On the occasion of Dr. Panjabrao alias Bhausaheb Deshmukh's 125th Birth Anniversary year, R. T. M. Nagpur University Centenary Year

Department of Botany in collaboration with Dept. of Microbiology, Shri Shivaji Science College Congress Nagar Nagpur.

WORKSHOP ON PLANT TISSUE CULTURE

9 February, 2024

10 am Onwords

Botany Research Lab

Objective :The primary goal of the Plant Tissue Culture Training Program was to provide a practical understanding of Plant Tissue Culture Techniques to B.Sc. III students. Through this program, participants were equipped with the necessary skills and knowledge to engage effectively in tissue culture methodologies..

Prof. Atul Bobdey
Coordinator
Dept. of Biotechnology

Prof. Mahendra Dhore
Chairman & Principal
Science College, Nagpur

Prof. Rajendra Deshmukh
Head
Dept of Botany

Coordinators

Dr. Pranita Gulhane **Prof. Punita Tiwari**

Organizers

Ms. Shruti Agrawal **Ms. Aishwarya Zure**
Ms. Mayri Bhad **Ms. Nupur Deshmukh**

NOTICE

All the students of B.Sc. SEM VI , Botany are here by informed that Department of Botany is organising Workshop on Plant Tissue Culture technique. Interested students can contact coordinator Dr Punita Tiwari.

Date: 9.2.2024
Venue : Department of Botany

Head, Dept of Botany
Prof. R.N.Deshmukh
HEAD
DEPARTMENT OF BOTANY
SHRI SHIVAJI EDUCATION SOCIETY
AMRAVATI'S SCIENCE COLLEGE
CONGRESS NAGAR, NAGPUR

Science College, Congress Nagar, Nagpur
SEAL

Coordinator
Prof. P.S.Tiwari

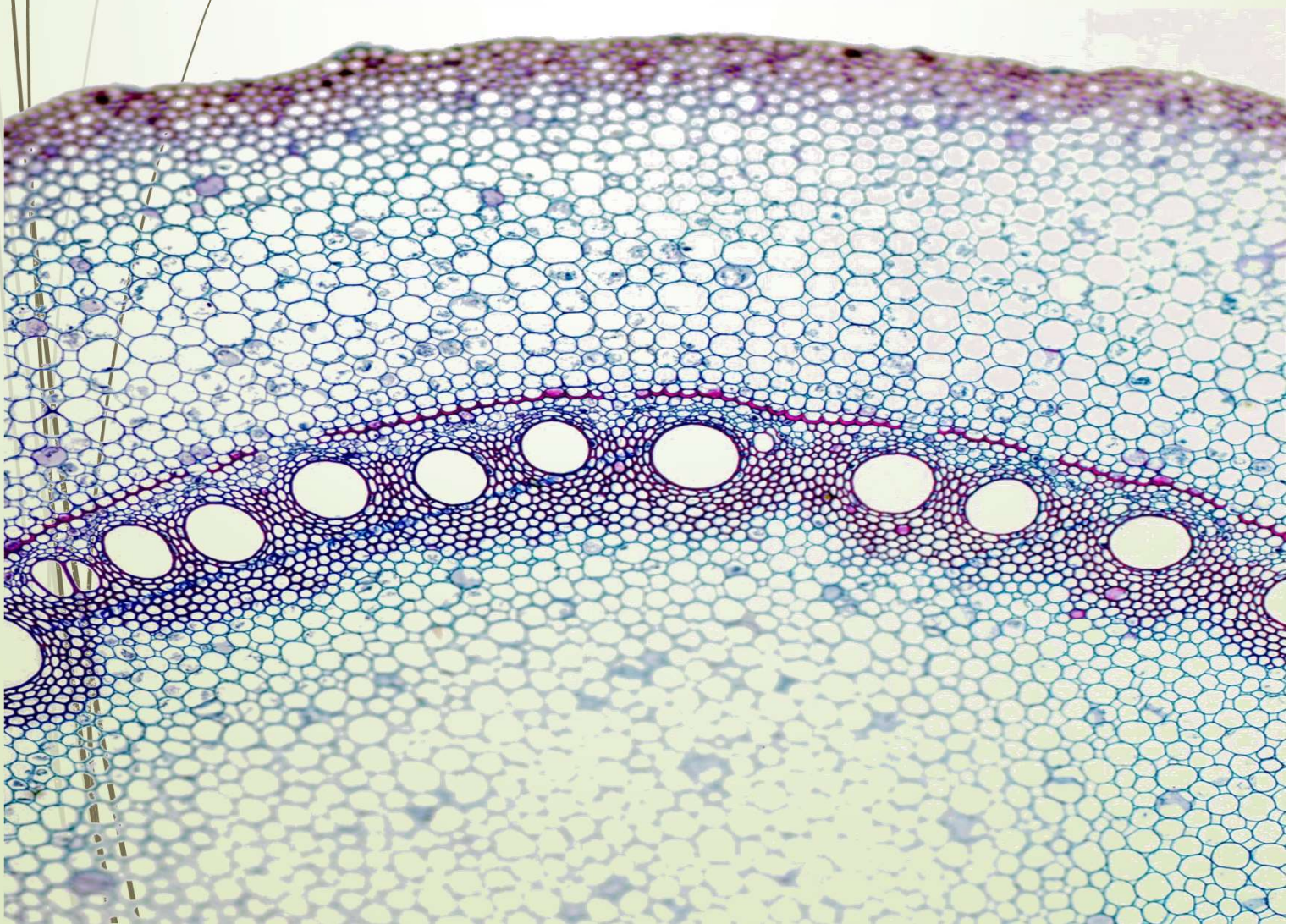
INTRODUCTION

Plant tissue culture refers to a set of techniques used to grow and maintain plant cells, tissues, or organs under sterile conditions in a nutrient medium, to proliferate cells, each one of which can be converted into whole plant.

This controlled environment allows researchers to manipulate plant growth and development in vitro culture, or outside of the organism's natural environment. Plant tissue culture is a valuable tool in plant biology, agriculture, horticulture, and biotechnology, enabling processes such as micropropagation (mass cloning of plants), genetic transformation, germplasm preservation, and production of secondary metabolites.

TOTIPOTENCY

- Totipotency is the ability of plant cells to regenerate into a whole plant under appropriate conditions. This means that a single plant cell, tissue, or even an organ has the potential to develop into an entire plant with shoots, roots, and leaves.
- **Haberlandt (1902)** developed initially the in vitro technique to demonstrate the totipotency of plant cells.



PREPARATION OF SUITABLE NUTRIENT MEDIA

Preparation of Murashige and Skoog (MS) media is a crucial step in plant tissue culture. MS medium is one of the most commonly used nutrient media for the growth and development of plant tissues in vitro

- Ingredients:
- MS Basal Medium Powder
- Sucrose (sugar)
- Vitamins (e.g., thiamine, pyridoxine, nicotinic acid)
- Plant Growth hormone
- Agar (if making solid medium)
- Distilled water

- Adjusting pH:

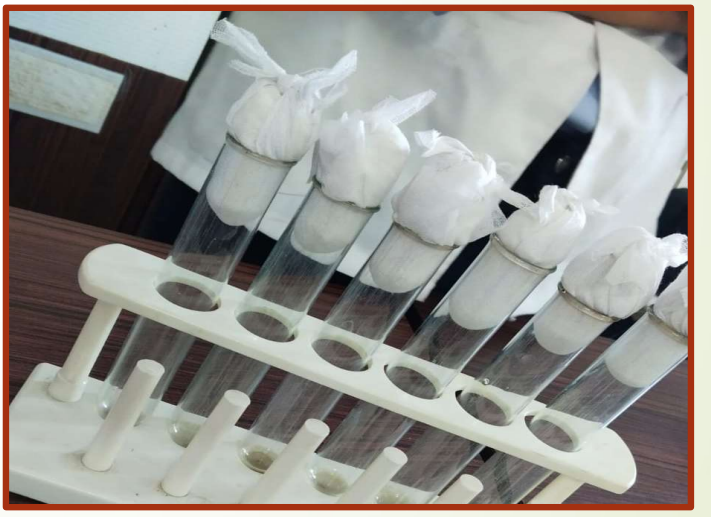
- Check the pH of the medium using a pH meter or pH indicator strips.
- Adjust the pH to the desired range (typically around pH 5.6 for MS medium) using potassium hydroxide (KOH) or hydrochloric acid (HCl) dropwise.

COMPOSITION OF MS MEDIUM FOR TISSUE CULTURE OF PLANT

Chemical	Formula	Concentration
Macronutrients (10 X)		100 mL/L
Ammonium nitrate	NH_4NO_3	16.5
Potassium nitrate	KNO_3	19.0
Calcium chloride	$CaCl_2.2H_2O$	4.4
Magnesium sulfate	$MgSO_4.7H_2O$	3.7
Potassium dihydrogen orthophosphate	KH_2PO_4	1.7
Micronutrients (100 X)		10 mL/L
Manganese sulphate	$MnSO_4.4H_2O$	2.23
Zinc sulphate	$ZnSO_4.7H_20$	0.86
Potassium iodide	KI	0.086
Cupric sulphate	$CuSO_4.5H_2O$	0.0026
Sodium molybdate	$Na_2MoO_4.2H_2O$	0.025
Cobalt (ous) chloride	$CoCl_2.6H_2O$	0.0026
Boric acid	H_3BO_3	0.62
Vitamin source (100 X)		10 mL/L
Nicotinic acid	$C_6H_5NO_2$	0.05
Thiamine hydrochloride	$C_{12}H_{17}CIN_4OS.HCl$	0.01
Pyridoxine hydrochloride	$C_8H_{12}N_2O_2.2HCl$	0.05
Glycine	$C_6H_{12}O_6$	0.2
Iron source (100 X)		10 mL/L
Sodium EDTA	$C_{10}H_{14}N_2O_8Na_2H_2O$	2.78
Ferrous sulphate	$FeSO_4.7H_2O$	3.72
Myo-inositol		0.1 g (freshly add)
Sucrose	$C_2H_5NO_3$	30 g
Phytagel		2 g

PREPARATION OF COTTON PLUGS

- Preparing a cotton plug for plant tissue culture involves ensuring sterility to prevent contamination of the tissue culture medium and plant samples.
- Take a small amount of cotton and roll it into a tight ball or cylinder shape.
- Ensure that the cotton plug is compact but not too tight to prevent hindering airflow



SOUND COMES WHILE REMOVING PLUG OF TEST TUBES

CALLUS CULTURE

- **Callus culture is a technique used in plant tissue culture where undifferentiated mass of cells, often arising from explants, is cultured on a nutrient medium. Here's an overview of callus culture:**
- 1. Initiation: The process begins by selecting and preparing the explants. These can be various plant tissues such as leaf, stem, root, or embryo. The explants are then placed onto a suitable nutrient medium containing plant growth regulators (auxins and cytokinins) that stimulate the formation of callus.
- 2. Callus Formation: Over time, the cells from the explant begin to proliferate and form an undifferentiated mass of cells known as callus. Callus formation is influenced by factors such as the type and concentration of plant growth regulators in the culture medium, as well as the genotype and physiological state of the explant.

- 3. Subculture: Once callus has formed, it can be sub-cultured onto fresh medium to promote further growth and proliferation. Subculturing involves transferring a portion of the callus onto a new medium to prevent overcrowding and maintain optimal growth conditions.

SELECTION OF EXPLANT

- Vigna radiata L., commonly known as mung bean, is often used as an explant in plant tissue culture for several reasons:

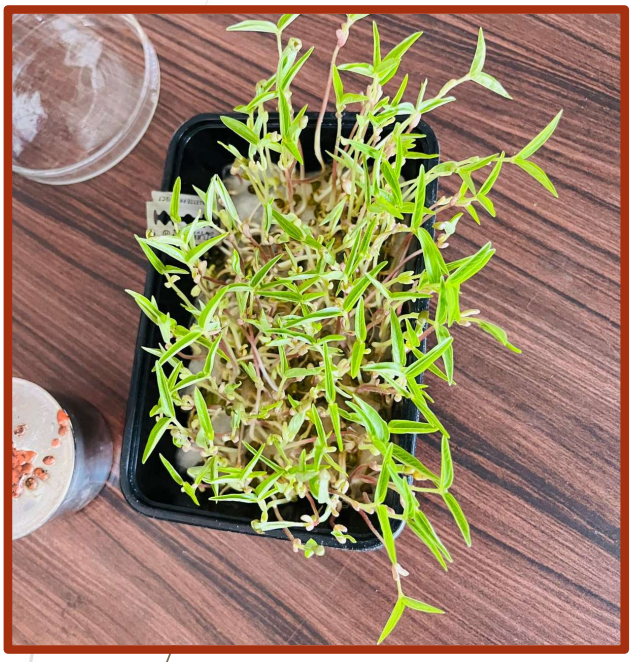
- 1. Ease of Culture: Mung bean is relatively easy to grow and maintain under laboratory conditions. It has a shoVigna radiata L., commonly known as mung bean, is often used as an explant in plant tissue culture for several reasons:

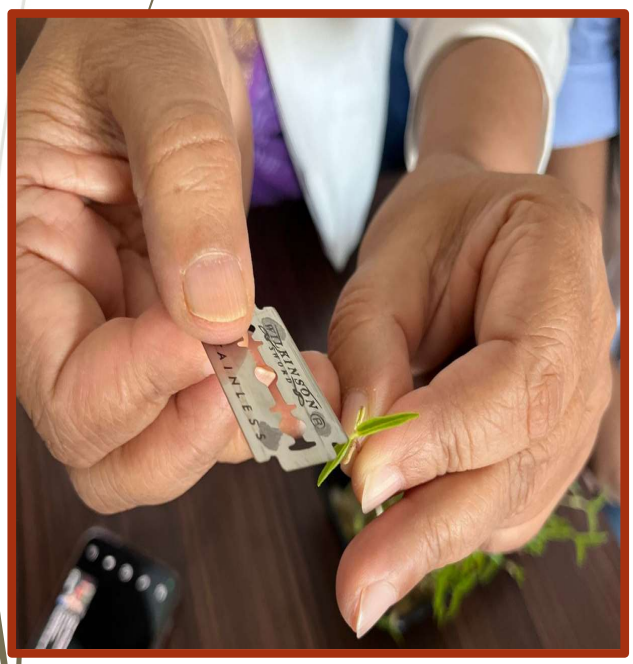
- 2. Ease of Culture: Mung bean is relatively easy to grow and maintain under laboratory conditions. It has a short life cycle and can be grown quickly, allowing for rapid turnover of tissue culture experiments.

- 3. High Regeneration Potential: Mung bean exhibits high regeneration potential from various explant sources, including cotyledonary nodes, hypocotyls, shoot tips, and embryogenic callus. This makes it suitable for a wide range of tissue culture applications, including somatic embryogenesis and shoot regeneration.

- 4. Genetic Stability: Mung bean is known for its genetic stability in tissue culture, meaning that the regenerated plants tend to retain the characteristics of the parent plant. This stability is essential for maintaining the desired traits during mass propagation or genetic transformation experiments.

EXPLANT SELECTION & PREPARATION





STERILIZATION OF EXPLANT

- Before initiating tissue culture, plant materials, such as explants (e.g., leaf segments, shoot tips, or embryos), are surface sterilized to eliminate external contaminants.

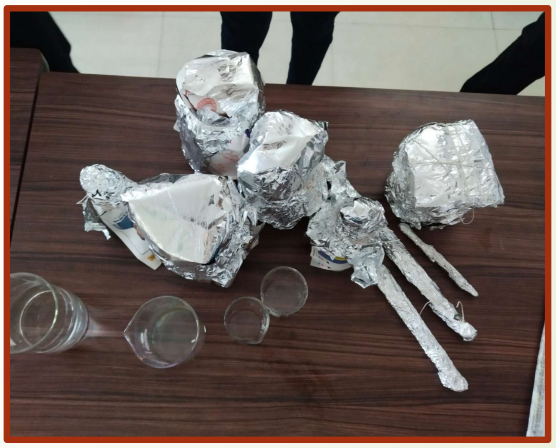
- This typically involves washing the plant material with a disinfectant solution i.e. 70% alcohol or HgCl2 followed by rinsing and.

- This helps step helps to ensure that the surface-sterilized plant material is free from any residual contaminants.

STERILIZATION

- **Method using for sterilization of all equipment's required for plant tissue culture is Autoclaving (steam under pressure) :**
- Autoclaves can sterilize a variety of materials commonly used in plant tissue culture, including culture media, glassware, surgical instruments, and other equipment. This versatility makes it a practical choice for sterilizing a range of items needed in tissue culture experiments.

- Autoclaving uses steam under pressure to sterilize materials, eliminating the need for harsh chemicals that can be harmful to the environment and lab personnel.
- Setting Parameters: Set the autoclave parameters including temperature, pressure, and duration of sterilization. The typical parameters for sterilizing plant tissue culture media and equipment are 121°C (250°F), 15 psi (1 atm), and 15-20 minutes. Adjust these parameters based on the specific requirements of the items being sterilized.



INOCULATION

1. Assembling the Workspace:

- Sterilize the workspace, including the laminar flow hood or sterile work area, by UV light or other suitable methods.
- Gather all necessary tools and materials, including sterile forceps, scalpels, culture vessels (e.g., Petri dishes, test tubes, or culture flasks), and culture medium.

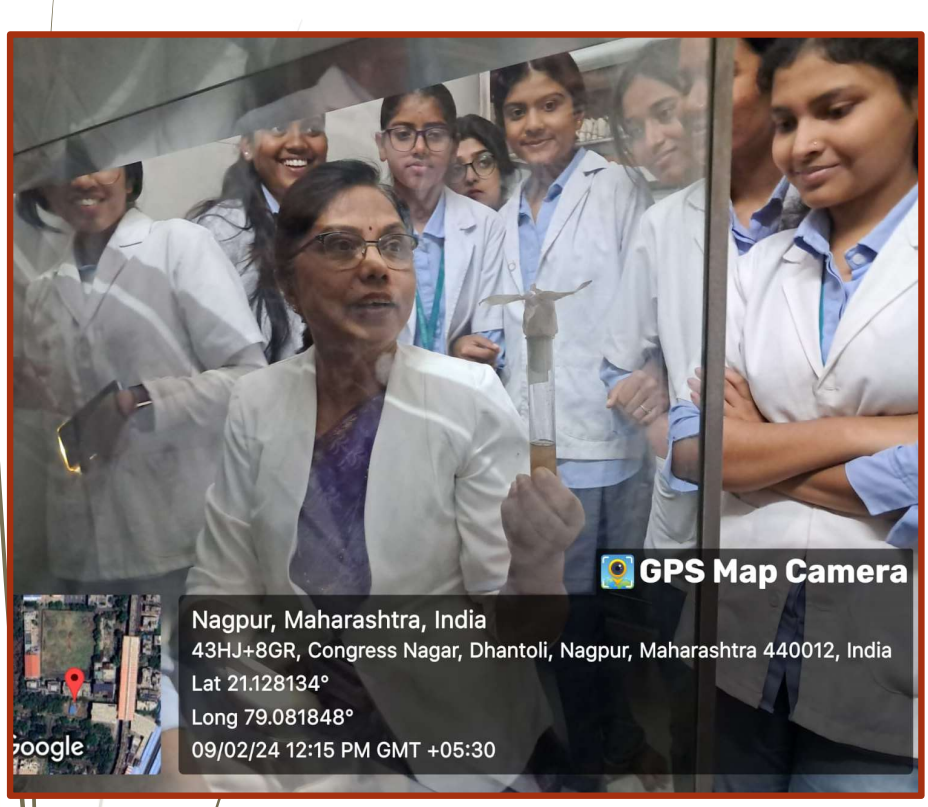
2. Inoculation:

- Open the culture vessel within the sterile workspace.
- Use sterile forceps or a scalpel to carefully transfer the sterilized explants onto the surface of the culture medium.
- Place the explants onto the medium in a predetermined arrangement, taking care to avoid overcrowding.
- Close the culture vessel immediately after inoculation to prevent contamination.

3. Plugging and Labeling:

- plug the culture vessel to maintain sterility and prevent evaporation.
- Label each culture vessel with relevant information such as the date of inoculation, type of explant, culture medium composition, and any other pertinent details

INOCULATION



INCUBATION

Incubation refers to the period during which the cultured explants or cells are placed in a controlled environment to promote their growth, development, and/or regeneration. Here's a detailed explanation of the incubation process:

1. Controlled Environment: Cultured explants or cells are placed in culture vessels (e.g., Petri dishes, test tubes, or culture flasks) containing a suitable nutrient medium. These vessels are then placed in a growth chamber or incubator where environmental conditions such as temperature, light, humidity, and gas composition can be precisely controlled.
2. Temperature: temperatures ranging from 25°C to 27°C are suitable for most plant tissue culture applications, but this may vary depending on the species and experimental conditions.
3. Lighting: Depending on the requirements of the cultured plant material. 16hrs light and 8 hrs dark

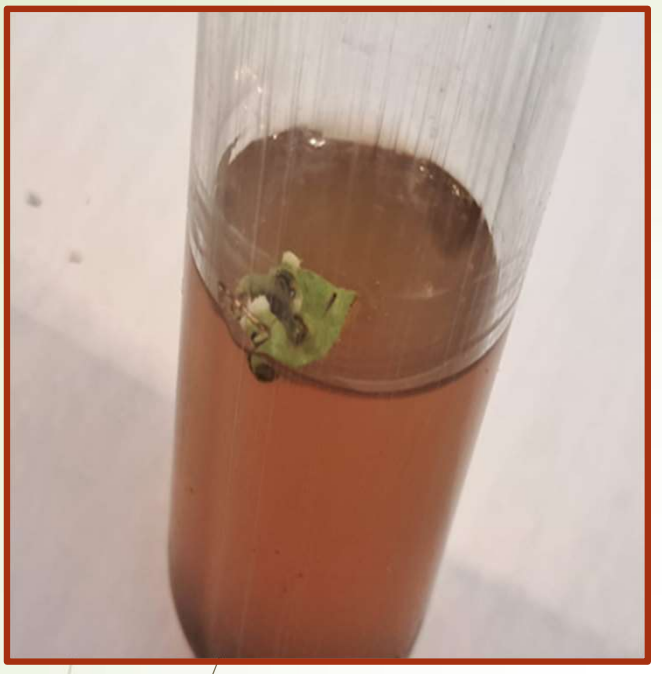
5. Duration: The duration of incubation varies depending on the specific objectives of the tissue culture experiment. It may range from a few days to several weeks or even months, depending on factors such as the rate of growth and development of the cultured tissues, the desired outcomes of the experiment, and the experimental protocol being followed.

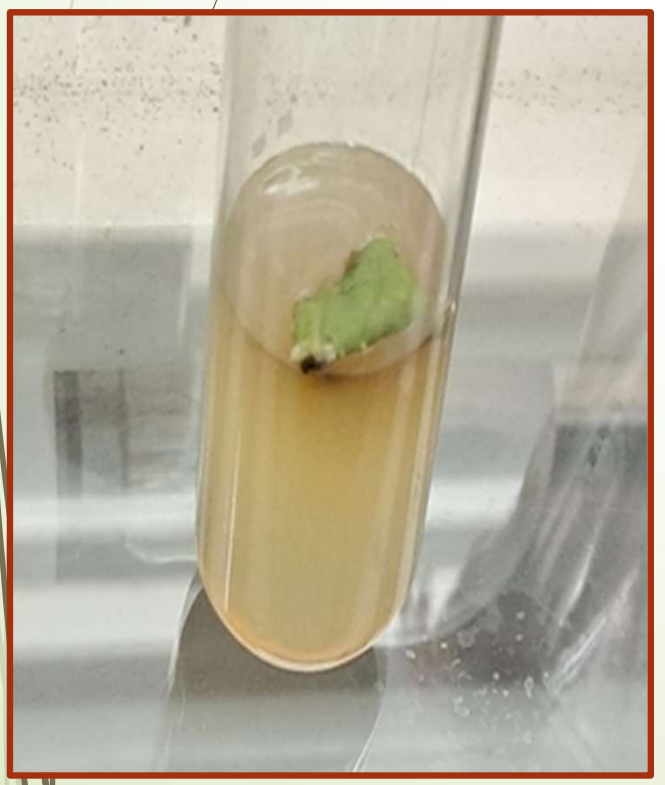
6. Monitoring: Throughout the incubation period, the cultured tissues are regularly monitored for signs of growth, contamination, or other abnormalities. This may involve visual inspection, measurement of growth parameters, and periodic subculture or maintenance procedures to ensure the health and viability of the cultures.

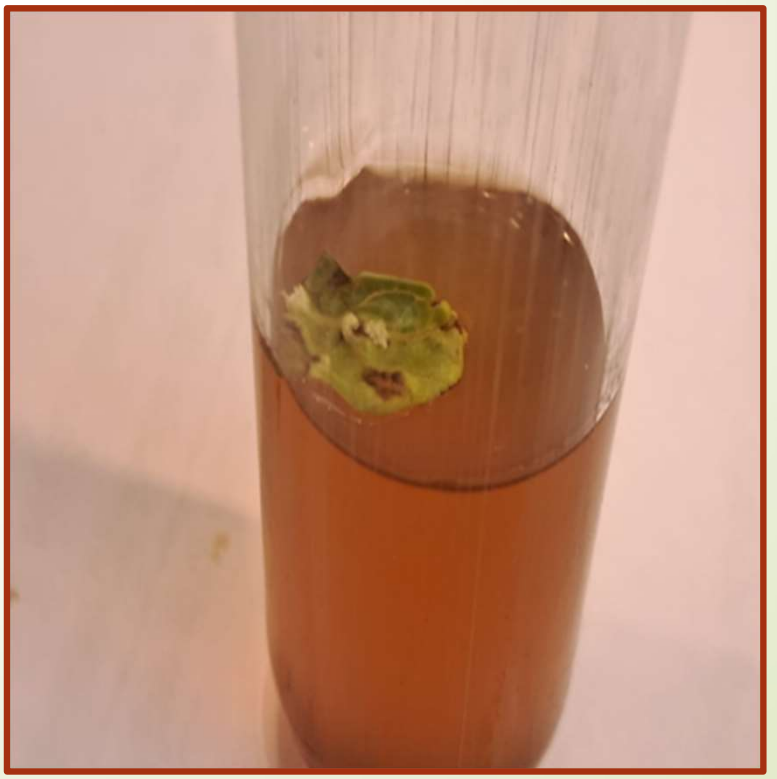
OBSERVATION

Sr.no	Growth hormone	Explant	observation	texture
1	5mg/L BPA	hypocotyl	Callus	White Friable
		leaf	Callus	Green Friable
2	0.1mg/L 2,4-d	hypocotyl	Callus	White Friable
		leaf	callus	Green friable

OBSERVATION







PLANT
TISSUE CULTURE

Preparation of cotton plugs

Preparation of MS media

Selection of explant

Inoculation

Callus development

CONCLUDING PROGRAM OF THE WORKSHOP

PRESENTATION OF WORKSHOP

CERTIFICATE DISTRIBUTION

WORKSHOP ARTICLE IN NEWSPAPER

प्लांट टिश्यू कल्चर पर वर्कशॉप

■ नागपुर, नगर प्रतिनिधि. शिवाजी साइंस कॉलेज के बॉटनी और माइक्रोबायोलॉजी विभाग द्वारा संयुक्त रूप से प्लांट टिश्यू कल्चर विषय पर वर्कशॉप आयोजित की गई. कॉलेज के हॉल में आयोजित वर्कशॉप का उद्घाटन प्राचार्य महेन्द्र ढोरे के हाथों किया गया. इस अवसर पर डीएनसी कॉलेज के प्राचार्य डॉ. ओमराज देशमुख की मुख्य अतिथि के तौर पर उपस्थिति रही. उन्होंने अपने संबोधन में प्रतिभागियों को आसान कार्यान्वयन के लिए टिश्यू कल्चर तकनीक का अपना प्रोटोकॉल तैयार करने का सुझाव दिया. प्राचार्य ढोरे ने 2 अलग विभागों के प्रतिभागियों द्वारा मिलकर आयोजित वर्कशॉप पर अभिनंदन किया.

सफलतार्थ प्रा. पुनीता तिवारी और डॉ. प्रणिता गुल्हाने प्रयासरत रहे. केतकी आर्य व वैदेही ने आभार प्रदर्शन किया. दोनों विभागों से प्रा. बोबडे, डॉ. महाखोडे, डॉ. सोलवालकर, डॉ. देशमुख, मयूरी भड, नुपुर देशमुख आदि की उपस्थिति रही.

CERTIFICATE

Shri Shivaji Education Society Amravati's

SCIENCE COLLEGE, CONGRESS NAGAR, NAGPUR

Accredited with CGPA of 3.51 at 'A+' Grade by NAAC, Bangalore
A College with Potential for Excellence
An Institutional Member of APQN
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A Mentor College under Paramarsh Scheme of UGC, New Delhi
A Mentor College under Paris Sparsh Scheme of Maharashtra State

Dept. of Botany in collaboration with Dept. of Microbiology

Workshop on Plant Tissue Culture Technique (WPTCT-2024)

9th February 2024

CERTIFICATE OF PARTICIPATION

This certificate is hereby awarded to ***Mr/Ms……*** **from Science College, Congress Nagar, Nagpur** for participating in ***"Workshop on Plant Tissue Culture Technique (WPTCT-2024)" organized by Dept. of Botany in collaboration with Dept. of Microbiology on 9th February 2024*** at Botany Research Lab., Science College, Congress Nagar, Nagpur.

Dr. Pranita Gulhane	Prof. Atul Bobdey	Prof. Mahendra Dhore	Prof. Rajendra Deshmukh	Prof. Punita Tiwari
WPTCT Coordinator	Cordinator- Microbiology	Principal	HoD- Botany	WPTCT Coordinator

ATTENDANCE OF STUDENTS PARTICIPANTS

DEPARTMENT OF BOTANY

	Name Of Students	Signature
Ku	ADMANE TANVI RAVINDRA	
Ku	BAWANKAR VAIDEHEE PRAKASH	
Ku	BHANDARKAR TORAL SURESH	
Ku	BHONDE SAKSHI PRASHANT	
Ku	BURADE JANHAVI NARENDRA	
Ku	CHAUDHARI KETAKI PRAKASH	
Ku	CHIPATE TANAYA PRAVIN	
Ku	CHUNCHUWAR SAKSHI RAJESH	
Ku	DIGERSE YASH ULHAS	
Ku	DHAPKE SAKSHI LALIT	
Ku	DURBULE RASIKA RAJESH	
Ku	GAIDHANE YOGITA KESHAV	
Ku	GOUTAM SHINJAN RUPESH	
Ku	INWATE DEVESHRI RANJEET	
Ku	KAKDE DIVYA YUVARAJ	
Ku	KHANDARE PRASHIK PRAKASHRAO	
Ku	KOTHE UTKARSHA VINOD	
	KOHAD HARSH SANJAY	
Ku	MAHALE SAKSHI GOPAL	
Ku	PANDEY JANVI DINESH	
Ku	PANDA VAISHNAVI PRAMOD KUMAR	
Ku	SINGH DIVYA RAKESH	
Ku	SONTAKKE SRUSHTI SIDHARTH	
Ku	WADE ARYA ULHAS	
Ku	BANGALE SWARUP VIJAY	
	BHADANGE SARANG SANJAY	

Science College, Congress Nagar, Nagpur SEAL

DEPARTMENT OF MICROBIOLOGY

	NAME OF STUDENTS	SIGNATURE
1	Astha. A. Sakharwade.	
2.	Akanksha. R. Bisen	
3.	Akanksha. V. Tekade	
4.	Anisha A. Shende	
5	Anuradha purushottam Khope	
6.	Arshiya shaikh mushtaque	Arshiya
7	Arya. S. walode	
8	Bhisvani Mahesh Dhurve	
9.	Ishita. Y. padgil	
10.	Ishwari. N. Gawande	Ishwari
11	Leena. N. Meher	
12	Manisha. G. Jilhare	Absent
13	Manisha. R. Roy.	Manisha
14.	Namrata. O. Nagose	
15.	Ojaswini. Bhagat	
16.	Rujuta. R. Ramteke	
17.	Shamim. Kausar	
18.	Snehal. Sahare	S.N sahare
19.	Vaishnavi. Ingle	
20	Vani masaram	Vani
21.	Yashoda. Ravindra wade	

Action Taken Report

The Plant Tissue Culture Training Program led by Prof. Punita Tiwari significantly enhanced B.Sc. III students' practical skills and theoretical understanding of tissue culture techniques. Key areas covered included media preparation, sterilization, explant handling, and observation. The program yielded successful callus proliferation results and fostered valuable intercollegiate collaboration and industrial exposure. Overall, it provided students with essential skills for further studies or careers in biotechnology and plant sciences.

FEEDBACK FORM

Sr.No.	Question	Response		
		Good	Better	Avera ge
1)	Overall effectiveness of the training program?			
1)	Relevance of practical sessions?			
1)	Clarity of experimental results?			
1)	Faculty support and guidance?