MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
V. N. Karazin KHARKIV NATIONAL UNIVERSITY

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OBTAINING AND USING CALLUS CULTURES

Educational and methodological complex

Electronic resource

Kharkiv – 2024

UDC 581.143.6:581.6(075.8)
A 95

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Approved for distribution in the Internet by the decision of the Scientific and Methodical Council of V. N. Karazin Kharkiv National University (Protocol № 12 of October 24, 2024)

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A 95 Obtaining and using callus cultures : educational and methodological complex [Electronic resource] / compilers O. O. Avksentieva, Y. D. Batuieva. – Kharkiv : V. N. Karazin KhNU, 2024. – (PDF 59 p.)

Methodical materials from the course «Obtaining and using callus cultures» are presented in the complex. The general structure of the course, the annotated content of the lecture material, the protocols of the laboratory workshop, the literature and internet resources for the course, the topics of the seminar classes, a set of test tasks for self-control of students, control questions for the course, and the principles of evaluating the success of students are given.

The educational and methodological complex is intended for students of higher education institutions studying disciplines in plant biotechnology.

UDC 581.143.6:581.6(075.8)

CONTENT

SECTION 1. Course aim and objectives

The aim of the course is to provide students with theoretical knowledge of the basics of in vitro cultivation of plant callus cultures and practical orientation skills necessary for professional activities in the field of plant biotechnology.

The main objectives of the course are to form a holistic view of the basic principles and methods of work organization in the laboratory in vitro culture of higher plants; to acquaint with the basic principles of obtaining and cultivating plant callus cultures, as well as with the variety of modern biotechnologies based on the use of in vitro callus culture of higher plants.

To know:

- historical information on the development, formation and modernity of methods of in vitro culture of higher plants;
- terminology (glossary) of modern plant biotechnology;
- features of the plant organism as an object of biotechnology;
- variety of methods for obtaining in vitro callus cultures of higher plants;
- main characteristics of callus cultures (morphological, cytological, genetic, biochemical, etc.);
- features of morphogenetic reactions of calluses;
- use of theoretical knowledge of the basics of cultivation of plant callus cultures in the practice of modern biotechnology.

Be able to:

- analyse, structure, integrate theoretical educational and lecture material;
- organise the work of the laboratory of in vitro culture of higher plants;

sterilise plant material;

- introduce plant objects into in vitro culture using different types of explants and obtain callus cultures;
- carry out work in a laminar flow box;
- to study callus cultures according to various characteristics;
- subcultivate callus cultures.

SECTION 2. Work programme of the course

2.1. General scheme and structure

Number of credits - 4, total number of hours - 120,
lecture - 20, practical - 20, individual work – 80

Titles of of sections and topics	Number of hours							
	Full-time form education				Part-time form education			
	Total	including			Total	including		
		lecture	pract/ lab	indiv. work		lect	pract/ lab	indiv work
Section 1. Basic principles of work in the laboratory of in vitro culture of higher plants								
Introduction. Topic 1: Organization of work in the plant biotechnology laboratory	14	2	2	10	15			15
Topic 2. General characteristics of culture media	14	2	2	10	19	2	2	15
Total for section 1	**28**	**4**	**4**	**20**	**34**	**2**	**2**	**30**
Section 2. Callus culture and its characteristics								
Topic 1: Primary callusogenesis	16	2	4	10	19	2	2	15
Topic 2. Mature callus culture	16	2	4	10	19	2	2	15
Topic 3: Morphological and genetic reactions of callus cultures	16	2	4	10	17	2		15
Total for section 2	**48**	**6**	**12**	**30**	**55**	**6**	**4**	**45**
Section 3. Modern plant biotechnology								
Topic 1: Biotechnology based on in vitro culture of cells, tissues and organs of higher plants	44	10	4	30	31	2		29
Total for section 3	**44**	**10**	**4**	**30**	**31**	**2**	**0**	**40**
Total hours	**120**	**20**	**20**	**80**	**120**	**10**	**6**	**104**

2.2. Annotated content of the lecture part

Introduction. Features of the plant organism as an object of modern phytobiotechnology. Totipotency is a unique property of a plant cell. History and development of in vitro culture methods of higher plants: main stages and achievements. Contribution of Ukrainian scientists to the development of world plant biotechnology.

Section 1. Basic principles of work in the laboratory of in vitro culture of higher plants

Topic 1: Organisation of work in the plant biotechnology laboratory. Basic principles and methods of work organisation in the laboratory of in vitro culture of plant cells, tissues and organs. Premises and equipment of the laboratory (devices, utensils, tools, materials). Basic safety regulations. Methods and techniques of sterilisation of dishes, instruments; maintaining sterile conditions in the room, working in a laminar flow box.

Topic 2. General characteristics of culture media. Macro- and microelements, carbon nutrition, additional organic compounds. The role of vitamins, phytohormones and growth regulators as components of nutrient media.

Section 2. Callus culture and its characteristics

Topic 1: Features of primary callusogenesis. Stages of in vitro introduction of higher plants. Ranking of systematic groups of plants, anatomical structures and tissues by their ability to form callus. Sterilisation of plant material - seeds, leaves, apical meristems, etc. Selection and isolation of explants. Concepts of competence, differentiation, de- and redifferentiation. Obtaining primary callus from different explants and aseptic plants. The phenomenon of physiological polarity. The role of the main classes of phytohormones - auxins and cytokinins in the processes of induction of callusogenesis. The role of the source plant genotype. Genetic and epigenetic control of callusogenesis. The main factors of dedifferentiation and callus formation (composition of the culture medium, chemical and physical conditions of cultivation, etc.)

Topic 2. Mature callus culture. Types of callus cultures and their morphological, cytological, physiological, biochemical and genetic characteristics. Classification of callus types. Callus heterogeneity is the main characteristic of callus tissue. Somaclonal variability in callus culture. Growth curve - characteristics of individual phases. Variability of growth rates and its possible causes. Mitotic rhythm as an indicator of the physiological state of callus cell populations. Dynamics and indicators of growth of callus cultures -

growth index (GI), subcultivation (the role of the ratio of phytohormones). Influence of cultivation conditions: duration of passage, composition of the culture medium, lighting conditions, temperature, etc. Callus culture is the main in vitro culture of higher plants. Relationship with other types of in vitro cultures of higher plants: suspension, single cells, isolated protoplasts, haploid cells, etc.

Topic 3. Morphogenetic reactions of callus cultures in vitro. Basic concepts: morphogenesis, cytogenesis, histogenesis, organogenesis, floral morphogenesis in vitro, etc. Induction of various pathways of morphogenesis and their interrelation. Direct and indirect pathways of morphogenesis. Somatic embryoidogenesis. Factors affecting differentiation in cell culture. Phytohormonal regulation of the direction of morphogenesis in vitro in higher plants. The classical Skoog-Miller rule. The main points of obtaining regenerating plants in vitro.

Chapter 3. Modern plant biotechnology

Topic 1: Biotechnologies based on the culture of cells, tissues and organs of higher plants. Use of callus tissues in basic research and biotechnology. Microclonal reproduction and production of virus-free plant material. Recovery of planting material by methods of chemotherapy and thermotherapy. Methods for obtaining cell cultures that produce valuable biologically active substances. In vitro cultures in plant breeding and genetic engineering. Use of plant cell cultures to preserve the gene pool of higher plants. Cryopreservation of cell cultures and meristems.

2.3 Topics of laboratory classes

Section 1. Basic principles of work in the laboratory of *in vitro* culture of higher plants

Laboratory work № 1. Introduction to the organisation of work and equipment in a biotechnology laboratory.

Laboratory work № 2. Preparation of the biotechnological laboratory for work.

Laboratory work № 3. Work in a laminar flow box.

Laboratory work № 4. Preparation of mother liquors of macro- and microsalts, vitamins and phytohormones.

Laboratory work № 5. Preparation of Murashige and Skoog basal medium (MS).

Laboratory work № 6. Sterilisation of instruments, equipment and materials. Sterilisation of plant material. Growing aseptic seedlings.

Section 2. Callus culture and its characteristics.

Primary callusogenesis

Laboratory work № 7. Obtaining primary callus from aseptic seedlings.

Laboratory work № 8. Obtaining primary callus from plant seeds.

Mature callus culture and its characteristics

Laboratory work № 9. Morphological characteristics of callus culture.

Laboratory work № 10. Cytological analysis of callus tissue.

Laboratory work № 11. Induction of various pathways of morphogenesis in vitro under the influence of phytohormones.

Section 3. Modern plant biotechnology

Laboratory work № 12. Clonal micropropagation of *Saintpaulia ionantha*.

2.4. Topics for the seminar sessions

Seminar session № 1. Features of the plant organism as an object of modern phytobiotechnology.

Seminar session № 2. Phytohormones - the main factors in the regulation of plant morphogenesis *in vitro*.

Seminar session №. 3. Genetic and epigenetic control of morphogenesis.

Seminar session № 4. Modern plant biotechnology.

2.5. Recommended literature and Internet resources for the course

1. Principles of plant biotechnology – [Principles-of-Plant-Biotechnology.pdf]

2. Chawla, H.S. Introduction to plant biotechnology.--3nd ed. 2009, 745 pp. Introduction to Plant Biotechnology PDF

3. Plant Biotechnology, Volume 1. Principles, Techniques, and Applications, 1st Edition. Edited by Bishun Deo Prasad, Sangita Sahni, Prasant Kumar, Mohammed Wasim Siddiqui. Apple Academic Press. 562 p. PLANT BIOTECHNOLOGY: Principles, Techniques, and Applications PDF

Internet resources

http://www.ncbi.nih.gov – (Center for Biotechnology Information – NCBI)

websites of scientific journals:

1. http://www.plantphysiol.org/
2. http://www.annualreviews.org/loi/arplant
3. https://www.crops.org/publications
4. https://journal.unisza.edu.my/agrobiotechnology
5. http://biotech.nature.com
6. https://link.springer.com/journal/11240

Videos - films (must watch)

1. Plant tissue culture overview
https://www.youtube.com/watch?v=dFrx-t5J0PA
2. Plant Biotech Lab Tour
https://www.youtube.com/watch?v=HHYDmfj4ojk
3. Plants BIOTECHNOLOGY.mp4 - YouTube
https://www.youtube.com/watch?v=bKe3YivIqJc
4. How to prepare MS medium for plant tissue culture
https://www.youtube.com/watch?v=eMv_PMNPYMc
5. Ep3: Plant tissue culture (Stages in plant tissue culture)
https://www.youtube.com/watch?v=AlbnKShDnBA
6. Rice callus induction protocol
https://www.youtube.com/watch?v=8F_dj1CUGoM
7. Tissue Culture
https://www.youtube.com/watch?v=xuwV3ywCxW8
8. Plant Tissue Culture - Suspension Culture
https://www.youtube.com/watch?v=D3fYFh41MM0
9. Plant cell culture Suspension culture 1 Initiation
https://www.youtube.com/watch?v=30gl73MWQ-g
10. Laminar air flow hood (LAF Hood): Design, Principle, Working
https://www.youtube.com/watch?v=43y3XGMOYEI
11. Organogenesis in plant tissue culture | Types: Direct and indirect organogenesis | Differences
https://www.youtube.com/watch?v=0Oy9ijxf5us

2.6. Control questions for the course 'OBTAINING AND USING CALLUS CULTURES'

1. Cell culture of higher plants is a unique model biological system.
2. Features of the plant organism as an object of modern plant biotechnology.
3. Totipotency is a unique property of a plant cell.
4. History and formation of in vitro culture methods of higher plants: main stages and achievements.
5. Contribution of Ukrainian scientists to the development of world plant biotechnology.
6. Fundamental and practical directions of using in vitro culture methods of higher plants.
7. Basic principles and methods of organising the work of the plant biotechnology laboratory.
8. Premises and equipment of the laboratory (dishes, tools, materials; maintaining sterile conditions).
9. General characteristics of nutrient media.
10. Macro- and microelements, carbon nutrition, additional organic compounds.
11. The role of vitamins, phytohormones and growth regulators as components of nutrient media.
12. Methods of sterilisation in plant biotechnology.
13. Sterilisation of plant material - seeds, leaves, apical meristems, etc. Growing aseptic seedlings in vitro.
14. Dedifferentiation of tissues of higher plants and obtaining primary callus.
15. The phenomenon of physiological polarity.
16. The concepts of competence, differentiation, de- and redifferentiation.
17. The role of the main classes of phytohormones - auxins and cytokinins in the processes of induction of callusogenesis.
18. The role of the source plant genotype and genetic control of callusogenesis.
19. The main factors of dedifferentiation and callus formation (composition of the culture medium, chemical and physical conditions of cultivation, etc.)
20. The main stages of obtaining callus culture.
21. Primary and mature callus culture.
22. Morphological and cytological characteristics of callus.

23. Physiological and biochemical characteristics of callus culture.

24. Genetic and epigenetic characteristics of callus.

25. Dynamics and indicators of growth of callus cultures, passage, subcultivation (role of phytohormones ratio).

26. Growth curve of callus culture: characteristics of individual phases.

27. Heterogeneity of callus - the main characteristic of callus tissue.

28. Somaclonal variability in callus culture.

29. Mitotic rhythm as an indicator of the physiological state of the population of callus cells.

30. Influence of cultivation conditions: duration of passage, composition of the culture medium, lighting conditions, temperature, etc.

31. Callus culture is the main in vitro culture of higher plants. Relationship with other types of in vitro cultures of higher plants: suspension, single cells, isolated protoplasts, haploid cells, etc.

32. Basic concepts: morphogenesis, cytogenesis, histogenesis, organogenesis, floral morphogenesis in vitro, etc.

33. Induction of various pathways of morphogenesis and their interrelation.

34. Direct and indirect pathways of morphogenesis in vitro.

35. Somatic embryoidogenesis.

36. Factors affecting differentiation in cell culture.

37. Phytohormonal regulation of the direction of morphogenesis in vitro of higher plants.

38. The classical rule of Skoog-Miller.

39. The main aspects of obtaining regenerative plants in vitro.

40. Culture of plant cells, tissues and organs as the basis of modern biotechnology.

41. Microclonal propagation of plants.

42. Obtaining healthy (virus-free) planting material.

43. Culture of plant cells, tissues and organs and genetic engineering of plants.

44. Cultures of plant cells - producers of valuable biologically active substances - substances of secondary metabolism.

45. Cryopreservation of plant cell, tissue and organ cultures.

SECTION 3. Protocols of laboratory classes

Student (full name)

_____

Course of study

_____

Work 1: Acquaintance with the arrangement and organisation of work in the biotechnological laboratory

The aim: to get acquainted with the organisation of work in a biotechnology laboratory, to learn the modes of operation of devices and equipment of the biotechnology laboratory, to undergo safety training.

Materials and equipment: drying cabinet, thermostat, distiller, autoclave, laminar flow box, etc.

Protocol

1. Familiarise yourself with the equipment of the biotechnology laboratory.

2. Take a safety briefing on working in a biotechnology laboratory.

Questions for theoretical preparation:

1. What is meant by 'methods of in vitro culture of plant cells, tissues and organs'?

2. What are the areas of application of in vitro methods?

Answers to the questions:

_____

_____

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Task: fill in the safety table (see Annex A)

Table 1.

Basic safety rules in the plant biotechnology laboratory

Sources of risk and specifics of work	**Safety rules that must be followed**
Electronic devices	
Fire danger (open flames)	
Chemical reagents	
UV irradiation	
Working with laboratory glassware	
Biological contamination	
Rules of conduct in the laboratory	

Work 2. Preparing the biotechnology laboratory for work

Materials and equipment: drying cabinet, thermostat, distiller, autoclave, laminar flow box, alcohol flask, sterilised instruments and utensils, alcohol.

The Biotechnology Laboratory is a specialised block of premises equipped in accordance with the work performed at each stage of the introduction and cultivation of plant explants *in vitro*.

Protocol

1. Familiarise yourself with the premises and equipment of the biotechnology laboratory.
2. Learn the method of sterilisation of premises, utensils, instruments, culture media.
3. Identify the basic rules of work in aseptic conditions.

Task: complete the table.

Table2.

Structure and equipment of the biotechnological laboratory

Facilities	Equipment
Room for washing up	
Sterilisation room (autoclave)	
Room for preparation of culture medium	
Sterile box	
Cultural room	
Climate control chambers	

Laboratory facilities	

Work 3. Working in a laminar box

Aim: to master the skills of working in a laminar flow box (pouring the culture medium into Petri dishes, isolating explants),

Materials and equipment: laminar box, alcohol flask, sterilised instruments and utensils, alcohol.

Protocol

1. Prepare the laminar box for operation.
2. Carry out the work in the laminar box in accordance with the objectives of the study
3. Clean up the laminar box after the work is completed

Write down the procedure and types of work in the laminar flow box in the plant biotechnology laboratory.

Work 4. Preparation of mother liquors of macro- and microsalts, vitamins, hormones for Murashige and Skoog (MS) medium

The aim: to prepare mother liquors of macro- and microsalts, vitamins, phytohormones, Fe-chelate.

Materials and equipment: reagents: NH_4NO_3, KNO_3, $CaCl_2$, $MgSO_4 \times 7H_2O$, KH_2PO_4, H_3BO_3, $MnSO_4 \times 5H_2O$, KI, $ZnSO_4 \times 7H_2O$, $Na_2MoO_4 \times 2H_2O$, $CuSO_4 \times 5H_2O$, $CoCl_2 \times 6H_2O$, $FeSO_4 \times 7H_2O$, $Na_2EDTA \times 2H_2O$, alcohol, 1n HCl and 1n KOH, equipment: scales, magnetic stirrer, spatulas, laboratory glassware - 1 litre beaker or flask, 500 ml and 100 ml measuring cylinders, 5 ml and 1 ml pipettes or pipette, dark glassware - 1 litre, 100 ml, 50 ml, 25 ml.

Protocol

Write a scheme for the preparation of macro- and microsalts, vitamins, phytohormones and Fe-chelate.

_____
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_____
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Work 5. Preparation of Murashige and Skoog (MS) medium

The aim: to prepare MS medium and sterilise it by autoclaving.

Materials and equipment: mother liquor of macro- and microsalts, Fe-chelate and vitamins, meso-inositol, sucrose, agar-agar, 1 n HCl and 1 n KOH, scales, spatulas, magnetic stirrer, pH meter, autoclave, 1 l beaker or flask, 500 ml and 100 ml measuring cylinders, 5 ml and 1 ml measuring pipettes or pipette, cotton plugs or foil.

Protocol

Write a scheme (stages) of preparation of hormone-free MS medium for induction of callusogenesis and microclonal propagation.

Answer the following questions:

1. What are the main requirements for the composition of culture media for in vitro plant cultures?

2. How do solid and liquid media differ in composition? For what purposes are they used?

Work 6. Sterilization of premises, equipment, instruments and materials

The aim: to get acquainted and master the methods of sterilization used in the biotechnological laboratory; to sterilize dishes, materials, tools, nutrient medium, water.

Materials and equipment: Petri dishes, flasks with distilled water, racks with test tubes filled with culture medium, dissecting needles, tweezers, scalpels, filter and parchment paper.

Protocol

Answer the questions:

1. What is the method of sterilization of metal instruments?

2. What is the mode of sterilization (autoclaving) of culture media used for the cultivation of plant cells and tissues?

_____
_____
_____
_____
_____
_____
_____
_____
_____

Work 6 a. Sterilization of plant material. Growing aseptic seedlings (continued)

The aim: to choose the concentration of the sterilizing solution and the time of seed sterilization that will ensure the highest efficiency of this process. To sterilize seeds and grow aseptic seedlings from them.

Materials and equipment: 70 % ethyl alcohol solution, sterile water, commercial solution of the drug 'whiteness' (concentration 15-30 %), chemical glass for sterilizing solutions, seeds of soybeans, wheat and other crops, tubes with sterile hormone-free MS medium, instruments, laminar flow box.

Protocol

Write a scheme (stages) of sterilization of plant material for the selected objects.

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

Based on the results of your work, fill in the table.

Object	Dilution of the sterilizing solution	Sterilization time, min	Number of seeds	Number of inf. seeds		Seed germination		Seed sterilization efficiency	
				pcs.	%	pcs.	%	pcs.	%
	1								
	1:1								
	1:2								
	1								
	1:1								
	1:2								

Determine the most effective conditions for sterilizing seeds of different crops. Draw conclusions based on the results.

_____
_____
_____
_____
_____

Answer the following questions:

1. What sterilization methods are used in a biotechnology laboratory?
2. What is sterilization and what are the types of sterilization?

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

Callus culture. *Primary callusogenesis*

Work 7. Obtaining primary callus from aseptic seedlings

The aim: to learn the technique of isolating explants from aseptic seedlings and obtaining primary callus tissue.

Materials and equipment: aseptic seedlings, Petri dishes with sterile medium for induction of primary callusogenesis (with the addition of 2,4-D), instruments (tweezers, dissecting needles, blades), laminar flow box.

Define the following terms

callus -

_____
_____
_____

passage (subcultivation) -

_____
_____
_____

Protocol

Draw a scheme of the experiment

Record your results in the table.

Object, type of explant	Number of explants, pcs.	The frequency of callusogenesis	
		pcs.	%

Draw conclusions based on the results of the experiment.

_____
_____
_____
_____

Answer the following questions

What plant organs can be used as explants for obtaining callus culture?

_____
_____
_____
_____

What is the difference between primary callus and mature callus culture?

_____
_____
_____
_____

Work 8. Obtaining of primary callus from plant seeds

The aim: to learn the methods of sterilisation of seeds of various crops, to obtain primary callus, to evaluate the effectiveness of sterilisation and the frequency of callusogenesis.

Materials and equipment: laminar flow box, petri dishes with nutrient medium for induction of callusogenesis MS + 2,4-D (10 mg/l), seeds of various plant representatives, tools: tweezers, scalpels, cotton wool, bandage, sterilizing solution, sterile distilled water, alcohol flasks, 70% alcohol.

Define the terms:

totipotency-

_____
_____
_____

dedifferentiation-

_____
_____
_____

Protocol

Draw a scheme of the experiment.

Record your results in the table.

Object	Number of seeds, pcs.	Number of aseptic seeds		Number of seeds that form a callus	
		pcs.	%	pcs.	%

Draw conclusions based on the results of the experiment.

_____
_____
_____

Answer the question:

What culture media are used to induce callusogenesis and subculture calluses?

_____
_____
_____
_____

What are the criteria for classifying callus tissues?

_____
_____
_____
_____

Mature callus culture and its characteristics

Work 9. Morphological characteristics and determination of callus water content

The aim: to learn and master the methods of morphological analysis of callus cultures of different representatives of plant objects and types of calluses, to learn methods for determining the degree of water content of callus cultures of different representatives of plant objects, to make appropriate conclusions and photographs.

Materials and equipment: cups with callus cultures of different representatives of plant objects (soybeans, wheat, tomatoes, mustard, etc.), calluses of different ages, origin, etc., a drying cabinet, tools: tweezers, dissecting needles, 96% alcohol, a laminar flow box, a rubbing alcohol.

Define the terms:

growth cycle -

_____
_____
_____

auxins -

_____

_____

_____

Protocol

Draw a scheme of the experiment.

According to the results of the measurement, determine the degree of water content of the callus culture using the formula:

$$WC = (M_{wet} - M_{dry} / M_{wet}) \times 100\ \%,$$ where

WC – the water content of the callus.

M_{wet} – the wet weight of callus.

M_{dry} – the dry weight of the callus.

Record the results in the table.

Object	Morphological characteristic				
	colour	structure	size	density	water content

Draw conclusions based on the results of the experiment.

_____

_____

_____

_____

_____

Answer the following questions:

What is the physiological and genetic heterogeneity of calluses?

_____
_____
_____
_____
_____
_____

Work 10: Cytological analysis of callus

The aim: to learn and master the methods of cytological analysis of callus cultures of different representatives of plant objects, to make appropriate conclusions and photographs.

Materials and equipment: dishes Petri with callus cultures of different representatives of plant objects, sterile Petri dishes, chromic acid, 96% alcohol, tweezers, dissecting needles, laminar flow box, shaker (rolling pin), water bath, alcohol, cytometer, microscope, scales, paper.

Define the terms:

cytokinins -

_____
_____
_____

transplant -

_____
_____
_____

proliferation -

_____
_____
_____

Protocol

Draw a diagram of the experiment.

Make the appropriate calculations using the formula

$$N = \frac{n \times 10^3 \times m}{0{,}2 \times d}$$, where

N - cell number in one gram;
n - the average number of cells in one large square;
m - volume of cell suspension in chromic acid, ml;
d - tissue weight, g;
0.2 - depth of the hemocytometer.

Draw conclusions based on the results of the experiment.

_____
_____
_____
_____

Answer the question:
What phases are distinguished in the 'growth curve' of a callus culture?

_____
_____
_____
_____
_____
_____

How often and why is it necessary to passivate callus cultures?

_____
_____
_____
_____
_____

Work 11. Induction of different pathways of morphogenesis in vitro of callus tissues under the influence of phytohormones

The aim: to identify phytohormonal regulation of the direction of morphogenesis in vitro.

Materials and equipment: mature callus cultures, flasks with MS media: for stem organogenesis (haemogenesis), somatic embryogenesis and induction of rhizogenesis, 96 % alcohol, sterile tweezers and dissecting needles, alcohol.

Define the terms.

Morphogenesis -

_____

_____

Rhizogenesis

_____

_____

haemogenesis -

_____

_____

somatic embryogenesis -

_____

_____

Protocol

Draw a diagram of the experiment.

Record the results in the table.

Object	PG composition of MS	Total number of explants	Morphogenesis pathways					
			haemogenesis		rhizogenesis		callusogenesis	
			pcs	%	pcs	%	pcs	%

Draw conclusions according to the results of the experiment.

_____
_____
_____
_____

Answer the question.

Draw a scheme of the morphogenesis pathways in vitro of a totipotent plant cell.

Formulate the classic Skoog–Miller rule:

_____
_____
_____
_____
_____

What is the difference between direct and indirect ways of morphogenesis?

_____
_____
_____
_____
_____
_____

Work 12: Clonal micropropagation of *Saintpaulia ionantha*

The aim: to master the method of clonal micropropagation of plants based on direct morphogenesis.

Materials and equipment: Saintpaulia leaves, sterile MS medium with half salt composition, sterile Petri dishes, instruments, laminar flow box, sterile water, ethanol, linen.

Define the terms.

clone -

_____

_____

_____

mericlon -

_____

_____

_____

somaclonal variability -

_____

_____

_____

pre-adaptation -

_____

_____

_____

Protocol

Draw a scheme of the experiment.

Write down the results in the table.

Object	P/h composition of MS	Total number of explants	Formation of meryclones				
			sterility		Direct morphogenesis		
			pcs.	%	explants with mericlones	%	piece/ explant

Draw conclusions based on the results of the experiment.

_____
_____
_____
_____

Answer the following questions:

What is called clonal micropropagation of plants?

_____
_____
_____
_____

What are the methods of clonal micropropagation of plants?

_____
_____
_____
_____
_____
_____

What is the advantage of the clonal micropropagation method compared to traditional methods of plant propagation?

_____
_____
_____
_____
_____
_____

SECTION 4. Individual work of students

4.1 Topics of essays (abstracts)

1. Culture of plant cells, tissues and organs as the basis of modern biotechnology.
2. Microclonal propagation of plants - the main stages and methods.
3. Features of microclonal propagation of woody plants.
4. Features of microclonal propagation of ornamental plants.
5. Plant organism as an object of modern biotechnology.
6. Obtaining healthy (virus-free) planting material.
7. Culture of plant cells, tissues and organs in plant breeding.
8. Culture of plant cells, tissues and organs and genetic engineering of plants.
9. Cultures of plant cells - producers of valuable biologically active substances - substances of secondary metabolism.
10. Cryopreservation of plant cell cultures, tissues, organs.
11. Genetic banks of in vitro cultures.
12. Use of in vitro cultures for biodiversity conservation.
13. History of the culture of plant cells, tissues and organs.
14. Cell culture of higher plants - a unique model system.
15. Features of the plant cell as an object for the creation of cell, tissue and organ culture.

4.2. Guidelines for writing abstracts (essays)

Abstracting is the process of processing and written presentation of a text, which results in the preparation of a secondary document - an abstract. The purpose of the abstract is to convey the content in the shortest, most concise form, highlighting particularly important or new information contained in the material under review.

Firstly, the student chooses one of the proposed topics for the essays in the course “Obtaining and using callus cultures” (see 4.1). Then he/she studies the scientific literature on this issue, searches for literature sources - textbooks, manuals, monographs, scientific articles in periodicals publications (journals ‘Plant Physiology’, ‘Plant Biotechnology Reports’, ‘Plant Cell’, ‘Plant Biotechnology Journal’, ‘Plant Cell, Tissue and Organ Culture’, etc.)

Having studied a sufficient number of literary sources and familiarised themselves with the basic information on the topic, the student must draw up a thorough plan of the abstract.

The structure of the essay should be as follows:

- title page;
- introduction
- table of contents or outline;
- main body (sections and subsections);
- conclusions;
- list of references.

The title page is drawn up in accordance with the rules of the university.

The page number is not indicated on the title page.

The table of contents or outline contains a list of sections and subsections (paragraphs and subparagraphs) and their page numbers.

The introduction can be from one paragraph to a page. The main purpose of the introduction is to introduce the essence of the problem, to justify the choice of the topic, its relevance and importance.

The main part is a statement of the main concepts, provisions, and aspects of the topic of the essay that are available in the literature. Before writing the main part, you should clearly define the titles of sections and subsections and build a logical chain of presentation of the material. When presenting the material, it is mandatory to make references to the authors and literary sources used in the work (indicated in square brackets as the number of the source from the list of references).

Conclusions summarise the topic of the essay in a concise form, and present the author's view of the problem and ways to solve it.

List of references is submitted in accordance with the rules of bibliographic description.

During the review of the essay, the following is assessed:

- knowledge of the factual material, mastery of general concepts, ideas, representations, etc;
- realisation of the purpose and objectives of the work;
- the degree of validity of generalisations and conclusions;
- use of literary sources;
- culture of written presentation of the material;
- culture of work design.

4.3. Methodological recommendations for preparing report presentations

Making presentations allows you to logically organise the material, systematise it, present it for defence, gain experience of speaking in front of an audience, and develops students' communication competences.

The number of slides corresponds to the content and duration of the presentation (for example, for a 5-minute presentation, it is recommended to use no more than 10 slides).

The first slide must contain the topic of the presentation and information about the authors. The following slides can be prepared using two different strategies:

1st strategy: the slides should contain a basic outline of the speech and keywords to be used as a plan for the speech. In this case, the slides should meet the following requirements:

- the amount of text on a slide should not exceed 7 lines;
- a bulleted/numbered list contains no more than 7 items;
- no punctuation at the end of lines in bulleted and numbered lists;
- significant information is highlighted with the help of colour, bolding, animation effects.

Be especially careful to check for grammatical and typographical errors in the text. The main mistake when choosing this strategy is that speakers replace their speech with reading the text from the slides.

2nd strategy: the slides contain factual material (tables, graphs, photographs, etc.) that is appropriate and sufficient to help reveal the main idea of the speech. In this case, the slides should meet the following requirements

- the chosen means of visualising information (tables, diagrams, graphs, etc.) correspond to the content;
- illustrations of good quality (high resolution), with a clear image (usually, no one present is interested in reading the text on your slides and peering at small illustrations);

Requirements for presentation design (Annex B)

It is recommended to use simple templates without animation to design presentation slides, and to adhere to a single style for all slides. It is not recommended to use more than 3 colours on one slide: one for the background, one for the headings, one for the text. You can change slides with a single swipe.

The font chosen for the presentation should provide expressiveness on the screen and be within the size range of 18-72 pt, which ensures the presentability of the information presented. The font on the presentation slides should match the selected design templates. Do not use different fonts in the same presentation. When copying text from Word to a slide, it should be pasted into the text frame on the slide.

The maximum amount of graphic information on one slide is 2 pictures (photographs, diagrams, etc.) with text comments (no more than 2 lines for each). The most important information should be placed in the centre of the screen.

A typical slide without animation effects should be displayed on the screen for at least 10-15 seconds. You should be especially careful about the presentation design. If possible, you should use the same design template for all slides of your presentation, with a font size of at least 24 points for headings and at least 18 points for information.

For better orientation in the presentation during the speech, you should number the slides. It is advisable to leave margins of at least 1 cm on each side of the slides.

After preparing a presentation, it is useful to check yourself with the following questions

- Did you achieve the ultimate aim of the presentation (what did you manage to define, explain, propose or demonstrate)?

- What features of the subject matter of the presentation managed to attract the audience's attention?

- Does the presentation distract from the oral presentation?

After preparing a presentation, you need to rehearse your speech.

4.4. Recommendations for seminars

A seminar is one of the main forms of organising the educational process, which is a collective discussion of theoretical issues by students under the guidance of a teacher. Seminars within specific sections of academic disciplines are intended primarily for in-depth study of theoretical material. Seminars develop students' skills of free discussion, primary research skills, and stimulate interest in independent search for new ideas and facts.

Seminars are based on a system of reports (messages) prepared by students on a pre-selected topic. When preparing a report (message, presentation) for a seminar, the goal is to conduct a comparative analysis of problem situations, as

well as the methods and specifics of solving these problems, where the main task for the student is to analyse them independently.

The seminar involves not only the presentation of students with reports prepared in advance, but also a detailed discussion after each of the presentations. That is why participation in a seminar is also participation in such a conversation, which involves asking questions related to the topic of the report, formulating answers to them, and polemics with both the authors of the reports and other students participating in the seminar. That is why preparing for a seminar class should include not only preparing your own speech, but also familiarising yourself with each of the issues proposed for discussion at the seminar. Questions to the speaker are primarily asked by students, not the teacher. It is necessary to require that the questions posed by students are profound, related to the topic, clearly and precisely formulated, relevant, and arousing a keen interest.

The procedure for conducting a seminar class involves the following sequence:

- introductory remarks by the lecturer (discussing the topic of the seminar, the main problems, the procedure);
- presentations by students;
- questions to the speakers and their answers;
- extended conversation - discussion of the problem;
- conclusion of the lecturer (general assessment of the class, brief analysis of the reports, information on unresolved issues, tasks for the next classes).

Seminar session 1.
Features of the plant organism as an object of modern phytobiotechnology

1.1. Totipotency is a unique property of plant cells.

1.2. Plant stem cells - features, comparison with animal cells.

1.3. Plant meristems. Processes of differentiation, dedifferentiation, redifferentiation.

1.4. History of the development of in vitro culture methods in plant research.

Recommended literature

1. Aggarwal S., Sardana C., Ozturk M. & Sarwat M. Plant stem cells and their applications: special emphasis on their marketed products. *Biotech*, (2020), 10(7), 291. https://doi.org/10.1007/s13205-020-02247-9

2. Hong L, Fletcher JC. Stem Cells: Engines of Plant Growth and Development. International *Journal of Molecular Sciences*. 2023; 24(19):14889. https://doi.org/10.3390/ijms241914889
3. Jin J., Lu P., Xu Y. et al. PCMDB: a curated and comprehensive resource of plant cell markers, *Nucleic Acids Research*. 2022, Vol. 50, Issue D1, P. D1448–D1455, https://doi.org/10.1093/nar/gkab949
4. Pierre-Jerome E., Drapek C. & Benfey P. N. Regulation of Division and Differentiation of Plant Stem Cells. *Annual review of cell and developmental biology*. 2018, 34, 289–310. https://doi.org/10.1146/annurev-cellbio-100617-062459
5. Satterlee J. W., Strable J. and Scanlon M. J. Plant stem-cell organization and differentiation at single-cell resolution, *PNAS*, 2020 117 (52) 33689 - 33699 https://doi.org/10.1073/pnas.2018788117
6. Stahl Y., Simon R. Plant primary meristems: shared functions and regulatory mechanisms. *Curr Opin Plant Biol*. 2010, 13(1):53-8. doi: 10.1016/j.pbi.2009.09.008.

Internet platform:
Pubmed, ScienceDirect, Google Scholar, ResearchGate

Seminar session 2.
Phytohormones - the main factors of plant morphogenesis regulation *in vitro*

2.1. Auxins are the main growth-stimulating phytohormones. Morphophysiological processes regulating auxins in vivo and in vitro.

2.2 Cytokinins. Morphophysiological processes that regulate in vivo and in vitro.

2.3. Phytohormonal system of plants. Receptors, synergistic and antagonistic actions.

2.4. Other classes of phytohormones - general characteristics.

2.5. Synthetic phytohormones and substances with hormonal action - regulators of morphogenesis in vitro - use in the practice of plant biotechnology.

Recommended literature

1. Aoki M., Kisiala Anna B , Rahman T. , Morrison E. N. , Neil Emery R.J. Cytokinins are pervasive among common in vitro culture media: An analysis of their forms, concentrations and potential sources. *Journal of Biotechnology.* 2021.Vol.334, P. 43-46. DOI: 10.1016/j.jbiotec.2021.05.005
2. Gao J., Gao J., Zhuang S., Zhang W. Advances in Plant Auxin Biology: Synthesis, Metabolism, Signaling, Interaction with Other Hormones and Roles under Abiotic Stress. *Plants.* 13(17):2523. DOI: 10.3390/plants13172523

https://doi.org/10.1016/j.aac.2023.07.008

3. Kieber J. J., & Schaller, G. E. Cytokinins. *The arabidopsis book.* 2014. 12, e0168. https://doi.org/10.1199/tab.0168

4. Liu Y., Zhang M., Meng Z., Wang B. & Chen M. Research Progress on the Roles of Cytokinin in Plant Response to Stress. *International journal of molecular sciences*. 2020. 21(18), 6574. https://doi.org/10.3390/ijms21186574
5. Mroue S., Simeunovic A., Robert H. S. Auxin production as an integrator of environmental cues for developmental growth regulation. *Journal of Experimental Botany*. 2018. Vol. 69, Iss. 2, P. 201–212. https://doi.org/10.1093/jxb/erx259
6. Phillips G. C., & Garda M. Plant tissue culture media and practices: an overview. *In Vitro Cellular & Developmental Biology Plant.* 2019. 55(3), 242–257. https://doi.org/10.1007/s11627-019-09983-5
7. Yan H, Yang Z., Chen S., Wu J. Exploration and development of artificially synthesized plant growth regulators. *Advanced Agrochem*. 2024. Vol. 3, Iss. 1. 47-56

Internet platform:
Pubmed, ScienceDirect, Google Scholar, ResearchGate

Seminar session 3.
Genetic and epigenetic control of morphogenesis

3.1. Epigenetic control of development in a plant organism - modification of DNA, histones, microRNAs.

3.2. Genes and transcription factors - regulators of plant development

3.3 Genetic control of morphogenesis in vitro.

3.4. Epigenetic control of morphogenesis in vitro.

Recommended literature

1. Fehér A. Callus, dedifferentiation, totipotency, somatic embryogenesis: what these terms mean in the era of molecular plant biology? *Front. Plant Sci*. Vol. 10. https://doi.org/10.3389/fpls.2019.00536
2. Hemenway E. A., & Gehring M. Epigenetic Regulation During Plant Development and the Capacity for Epigenetic Memory. *Annual review of plant biology*. 2023. 74, 87–109. https://doi.org/10.1146/annurev-arplant-070122-025047
3. Lee K., Seo P. J. Dynamic Epigenetic Changes during Plant Regeneration. *Trends Plant Sci*, 2018. 23 (3) P. 235–247. DOI: 10.1016/j.tplants.2017.11.009
4. Liu L., White M. J., MacRae T. H. Transcription factors and their genes in higher plants. Functional domains, evolution and regulation. *European Journal of Biochemistry*. 1999. Vol.262, Is.2. P. 247-257. https://doi.org/10.1046/j.1432-1327.1999.00349.x
5. Long Y., Yang Y., Pan G., & Shen, Y. New Insights Into Tissue Culture Plant-Regeneration Mechanisms. *Frontiers in plant science*, 2022, 13, 926752. https://doi.org/10.3389/fpls.2022.926752
6. Momoko Ikeuchi, Keiko Sugimoto, and Akira Iwase Plant Callus: Mechanisms of Induction and Repression. *The Plant Cell*. 2013. Vol. 25. P. 3159–3173. https://doi.org/10.1105/tpc.113.116053
7. Strader L., Weijers D., Wagner D. Plant transcription factors — being in the right place with the right company. *Current Opinion in Plant Biology*. 2022. Vol. 65. 102136. https://doi.org/10.1016/j.pbi.2021.102136

8. UsCamas R., Rivera-Solís G. Duarte-Aké F., De-la-Peña C. In vitro culture: an epigenetic challenge for plants. *Plant Cell Tiss Organ Cult*. 2014. 16 p. DOI 10.1007/s11240-014-0482-8
9. Zou X., Sun H. DOF transcription factors: Specific regulators of plant biological processes. *Front. Plant Sci.* 2023. Vol.14. https://doi.org/10.3389/fpls.2023.1044918

Internet platform:

Pubmed, ScienceDirect, Google Scholar, ResearchGate

Seminar session 4.

Modern plant biotechnology

4.1 Microclonal plant propagation.

4.2 Production of 'artificial seeds'.

4.3 Methods of obtaining healthy (virus-free) plant material

4.4 Methods of obtaining cell cultures - producers of valuable biologically active substances.

4.5 Preservation (cryopreservation) of the gene pool - gencollections of cultures *in vitro*.

4.6 3D bioprinting of plant cells

Recommended literature

1. Bapat V.A., Kavi Kishor P.B., Jalaja N., Jain S.M., Penna S. Plant Cell Cultures: Biofactories for the Production of Bioactive Compounds. *Agronomy*. 2023, 13, 858. https://doi.org/10.3390/agronomy13030858
2. Hasnain A., Naqvi S., Ayesha et al. Plants in vitro propagation with its applications in food, pharmaceuticals and cosmetic industries; current scenario and future approaches. *Frontiers in plant science* 2022. 13, 1009395. https://doi.org/10.3389/fpls.2022.1009395
3. Rihan H.Z., Kareem F., El-Mahrouk M.E., Fuller, M.P. Artificial Seeds (Principle, Aspects and Applications). *Agronomy* 2017, 7, 71. https://doi.org/10.3390/agronomy7040071
4. Roque-Borda C. A., Kulus D., Vacaro de Souza A., Kaviani B., & Vicente E. F. Cryopreservation of Agronomic Plant Germplasm Using Vitrification-Based Methods: An Overview of Selected Case Studies. *International journal of molecular scienc*es. 2021. 22(11), 6157. https://doi.org/10.3390/ijms22116157
5. Wang M. R., Cui Z. H., Li J. W., Hao X. Y., Zhao, L. & Wang, Q. C. In vitro thermotherapy-based methods for plant virus eradication. *Plant methods*. 2018. 14, 87. https://doi.org/10.1186/s13007-018-0355-y

Internet platform:

Pubmed, ScienceDirect, Google Scholar, ResearchGate

4.5. Tasks for self-control

Group A tests (one correct answer)

1. The optimal pH of the culture medium for plant cells is

А) 5.0-5.5

Б) 6.5-7.0

В) 9.0-10.0

Г) 11.0-12.0

2. The most versatile and widespread culture medium:

A) Murashige-Skoog (MS)

B) Schenck-Hildenbrant medium (SH)

C) Gamborg medium (B5)

D) White's medium

3. The nutrient medium for induction of callusogenesis must contain:

A) cytokinins

B) auxins

D) gibberellins

E) hormone-free

4. For sterilisation of plant material use:

A) UV irradiation

B) autoclaving

C) chloramine

D) alcohol + burner flame

5. Aseptic seedlings are cultivated on the medium:

A) MS + 2.4 D

B) hormone-free MS

C) liquid MS

D) solid MS + 6 BAP

6. Loose, hydrated callus is used for:

A) obtaining a cell suspension

B) plant regeneration

C) obtaining isolated protoplasts

D) GM plants

7. To obtain callus tissue as an explant it is better to use

A) meristem

B) stomata

C) root cap

D) sclerenchyma

8. The cause of death of primary explants is usually the accumulation of

A) auxins

B) cytokinins

C) phenols

D) carbohydrates

9. Belong to cytokinins:

A) BAP

B) NAA

C) ABA

D) 2.4 D

10. What degree of viability is acceptable for a suspension culture:

А) 30 %

Б) 50 %

В) 70 %
Г) 90 %

11. Suspension cultures in modern biotechnology are used for:
A) obtaining secondary metabolites
B) preservation of the gene pool
C) microclonal reproduction
D) obtaining transgenic plants

12. For the industrial production of secondary metabolites, suspension cultures are used in the
A) stationary phase
B) logarithmic phase
C) lag phase
D) degradation phase

13. For the industrial production of biomass use suspension cultures in the stage of:
A) stationary phase
B) logarithmic phase
C) lag phase
D) degradation phase

14. To create a 'feeding layer' use:
a) cell suspension
b) callus tissue
c) rich nutrient medium
e) depleted medium

15. Conditioning factor can be replaced by phytohormones:
a) possible
b) impossible
c) possible with the addition of other substances
d) possible for some time

16. Protoplasts of plant cells were first isolated enzymatically:
a) Selton
б) Cocking
в) Clerk
г) White

17. Apical dominance can be overcome by adding the following to the culture medium:
a) auxins
b) abscisic acid
c) cytokinins
d) gibberellins

18. Rhizogenesis is stimulated at a ratio of cytokinins / auxins:
А) 10:1
Б) 1:1
В) 1:10
Г) 100:1

19. Microclonal reproduction is:
A) asexual reproduction
B) sexual reproduction
C) vegetative reproduction in vitro
D) apomixis

20. In the case of microclonal reproduction, the offspring are:
a) genetically homogeneous
b) genetically heterogeneous
c) haploid

d) aneuploid

21. Differentiation of germ-like structures from somatic cells is called:

A) embryogenesis
B) hematogenesis
C) hemorrhizogenesis
D) gynogenesis

22. An amorphous mass of thin-walled, parenchymal, strongly vacuolated cells, prone to constant proliferation, is called:

A) callus
B) passage
C) inoculum
C) explant

23. A regenerating plant containing cytoplasm of both parents and nucleus of one of them is called:

A) cybrid
B) hybrid
C) clone
D) transgene

24. The production of haploids from pollen or anthers is called:

A) androgenesis
B) gynogenesis
C) haemogenesis
D) callusogenesis

25. The part of a tissue or organ that is transferred to the culture medium is called:

A) explant
B) callus
C) inoculum
D) transplant

26. Loss of specialisation (structural and functional properties) by cells is called:

A) dedifferentiation
B) specialisation
C) redifferentiation
D) determination

27. Treatment of planting material by increasing temperature is called:

A) chemotherapy
B) thermotherapy
C) radiotherapy
D) transgenesis

28. Isolated protoplasts biological properties of the cell:

A) preserve
B) do not preserve
D) change
E) modify

29. The medium for cultivation of isolated protoplasts must contain:

A) phytohormones
B) carbohydrates
C) osmotic stabilisers
D) enzymes

30. Direct and indirect pathway of morphogenesis differ by:

A) the presence of the callus tissue stage
B) the possibility of formation of regenerating plants

C)obligatory stage of ebmrioidogenesis
D) obligatory stage of haemogenesis

31. Microclonal reproduction of plants is based on:
A) activation of the axillary meristems
B) activation of apical meristems
C) formation of embryos from explant tissues
D) all answers are correct

32. Activated carbon is added to the nutrient medium for:
A) absorption of phenols and other plant growth inhibitors
B) to stimulate callus formation
C) to stimulate rhizogenesis
D) to inactivate the growth of endophytic microflora

33. Antibiotics are added to the nutrient medium for:
A) absorption of phenols and other plant growth inhibitors
B) to stimulate callus formation
C) surface sterilisation of explants
D) to inactivate the growth of endophytic microflora

Group B tests (multiple choice)

34. For the successful cultivation of plants in vitro, a prerequisite is:
A) sterility
B) selective medium
C) lighting
D) access to oxygen

35. In vitro culture techniques are used to:
A) production of secondary metabolites
B) preservation of the gene pool
C) microclonal reproduction
D) obtaining transgenic plants

36. Biotechnological laboratory necessarily contains:
A) a room for storage of reagents
B) laminar flow box
C) culture (light) room
D) centrifuge room

37. Laminar flow box is:
A) a box for creating sterile conditions
B) a box for isolation of explants
C) a box for weighing reagents
D) a box for growing cultures

38. The necessary components of nutrient media are:
A) macro- and microsalts
B) agar-agar
C) phytohormones
D) sucrose

39. To the BAP (biologically active substances) in the composition of nutrient media are:

A) FG (phytohormones)

B) vitamins

C) synthetic growth regulators

D) Fe-chelate

40. Callus cells of plants from tumour cells morphologically:

a) differ

b) do not differ

c) similar

d) dissimilar

41. Plant tumour cells in culture in vitro:

a) hormone-dependent

b) hormone independent

c) hormone-sensitive

e) hormone insensitive

42. For the induction of primary callusogenesis, the medium is used:

A) hormone-free MS

B) MS + kinetin

C) MS + IAA

D) MS + 2.4 D

43. The property of totipotency of a plant cell is the basis for obtaining:

A) biologically active substances

B) regenerating plants

C) microclones

D) GM plants

44. Callus tissue is

A) heterogeneous

B) homogeneous

C) heterotrophic

D) mixotrophic

45. Dense callus with meristematic zones is used for:

A) obtaining a cell suspension

B) plant regeneration

C) obtaining isolated protoplasts

D) GM plants

46. Auxins include:

A) BAP

B) NAA

C) ABA

D) 2,4 E

47. Synthetic growth regulators include:

A) BAP

B) NAA

C) ABA

D) 2.4 D

48. What are the criteria for classifying callus tissue:

A) colour

B) density

C) morphology

D) synthetic activity

49. Suspension culture can be obtained from:

A) an intact plant

B) callus culture

C) single cell culture

D) culture of isolated protoplasts

50. Suspension cultures are characterised by:

a) high aggregation

b) formation of groups of 25-50 cells

c) formation of groups of 5-10 cells

c) single cells

51. In the physical method of protoplast fusion, the active force is:

a) polyethylene glycol

b) a constant electric field

c) alternating electric field

d) osmotic solution

52. In cibrides, both partners have cytoplasmic status:

a) equal

b) unequal

c) the same

d) unequal

53. Haploid plants:

a) fertile

b) sterile

c) non-viable

d) viable

54. In pollen culture, the appearance of digaploid plants:

a) possible

b) impossible

c) spontaneous

d) induced

55. Auxins in in vitro culture stimulate:

A) cell growth by stretching

B) cell proliferation

C) adventitious shoot formation

D) rhizogenesis

56. Cytokinins in in vitro culture stimulate:

A) cell growth by stretching

B) cell proliferation

C) hematogenesis

D) rhizogenesis

57. Adventitious buds are formed when the ratio of cytokinins/auxins:

A) 10:1

Б) 1:1

В) 1:10

Г) 100:1

58. Different ways of realisation of morphogenesis *in vitro* include

A) hemorrhizogenesis

B) thermogenesis

C) callusogenesis

D) embryoidogenesis

59. The main methods of microclonal reproduction include:

A) activation of axillary meristems

B) activation of adventitious shoot formation

C) direct embryogenesis

D) indirect embryogenesis

60. List the main stages of microclonal reproduction:

1)

2)
3)
4)

Group C tests (sequence, conformity, definition)

61. Correlate the in vitro culture (or morphogenesis) with the composition of the culture medium.

А) primary callusogenesis	1) MS + 0,5 mg/l BAP + 1 mg/l NAA
Б) microclonal reproduction	2) MS + 2–10 mg/l 2,4 D
В) aseptic seedlings	3) ½ MS + 3 mg/l BAP + 0,5 mg/l NAA
Г) rhizogenesis	4) MS

62. Establish a sequence of steps for obtaining transplantable callus culture.

А) explant removal	1)
Б) obtaining primary callus	2)
В) subcultivation	3)
Г) surface sterilization	4)

63. Match the in vitro culture and its characteristics

А) callus culture	1) microspore explant
Б) single cell culture	2) surface cultivation
В) suspension culture	3) the ‘conditioning’ factor
Г) androgenesis	4) continuous shaking

64. Match the class of biologically active substances with their representative.

А) auxins	1) BAP
Б) cytokinins	2) 2,4 D
В) vitamins	3) IAA
Г) synthetic growth regulators	4) meso-inositol

65. The process of transferring a transplant or inoculum to fresh culture medium is called _____

66. A group of dedifferentiated cells resulting from disorganised proliferation in vivo or in vitro is _____

67. The _____ property is the ability of somatic cells to fully realise the genetic potential of an entire fertile plant organism.

68. The medium for cultivation of plant cells, tissues and organs under sterile conditions, containing a combination of macro- and microelements, a source of carbohydrate nutrition, vitamins and growth regulators, can be liquid or solid - is called _____

69. A plant cell obtained by the fusion of two isolated protoplasts, the nuclear material of one of which is completely eliminated, is called _____

70. The set of genetic information of the plastid system of a plant cell is _____

71. The buds (shoots) that have arisen from plant tissues that do not normally form them are called _____

72. The process of obtaining regenerative plants from germ sacs (female plant germ cells) is _____

73-80. Define the following terms:

Gemogenesis -

_____

_____

Dedifferentiation

_____

_____

Gynogenesis

_____

_____

Embryogenesis -

_____

_____

Microclonal propagation

_____

_____

Androgenesis

_____

_____

Teratoma -

_____

_____

Isolated protoplast

_____

_____

81-85. Explain the abbreviations

NAA_____

BAP_____

2,4-D_____

IAA_____

MS_____

Open questions

86. What is meant by ‘in vitro culture methods’?

87. What are the methods of sterilisation of chemical glassware and instruments used in a biotechnology laboratory?

88. What are the basic requirements for the composition of nutrient media for in vitro plant cultures?

89. What are the indicators for analysing the growth response of callus tissue?

90. What is the difference between primary callus and mature callus culture?

91. What are the morphological characteristics of callus cultures are divided into types?

92. What is the ‘conditioning factor’?

93. What is somatic hybridization?

94. What is a hybrid? What is the difference between a symmetrical and an asymmetrical somatic hybrid?

95. What is a cybrid?

96. What are haploids used for in breeding and genetics?

97. What is the difference between direct and indirect ways of morphogenesis in vitro?

98. Formulate the rule of Skoog-Miller.

99. What is apical dominance? What phytohormones regulate this process?

100. How is the planting plant material health improvement carried out by in vitro methods?

SECTION 5. Criteria for evaluating students' knowledge

The grade "excellent" deserves a student who, during his studies, received 90-100 points in all forms of educational activity, showed systematic, deep and versatile knowledge of the material according to the discipline program from the sections:

- basic principles of organizing the work of a biotechnological laboratory;
- characteristics of callus cultures: obtaining and cultivation - primary callusogenesis;
- mature callus culture - main characteristics and growth regulation;
- modern biotechnologies based on callus cultures.

Completed all tasks planned by the program, mastered the main literature and familiarized myself with the additional literature, completed the laboratory practicum in the discipline in full and actively participated in independent work. The grade "excellent" is assigned to a student who knows how to establish the relationship between the main sections and concepts of the discipline, shows a creative approach in understanding, interpreting and using the program material.

A student who received 70–89 points during his studies, showed full knowledge of the program material, successfully completed the program tasks, mastered the basic literature, completed a full laboratory practicum in the discipline and participated in individual work deserves a "good" rating. A grade of "good" is awarded to a student who has demonstrated systematic knowledge of the discipline and the ability to independently replenish and update it in further academic work and professional activity.

A student who received 50–69 points during his studies, showed knowledge of the main program material in the amount necessary for further study, completed the program tasks, and familiarized himself with the main literature of the program deserves a "satisfactory" grade. A "satisfactory" grade is given to a student who made mistakes during the preparation of current and final control tasks, missed up to 30% of the laboratory practice classes for an improper reason and did not participate in individual work.

An "unsatisfactory" grade is given to a student who does not have knowledge of individual sections of the main program material and general ideas about the basic principles of organizing the work of a biotechnological laboratory; characteristics of callus cultures: obtaining and cultivation - basics

of primary callusogenesis, mature callus culture - main characteristics and growth regulation; modern biotechnologies based on callus cultures.

An "unsatisfactory" grade is given to a student who made fundamental mistakes during the implementation of the program tasks of the current and final control, missed more than 40% of the laboratory practice classes for an improper reason, and did not participate in individual work.

GLOSSARY OF TERMS

Adventitious buds (shoots) are buds (shoots) that arose from the tissues and cells of the plant, which usually do not form them.

Androgenesis is the process of obtaining regenerating plants from anthers or pollen (that is, male reproductive cells of a plant).

Aneuploids are a nucleus, a cell, an organism with the number of chromosomes deviating from n and multiples of n.

Apex is the top part of a stem or root.

Apical dominance is the phenomenon of suppressing the growth of lateral buds of a shoot, if there is an apical bud.

Auxins are phytohormones (IAA, NAA, 2,4-D) that activate growth by "stretching" and stimulate the formation of roots in seedlings.

Haploid is a nucleus, cell, organism characterized by a half set of chromosomes characteristic of a species (symbol - n).

Hemogenesis is the process of formation and development of buds in vitro on callus tissues or directly on explants.

Gibberellins are phytohormones that activate the growth of stems by lengthening the internodes, stimulate seed germination.

Somatic hybridization is the process of fusion of isolated protoplasts, that is, somatic cells.

Gynogenesis is the process of obtaining regenerating plants from embryo sacs (i.e. female germ cells of a plant)

Histogenesis is one of the forms of manifestation of in vitro morphogenesis - the process of tissue formation.

Dedifferentiation is the transition of specialized cells to proliferation and disorganized callus growth (loss of specialization by cells).

A diploid is a nucleus, cell, organism, characterized by a double set of homologous chromosomes, represented in the amount typical for this species (symbol - 2n).

Differentiation is a set of processes leading to differences between cells.

An explant is a tissue or organ fragment cultivated on a nutrient medium independently or used to obtain a primary callus.

An embryoid is an embryo-like structure arising from somatic cells

Embryoidogenesis (embryogenesis) is the process of formation of embryo-like structures (embryoids) asexually in the culture of tissues and cells in vitro.

Epigenetic changes are changes in gene expression or cell phenotype caused by mechanisms that do not disrupt the DNA sequence; they are stored in a series of mitotic divisions of somatic cells, and can also be transmitted to subsequent generations.

Euploid is nucleus, cell, organism with the number of chromosomes that is a multiple of n.

An isolated protoplast is a plant cell that has been stripped of its cell wall by enzymatic or mechanical destruction.

An inoculum is a part of a cell suspension used for transfer to a fresh nutrient medium.

A callus is a group of dedifferentiated cells that arose in vivo or in vitro by disorganized proliferation.

Clonal micropropagation or microclonal propagation is the in vitro asexual production of plants that are genetically identical to the original plant (method of vegetative propagation of plants in in vitro culture).

A clone is a group of genetically identical organisms or cells

Competence is the ability of a cell, tissue, or organ to perceive an inducing influence and specifically react or not react by changing the developmental program.

Culture of zygotic embryos in vitro is aseptic cultivation of immature or mature isolated germs on an artificial nutrient medium.

The culture of isolated protoplasts is the cultivation of cells without walls (shells) in a liquid or agarized nutrient medium.

Root culture in vitro is aseptic cultivation on an artificial nutrient medium in the transplant mode of isolated roots.

Meristem culture in vitro is aseptic cultivation on an artificial nutrient medium of an isolated apex or axillary bud of a growth cone shoot with one or two leaf primordia.

A "nurse" culture is a callus culture used as a substrate (a source of "conditioning factor") during the cultivation of a single cell culture.

Organ culture in vitro is aseptic growing on an artificial nutrient medium in the transplant mode of isolated roots, stem apices, immature flower parts, immature fruits.

Suspension culture or in vitro cell culture is the aseptic cultivation of individual cells or their small groups (aggregates) in a liquid nutrient medium.

In vitro tissue culture is growing in a long-term transplant culture of tissues that have arisen through the proliferation of cells of isolated segments of various organs or plant organs.

A meristem is a tissue with small, actively proliferating cells.

In vitro morphogenesis is the process of formation, i.e. the establishment, growth and development of cells (cytogenesis), tissues (histogenesis) and organs (organogenesis) in the culture of cells and tissues in vitro.

Organogenesis is one of the forms of in vitro morphogenesis, the process of organ formation.

Passivation (subcultivation) is the process of periodically transplanting the callus to a fresh nutrient medium to maintain the transplanted callus culture.

A plastome is a set of genetic information of the plastid system of a plant cell.

Polyploidy is a nucleus, cell, organism characterized by a multiple increase in the basic set of chromosomes (symbol – 3n, 4n, etc.).

Proliferation is the formation of new cells and tissues by reproduction (division) of existing ones.

Regenerant plant is a plant obtained as a result of in vitro morphogenesis.

Regeneration is the restoration of a whole organism from a cell, tissue, or organ.

Redifferentiation is the transition of specialised cells from one state of differentiation to another with preliminary divisions or directly.

Rhizogenesis is the process of root establishment, growth and development.

Growth cycle is the growth of a population of cells in a cycle of periodic cultivation, characterised by an S-shaped curve. The phases of the growth cycle are latent (lag phase), exponential (logarithmic growth phase), growth retardation phase, stationary and degradation phase.

Fusion of isolated protoplasts is the formation of one cell from two or more by combining their surface membranes.

Somatic (parasexual) hybridisation is a method of creating hybrid cell lines and somatic plant hybrids by fusing somatic (asexual) cells, for example, by fusing isolated protoplasts.

A somatic hybrid is a plant obtained by hybridising isolated protoplasts.

Somaclonal variability (variability) is epigenetic or genetic changes in cells or groups of cells developing in culture that lead to changes in the phenotype of the regenerating organism.

Nutrient medium is a medium for cultivation of plant cells and organ tissues under sterile in vitro conditions, containing a set of macro- and microelements, a source of carbohydrate nutrition, vitamins and growth regulators; it can be liquid or solid.

Subcultivation is the process of transferring a transplant or inoculum into a culture vessel on fresh culture medium.

Teratomas is the formation of morphologically abnormal plant organs during in vitro cultivation.

Totipotency is the ability of somatic cells to fully realise the genetic potential of an entire plant organism.

Transplant is a part of the callus tissue used for transfer to a new nutrient medium.

Phytohormones (plant hormones) are biologically active compounds produced in plants in small quantities that cause a specific growth or morphogenetic effect.

Chimera is a mosaic organism that combines cells, tissues, organs of different organisms (example: animal + plant).

Cybrid is a plant obtained by fusing two isolated protoplasts, the nuclear material of one of which has been completely eliminated.

Cultivation cycle is the period from passivation of inoculum or callus transplant on the culture medium to further subcultivation.

Cytokinins are phytohormones (kinetin, 6-BAP) that activate meristem development, cell division, and stimulate bud formation.

Ex planta 'outside the plant' is a biological process, a form of biological analysis in an organ, part of an organism, cell separated from the plant.

In planta 'inside the whole plant' is a biological process or biological analysis in the whole plant.

Ex vitro is a transfer of in vitro cultures to non-aseptic conditions

In vitro - the cultivation of plant objects 'in glass' (test tube, flask, bioreactor) on artificial nutrient media, under aseptic conditions.

In vivo 'inside a living organism' - a biological process or biological analysis in a whole living organism.

ANNEXES

Annex A

RULES FOR WORKING IN THE BIOTECHNOLOGY LABORATORY

When working in the biotechnology laboratory, the requirements set out in the safety instructions must be strictly observed. If a higher education student is not familiar with these requirements, he or she must inform the teacher. The higher education student is personally responsible for his/her own safety while in the laboratory, which is confirmed by signing the safety log during the briefing. It is forbidden to enter the laboratory in outerwear. All higher education students must wear clean cotton gowns, which must be buttoned up. Hair must be removed from the face and hidden under a cap. The workplace must be kept in an exemplary manner. Personal belongings should be kept in a specially designated place. Food and drink are not allowed in the laboratory. Students with open skin lesions that have not been treated and covered with a bactericidal plaster are not allowed to work. When working with an open flame (gas burner, alcohol bottle), the following requirements must be observed: ignite the alcohol bottle and gas burner only with a match. Extinguish a lit alcohol bottle by closing the air supply with a special cap, and a gas burner by shutting off the gas supply. Place the alcohol bottle at a distance of at least 20 cm from the edge of the work table. Do not move a lit alcohol bottle from one place to another. When you finish working with gas burners, make sure that the gas outlet is shut off. Work in the laminar box is allowed only after additional safety training and wearing appropriate protective clothing (gown, cap, face shield and goggles). It is strictly forbidden to enter the box when the germicidal lamp is switched on. Do not use glassware with chips, cracks, or sharp edges. In the laboratory, you must follow safety rules when working with chemicals. If necessary (when working with concentrated chemicals), use personal protective equipment (gloves, respirators, rubber apron, goggles). When diluting a concentrated acid, add the acid to the solvent, not vice versa. If any chemicals come into contact with the skin, rinse off the reagent with plenty of water; neutralise the acid with a weak soda solution, and the alkali with a weak acetic acid solution. Work with concentrated and volatile chemicals must be carried out under a fume hood. Electrical safety requirements must be strictly observed. It is forbidden to use faulty electrical equipment and switch on devices without the permission of the teacher or laboratory assistant, as well as touch the surface of the device with wet hands.

At the end of the work, the higher education student must tidy up the workplace, wash his/her hands thoroughly, and if necessary, treat them with a disinfectant solution. Have an individual towel or napkins for drying hands.

Annex B

REQUIREMENTS FOR THE DESIGN OF PRESENTATION SLIDES

Style	▪ stick to a single design style ▪ avoid styles that will distract from the presentation itself ▪ supporting information (control buttons) should not dominate the main information (text, illustrations)
Background	Cool tones (blue, green) are preferable for the background
Using colour	▪ it is recommended to use no more than three colours on one slide: one for the background, one for the title, one for the text ▪ use contrasting colours for the background and text
Animation effects	▪ Do not overuse various animation effects, they should not distract attention from the content of the information on the slide

Presentation of information

Content of information	▪ use short words and sentences ▪ minimise the number of prepositions, adverbs, adjectives ▪ headlines should attract the attention of the audience
Information location on the page	▪ preferably horizontal placement of information ▪ the most important information should be placed in the centre of the screen ▪ if there is a picture on the slide, the photo caption should be placed below it
Script	▪ for headlines - not less than 24 ▪ for information - at least 18 ▪ do not mix different types of fonts in one presentation ▪ use bold, italics or underlining to highlight information ▪ do not overuse capital letters (they are read worse than lowercase letters)
Ways to highlight information	You should use: ▪ frames; borders, fill; shading, arrows ▪ drawings, charts, diagrams to illustrate the most important facts
Amount of information	▪ do not fill one slide with too much information: people can remember no more than three facts, conclusions, definitions at a time ▪ The greatest effectiveness is achieved when the key points are displayed one by one on each separate slide
Types of slides	Use different types of slides to provide variety: ▪ with text ▪ with tables ▪ with diagrams

Annex C

CONDITIONS FOR STERILISATION OF DISHES, INSTRUMENTS, CULTURE MEDIA AND EQUIPMENT

Temperature and pressure ratios during autoclaving

Saturated steam pressure in the autoclave, kPa	Temperature, °C
34,47	108
68,95	116
103,42	121
137,90	127
172,37	131
206,84	134
1 atm = 101.325 kPa	

Temperature conditions for sterilization of culture media, dishes, instruments and equipment

Type of sterilization	Object of sterilization	Temperature/ pressure, °C/atm.	Sterilization time, min
Steam treatment under pressure (autoclaving)	medium	115–121 / 0,7–1	15–30
	glassware (Petri dishes, heat-resistant flasks)	110–115 / 0,5	20–40
		121 / 1	15
	equipment (cotton wool, gauze, cotton plugs, filter paper)	134 / 2	25–30
Dry method (dry heat sterilizer)	glassware (Petri dishes, heat-resistant flasks)	140	120
		160	80
		170	60
	instruments* (scalpels, tweezers, needles, etc.)	140	120
		160	80

*Syringes, scissors, etc. are best boiled. Metal objects should not be autoclaved: the steam will rust and dull them.

Annex D

COMPOSITION OF MURASHIGE-SKOOG CULTURE MEDIUM (MS) FOR CULTIVATION OF PLANTS *IN VITRO*

Components	Quantity per 1 litre			
	hormone-free for aseptic seedlings	for the induction of callusogenesis	½ MS for clonal micropropagation	for suspension culture
Mother liquor of macro salts	100 ml	100 ml	50 ml	100 ml
Mother liquor of micro salts	1 ml	1 ml	0,5 ml	1 ml
Fe-chelate	5 ml	5 ml	2,5 ml	5 ml
Vitamins: PP	0,5 mg	0,5 mg	0,5 mg	0,5 mg
B1	1 mg	1 mg	1 mg	1 mg
B6	1 mg	1 mg	1 mg	1 mg
Meso-inositol	100 mg	100 mg	50 mg	100 mg
PH: NAA	-	-	0,5-1 mg	-
IAA	-	-	0,5-1 mg	0,5-1 mg
2,4-D	-	2-10 mg	-	2-5 mg
Kinetin	-	-	1-3 mg	-
BAP	-	-	1-3 mg	1-3 mg
Sucrose	30 g	30 g	15 g	30 g
Agar	7 g	7 g	7 rg	-

Annex E

COMPOSITION OF CULTURE MEDIUM FOR CLONAL MICROPROPAGATION OF DIFFERENT PLANTS

Media components per 1 l	Object (plant species/genus, variety)					
	Potato *Solanum tuberosum*	Strawberry *Fragaria sp.*	Cabbage *Brassica oleracea*	Rose *Rosa sp.*	Lily *Lilium sp.*	Violet *Saintpaulia ionantha*
Mother liquor of macro salts	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml
Mother liquor of micro salts	0,5 ml	0,5 ml	0,5 ml	0,5 ml	0,5 ml	0,5 ml
Fe-chelate	2,5 ml	2,5 ml	2,5 ml	2,5 ml	2,5 ml	2,5 ml
Vitamins: PP	0,5 мг	0,5 mg	0,5 mg	0,5 mg	0,5 мг	0,5 mg
B1	1 mg	1 mg	1 mg	1 mg	1 mg	1 mg
B6	1 mg	1 mg	1 mg	1 mg	1 mg	1 mg
Meso-inositol	50 mg	50 mg	50 mg	50 mg	50 mg	50 mg
PH: NAA	0,5 mg	0,5 mg	0,5 mg	0,5 mg	0,5 mg	0,1–0,5 mg
IAA	0,5 mg	0,5 mg	0,5 mg	0,5 mg	0,5 mg	0,1–0,5 mg
Kinetin	1–3 mg	1–3 mg	1–3 mg	1–3 mg	1–3 mg	1–3 mg
BAP	1–3 mg	1–3 mg	1–3 mg	1–3 mg	1–3 mg	1–3 mg
Sucrose	15 g	20 g	20 g	20 g	20 g	20 g
Agar	7 g	7 g	7 g	7 g	7 g	7 g

Annex F

COMPOSITION OF THE CULTURE MEDIUM FOR INDUCTION OF DIFFERENT PATHWAYS OF MORPHOGENESIS OF CALLUS CULTURES

Media components per 1 l	Manifestation of organogenesis		
	Gemogenesis	Rhizogenesis	Embryogenesis
Mother liquor of macro salts	50 ml	50 ml	50 ml
Mother liquor of micro salts	0,5 ml	0,5 ml	0,5 ml
Fe-chelate	2,5 ml	2,5 ml	2,5 ml
Vitamins: PP	0,5 mg	0,5 mg	0,5 mg
B1	1 mg	1 mg	1 mg
B6	1 mg	1 mg	1 mg
Meso-inositol	50 mg	50 mg	50 mg
PH: NAA	-	0,5–1 mg	0,2 mg
IAA	-	0,5–1 mg	0,2 mg
2,4-D	-	-	0,2–1 mg
Kinetin	1–3 mg	0–0,5 mg	0–0,1 mg
BAP	1–3 mg	0–0,5 mg	0–0,1 mg
Sucrose	20 g	20 g	20–30 g
Agar	7 g	7 g	7 g

Електронне навчальне видання комбінованого використання
Можна використовувати в локальному та мережному режимі

Авксентьєва Ольга Олександрівна
Батуєва Євгенія Дмитрівна

ОТРИМАННЯ ТА ВИКОРИСТАННЯ КАЛУСНИХ КУЛЬТУР

Навчально-методичний комплекс

(Англ. мовою)

Комп’ютерне верстання *О. О. Авксентьєва*

В авторській редакції

Підписано до розміщення 24.10.2024. Гарнітура Times New Roman.
Ум. друк. арк. 3,36. Обсяг 0,557 Мб. Зам. № 281/24.

Харківський національний університет імені В. Н. Каразіна,
61022, м. Харків, майдан Свободи, 4.
Свідоцтво суб’єкта видавничої справи ДК № 3367 від 13.01.2009
Видавництво ХНУ імені В. Н. Каразіна