# 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

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

**Dept. of Botany in Collaboration with Dept. of Microbiology,**
**Science College, Congress Nagar, Nagpur**
*Organizes*

## workshop
*on*
# Plant Tissue Culture Technique

***Workshop Objective:*** Plant Tissue culture is an important tool for both basic and applied aspects of plant biotechnology as well as its commercial applications. All techniques are skill based and upon systematic learning, can equip a person to effectively utilize the techniques in various areas like basic research, environmental issues and commercial applications. It is a valuable tool for research on crop improvement by biotechnology. Plant Tissue Culture is a practice used to propagate plants under sterile conditions, often used to produce clones of a plant. Different techniques in Plant Tissue culture offer advantages over traditional methods of propagation which includes the production of multiple clones of plants in the absence of seeds or pollinators necessary to produce seeds and mature plants. This workshop offers a comprehensive hands-on training for learning the basics with an insight to laboratory.

**Venue:** Botany Research Lab.
**Date:** 9th February 2024
**Time:** 10:00 AM onwards

**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**

# PLANT TISSUE CULTURE


- PRESENTED BY : JANHAVI N. BURADE
- SHRI SHIVAJI SCIENCE COLLEGE,
- Congress nagar, Nagpur.
- Janhvi.burade01@gmail.com

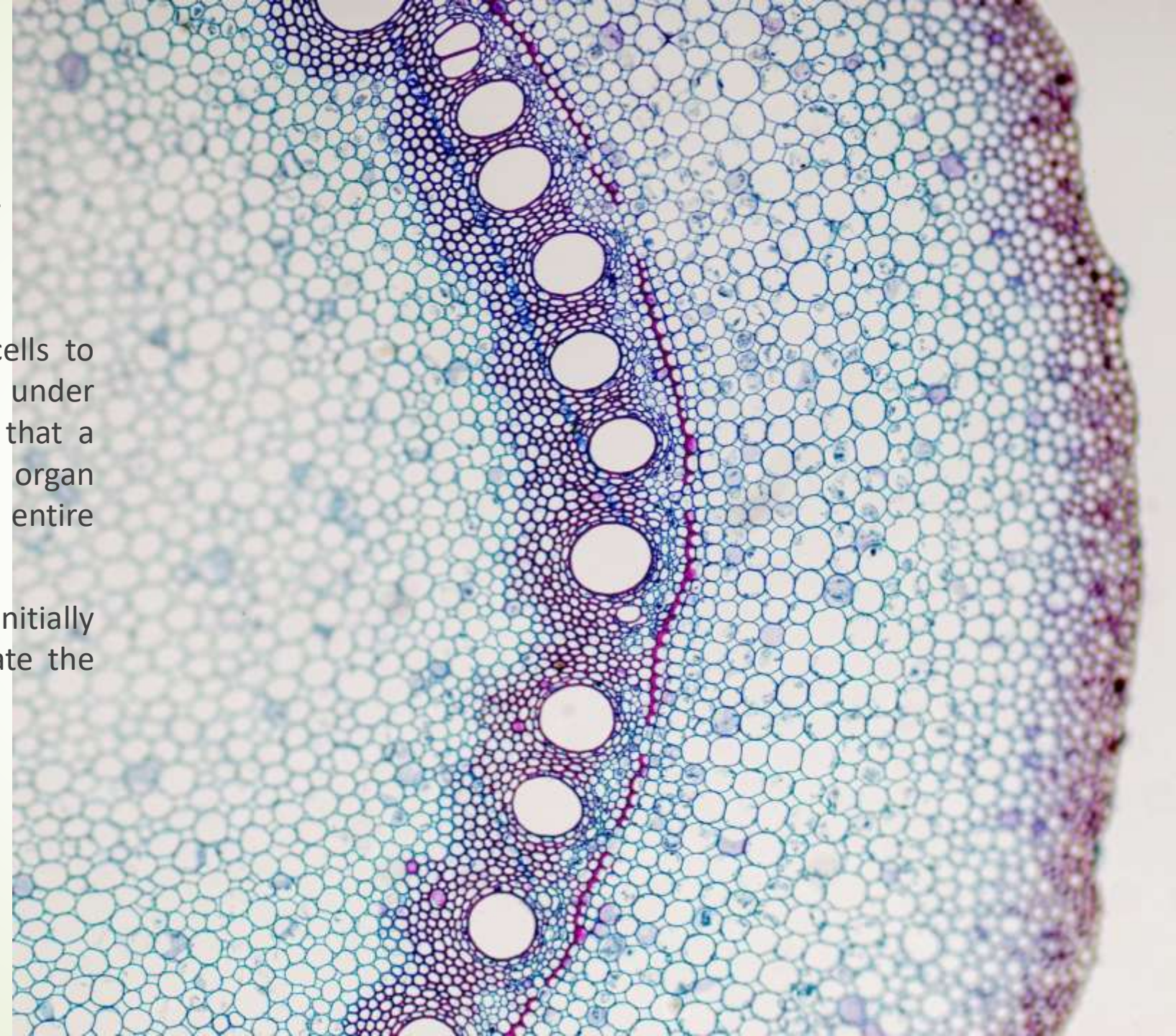
# INTRODUCTION

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

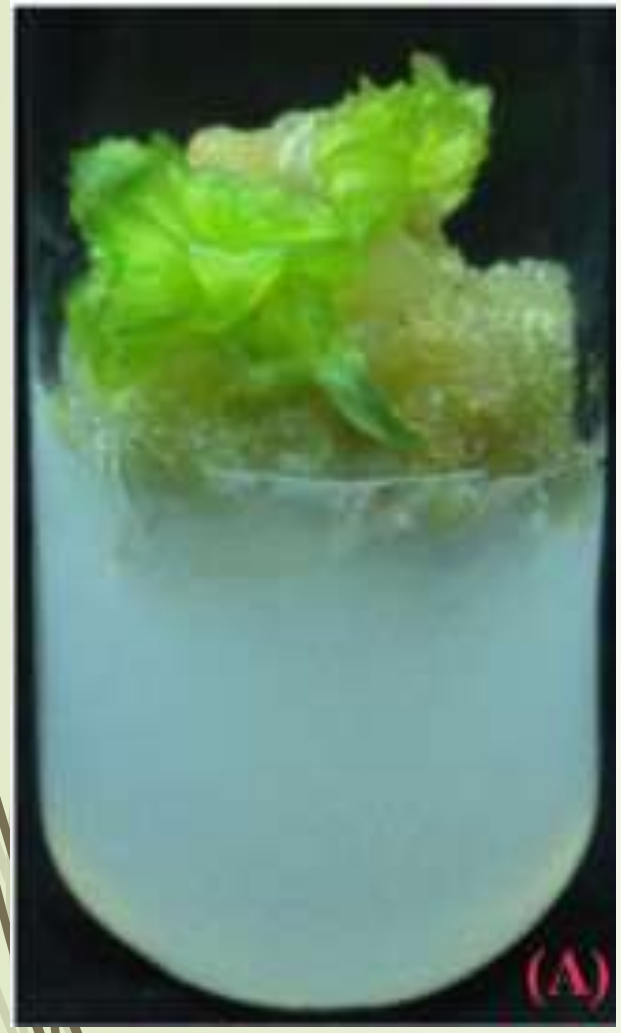

| Chemical | Formula | Concentration |
|---|---|---|
| Macronutrients (10 X) | | 100 mL/L |
| Ammonium nitrate | $NH_4NO_3$ | 16.5 |
| Potassium nitrate | $KNO_3$ | 19.0 |
| Calcium chloride | $CaCl_2.2H_2O$ | 4.4 |
| Magnesium sulfate | $MgSO_4.7H_2O$ | 3.7 |
| Potassium dihydrogen orthophosphate | $KH_2PO_4$ | 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 subcultured 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.

# 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.

# STERLIZATION

- **Method using for sterilization of all equipments required for plant tissue culture is Auotoclaving (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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# 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 |

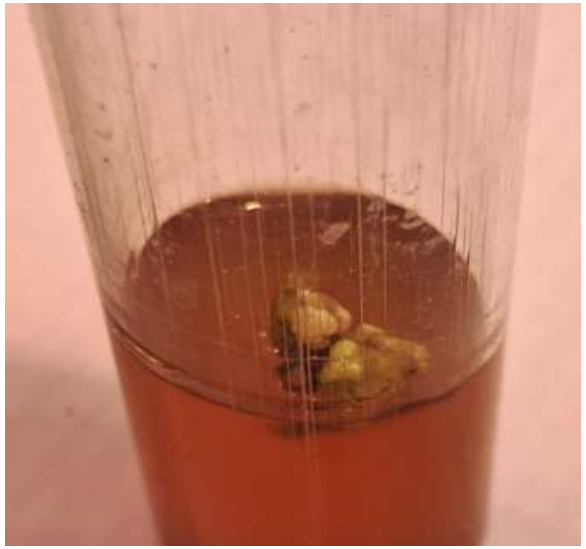

## Callusing of different pattern


Preparation of cotton plugs

Preparation of MS media

Selection of explant


Inoculation



Callus development


GPS Map Camera
Nagpur, Maharashtra, India
43HJ+8GR, Congress Nagar, Dhantoli, Nagpur, Maharashtra 440012, India
Lat 21.128133°
Long 79.081845°
09/02/24 11:52 AM GMT +05:30
Google


GPS Map Camera
Nagpur, Maharashtra, India
43HJ+CG3, opposite Ajni Railway Station, Congress Nagar, Dhantoli, Nagpur, Maharashtra 440012, India
Lat 21.128352°
Long 79.081781°
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GPS Map Camera
Nagpur, Maharashtra, India
43HJ+8GR, Congress Nagar, Dhantoli, Nagpur, Maharashtra 440012, India
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Long 79.081845°
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Google

# Concluding Program of the workshop


# PRESENTATION OF WORKSHOP


# Certificate distribution

के अनुसार टास्क दिया
ारोपी ने उनके खाते में
. मयूर को विश्वास हो गया
वेश करके टास्क पूरे करता
के के साथ रकम जमा
कम दोबारा निवेश होती थी.

आरोपियों से और रकम डिपॉजिट नहीं करने को कहा और अपनी रकम वापस मांगी. आरोपियों ने संपर्क ही तोड़ दिया. मोटी रकम हाथ से जाने पर मयूर ने मामले की शिकायत पुलिस से की. पुलिस ने धोखाधड़ी और आईटी एक्ट की धाराओं के तहत मामला दर्ज कर जांच आरंभ की है.

# 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.

## उदयनगर, आशीर्वादनगर, हुड़केश्वर में विकट हो रही स्थिति

Shri Shivaji Education Society Amravati's

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**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 |

SHRI SHIVAJI EDUCATION SOCIETY AMRAVATI'S

SCIENCE COLLEGE, CONGRESS NAGAR, NAGPUR

**Department of Botany**

in Collaboration with

**Department of Microbiology**

Organizes

**Workshop on 'Plant Tissue Culture Techniques"**

Dt: 09/02/2024

## B.Sc. Sem VI Students

Plant Tissue Culture Candidates CBM(B2)

| Name | 100 Rs. (Paid/unpaid) | Sign |
|---|---|---|
| 1) Ketaki Chaadhani | Paid | |
| 2) Shinjan Goutam | Paid | |
| ~~3) Tanal Bhandankar~~ | | |
| 4) Divya Singh | Paid | |
| 5) Yash Digrase | Paid | |
| 6) Vaidehee Bawankar | Paid | |
| ~~7) Yogita Gaidhane~~ | | |
| 8) Utkansha Kothe | Paid | |
| 9) Sakshi Mahale | Paid | |
| ~~10) Sakshi Dhapke~~ | ~~Paid~~ | Retrieved |
| 11) Prashik Khandare | Paid | |
| 12) Janhavi Bunade | Paid | |

PTC Candidates CBG(G12)

1) Gauri Panchbudhe
2) Falguni Kumbhare

## M.Sc. Sem II Students

| Sr. No | Name of Student |
|---|---|
| 1. | Astha A. Sakharwade |
| 1. | Akanksha R. Bisen |
| 1. | Akanksha V. Tekade |
| 1. | Anisha A. Shende |
| 1. | Anuradha P. Khope |
| 1. | Arshiya S. Mushtaque |
| 1. | Arya S. Walode |
| 1. | Bhisvani M. Dhurve |
| 1. | Ishita Y. Padgil |
| 1. | Ishwari N. Gawande |
| 1. | Leena N. Meher |
| 1. | Manisha G. Lilhare |
| 1. | Manisha R. Roy |
| 1. | Namrata O. Nagose |
| 1. | Ojaswini R. Bhagat |
| 1. | Rujuta R. Ramteke |
| 1. | Shamim M. Shahzad |
| 1. | Snehal N. Sahare |
| 1. | Vaishnavi V. Ingle |
| 1. | Vami K. Masram |
| 1. | Yashoda R. Wade |

# Thank you