وزارة التعليم العالي والبحث العلمي
الجامعة التقنية الشمالية
الكلية التقنية الزراعية

الحقيبة التعليمية

القسم العلمي:	**تقنيات الإنتاج النباتي**
اسم المقرر:	زراعة الانسجة النباتية
المستوى:	الرابع
الفصل الدراسي:	**الاول**
السنة الدراسية:	2026-2025

Plant Tissue Culture	**Course Name:**
Plant Production Techniques	**القسم/ Department**
Agricultural Technology	**الكلية/ College**
Fourth	**المستوى/ Level**
First	**الفصل الدراسي/ Semester**
2 practical 2 theore	**Number of Weekly Hours:**
3	**Number of Academic Units**
PLP 406	**Code**
** كلهما practical theoretical	**Type of Course**
no	**Is there a counterpart to the course in other departments?**
	Name of the counterpart course
	Department
	Department
Subject teaching information	
Dr. Khawla Mahmoud Al-Nouh	**Name of the course instructor(s):**
مدرس	**Academic title**
2023	**Year of obtaining the title**
Ph.D.	**Certificate:**
2013	**Year of obtaining the certificate**
7	**Number of years of experience (teaching)**

General description of the course

Introducing and training the student on plant tissue culture techniques, the use of agricultural media, the most important additions to the agricultural media, sterilization methods used in tissue culture laboratories, and the prospects for propagating plant varieties in the laboratory. In addition .to introducing the student to bioreactors and the prospects for their use

General objectives

- The student will be aware of the importance of plant tissue culture and the prospects of its use
- The student will be familiar with the types of culture media and methods of preparing the culture media
- The student will be familiar with sterilization methods and types of sterilizers used and sterilization devices
- The student will be able to use tissue culture technology in propagating different plants.
- The student will have learned the importance of cell suspensions and the prospects of their uses
- The student will know the methods of propagating plants free of disease infections

Specific Objectives

- The student will be able to use tissue culture technology in the commercial propagation of different plants.
- The student will be able to perform sterilization operations using special devices
- Produce plants free from disease infections

Behavioural objectives or learning outcomes

• The student will be able to distinguish the parts of the tissue culture laboratory and know the importance of each part.
• The student will be able to distinguish the types of sterilizers used in the laboratory and the most important sterilization methods.
• The student will be able to prepare agricultural media.
• The student will be able to identify the stages of tissue propagation.

• The student will be able to acclimatize plants resulting from tissue culture.
• The student will be able to prepare cell suspensions..

Prerequisites

The student must be familiar with general botany, plant anatomy, plant growth regulators, their types and mechanism of action

Behavioral objectives or basic learning outcomes		
Evaluation mechanism	Detailing the behavioral objective or learning outcome	ت
Daily exams, weekly reports	**The importance of plant tissue culture and its prospects of use**	**1**
Daily exams, practical applications	Knowledge of the types of culture media, methods of preparing the culture media and zeroing the pH of the medium	**2**
Daily exams, practical applications	**Knowledge of sterilization methods, types of sterilizers used and sterilization devices**	**3**
Daily exams, practical applications	Application of tissue culture technology in .propagating different plants	**4**

Daily exams, discussion reports	Identifying bioreactors (cell suspensions) and their prospects of use	5
Daily exams, practical applications	Propagation of plants free from disease infections	6

TEACHING METHODS

Justifications for selection	METHOD OR APPROACH
Conveying an introductory overview of each topic in the curriculum	**LECTURES .1**
Motivating the student to find information and the method of presenting the information	**DISCUSSION GROUPS.2**
Encouraging the student to do field work on tissue culture technology, from preparing the medium to sterilization to micro-cultivation, with follow-up of the crops in the development room	**LABORATORY APPLICATION.3**
to evaluate the student's scientific reality.	**DAILY AND SEMESTER EXAMS.4**
	.5
	.6

Chapter One of the Scientific Content						
Introduction to tissue culture, introducing the student to the importance of tissue culture and its potential uses				the time		Chapter Title
Techniques	Teaching Method	Teaching Method Techniques	Subtitle	practical	Theoretical	Time distribution
Daily exam	Presentation, explanation, questions and answers, discussion	Lectures	History and present situation of plant tissue culture and application	2	2	First week
Daily exam	Presentation, explanation, questions and answers, discussion	Lectures + slides + practical preparation in the lab	Subtitles Benefits, features terms in plant tissue culture	Benefits, features and terms in plant tissue culture 2	2	Second week
Daily exam	Presentation, explanation, questions and answers, discussion	Lectures + slides + practical preparation in the lab	Main components of the growing medium Types of energy sources added to the environment	the growing medium 2	2	The third week

Chapter Two of the Scientific Content							
Growth regulators and their role in plant tissue culture Sterilization and its importance in plant tissue culture					the time		Chapter Title
Techniques	Teaching Method	Teaching Method Techniques	Subtitle		practical	Theoretical	Time distribution
Daily exam	Presentation, explanation, questions and answers, discussion	Lectures + slides + practical preparation in the lab	Organic additions to the medium Hardening of the growing medium	the growing medium	2	2	Week 4
Daily exam	Presentation, explanation, questions and answers, discussion	Lectures + slides + practical preparation in the lab	The importance of plant growth regulators and their role in tissue culture Auxins and Cytokinins Growth inhibitors and Gibberellins	Growth regulators and their role in plant tissue culture	2	2	Week 5
Daily exam		Lectures + slides +	Wet Sterilization Dry Sterilization	Methods of sterilizing	2	2	Week 6

	Presentation, explanation, questions and answers, discussion	practical preparation in the lab	Sterilization Using Filters				
Daily exam	Presentation, explanation, questions and answers, discussion	Lectures + slides + practical preparation in the lab	Types of sterilizers used to sterilize the plant part (the negatives and positives of each)	tissue culture and methods of sterilization	2	2	Week 7
Chapter Three of the Scientific Content							
tissue culture					the time		Chapter Title
Techniques	Teaching Method	Teaching Method Techniques	Subtitle		practical	Theoretical	Timeline
Daily exam	Presentation, explanation, questions and answers, discussion	Lectures + slides + practical preparation in the lab	Definition of callus and prospects for its uses	Callus farms development	2	2	Week 8
			Nutritional and organic additives to the culture medium that stimulate callus initiation and growth				
Daily exam	Presentation, explanation, questions and answers, discussion	Lectures + slides + practical	Types of callus farms		2	2	Week 9
			Callus specialization				

		preparation in the lab					
Daily exam	Presentation, explanation, questions and answers, discussion	Lectures + slides + practical preparation in the lab	Callus death		2	2	Week 10
Chapter Four of the Scientific Content							
Cell suspensions, tissue culture stages					the time		Chapter Title
Techniques	Teaching Method	Teaching Method Techniques	Subtitle		practical	Theoretical	Timeline
Daily exam	Presentation, explanation, questions and answers, discussion	Lectures + slides + practical preparation in the lab	Cell suspensions, culture Mechanism of creating cell suspensions	Cell suspensions, culture	2	2	Week 11
Daily exam	Presentation, explanation, questions and answers, discussion	Lectures + slides + practical preparation in the lab	Bioreactors Secondary Metabolism		2	2	Week 12
Daily exam	Presentation, explanation, questions and answers, discussion	Lectures + slides + practical preparation in the lab	Zero Phase Emergence Phase	Tissue culture stages Tissue culture stages	2	2	Week 13

Daily exam	Presentation, explanation, questions and answers, discussion	Lectures + slides + practical preparation in the lab	Duplicate Phase Rooting Phase Acclimatization		2	2	Week 14
Daily exam	Presentation, explanation, questions and answers, discussion	Lectures + slides + practical preparation in the lab	Apical Meristem Culture	Production of plants free from viral infection	2	2	Week 15

المحتوى العلمي

Approved measurement map

Number of paragraphs	Behavioral objectives					relative importance	Chapter Titles	Educational Content
	Evaluation	Analysis	Application	Understanding	Knowledge			
9	0	0	1	3	5	%19	Introduction to tissue culture, introducing the student to the importance of tissue culture and its uses	Chapter One
14	0	0	1	5	8	%29	Growth regulators and their role in plant tissue culture	Chapter Two
10	0	0	1	5	4	21	Sterilization and its importance in plant tissue culture	Chapter Three
15	0	0	1	5	9	%31	Tissue cultures Cell suspensions, stages of tissue culture	Chapter Four Educational Content
48	0	0	4	18	26			

Contents (for each chapter in the course)

Lecture No.:	**The first lecture**
Lecture Title:	**History and present situation of plant tissue culture**
Teacher's Name:	**Dr. Khawla Mahmoud Al-Nouh**
Target Group:	**Level 4**
General Objective of the Lecture:	**Know the general history of tissue culture.**
Behavioral Objectives or Learning Outcomes:	**1- The student should know the concept of plant tissue culture.** **2- The student should learn about the first scientists in plant tissue culture.** **3- The student should know on what characteristics the plant cell and tissue culture technique depends.**
Facilitation Strategies Used	**Explaining the lecture, discussing ideas and sharing experiences**
Acquired Skills	**Know the history of plant cell and tissue culture technology**
Accepted Measurement Methods	**Daily tests, brainstorming, and assignments.**

Introductory questions

What do you know about tissue culture?

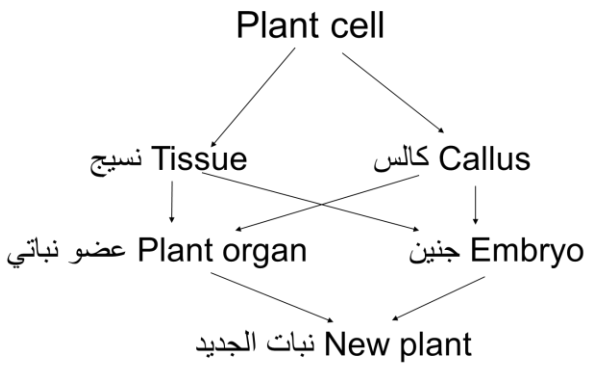
Plant tissue culture concept

It means the growth of different plant cells, tissues or parts in glass and sometimes plastic containers containing artificial nutrient environments consisting of the nutrients the plant needs, and this is done under completely sterile conditions.

Cells and tissues rely on totipotency to produce a complete plant.

Totipotency: The ability of cells to differentiate and develop into a complete plant when the conditions are suitable for growth. This is because each cell has a latent genetic potential from the mother plant.

Totipotency



Historical overview

In 1838, the two scientists (Schleiden and Schwann) developed a theory called Cell Totipotency, which is the ability of the plant cell to grow, unfold and form a new plant . This theory was the basis for plant tissue culture..

In 1902, Haberlandt made the first attempt at tissue culture and identifying its problems.

In 1904, the scientist (Hanging) cultivated embryos of plants from the cruciferous family.

In 1922, Robbins succeeded in obtaining tissue cultures of the tips of growing roots.

In 1933, scientists (Gauther, Nobcourt and White) succeeded in obtaining a callus that continued to grow.

In 1934, the scientist (White) succeeded in obtaining tissue cultures of tomato plant roots.

In 1944, Skoog studied the development of broad-bud smoke in the laboratory.

In 1945, Loo cultivated the growing tip of the Asparagus plant.

In 1946, Ball obtained the first complete plant from the growing tip culture.

In 1948, Skoog and Tsui succeeded in forming stems and roots in tobacco plant tissue cultures by adjusting the ratio of auxin to adenine in the nutrient medium.

In 1962, Murashige and Skoog announced the composition of the nutrient medium (MS), which is considered the most famous medium used in plant tissue culture.

In 1970, the scientist (Power et al.) made the first successful attempt to fuse protoplasts.

Plant tissue culture has been applied to propagate many economic plant varieties and has had an effective impact in producing new plants and varieties free from diseases and insects.

Post-questions

1- Define plant cell and tissue culture?
2- What does the term Totipotency mean?
3- Mention five scientists in the field of plant cell and tissue culture.?

Lecture No.:	Lecture 2
Lecture Title:	**In vitro**
Teacher's Name:	**Dr. Khawla Mahmoud Al-Nouh**
Target Group:	**Level 4**
General Objective of the Lecture:	**Know the advantages of tissue culture**
Behavioral Objectives or Learning Outcomes:	**مخرجات التعلم** **1- The student should know the advantages of tissue culture.** **2- The student should know the benefits of tissue culture.** **3-The student should be familiar with some scientific terms related to plant tissue culture.**
Facilitation Strategies Used	**Explaining the lecture, discussing ideas and sharing experiences**
Acquired Skills	**To familiarize the student with the advantages and benefits of plant tissue .culture**
Accepted Measurement Methods	**Daily tests, brainstorming, and assignments.**

Introductory questions

What is the agricultural medium?

In vitro : Plant tissue culture or micro propagation or called in vitro is a collection of techniques used to maintain or grow plant cells , tissues or organs under sterile conditions on a nutrient culture medium of known composition .

An alternative term of tissue culture is In vitro .

Distinctive characteristics of tissue culture techniques:

1- It is done in a relatively small space compared to traditional agriculture

2- It is possible to provide the optimal environmental conditions that the plant needs

3- It is done away from all living organisms such as fungi, bacteria, viruses and insects

4- It is not related to the natural pattern of plant development and life cycle.

5- Ease of handling cell and tissue cultures for plants that are difficult to handle or process.

6- One of the most important characteristics is that it is done in glassware and under completely sterile conditions

Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation , including ;

1-The production of exact copies of plants that produce particularly good flowers , fruits or have other desirable traits .

2-To quickly produce mature plants .

3-The production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds .

4-The regeneration of whole plants from plant cells that have been genetically modified.

5- The production of plants in sterile containers that allows them to be moved with greatly reduced chances of transmitting diseases , pests and pathogens.

6- The production of plants from seeds that otherwise have very low chances of germination and growing ,i.e.; orchids and Nepenthes .

Explant: It is a tissue or plant part that has been separated from the mother plant to be used in agriculture using the plant cell and tissue culture technique .it Includes Shoot tips , root , leaf primordial , Flower primordial , Embryos , Protoplast .

Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant (Totopotiency) Single cells

protoplasts: plant cells without cell walls.

A Protoplast is a plant , bacterial or fungal cell that had its cell wall completely or partially removed using either mechanical or enzymatic means.

Tribal questions

Q1/ What are the advantages of plant cell and tissue culture?

Q2/ What are the areas of use of plant cell and tissue culture?

Q3/ Define each of the following: protoplasts , Explant

Lecture No.:	Lecture 3
Lecture Title:	**Preparing the growing medium**
Teacher's Name:	**Dr. Khawla Mahmoud Al-Nouh**
Target Group:	**Level 4**
General Objective of the Lecture:	**Identify the components of the growing medium**
Behavioral Objectives or Learning Outcomes:	1- **The student should know the most important agricultural media used in the cultivation of plant cells and tissues.** 2- **The student should be familiar with the most important nutritional elements used in preparing the growing medium.** 3- **The student should be familiar with the energy sources added to the agricultural environment.**
Facilitation Strategies Used	**Explaining the lecture, discussing ideas and sharing experiences**
Acquired Skills	**The student can prepare a growing medium.**
Accepted Measurement Methods	**Daily tests, brainstorming, and assignments.**

Introductory questions

What does the plant need to grow?

Plant Tissue culture Media

Best growth and morphogenesis of tissues may vary for different plants according to their nutritional requirements . Moreover , tissues from different parts of plants may also have different requirements for satisfactory growth . Tissue culture media were first developed from nutrient solutions used for culturing whole plants . e.g. root culture medium of White and callus culture medium of Gautheret . White's medium was based on Uspenski and Uspenska's medium for algae , Gathered's medium was based on Knop's salt solution . Basic that are frequently **used include Murashige and Skoog (MS) Medium ,**

Linsmaier and Skoog (LS)medium , Gamborg (B 5) medium and Nitsch and Nitsch (NN)medium.

Media composition

Plant tissue culture media should generally contain some or all of the following components.

1-Macronutrients :

The essential elements in plant cell or tissue culture media include , besides C , H , and O

Macronutrients : nitrogen (N) , phosphorus (P) , potassium (K) ,calcium (Ca) ,magnesium (Mg) and sulphur (S) for satisfactory growth and morphogenesis . Culture media should contain at least 25-60 mM of inorganic nitrogen for satisfactory plant cell growth . Potassium is required for cell growth of most plant species . most media contain K in the form of nitrate chloride salts .

Micronutrients

The essential Micronutrients (minor elements)for plant cell and tissue growth include iron (Fe) , manganese (Mn) , zinc (Zn) , boron (B), copper (Cu), and molybdenum (Mo).

Iron is usually the most critical of all the Micronutrients . There exist some problems with these compounds for their difficulty to dissolve and precipitate after media preparation. There has been trials to solve this problem by using ethylene diaminetetraacetic acid (EDTA) –iron chelate (Fe EDTA) Cobalt (Co) and iodine (I) may be added to certain media , (Na) and chlorine (Cl)are also used in some media .

3-Carbon and energy sources

In plant tissue culture media , besides the sucrose frequently used as carbon sources at concentration of 2-5 % , other carbohydrates are also used . these include lactose , galactose , maltose and starch and they were reported to be less effective than either sucrose,

It was frequently demonstrated that autoclaved sucrose was better for growth than filter sterilized sucrose .

Autoclaving seems to hydrolyze sucrose in to more efficiently utilizable sugars such as fructose .

It was found that supplements of sugar cane molasses , banana extract and coconut water to basal media can be a good alternative for reducing medium costs . These substrates in addition to sugars, they are sources of vitamins and inorganic ions required for growth .

Lecture No.:	**Lecture 4**
Lecture Title:	**Preparing the growing medium**
Teacher's Name:	**Dr. Khawla Mahmoud Al-Nouh**
Target Group:	**Level 4**
General Objective of the Lecture:	**Preparing the growing medium**
Behavioral Objectives or Learning Outcomes:	**1- The student should know the most important vitamins added to the agricultural medium.** **2- 1- The student should know the most important amino acids added to the agricultural medium.** **3- 1- The student should understand how to harden the growing medium.** **4- The student understands the role of organic additives in the medium.**
Facilitation Strategies Used	**Explaining the lecture, discussing ideas and sharing experiences**
Acquired Skills	**Ability to prepare a complete culture medium**
Accepted Measurement Methods	**Daily tests, brainstorming, and assignments.**

Introductory questions

What are the additional components of the growing medium?

4-Vitamins and myo-inositol

Some plant are able to synthesize essential requirements of vitamins for their growth .

some vitamins are required for normal growth and development of plants ,

they are required by plants as catalysts in various metabolic processes .

They may act as limiting factors for cell growth and differentiation when plant cells and tissues are grown *In vitro*

The vitamins most used in the cell and tissue culture media include ;

Thiamin (B1) , nicotinic acid and pyridoxine(B6)

Other vitamins such as biotin , folic acid , ascorbic acid , pantothenic acid , tocopherol (vitamin E) , riboflavin , p-amino – benzoic acid are used in some cell culture media .

Myo-inositol is added in small quantities to stimulate cell growth of most plant species . myo- inositol is believed to play a role in cell division It is generally used in plant cell and tissue .

5-Amino acids ;

The required amino acids for optimal growth are usually synthesized by most plants , however, the addition of certain amino acids mixtures is particularly important for establishing cultures of cells and protoplasts.

Amino acids provide plant cells with a source of nitrogen that is easily assimilated by tissues and cells faster than inorganic nitrogen sources .

Amino acid mixtures such as casein hydro lysate , L-glutamine , L-asparagine , and adenine. are frequently used as sources of organic nitrogen in culture media .

6-Undefined organic supplements :

Some media were supplemented with natural substances or extracts such as coconut milk , malt extract , banana extract , orange juice and tomato juice , to test their effect on growth enhancement are now commonly added to culture media .

The addition of activated charcoal is sometimes added . On the other hand , an inhibition of cell growth was noticed on addition of activated charcoal to

culture media of soybean . Explanation of the mode of action of activated charcoal was based on adsorption of inhibitory compounds from the medium, adsorption of growth regulators from the culture medium or darkening of the medium .

7- Solidifying agents :

Hardness of the culture medium greatly influences the growth of cultured tissues .

There are a number of gelling agents such as agar.

Agar- solidified medium supporting plant growth .

Agar, a polysaccharide Obtained from seaweeds , use as a gelling agent for preparing semi-solid and solid plant tissue culture media .

Agar has several advantages over other gelling agents ;mixed with water , it easily melts in a temperature range 60-100°C and solidifies at approximately 45 °C and it forms a gel stable at all feasible incubation .

Lecture No.:	**Lecture 5**
Lecture Title:	**growth regulators**
Teacher's Name:	**Dr. Khawla Mahmoud Al-Nouh**
Target Group:	**Level 4**
General Objective of the Lecture:	**Identify the role of growth regulators in tissue cultures.**
Behavioral Objectives or Learning Outcomes:	1- **The student should know the role of growth regulators in tissue cultures.** 2- **The student should know the most important groups of growth regulators that can be added to tissue cultures.** 3- **The student should be able to prepare basic solutions of growth regulators.**

	4- **The student should distinguish the role of cytokinin in the culture medium.**
Facilitation Strategies Used	**Explaining the lecture, discussing ideas and sharing experiences**
Acquired Skills	**The student understands the role of growth regulators in the culture medium.**
Accepted Measurement Methods	**Daily tests, brainstorming, and assignments.**

Introductory questions

What are growth regulators?

growth regulators

Plant growth regulators are important in plant tissue culture since they play vital roles in stem elongation , tropism , and apical dominance . They are generally classified in to the following groups ; auxins , cytokinins , gibberellins and abscisic acid . Moreover , proportion of auxins to cytokinins determines the type and extent of organogenesis in plant cell cultures.

1-Auxine ;

The common auxins used in plant tissue culture media include:

Indole-3- acetic acid (IAA)

Indole-3- butric acid (IBA)

2,4-dichlorophenoxy - acetic acid (2,4-D)

Naphthalene- acetic acid (NAA)

IAA is the only natural auxin occurring in plant tissues .There are other synthetic auxine used in culture media such as(picloram) .

Auxine differ in their physiological activity and in the extent to which they translocate through tissue and are metabolized . prepare fresh IAA solution each time during medium preparation , however IAA solutions can be stored in an amber bottle at 4 °C for no longer than a week .Generally IAA and 2,4-D are dissolved in a small volume of 95% ethyl alcohol . NAA , 2,4D and IBA can be dissolved in a small amount of 1N NaOH .

2-Cytokinins ;

Cytokinins commonly used in culture media include:

BAP (6-benzyloaminopurine),

2ip (6- dimethylaminopurine) ,

kinetin (N-2-furanylmethyl – H-purine -6-amine) ,

Zeatin (6-4- hydroxyl- 3-methyl-trans-2-butenylaminopurine) .

TDZ (thidiazuron-N-phenyl-N-1,2,3)

Thidiazuron , Zeatin and 2ip are naturally cytokinin , and zeatin is more effective . In culture media cytokinin proved to 1- stimulate cell division , 2- induce shoot formation and 3- axillary shoot proliferation and to 4- retard root formation .

The cytokinins are relatively stable compounds in culture media and can be stored desiccated at 20 °C .

Cytokinins are frequently reported to be difficult to dissolve and sometimes addition of few drops of 1N HCL or1N NaOH facilitate their dissolution .

Gibberellins ;

3-Gibberellins comprise more than fifty compounds , of which GA_3 is the most frequently used gibberellins , these compounds enhance growth of callus and help elongation of dwarf plantlets .

4-Othere growth regulators ;

Sometimes added to plant tissue culture media as abscisic acid , a compound that is usually supplemented to inhibit or stimulate callus growth , depending up on the species . It enhances shoot proliferation and inhibits later stages of embryo development .

Although growth regulators are the most expensive medium ingredients , they have little effect on the medium cost because they are required in very small concentrations .

Post-questions

1-What are the types of auxins and what is natural auxin?

2- What are the types of cytokinins?

4- How can you prepare basic solutions of cytokinins and auxins?
5- What is the role of cytokinin in the culture medium?

Lecture No.:	Lecture 6
Lecture Title:	**Sterilization**
Teacher's Name:	**Dr. Khawla Mahmoud Al-Nouh**
Target Group:	**Level 4**
General Objective of the Lecture:	**Introducing the student to the importance of sterilization**
Behavioral Objectives or Learning Outcomes:	1- **The student understands the importance of sterilization in plant tissue culture.** 2- **The student should know the types of sterilization.** 3- **The student should know the types of sterilizers.**
Facilitation Strategies Used	**Explaining the lecture, discussing ideas and sharing experiences**
Acquired Skills	**The student should be able to work in completely sterile conditions.**
Accepted Measurement Methods	**Daily tests, brainstorming, and assignments.**

Introductory questions

What is meant by sterilization?

Sterilization

Tissue culture requires high levels of sterility in all steps of the work, as plant cells and microorganisms have the same growth requirements. In commercial tissue culture production units, contamination of a batch of cultures may cause significant financial loss and even the loss of an entire plant strain. Therefore, strict preventive measures are imposed for the entry of people and living materials into such laboratories.

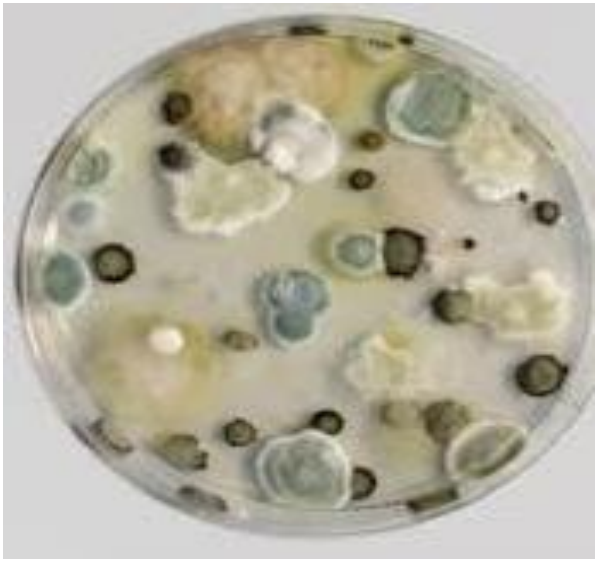
Contamination is one of the most important problems that must be taken into consideration in tissue culture laboratories because it is the ideal environment

for the growth of all types of bacteria and fungi. Contamination hinders the growth of plant cells in several ways, including:

Release of some enzymes and toxins that inhibit cell growth

Selective absorption of certain nutrients from the culture medium

Infection and contamination of some plant tissues



Lab Cleanliness

Precautions to be taken in the tissue culture laboratory:

1- Reduce the air flow in the work area to avoid the movement of microorganisms and prevent the use of fans in the transfer room containing the laminar
2- Store the prepared media, nutrient solutions and tools in a special cabinet
3- Use an isolated area for cleaning, washing and preparing the media

In general, three methods of sterilization are used in the laboratory:

1- Dry heat sterilization
2- Wet heat sterilization
3- Filtration sterilization

Post-questions

1- What are the types of sterilization?
2- Mention three compounds that can be used in the sterilization process?
3- What is the role of the spreading agent in the sterilization process?

Lecture No.:	**Lecture 7**
Lecture Title:	**Sterilization**
Teacher's Name:	**Dr. Khawla Mahmoud Al-Nouh**
Target Group:	**Level 4**
General Objective of the Lecture:	**importance of sterilization**
Behavioral Objectives or Learning Outcomes:	1- **The student understands the importance of sterilization in plant tissue culture.** 2- **The student should know the types of sterilization.** 3- **The student should know the types of sterilizers.**
Facilitation Strategies Used	**Explaining the lecture, discussing ideas and sharing experiences**
Acquired Skills	**The student should be able to work in completely sterile conditions.**
Accepted Measurement Methods	**Daily tests, brainstorming, and assignments.**

Introductory questions

Is there more than one method of sterilization in tissue culture?

Dry heat sterilization

Glassware, metal utensils and other materials that do not burn at high temperatures are placed in containers or wrapped in aluminum foil and placed in a dry oven and sterilized for at least three hours at a temperature of 140−160 degrees Celsius.

Wet heat sterilization

One of the most common methods for sterilizing media is to use an Autoclave at a temperature of 121°C and a pressure of 1.04 kg/cm2 for 15-20 minutes. These data vary according to the sizes of the medium. The bottles containing the medium must be placed vertically and not filled more than 50%.

Filtration sterilization

It is used when heat sterilization of the solution is not possible, as the filter porosity, which is usually between 0.22 and 0.45 micrometers, traps microorganisms. This method is generally suitable for heat-unstable compounds or heat-sensitive chemicals such as plant growth regulators (Zeatin, IAA, IBA, GA3), enzymes, antibiotics, etc.

Sterilization procedures ; خطوات التعقيم

1- Placing the material in 70% ethyl alcohol solution prior to treatment with another disinfectant solution .The use of two-step (two source) sterilization procedure has proven beneficial with certain species.
2- Using a wetting agent ,(such as TWEEN 20) , can be added to the disinfectants to reduce surface tension and allow better surface contact
3- Conducting the sterilization process under vacuum . This results in the removal of air bubbles and provides a more efficient sterilization process.

Disinfectant used in sterilization

Disinfectant	Concentration %	Exposure (min)
Calcium hypochlorite	9-10	5-30
Sodium hypochlorite	0.5-5	5-30
Hydrogen peroxide	3-12	5-15
Ethyl alcohol	70-95	0.1-0.5
Silver nitrate	1	5-30
Mercuric chloride	0.1-1.0	2-10

Benzalkonium chloride	0.01−0.1	5−20

Orchid Seed Sterilization ;

Orchid seeds are very small and contain little to no food reserves .A single seed capsule may contain 1500− 3000000 seeds . Sowing the seed in vitro makes it possible to germinate immature seed (green pods).It is much easier to sterilize green capsules than individual seeds after the capsule has split open . Lucke (1971) indicated that orchid seed can be sterilized when the capsule is about two –thirds ripe Green.

Questions

1- What are the types of sterilization?
2- What are the chemicals used to sterilize plant parts?
3- What is the relationship between the concentration of the sterilizing substance and the length of the sterilization period?

Lecture No.:	**Lecture 8**
Lecture Title:	**Callus**
Teacher's Name:	**Dr. Khawla Mahmoud Al-Nouh**
Target Group:	**Level 4**
General Objective of the Lecture:	**Introducing the student to callus cells and methods of creating them.**
Behavioral Objectives or Learning Outcomes:	1-The student understands what callus is 2-The student should know how to create callus. **3-** The student should know the media used in the creation of callus**.**
Facilitation Strategies Used	**Explaining the lecture, discussing ideas and sharing experiences**

Acquired Skills	**Full understanding of the prospects of using callus cells**
Accepted Measurement Methods	**Daily tests, brainstorming, and assignments.**

Introductory questions

What are callus farms?

Callus

Callus : is a mass of unorganized parenchyma cells. derived from plant tissue (explant) for use in biological research and biotechnology .plant callus can differentiate into a whole plant process called **regeneration** , through addition of plant hormones in culture medium . Callus cells are not necessarily genetically homogeneous because callus is often made from structural tissue , not individual cells.

biology , callus cells are those cells that cover a plant wound.فراغات

Calls formation is induced from plant tissues after surface sterilization and plating onto in vitro tissue culture medium . plant growth regulators , such as auxins , cytokinins , are supplemented into the medium to initiate callus formation or somatic embryogenesis . callus initiation has been described for many plant taxonomic divisions :

Callus induction and tissue culture

A callus cell culture is usually sustained on **gel** medium . calls induction medium consists of agar and a mixture of macronutrients and micronutrient for the given cell type . there are several types of basal salt mixtures used in plant tissue culture , but most notably modified Murashige and Skoogs medium, White's medium , and woody plant medium . Vitamins are also provided to enhance growth such as Gamborg B5 vitamins.

Callus morphology :

Plant callus is usually derived from somatic tissues . The tissues used to initiate callus formation depends on **plant species** and **which tissues are available for explant culture .**

The cells that give rise to callus and somatic embryos usually undergo **rapid division** or are partially **undifferentiated** such as meristematic tissue . In alfalfa, *Medicago truncatula* , however callus and somatic embryos are derived

from mesophyll cells that undergo dedifferentiation . Plant hormones are used to initiate callus growth . Specific auxin to cytokinin ratios in plant tissue culture medium give rise to an unorganized growing and dividing mass of callus cells . Callus cultures are often broadly classified as being either **compact or friable** . friable calluses fall apart easily , and can be used to generate **cell suspension cultures . Callus can directly** undergo direct organogenesis and / or embryogenesis where the cells will form an entirely new plant .

Death of Callus cells :

Callus can turn brown and die during culture , but the causes for callus browning are not well understood. In *Jatropha curcas* callus cells , small organized callus cells became disorganized and varied in size after browning occurred . Browning has also been associated with oxidation and phenolic compounds in both explant tissues and explant secretions .

Chimera :

Regeneration of a whole plant from a single cell allows researchers to recover whole plants that have a copy of the transgene in every cell .

Chimaera as a definition , in botany , a plant or plant part that is a mixture of two or more genetically different types of cells .

Questions

1- Define each of the following: callus, camera?
2- What are the additives required in the callus initiation medium?
3- What are the forms of callus?

Lecture No.:	**Lecture 9**
Lecture Title:	**Callus morphology**
Teacher's Name:	**Dr. Khawla Mahmoud Al-Nouh**
Target Group:	**Level 4**
General Objective of the Lecture:	**Introducing the student to callus cells callus morphology**
Behavioral Objectives or Learning Outcomes:	**1- The student should know where callus is formed.**

	2- The student should understand the features and benefits of callus. **3- The student understands the uses of callus.**
Facilitation Strategies Used	**Explaining the lecture, discussing ideas and sharing experiences**
Acquired Skills	**The student can create a callus farm.**
Accepted Measurement Methods	**Daily tests, brainstorming, and assignments.**

Introductory questions

can you create a callus farm?

Callus morphology :

Callus : is a mass of unorganized parenchyma cells. derived from plant tissue (explant) for use in biological research and biotechnology .plant callus can differentiate into a whole plant process called **regeneration** , through addition of plant hormones in culture medium . Callus cells are not necessarily genetically homogeneous because callus is often made from structural tissue , not individual cells.

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The cells that give rise to callus and somatic embryos usually undergo **rapid division** or are partially **undifferentiated** such as meristematic tissue . In alfalfa, *Medicago truncatula* , however callus and somatic embryos are derived from mesophyll cells that undergo dedifferentiation . Plant hormones are used to initiate callus growth . Specific auxin to cytokinin ratios in plant tissue culture medium give rise to an unorganized growing and dividing mass of callus cells . Callus cultures are often broadly classified as being either **compact or friable** . friable calluses fall apart easily , and can be used to generate **cell suspension cultures . Callus can directly** undergo direct organogenesis and / or embryogenesis where the cells will form an entirely new plant .

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Lecture No.:	**Lecture 10**
Lecture Title:	**Death of Callus cells**
Teacher's Name:	**Dr. Khawla Mahmoud Al-Nouh**
Target Group:	**Level 4**
General Objective of the Lecture:	**Identifying some of the problems facing callus farms.**
Behavioral Objectives or Learning Outcomes:	**1- The student knows the reasons for the death of callus farms.** **2- The student learns about the symptoms of callus death.** **3- The student learns about the treatments that can be used for the success of callus farms.**
Facilitation Strategies Used	**Explaining the lecture, discussing ideas and sharing experiences**
Acquired Skills	**المهارات المكتسبة**
Accepted Measurement Methods	**Daily tests, brainstorming, and assignments.**

Introductory questions

Is it possible to lose a callus farm?

Death of Callus cells :

Callus : is a mass of unorganized parenchyma cells. derived from plant tissue (explant) for use in biological research and biotechnology .plant callus can differentiate into a whole plant process called **regeneration** , through addition

of plant hormones in culture medium . Callus cells are not necessarily genetically homogeneous because callus is often made from structural tissue , not individual cells.

Callus can turn brown and die during culture , but the causes for callus browning are not well understood. In *Jatropha curcas* callus cells , small organized callus cells became disorganized and varied in size after browning occurred . Browning has also been associated with oxidation and phenolic compounds in both explant tissues and explant secretions .

Lecture No.:	**Lecture 11**
Lecture Title:	**Cellular suspensions**
Teacher's Name:	**Dr. Khawla Mahmoud Al-Nouh**
Target Group:	**Level 4**
General Objective of the Lecture:	**The student understands what cell suspensions are and their potential uses.**
Behavioral Objectives or Learning Outcomes:	**1- The student understands what cell suspensions are.** **2- The student knows where cell suspensions can be used.** **3- The student knows what are the products of cell suspensions.**
Facilitation Strategies Used	**Explaining the lecture, discussing ideas and sharing experiences**
Acquired Skills	**The student will be familiar with the concept of cell suspensions.**
Accepted Measurement Methods	**Daily tests, brainstorming, and assignments.**

Introductory questions

Suspension and protoplast cultures

Suspension and protoplast cultures are cultures of plant cells taken from their natural environment and placed under controlled conditions .

Cell suspension denotes cultures consisting of single cells or the smallest cellular . Association without differentiation , submersed in shaking or turbulent medium .

Protoplasts : are naked cells of varied origin without cell walls , which are cultivated in liquid as well as on solid media .

Totipotency : The ability of a single cell to divide and produce all the dedifferentiated cells in an organism including extra embryonic tissues .

formed during sexual and asexual reproduction include spores and zygotes . Plant cuttings or callus may dedifferentiate and regain their totipotency to form entire plant .

Secondary metabolites :

عمليات الايض الثانوي

Plant cell and tissue cultures can be established routinely under sterile condition from explants , such as plant leaves or stems . Strain improvement , methods for the selection of high –producing cell lines , and medium optimizations **can lead to an enhancement in secondary metabolites must be considered .**

One of the main problems is the lack of basic knowledge of the biosynthetic routes , and mechanisms responsible for the production of plant metabolites .

The lack of particular precursors , biotransformation using an exogenous supply of biosynthetic precursors may improve the accumulation of compounds.

Steps for choosing explant

-Physiological age of the explant :

- Stage of development .
- Younger parts of plants .
- Time of sampling explants during a year or a day
- Different light / dark condition .
- Different temperature conditions .

- Characterized by different degrees of lignification PH optimum conditions are 5.5 to 6.6

Plant cell suspension cultures : زراعة المعلقات الخلوية

Plant cell suspension cultures are widely used in plant biology as a convenient tool for the investigation of a wide range of the structure of the plant .

Moreover , plant cell cultures provide a valuable platform for the production of high – value secondary metabolites and other substances of commercial interest .

Post-questions

1- **What are cell suspensions?**
2- **What are the uses of cell suspensions?**
3- **What are the factors determining the use of plant parts to create cell suspensions?**

Lecture No.:	**Lecture 12**
Lecture Title:	**Protoplast Isolation and Culture**
Teacher's Name:	**Dr. Khawla Mahmoud Al-Nouh**
Target Group:	**Level 4**
General Objective of the Lecture:	**Introducing the student to the meaning of protoplast and its uses**
Behavioral Objectives or Learning Outcomes:	1- The student understands the meaning of physical hybrids. 2- The student knows the benefits of the somatic hybrid. **3-** The student knows the methods of stimulating the formation of somatic .hybrids
Facilitation Strategies Used	**Explaining the lecture, discussing ideas and sharing experiences**
Acquired Skills	**Advantages of protoplast fusion technology that may be impossible with conventional methods**

Accepted Measurement Methods	**Daily tests, brainstorming, and assignments.**

Introductory questions

What is protoplast?

Somatic Hybridization

The theory of producing vegetative hybrids focuses on the ability of genetically different protoplasts to merge together and develop into a complete plant through the different developmental stages. In order to obtain a vegetative hybrid, we must go through two steps:

1. Protoplast fusion

2. Inducing the fused protoplast to enter the developmental stages that lead to the formation of a vegetative hybrid

The theoretical basis for the formation of vegetative hybrids is due to:

The separate protoplast cannot grow due to a defect in some physiological or chemical functions, but when the protoplasts merge, the necessary factors are completed and thus the hybrid continues to grow. In general, the process of selecting the resulting hybrids depends on their ability to grow, unlike other cells that do not have this ability.

Advantages of protoplast fusion technology that may be impossible with conventional methods

1- The possibility of transferring important traits such as resistance to environmental or biological stress from one plant to another that may not be achieved by sexual hybridization.
2- .2 Possibility of obtaining tetraploid plants in case of difficulty using colchicine.
3- Obtaining hybrids from plants that carry the male sterility trait or from plants with incompletely formed sexual organs
4- Hybridization between immature plants that reach sexual maturity after a long period is an important point in plant breeding.
5- It is known that in sexual reproduction only the mother's cytoplasm is transferred to the offspring, but in protoplast fusion the cytoplasm of both parents is transferred to the offspring, thus some traits that may be present in the cytoplasm of the plant that forms pollen grains can be transferred,

such as resistance to some pesticides or male sterility, as the nucleus of one of the parents is excluded by radiation, for example, and the result in this case is called (Cybrid).

Post-questions

1- Define somatic embryos
2- What are the benefits of somatic embryos?

Lecture No.:	**Lecture 13**
Lecture Title:	**Tissue culture stages**
Teacher's Name:	**Dr. Khawla Mahmoud Al-Nouh**
Target Group:	**Level 4**
General Objective of the Lecture:	**The student learns about the stages of preparing a tissue culture.**
Behavioral Objectives or Learning Outcomes:	**1- The student learns about the stages of preparing a tissue culture.** **2- The student knows the concept of the zero stage.** **3- The student knows how to create a sterile tissue culture.**
Facilitation Strategies Used	**Explaining the lecture, discussing ideas and sharing experiences**
Acquired Skills	**The student will be able to establish a tissue culture.**
Accepted Measurement Methods	**Daily tests, brainstorming, and assignments.**

Introductory questions

How do I start a tissue culture farm?

Scientists divided the stages of tissue culture into four stages. Which includes

1- The zero stage, which includes preparing the mother plant before taking the plant parts from it.
2- The developmental stage, during which a tissue culture free of any bacterial or fungal infection can be obtained.
3- The multiplication stage, during which the plant parts can be multiplied by adding growth regulators to the culture medium, which stimulates the multiplication process.

4- Rooting stage, during which the plant parts are stimulated to form roots by adding auxins to the growing medium to stimulate the rooting process.
5- Regionalization

Selection and preparation of stock plants

The mother plants from which the plant parts are taken must have the following characteristics:

1- To be healthy and free from disease and insect infestations.
2- To have good specifications
3- to be actively growing
4- The mother plant must be suitable for the environmental conditions of the region.
5- You must know the best time to take plant parts.

Tissue culture acquisition stage مرحلة الحصول على مزرعة نسيجية معقمة

The success rate of plant cell and tissue cultures depends primarily on the type of plant part and the efficiency of the sterilization process. The main goal is to obtain a sterile tissue culture free of any pathological contaminants. This stage includes:

1- Separation and preparation of the plant part.
2- Sterilization of the plant part and sterilization of the tools used in agriculture in order to eliminate all microorganisms and all types of pollution.

The efficiency of sterilizing the plant part depends on the following factors:

1- The nature of the mother plant from which the plant parts are taken
2- Source of cultivated plant part: leaf, node, growing tip
3- Type and efficiency of sterilizer.
4- Duration of sterilization.
5- Concentration of sterilizer solution.

General questions

What are the stages of propagation by plant tissue culture?

What is meant by the zero stage

How can I obtain a sterile culture?

Lecture Title:	**Tissue culture stages**
Teacher's Name:	**Dr. Khawla Mahmoud Al-Nouh**
Target Group:	**Level 4**
General Objective of the Lecture:	**The student learns about the stages of preparing a tissue culture.**
Behavioral Objectives or Learning Outcomes:	**1- The student should know how the plant part is stimulated to multiply.** **2- The student should understand how multiplication occurs.** **3- The student should understand the physiological changes during the acclimatization process.**
Facilitation Strategies Used	**Explaining the lecture, discussing ideas and sharing experiences**
Acquired Skills	**The student will be able to produce .tissue culture propagated plants**
Accepted Measurement Methods	**Daily tests, brainstorming, and assignments.**

Introductory questions

How do I stimulate the plant part to multiply?

Multiplication of tissues

The aim of this stage is to cause a rapid multiplication of the bud and cell clusters as a result of cultivating the plant part in a nutrient medium prepared for multiplication, which leads at the end of this stage to the formation of vegetative growths or complete plants**.**

There are several methods followed in the multiplication stage, which are:

1- formation of somatic embryos,
2- stimulation of the growth of axillary buds,
3- cultivation of meristematic tissue,
4- formation of lateral branches.

Root formation stage: This stage aims to form roots on the vegetative branches that were formed in the multiplication stage through secondary cultivation on a nutritional medium equipped with one of the auxins that encourage root formation or in a medium free of growth regulators. This process may take 3-4 weeks.

The stage of the Acclimatization

Acclimatization means preparing plants to face external environmental changes by encouraging the plant to produce food through photosynthesis and rely on itself and stimulating the formation of a waxy layer on the surface of the plant, which reduces water evaporation from the plant.

Questions:

What are the stages of tissue propagation?

What does multiplication mean?

What are the physiological changes that occur in the plant during acclimatization?

Lecture No.:	**Lecture 15**
Lecture Title:	**Growing meristematic tips**
Teacher's Name:	**Dr. Khawla Mahmoud Al-Nouh**
Target Group:	**Level 4**
General Objective of the Lecture:	**Introducing the student to meristematic tips, their characteristics and why they are used in agriculture.**
Behavioral Objectives or Learning Outcomes:	**1- The student will understand the location of the meristematic tips.** **2- The student will know why the meristematic tips are used in seedling production.** **3- The student will know how to produce plants free of pathogens.**
Facilitation Strategies Used	**Explaining the lecture, discussing ideas and sharing experiences**
Acquired Skills	**The student knows how to produce pathogen-free plants.**

Accepted Measurement Methods	**Daily tests, brainstorming, and assignments.**

Introductory questions

how can you produce pathogen-free plants?

Shoot tip and meristem culture

Most of the horticultural and forest crops are infected by systemic disease caused by fungi , viruses , bacteria , and nematode . While plant infected with bacteria and fungi may respond to treatments with bactericidal and fungicidal compounds , But there is no commercially available treatment to cure virus infected plants . It is possible to produce disease free plants through tissue culture .

Apical meristems in the infected plants are generally either free or carry a very low concentration of the viruses . The various reasons attributed to the escape of the meristems by virus invasion are ;

a/ Viruses move readily in a plant body through the vascular system which in meristems is absent ,

b/ A high endogenous auxin level in shoot apices may inhibit virus multiplication .

Meristem tip cultures has also enabled plants to be freed from other pathogens including Virus , mycroplasmas , bacteria and fungi . Therefore , shoot tip and meristem tip culture is the production of disease free plants through micro propagation.

Meristem Culture in plants ; Agriculture of axillary or apical shoot meristems , is known as meristem culture .

Shoot tip culture is widely used for rapid clonal propagation for which much larger 5-10 mm explant are used . Therefore , most cases of meristem culture are essentially shoot – tip culture Nodal explants of various sizes are also commonly employed for rapid clonal propagation .

When the objective is vegetative propagation , the size of shoot tip used for culture is not important . But when the objective is to free the stock from a virus , the apical meristem should be excised with a minimum part of the surrounding tissue .

Generally , explants taken from activity growing plants at the beginning of growing season are the most suitable .

Questions:

1- How to produce plants free from viral infections?
2- What is the difference between the growing tip and the apical meristems?