution of potassium permanganate, and then planted in a substrate: perlite: peat (1:1). Vessels with planted plants were placed in conditions of high humidity for better rooting. After 10-12 days, as the plants adapted, they took root and began to grow. The results of survival of regenerating carnation plants are presented in table. 10.

On the 30th day of cultivation, the seedlings had a height of 8-10 cm and 6-10 real leaves and were suitable for planting in open ground in a greenhouse. Thus, as a result of research conducted on the adaptation of regenerating carnation plants, we established that the survival of plants of the "Raffino Linde"

10. Rooting of regenerative plants of different carnation varieties

Variety	Plants planted in the substrate	Plants that have taken root	
		units	%
"Raffino Linde"	30	27	90 ± 3.56
"Tiya"	30	25	83 ± 3.96

variety was 90%, while under the same conditions, the variety "Tiya" - 83%, respectively.

Conclusions.

It was established that when introduced into the *in vitro* culture aseptic processing of explants using the Thimerosal scheme - 2 min, 70% ethyl alcohol - 0.5 min and 0.08% $AgNO_3$ - 1 min is the most effective. In addition, the use of 0.08% $AgNO_3$ reduces the level of fungal infection contamination under the conditions of lengthening the exposure time. Within carnation varieties, there were almost no significant differences in the course of callusogenesis processes, and its frequency was 100%. It should be taken into account that the regenerative capacity of callus tissues of different carnation genotypes depends not only on the genotype of the plants, but also on the age category for obtaining indirect morphogenesis. With age, especially after the ninth passage, a decrease in regenerative capacity is observed. Addition of cytokinins to the MC nutrient medium , and 6-BAP directly, enables the development of apical meristems and intensive carnation shoot formation . According to the conducted research, the best for rooting was the MS medium with half the concentration of macro- and microsalts and supplemented with 0.5 mg/l of NAA, which we recommend for rooting regenerating carnation plants of various varieties. It was established that the survival of plants of the variety "Raffino Linde" was 90%, while under the same conditions, the variety "Tiya" - 83%, respectively.

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Анотація. *Для отримання безвірусного посадкового матеріалу гвоздики використано метод культури апікальних меристем та прямий і непрямий морфогенез in vitro. Розроблена схема отримання асептичного матеріалу, яка полягає в поетапній обробці експлантатів: Thimerosal – 2 хв, 70% етиловий спирт – 0,5 хв і 0,08% AgNO3 - 1 хв, що знижує рівень контамінації грибної інфекції. Наведено результати досліджень калюсогенезу та прямого і непрямого морфогенезу в культурі in vitro експлантатів гвоздики голандської, їх залежність від вмісту регуляторів росту в живильному середовищі. Встановлено, що межах сортів гвоздики майже не спостерігалися значні відмінності у протіканні процесів калюсогенезу. При цьому частота калюсогенезу становила 100%. За умов отримання непрямого морфогенезу необхідно враховувати вік калюсних тканин. Ріст та інтенсивне пагоноутворення гвоздики відмічали на живильному середовищі Мурасіге-Скуга, доповненому БАП у концентрації 0,5 мг/л. Найкращим для укорінення виявилось середовище МС з половинною концентрацією макро- і мікросолей з добавлянням 0, 5 мг/л НОК, яке рекомендоване нами для укорінення рослин-регенерантів гвоздики різних сортів. Як субстрат для адаптації рослин-регенерантів використовували торф : перліт у співвідношенні 1:1. Приживаність рослин гвоздики до умов in vivo для сорту «Raffino Linde» становила 90%, тоді як для сорту «Tiya» - 83% відповідно.*

Ключові слова: *гвоздика, калюсогенез, морфогенез, пагоноутворення, різогенез.*