



**Department of Horticulture**

**College of Agriculture**

**University of Salahaddin**

**Subject: Plant Tissue Culture (Theory and Practice)**

**Lecturer's name: Dr. Media Ezaddin Mohammed-Amin**

**Course Book – 4<sup>th</sup> Year Students**

**Course Level: Undergraduate**

**Academic Year: 2023-2024**

## Course Book

<b>1. Course name</b>	<b>Fall Semester</b>
<b>2. Lecturer in charge</b>	<b>Mrs. Media Ezaddin MohammedAmin</b>
<b>3. Department/ College</b>	<b>Horticulture/Agriculture</b>
<b>4. Contact</b>	<b>e-mail: <a href="mailto:media.mohammedamin@su.edu.krd">media.mohammedamin@su.edu.krd</a></b>
<b>5. Time (in hours) per week</b>	<b>Theory: 2 Practical: 2</b>
<b>6. Office hours</b>	
<b>7. Course code</b>	
<b>8. Teacher's academic profile</b>	<b><a href="http://119.13.111.19/profile-admin/index.php?p=teaching">http://119.13.111.19/profile-admin/index.php?p=teaching</a></b>
<b>9. Keywords</b>	<b>Plant tissue culture, in vitro culture, micropropagation, plant tissue culture methods.....</b>
<b>10. Course overview:</b>	
<p>This course provides graduate-level knowledge of and expertise in plant tissue culture theory and practice. This course has a vocational focus and introduces the student to the theory and practice of plant tissue culture and their role from the propagation of endangered plants and from modifying cell lines in biotechnology to the propagation of all lines for use in agricultural, medical, microbiological and biochemical research. It prepares the students in particular for a career with plants, both in plant biotechnology and in environmental biotechnology. Students study media, sterilisation, explants, micro propagation, callus culture, organogenesis, embryogenesis, somatic variation, doubled haploids, interspecific hybrids, protoplast fusion and environmental conditions required. These are related to uses of tissue culture and compared with traditional techniques.</p>	
<b>11. Course objective:</b>	
<ol style="list-style-type: none"> <li>1. Learn the techniques for rapid clonal propagation <i>in vitro</i>.</li> <li>2. Explain the nature of plant growth processes, in the tissue culture environment.</li> <li>3. Determine growing media to use for tissue culture.</li> <li>4. Specify appropriate micropropagation procedures for different purposes.</li> <li>5. Explain the management of environmental control equipment used in tissue culture.</li> <li>6. Design a layout for a commercial tissue culture facility.</li> <li>7. Determine appropriate commercial applications for tissue culture.</li> <li>8. Knowing the importance of plant tissue culture in the improvement of useful crop plants and also the ways in which it has helped mankind.</li> </ol>	

9. Introduction of important techniques useful in plant tissue culture.
10. Development of practical skills in students.
11. To make student competent in plant tissue culture sector.
12. Explain the nature of plant growth processes, in the tissue culture environment.

## **12. Student's Duties & obligation**

The media often focuses on a lecturer's role in making sure that students obtain a quality education. While lecturers do have a strong impact on the learning process, students also have obligations to their lecturers, their classmates and themselves. By fulfilling their fundamental duties, students can contribute to a positive learning experience for everyone in the classroom.

### **Attendance**

Students should make every effort to maintain good attendance in their classes. Illnesses and emergencies do occur, so it may not be possible to show up every time. Nevertheless, students should do their best to consistently attend their lectures and get there before class begins. Missing college can lower attendance grades and result in missed quizzes or assignments. If a student misses a class, it is their responsibility to ask for a friend's notes and talk to the lecturer to determine whether an important announcement was made.

### **Participation**

Each student should participate in the classroom. Discussing relevant subjects at appropriate times can spark new conversations and produce valuable debates. If lecturers ask students to share thoughts with their respective groups, each student should contribute to the assignment. Students who are shy do not have to take a leadership role, but they can offer to take notes and add a few ideas.

### **Questions**

Asking questions about unclear material is an important part of the classroom experience. It is not uncommon for students to have similar difficulties, so speaking up will help everyone understand the discussed information. Lecturers can also benefit from a student's questions. By finding out what subjects are hard to understand, lecturers can adjust their lectures to clear up confusing topics.

### **Respect**

Students need to respect the ideas and opinions of their classmates in and outside of the classroom. They should not ridicule someone for having a different viewpoint, and they should be willing to listen to alternative perspectives. Students can debate the merits and drawbacks of diverse viewpoints as long as it is done in a constructive manner. Not everyone will agree, but listening to new ideas can help people develop their thoughts and see subjects in a new light.

### **Preparation**

Lecturers expect students to study outside of the classroom. Students should complete assigned reading before class begins, so they can contribute their thoughts to new discussions. Working on assignments during a lecture can distract other students and interfere with a lecturer's lesson plan. Students who attempt to work during class may also miss out on valuable information.

**13. Forms of teaching**

The course would be taught through lectures, demonstrations(White board presentation) , Data show for powerpoint presentation, practicals and vedio show of different techinques in plant tissue culture.

**14. Assessment scheme**

Lecture and Seminar

Theoretical part: Two exams: 15% & final exam 50%

Practical part: Two exams 20% Students scientific debate 5% & student activity 5%.

**15. Student learning outcome:**

This course will develop the graduate capabilities of knowledge ability, comprehension and applications of plants in cell and tissue culture systems, and how cell and tissue culture contributes to global sustainability. It will also develop the practical skills and confidence of students to successfully culture plant cells and tissues.

*By the end of the course, students should be able to:*

1. Understand the basic concepts in plant tissue culture
2. Discuss the process involved in micro propagation
3. Analyse the practical use of tissue culture in life
4. Explain the various components of plant tissue culture media, e.g. minerals, growth factors, hormones, and what governs the choice of components.
1. Establish and maintain plants in tissue culture and micropropagation, including morphogenesis.
2. The capacity to regenerate plants in sterile environments
3. The use of plant tissue culture techniques for both research and commercial purposes
4. Focuses on course work that prepares the student for immediate employment in the plant tissue culture industry.
5. The students also:
  - a. Would be able to initiate tissue culture and establish their own lab.
  - b. Would be skilled to execute/ accomplish micro propagation through budculture, organogenesis and somatic embryogenesis.
  - c. Can develop/produce artificial seeds.

**16. Course Reading List and References:**

Students will be provided with lists of relevant texts, library resources (Including appropriate journals) and freely accessible Internet sites. Other material will be provided in class.

Students will be able to access course information and learning material through the

Recommended texts for this course are:

- Dixon, R.A., and Gonzalez, R.A. (Eds) (1995). Plant Cell Culture: a Practical Approach, 2nd edn. IRL Press at Oxford University Press, Oxford, UK.
- Collin, H.A., Edwards, S.(1998). Plant Cell Culture. BIOS Scientific Publishers, Oxford, UK.
- Hudson T Hartmann : Plant Propagation-Principle and Practices
- Chopra V L, Sharma R P & Swaminathan M S: Agricultural Biotechnology
- Kalyan Kumar D : An introduction to Plant Tissue Culture
- Hamish A, Collin & Sue Edwards: Plant Cell Culture
- Razdan M K : An Introduction to Plant Tissue Culture Gupta P K: Elements of Biotechnology
- Robert N. Trigiano and Dennis J. Gray. 2011. Plant Tissue Culture, Development, and Biotechnology
- Cell Biology (India Ed.)-C. B. Powar (2003).
- 2. An Introduction to Plant Tissue Culture M. K.Razdan, Oxford and IBII publishing Co. pvt. Ltd., New Delhi.
- 3. Fundamentals of plant Biotechnology by AmlaBatra Capital Publishing Company.
- 4. Introduction to Plant Tissue Culture, Bhojwani and Rozdan.
- 5. A textbook of Biotechnology by R. C. Dubey.
- 6. Plant Biotechnology by K. G. Ramawat.
- 7. Plant Tissue Culture: K. K. De (New central Book Agency)
- 8. Applied and fLndamental aspects of plant cell, tissue and organ culture:J.Reinert and Y. P. S. Bajaj.

**Suggested Reading** : Experiments in Plant Tissue Culture (Dodds, J.H. and Roberts, L.W.) 1985; Plant Tissue Culture methods and application in agriculture (Thorpe, T.A.) 1981; Journal of Plant Cell & Tissue Culture; Plant Cell, Tissue & Organ Culture; Journal of Biotechnology.

**17. The Topics:**  
**Lecturer's name:**

**Dr. Media Ezaddin M.-Amin**

**Lecture 1**

Brief History of plant tissue culture, Introduction, Concept of cellular totipotency, Dedifferentiation, Redifferentiation, Regeneration present status and future.

**Lecture 2**

Plant tissue culture laboratory & Aseptic Manipulation

**Lecture 3**

Composition of Plant Tissue Culture Media and the role of growth regulators in plant tissue culture.

**Lecture 4**

Micropropagation and the Growth Factors affected it.

**1<sup>st</sup> Exam**

**Lecture 5**

**Types of Plant Tissue Culture Technique:** Meristem culture- Virus elimination

**Lecture 6**

**Types of Plant Tissue Culture Technique:** Callus Culture

**Lecture 7**

**Types of Plant Tissue Culture Technique:** Anther/Pollen culture

**Lecture 8**

**Types of Plant Tissue Culture Technique:** Embryo culture

**2<sup>nd</sup> Exam**

**Lecture 9**

**Types of Plant Tissue Culture Technique:** Suspension culture, Protoplast isolation, protoplast culture and fusion.

**Lecture 10**

**Types of Plant Tissue Culture Technique:** Plant Cell Culture

**Lecture 11**

**Somaclonal variation**

<p><b>Final examination</b></p>	
<p><b>18. Practical Topics</b>  <b>Lecturer's name:</b>  <b>Dr. Media Ezaddin M.-Amin</b></p>	
<p><b>Course objective:</b></p> <ol style="list-style-type: none"> <li>1. To acquaint students with the principles, technical requirement, scientific and commercial applications of plant tissue and organ culture.</li> <li>2. Work under aseptic conditions to cultivate different plant species and/or parts <i>in vitro</i>. Learn how to subculture and follow the growth pattern of the cultures.</li> <li>3. To motivate students to set goals towards pursuing graduate school and higher level positions, such as lab manager and key scientist in plant biotechnological research institutes and industries.</li> <li>4. Practice scientific thinking in analyzing the experiments, keeping records, and presenting results.</li> </ol> <p><b>Note:</b> All lectures were applied practically in plant tissue culture lab. In Horticulture Dept. –College of Agriculture/Salahaddin University.</p> <p><b>PRACTICALS:</b></p> <ol style="list-style-type: none"> <li>1. Laboratory Safety and Basic Requirements for Plant Tissue culture.</li> <li>2. Plant Tissue Culture Lab Organization &amp; Sterilization Techniques.</li> <li>3. Preparation of Plant Tissue Culture Media.</li> <li>4. <b>Sterilization of Plant Tissue Culture components:</b> Media, Explant, glassware.</li> </ol> <p><b>1<sup>st</sup> Exam</b></p> <ol style="list-style-type: none"> <li>5. <b>Clonal propagation:</b> Meristem, Shoot-tip and axillary bud culture.</li> <li>6. Sub culturing and Regeneration of roots, shoots and plants.</li> <li>7. <b>Inoculation of explants,</b> Callus Induction &amp; micropropagation.</li> <li>8. Preparation of regenerated plants for hardening.</li> </ol> <p><b>2<sup>nd</sup> Exam</b></p> <ol style="list-style-type: none"> <li>9. <b>Somatic embryogenesis</b> from suitable explant</li> <li>10. <b>Anther culture:</b> Regeneration of plants from anthers/pollens.</li> <li>11. <b>Embryo culture:</b> culturing bean embryos in MS media.</li> </ol> <p><b>Final Exam</b></p>	

19. Examinations:

**1. Compositional:** In this type of exam the questions usually starts with Explain how, What are the reasons for...?, Why...?, How....?

**For example:**

- What is the reason behind the following:-
- What are the advantages and disadvantages of ( )
- Identify and explain two problems that might occur as a result of the use of (.....)
- List three ways of ( )

**2. True or false type of exams:**

• Put (T) for true statement and (F) for false statement then correct the mistakes. In this type of exam a short sentence about a specific subject will be provided, and then students will comment on the trueness or falseness of this particular sentence. Examples should be provided

For instance:

- Fill in the blanks with suitable terms:
- **Fill in the blank:** Plant tissue culture involves the growth of plant cells, tissues, or organs in a \_\_\_\_\_ environment.
- Correct the underlined parts if they are false:
- Compare ( ) with ( )
- Draw distinctions/differences between ( ) and ( )

**3. Multiple choices:**

In this type of exam there will be a number of phrases next or below a statement, students will match the correct phrase. Examples should be provided.

For example:

Choose correct answer (single choice or multiple choice): for example:

**Q/** The pair of hormones required for a callus to differentiate are\_\_\_\_\_.

- (a) Ethylene and Auxin
- (b) Auxin and cytokinin
- (c) Auxin and Abscisic acid
- (d) Cytokinin and gibberellin

**Sol: (b) Auxin and cytokinin**

**4. Problem-solving:** Design an experiment to test the effectiveness of a specific plant tissue culture technique for improving the growth of a certain crop plant.

**5. Another styles of Questions may serve students:**

- Define following terms?
- Draw a scheme or picture of ( )
- Describe two ways of ( )
- Name the substance added to the culture medium to induce callus induction.
- Describe three different practices that can be used to ( )



- **Matching:** Match the following plant tissue culture techniques with their descriptions: a) Micropropagation b) Somatic embryogenesis c) Callus culture
- Production of large numbers of identical plants
- Formation of embryos from somatic cells
- Growth of undifferentiated cells on a solid medium

### SHORT QUESTIONS

- Discuss the role of meristematic tissue in plant tissue culture.
- How can plants in tissue culture be prepared for transfer to the natural environment?
- What are the components of a typical plant tissue culture medium?
- Describe the roles of auxin in plant development and plant tissue culture.
- Describe the role auxins and cytokines play in plant tissue culture.
- How may embryo culture be used for plant conservation?
- Outline the role played by Arabidopsis thaliana in plant science research.
- Describe the role auxins and cytokinins play in plant tissue culture.
- Define the following three terms as they relate to plant tissue culture:
  - Totipotency
  - Dedifferentiation
  - organogenesis.
- Describe two possible methods of protoplast fusion.
- Write short notes on two of the following:
  - (a) The value of plant secondary metabolites to industry.
  - (b) The typical components of a plant tissue culture medium.
  - (c) The advantages of micro propagation over other forms of plant propagation.
- Describe the role of protoplasts and protoplast fusion in artificially overcoming barriers to sexual reproduction in plants.
- Outline the options available for culturing protoplasts in vitro.
- Briefly describe the roles of auxin and cytokinin in plant tissue culture.

### LONG QUESTIONS

- Discuss the role of auxins and cytokinins in plant tissue culture. How does the balance of these two plant growth hormones impact on plant development?
- Micro propagation is one of the most important uses of plant tissue culture techniques. Describe the typical stages for establishing and successfully propagating a plant in vitro. For each stage, indicate the use, where relevant, of plant growth regulators. [50 marks]
- Outline how plant growth regulators can be used in plant tissue culture systems. How does the use of plant growth regulators differ with each stage of micro propagation?