NATIONAL UNIVERSITY OF LIFE AND ENVIRONMENTAL SCIENCES OF UKRAINE

Kolomiiets Yu.V., Klyachenko O.L.

BIOTECHNOLOGY

UDC 576:58:378.14 (073) BBK 28.55.-73 K 69

Recommended for publication by the Scientific Council of the National University of Life and Environmental Sciences of Ukraine (protocol №3 from 27.10.2021)

Reviewers:

- V. Postoenko Doctor of Agrcultural Sciences, Senior Researcher;
- S. Prylutska Doctor of Biological Sciences, Senior Researcher;
- **L. Klymenko** Senior teacher, Department of English Philology of Humanities Pedagogical Faulty NUBiP Ukraine

Yu. Kolomiiets, O. Klyachenko

Biotechnology. – K.: Comprint, 2021. – 260 p.

ISBN

The textbook outlines the most informative methods and techniques of biotechnological work with cultivated plants. Methods of introduction into culture *in vitro*, microclonal propagation, obtaining callus cultures, regeneration and adaptation *in vivo* of plants and modern technological genetic engineering approaches are presented. The definition and interpretation of the most commonly used terminology in biotechnology are given.

For students of agrobiotechnological specialties, scientists, teachers, graduate students, specialists specializing in biotechnology, selective breeding, cell biology, genetics and plant physiology.

UDC 576:58:378.14 (073) BBK 28.55.я73

ISBN

© Yu. Kolomiiets, O. Klyachenko, 2021

© National University of Life and Environmental Sciences of Ukraine, 2021

CONTENTS

Introduction	7
Biotechnology laboratory: structure and equipment	8
PART 1. PRINCIPLES AND METHODS OF GROWING ISOLATED	
CELLS AND TISSUES OF PLANTS	11
1. Methods of sterilization of laminar box, utensils, nutrient media and plant	
material	12
2. Preparation of stock solutions for Murashige and Skoog medium (MS)	15
PART 2. INFLUENCE OF MINERAL COMPONENTS OF NUTRIENT	
MEDIA ON CELL DEVELOPMENT.	18
3. Preparation of Murashige and Skoog medium. Sterilization of the	
medium by autoclaving	50
4. Sterilization of liquid media by passing through a bacterial filter (cold	
sterilization)	52
5. Preparation of nutrient media for culturing isolated plant cells and tissues	
	54
6. Sterilization of soybean seeds to obtain sterile seedlings	57
7. Sterilization of carrot roots and potato tubers and their introduction into	
in vitro culture	58
8. Study of the phenomenon of physiological polarity	60
PART 3. CULTURE OF CALLOUS TISSUE	62
9. Obtaining and cultivating callus tissue of soybeans	64
10. Transfer (passage) of callus tissue on fresh nutrient medium	66
PART 4. REMOVAL OF GROWTH CHARACTERISTICS OF CALL	
CULTURE	67
11. Obtaining callus tissue from tobacco leaves	67
12. Obtaining calluses from immature embryos and nodes of wheat	
tillering	69
13. Getting calluses from bean roots	71
14. Counting cells by the Brown's method	75

15. Transplantation of callus tissue on a fresh nutrient medium with	
different composition of hormones	77
PART 5. MORPHOGENESIS AND REGENERATION IN CULTURE OF	
CALLUS TISSUES. OBTAINING PLANT REGENERANTS	79
16. Induction of organogenesis in callus tissue of potatoes	86
17. Induction of stem organogenesis in the culture of callus tissue of	
tomatoes. Obtaining plant regenerants (indirect morphogenesis)	88
18. Induction of somatic embryogenesis in callus tissue of alfalfa leaves	90
PART 6. SUSPENSION CELL CULTURE	93
19. Obtaining a suspension culture of callus tissue of ginseng, carrots,	
Jerusalem artichokes, cloves, tomatoes	97
20. Evaluation of cell viability and the degree of aggregation of the	
suspension	98
21. Detection of suspension culture density and evaluation of its growth	
activity	99
22. Sowing suspensions on a solid agar medium to obtain unicellular	
clones	101
PART 7. APPLICATION OF TISSUE CULTURE METHOD IN PLANT	
SELECTION (NON-TRADITIONAL SELECTION METHOD)	104
23. Sowing the suspension on a selective nutrient medium	106
PART 8. CULTURE F ISOLATED PROTOPLASTS	108
24. Isolation and cultivation of protoplasts	112
PART 9. MICROCLONAL PROPAGATION OF PLANTS	116
25. Isolation and cultivation of apical meristems (cloves, potatoes, roses,	
currants)	120
26. Isolation and cultivation of apical meristems of potatoes	121
27. Obtaining virus-free planting material with thermotherapy method in	
combination with cultivation of apical meristems	123
28. Obtaining potato micro-bulbs in vitro	125
29. Micropropagation of cloves (potatoes) by cuttings	127
30. Induction of rooting in microclonal reproduction of gerberas	128
31. Accelerated microclonal reproduction (cloves, currants, potatoes)	130

	32.	Cultiva	ation of plant regener	ants		• • • • • • • • • • • • • • • • • • • •	1
	33.	Record	of growth characteri	istics of	plant regene	erants	1
PAI	RT	10.	REGULATORS	OF	PLANT	GROWTH	AND
DE	VEL	OPME	NT				1
	34.	Isolate	d soybean tissue as a	test sys	tem for cytol	kinins	1
	35.	Isolate	d Jerusalem artichoko	e tissue	culture as a t	test system for a	uxins 1
	36.	The ef	ffect of auxins, cytol	kinins a	nd gibberell	ins on the grov	vth and
deve	elopn	nent of	micro cuttings of ste	via, pot	atoes, cloves	S	1
PAI	RT 1	1. CUI	TURE OF ISOLAT	TED CI	ELLS AND	TISSUES IN P	LANT
SEI	LEC	ΓΙΟΝ.		• • • • • • • • • • • • • • • • • • • •			1
	37.	Growtl	n and development of	f anthers	in culture ii	n vitro (androge	nesis). 1
	38.	Obtain	ing haploids from a f	emale g	ametophyte	(gynogenesis).	1
	39.	Embry	o culture	• • • • • • • • • • • • • • • • • • • •	• • • • • • • • • • • • • • • • • • • •		1
PAI	RT 1:	2. MO	LECULAR BIOLO	GY AN	D GENETI	C ENGINEER	ING 1
	40.	Prepar	ration of nutrient n	nedium	for cultivat	ion of <i>Agroba</i>	cterium
tume	efaci	ens				• • • • • • • • • • • • • • • • • • • •	1
	41.	Transf	ormation of plant cel	ls of car	rots and Jeri	usalem artichok	e tubers
unde	er the	e effect	of Agrobacterium tu	mefacie	<i>ns</i> (natural g	genetic engineer	ing) 1
	42.	Transfo	ormation of tomato p	lant cell	s under the e	effect of Agroba	cterium
tume	efaci	ens		•••••	•••••		1
	43.	Transf	formation of a plant	cell und	er the effect	t of the Agroba	cterium
tume	efaci	ens Ti-	plasmid	• • • • • • • • • • • • • • • • • • • •			1
	44.	Isolatio	on of nuclear DNA fr	om plan	t tissues		1
	45.	Plasmi	d amplification				1
	46.	Isolatio	on of plasmid DNA.	• • • • • • • • • • • • • • • • • • • •			1
	47.	Isolatio	on of plant RNA				1
	48.	RNA g	gel electrophoresis				1
	49.	Quant	itative analysis of DN	NA or R	NA		1
	50.	Isolatio	on of DNA				1
	51.	Isolatio	on of viral DNA from	n plant n	naterial		1
	52.	Isolatio	on of viral RNA from	plant n	naterial		1
	53.	Realiza	ation of the reverse tr	anscript	ion reaction		1

54. Enzyme-linked immunosorbent assay for viral infections	180						
55. Realization of the polymerase chain reaction with the purpose of							
qualitative diagnostics and identification of viruses							
56. Agarose gel electrophoresis	186						
PART 13. REDUCTION-OXIDATION ENZYMES							
57. Peroxidase activity analysis	196						
58. Study of the electrophoretic spectrum of peroxidases	198						
59. Finding polyphenol oxidase activity	200						
PART 14. CRYOPRESERVATION OF PLANT MATERIAL							
60. Study of the protective effect of cryoprotectants on plant resistance cells							
to low temperatures	205						
61. Influence of cryoprotectants on cytoplasm proteins of plant cells							
exposed to negative temperatures							
PART 15. BIOTECHNOLOGY OF GROWING EDIBLE							
MUSHROOMS	208						
62. Obtaining a pure culture of mycelium (button, oyster mushrooms)	208						
63. Growing mycelium of oyster mushrooms, button mushrooms on grain							
substrate	209						
64. Obtaining fruiting bodies on the substrate	209						
Appendix	211						
Glossary	213						
References	252						