PARVATHANENI BRAHMAYYA
SIDDHARTHA COLLEGE OF ARTS & SCIENCE
Autonomous
Siddhartha Nagar, Vijayawada–520010
Re-accredited at 'A+' by the NAAC

Course Code				**23BOMAP234**			
Title of the Course				**Plant Biotechnology**			
Offered to: (Programme/s)				**B.Sc. Hons Botany**			
L	**0**	**T**	**0**	**P**	**2**	**C**	**1**
Year of Introduction:	**2024-25**			**Semester:**			**3**
Course Category:	**MAJOR**			**Course Relates to:**	**GLOBAL**		
Year of Introduction:	**2024**			**Percentage:**	**NA**		
Type of the Course:				**SKILL DEVELOMENT, Employability**			
Crosscutting Issues of the Course :				**Environment and Sustainability**			
Pre-requisites, if any				**Basics of Plant Tissue Culture techniques**			

Course Description:

The course deals with the study of plant life and application of technical approaches to biological environments and living organisms.

Students undertaking this course will be introduced to concepts and applications of modern plant biotechnology in agriculture. Areas to be covered include: Introduction to plant biotechnology; Tissue culture media and preparation; Sterilization techniques; In vitro micropropagation; Application of tissue culture to plant breeding; Introduction to molecular biology; Genome organization, structure and function; Basic molecular techniques; PCR based techniques; Genetic markers; Applications of molecular; Gene Cloning; Gene transfer in plants; Transgenics in crop improvement; and Impact of recombinant DNA technology.

Course Aims and Objectives:

S. No	COURSE OBJECTIVES
1	To familiarize the students with the key developments in the sphere of Plant Biotechnology.
2	To understand the basics principles of Plant Tissue culture Techniques.
3	To Learn Basic Sterilization Techniques used in Plant Tissue culture.
4	To acquire Knowledge of secondary metabolites and Biotransformation Techniques
5	To Know the Applications of Transgenic plants

Course Outcomes

At the end of the course, the student will / will be…

NO	COURSE OUTCOME	BTL	PO	PSO
CO1	To understand the basics principles of plant sciences and molecular biology.	**K2**	**2**	**1**
CO2	To have a knowledge of laboratory techniques used in plant biotechnology.	**K1**	**2**	**1**
CO3	To understand the industrial applications of biotechnology in developing new products.	**K2**	**2**	**1**
CO4	To gain research knowledge in plant biotechnology.	**K1**	**2**	**1**
CO5	Gain basic knowledge on trait improvement in plants.	**K1**	**2**	**1**

CO-PO-PSO MATRIX									
CO NO	**PO1**	**PO2**	**PO3**	**PO4**	**PO5**	**PO6**	**PO7**	**PSO1**	**PSO2**
CO1		**3**						**3**	
CO2		**3**						**3**	
CO3		**3**						**3**	
CO4		**3**						**3**	
CO5		**3**						**3**	

Course Structure:

Unit – I: (6 Hrs)

Practical 1 Equipment used in plant tissue culture

Aim: Requirements for Plant Tissue Culture Laboratory.

Laboratory Requirements: 'Plant tissue culture' or in vitro cultivation of plants basic requirements:

(a) Cultivation should be done under aseptic conditions.

(b) The isolated plant part should get an appropriate environment which will help to divide the cell and to get an expression of internal potential.

Basic facilities for plant tissue culture operations involving any type of in-vitro procedures must include:

(a) Washing and storage facilities;

(b) Media preparation, sterilisation and storage room;

(c) Transfer area for aseptic manipulations;

(d) Culture rooms or incubators for maintenance of cultures under controlled conditions of temperature, light and humidity;

(e) Observation or data collection area;

(f) Transplantation area.

Washing and Storage Facilities:

An area with large sink (lead lined to resist acids and alkalis) and draining area is necessary with provision for running water, draining-boards or racks and ready access to a de-ionized, distilled and double-distilled apparatus. Space should also be available to set up drying ovens, washing machines, plastic or steel buckets for soaking labware, acid or detergent baths, pipette washers, driers and cleaning brushes. For storage of washed and dried labware, the laboratory should be provided with dustproof cupboards or storage cabinets.

Media Preparation Room or Space:

This part is the central section of the laboratory where most of the activities are performed i.e., media preparation and sterilisation of media and glassware's needed for culture. There should be sufficient working bench as well as storage space.

The following items are essential in the room:

(i) Different types of glassware

(ii) Different kinds of balances

(iii) Required chemicals

(iv) Hot plates and Stirrer

(v) Water bath

(vi) pH meter

(vii) Autoclave and Hot air oven

(viii) Microwave oven

(ix) Vortex, Shaker

(x) Centrifuge

(xi) Refrigerator and Freezer

(xii) Storage cabinet (Dust-free)

Transfer Area:

Tissue culture techniques can only be successfully carried out in a very clean laboratory having dry atmosphere with protection against air-borne microorganisms. For this purpose a sterile dust-free room/cabinet is needed for routine transfer and manipulation work.

The 'laminar air flow cabinet' is the most common accessory used for aseptic manipulations now-adays. The cabinet may be designed with horizontal air flow or vertical air flow where the air is forced into the cabinet through a bacterial HEPA (High Efficiency Particulate Air) filter. The air flows over the working bench at a constant rate which prevents the particles

(microorganisms) from settling on the bench. Before operation in the laminar air flow cabinet, the interior of the cabinet is sterilised with the ultraviolet (UV) germicidal light and wiping the floor of cabinet with 70% alcohol. Inoculation chamber, a specially designed air tight glass chamber fitted with UV light, may also be used as transfer area.

Culture Room:

Plant tissue cultures should be incubated under conditions of well-controlled temperature, illumination, photoperiod, humidity and air circulation. Incubation culture rooms, commercially available incubator cabinets, large plant growth chambers and walk-in-environmental rooms satisfy these requirements. Culture rooms are constructed with proper air-conditioning; perforated shelves to support the culture vessels, fitted with fluorescent tubes having a timing device to maintain the photoperiod, black curtains may be used to maintain total darkness. For the suspension cultures, gyratory shakers are used. Air conditioners and heaters are used to maintain the temperature around 25 ± 2°C and humidity is maintained by uniform forced air-ventilation.

Data Collection Area:

The growth and development of tissues cultured in vitro are generally monitored by observing cultures at regular intervals in the culture room or incubators where they have been maintained under controlled environmental conditions. Arrangement should be there where the observations can be done under aseptic conditions using microscope. Special facilities are required for germplasm conservation i.e., cryopreservation accessories should be there.

Transplantation Area:

Plants regenerated from in vitro tissue culture are transplanted to soil in pots. The potted plants are ultimately transferred to greenhouse but prior to transfer the tissue culture grown plants are allowed for acclimatization under well humid condition and controlled temperature and under controlled entry of sunlight.

Practical 2: Sterilization Techniques in plant tissue culture laboratory.
Sterilization Procedure:

Principle: The culture medium, especially when it contains sugar, will also support the growth of micro-organisms like bacteria, fungi etc. So if they come in contact with medium either in cellular form or in spore form, the micro-organisms grow faster than the higher plant tissue due to their brief life cycle and will kill the tissue. The micro-organisms may come from glass vials, instruments, nutrient medium used for culture and even from plant material itself. Therefore, the surface of plant tissue and all non-living articles including nutrient medium should be sterilized.

Procedure:

(i) Sterilization of non-living Articles: The routine sterilization procedure of non-living articles such as nutrient medium, glass goods, distilled water, instruments (wrapped with brown paper) is by autoclaving under steam at a pressure of 15 lb/in2 and a temperature of 120°C for 15 minutes. Thermolabile compounds are often added in the medium and such medium is sterilized either at room temperature or in cold by passing through bacterial filter.

An alternative method of sterilizing glass goods and instruments is by heating in an oven at 150°C for 34 hrs. It should be noted that when autoclaving screw capped glass vials, care should be taken to ensure that the caps are not closed too tightly so that gases can expand without the risk of explosion.

Unit – II: (6 Hrs)

Practical 3. Preparation of culture media

Principle: Isolated cell, tissues and organs need nutrients for their in vitro growth and development. So, nutrients are supplied artificially according to the medium formulated by several workers. The main objective of medium preparation is to culture the cell, tissue and organ in vitro.

Procedure:

Media should be prepared with care and the following procedure is recommended. To make 1litre of MS medium: (i) Dissolve 30gms cane sugar in 200 ml DDH2 O. Mix 1-2gms activated charcoal and filter through filter paper, set inside the Buchner funnel fitted on a special conical flask with small side arm attachment. Filtering is done by using a suction pump. (ii) Take DDH2 O in another flask and add in sequence the appropriate amount of stock solution as follows

Stock solution of macrosalts	50 ml
Stock solution of microsalts	1 ml
Stock solution of KI	1 ml
Stock solution of Fe-EDTA	5 ml
Stock solution of MS 3 vits	1 ml
Stock solution of Glycine	1 ml
Stock solution of meso-inositol	2 ml

Desired concentrations of auxin and/or cytokinin are added from stock solution according to the formula: Desired concentration/Stock concentration = amount (ml) of stock solution to be taken for one litre medium.

If the quantity of the medium is less than one litre, then hormones are added using another formula— Required concentration X Volume of medium/Stock concentration X1, 000 = amount (ml) of stock solution to be added.

(iii) Pour filtered sucrose solution and salt, vitamins, amino acid, hormone solution mixture into a one litre measuring cylinder. Make the final volume to one litre with DDH2 O. Shake well to mix up uniformly.

(iv) Adjust the pH of the liquid medium 5.6-5.8 with the aid of 0.1(N) HCl or 0.1(N) NaOH. This operation is done using the pH metre.

(v) Add 5% to 8% agar to the liquid medium to make solid medium. Heat to 60°C to dissolve the agar completely. Otherwise, without adding agar, liquid medium can be used for culture.

(vi) Dispense the culture medium into culture tube (20 ml/tube) or wide mouth conical flask (25-40 ml/flask). Insert non-absorbent cotton plug wrapped with gauge cloth. Cover the plug with the help of brown paper and rubber band.

(vii) Medium is finally sterilized by autoclaving.

Practical 4: Callus induction and sub culturing

Aim:- To induce callus from explant. Callus is an actively-dividing non-organized mass of undifferentiated and differentiated cells often developing either from injury or in tissue culture in the presence of growth regulators. Explants from both mature and immature organs can be induced to form callus. However, explants with mitotically active cells (young, juvenile cells) are generally good for callus initiation. Callus is produced on explants invitro from peripheral layers as a result of wounding and in response to growth regulators,either endogenous or exogenously supplied in the medium. The season of the year, donor conditions of the plant, the age and physiological state of the parent plant contribute to the success of organogenesis in cell cultures.Growth regulator concentration in the culture medium is critical for morphogenesis. Auxin, at a moderate to high concentration, is the primary hormone used to produce callus. In some species, a high concentration of auxin and a low concentration of cytokinin in the medium promotes abundant cell proliferation with the formation of callus. Callus may be serially subcultured and grown for extended periods, but its composition and structure may change with time as certain cells are favoured by the medium and come to dominate the culture.

Reagents and other requirements

1. Culture tubes or conical flasks containing media

2. Sterile Petri dishes

3. Scalpel, blades, forceps and steel dissecting needles

4. Sterile distilled water

5. Alcohol

6. Detergent (Tween 20, Teepol, etc.)

7. Sterilants – HgCl2, Sodium Hypochlorite

8. Nutrition medium reagents – MS basic salts and vitamins Growth regulators – 2, 4-D

Plant material – Green gram

Media

Seed Germination: MS Medium Callus Induction: MS + 2, 4-D (2mg/lL) I. Seed Germination

1. The seeds washed by submerging in water with a few drops of detergent in a beaker with vigorous shaking.

2. The seeds were submerge in 70% alcohol for 40 s after which the alcohol was decanted.

3. The seeds were transfer to a flask containing 20% commercial sodium hypochlorite solution and left there for 20 min for surface sterilization. Later they were rinsed thrice with sterile distilled water.

4. 2-3 seeds were placed on the surface of MS medium and incubated at 25oC for 16h photoperiod with 250µE/m2/ s light intensity for 2 weeks.

5. Observe regularly for germination. If need be, transfer the individual plantlets to half MS medium.

II. Callus Induction

1. The leaves were removed from in vitro germinated seeds and were cut into pieces and placed on the MS medium. As a control measure, some explants should be inoculated on MS medium without hormones.

2. The cultures were incubated in dark at 25oC. Callus started appearing within 2 weeks and good callus growth can be observed in 3-4 weeks.

3. Callus can be sub-cultured after the 4th week on fresh medium with the same composition.

Result: The undifferentiated mass of cells was formed from the inoculated leaf explant.

Unit – III: (6 Hrs)

Practical 5: Organogenesis using PGRs

Practical 6: Demonstration of cell protoplast cultures

Unit – IV: (6 Hrs)

Practical 7: Demonstration of organ culture

Unit – V: (6 Hrs)

Practical 8 **:** Demonstration of another and pollen culture.

Virtual labs/demos

RFERENCE WEB LINKS:

https://www.onepointesolutions.com/blog/tissue-culture-lab-equipment/

https://labassociates.com/4-methods-of-sterilization-used-in-plant-tissue-culture

Question Paper Pattern for Practical Course

(A) Semester End Lab Examination

23BOMAP234: Plant Biotechnology **Offered to: B.Sc. Hons Botany**

Semester: III **Max.Marks: 50 (CIA+SEE)**

Max. Time: 3 Hrs

I. Answer the following. **Max. Marks: 30 Marks**

Q1. Perform the given experiment '**A**' and write the preparation procedure. 8M

Q2. Write the procedure for given experiment **'B'**. 8M

Q3. Write the procedure for given experiment **'C'**. 8M

Q4. Identify and write a note on '**D**' & '**E**'. 3M

Q5. Identify and write a note on '**F**'. 3M

II Viva **3 Marks**

III Record **2 Marks**

(B) CONTINUOUS ASSESMENT(Internal) **15 MARKS**

15 marks for the continuous assessment (Day to day work in the laboratory shall be evaluated for 15 marks by the concerned laboratory teacher based on the regularity/ record/viva). Laboratory teachers are mandated to ensure that every student completes 80%-90% of the lab assessments.

TOTAL: (A)+(B) = **50 MARKS**

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