Practical Manual

Forest Biotechnology

Course No. FBT 412 ; Credit Hrs. 3(2+1)

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College of Horticulture & Forestry
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Syllabus:

Requirements for Plant Tissue Culture Laboratory; Techniques in Plant Tissue Culture; Media components and preparations; Sterilization techniques and Inoculation of various explants; Aseptic manipulation of various explants; Callus induction and Plant Regeneration; Micro propagation of important crops; Anther, Embryo and Endosperm culture; Hardening / Acclimatization of regenerated plants; Somatic embryogenesis and synthetic seed production; Isolation of protoplast; Demonstration of Culturing of protoplast; Demonstration of Isolation of DNA; Demonstration of Gene transfer techniques, direct methods; Demonstration of Gene transfer techniques, indirect methods; Demonstration of Confirmation of Genetic transformation; Demonstration of gel-electrophoresis technique.

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Roll No. ....

Batch...

Session...

Semester...

Course Name: ....

Course No. : ....

Credit...

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This is to certify that Shri./Km. ....ID No....................................has completed the practical of course...course No. .... as per the syllabus of B.Sc. (Hons.) Forestry .... semester in the year.....................in the respective lab/field of College.

Date: Course Teacher

INDEX

Ex. No.	Title	Page No.	Remarks
1	To study of techniques in plant tissue culture		
2	To study of media components and preparations		
3	To study of sterilization techniques and inoculation of various explants		
4	To study of aseptic manipulation of various explants		
5	To study of callus induction and plant regeneration		
6	To study of micro propagation of important crops		
7	To study of anther culture		
8	To study of embryo culture		
9	To study of endosperm culture		
10	Hardening / Acclimatization of regenerated plants		
11	To study the somatic embryogenesis		
12	To study the synthetic seed production		
13	To demonstration of isolation of protoplast		
14	To demonstration of culturing of protoplast		
15	To demonstration of isolation of DNA		
16	To demonstration of gene transfer techniques by direct methods		
17	To demonstration of gene transfer techniques by indirect methods		
18	To demonstration of confirmation of genetic transformation		
19	To demonstration of gel-electrophoresis technique		
	Annexure		
	Glossary		

Practical No. 1

Objective: To study techniques in plant tissue culture

Material Required: …………………………………………………………………………………………………

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Procedure: ………………………………………………………………………………………………………

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Practical No. 2

Objective: To study of media components and preparations

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Calculations: ………………………………………………………………………………………………

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Practical No. 3

Objective: To study of sterilization techniques and inoculation of various explants

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Draw the picture of laminar air flow

Practical No. 4

Objective: To study of aseptic manipulation of various explants

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Conclusion: …………………………………………………………………………………………………

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Practical No. 5

Objective: To study of callus induction and plant regeneration

Material Required: ....

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Practical No. 6

Objective: To study of micro propagation of important crops

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Conclusion: ……………………………………………………………………………………………………

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Practical No. 7

Objective: To study the anther culture

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Practical No. 8

Objective: To study the embryo culture

Material Required: ……………………………………………………………………………………………

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Conclusion: ……………………………………………………………………………………………

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Practical No. 9

Objective: To study the endosperm culture

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Procedure: …………………………………………………………………………………………………

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Conclusion: ………………………………………………………………………………………………

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Practical No. 10

Objective: To study of hardening / acclimatization of regenerated plants

Material Required: …………………………………………………………………………………………………

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Procedure: …………………………………………………………………………………………………

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Conclusion: ………………………………………………………………………………………………

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Practical No. 11

Objective: To study of somatic embryogenesis

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Procedure: …………………………………………………………………………………………………

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Practical No. 12

Objective: To study procedure of synthetic seed production

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Conclusion: ……………………………………………………………………………………………………

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Practical No. 13

Objective: To study Isolation of protoplast

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Procedure: ……………………………………………………………………………………………………

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Observation: …………………………………………………………………………………………………

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Conclusion: …………………………………………………………………………………………………

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Practical No. 14

Objective: To demonstrate culturing of protoplast

Material Required: …………………………………………………………………………………………………

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Conclusion: ……………………………………………………………………………………………………

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Practical No. 15

Objective: To demonstrate isolation of DNA

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Practical No. 16

Objective: To demonstrate gene transfer techniques: Direct Methods

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Practical No. 17

Objective: To demonstrate gene transfer techniques: Indirect Methods

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Practical No. 18

Objective: To demonstrate confirmation of Genetic Transformation

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Practical No. 19

Objective: To demonstrate gel electrophoresis techniques

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Appendices

GENERAL LAB SAFETY RULES FOR PLANT TISSUES CULTURE

The following rules are very important as for as concern to works in the any laboratory since you need to work with different equipment, lab therefore it must follow during working in the laboratory as mention below:

1. Before entering the lab bears the lab coat, gloves, mask and goggle etc and be sure you are aware of all safety rules instructions, procedures and fully aware of your facilities.
2. You should know about first aid kit, fire extinguishers and emergency phone numbers, your labels exit and fire alarms are located and all essential chemicals.
3. You should not talk, not eat, and not drink during working in the lab
4. Keep proper distance while working in the lab and work in properly-ventilated areas.
5. Be sure after finishing your experiment, keep everything as it is
6. Never use lab equipment that you are not aware of the same.
7. End of your work switch off all electric appliances of the lab
8. Never leave an ongoing experiment without completions.
9. Never smell or taste chemicals even do not pipette by mouth.
10. Follow the proper procedures for disposing the lab waste.
11. If you notice any unsafe conditions in the lab, immediately inform to senior authority
12. Always keep your work area(s) tidy and clean.
13. Only required materials keep on the working bench
14. You should not bear the loose cloth while working in the lab
15. Before leaving the lab or eating, always wash your hands.
16. Before stating experiment keep all requirements on workings bench

Chemical safety rules

1. Do not allow any solvent to come into contact with your skin.
2. All chemicals should always be clearly labelled with the name an receiving date
3. Before removing any of the contents from a chemical bottle, read the instructions twice.
4. Do not put unused chemicals back into their original container.

ESSENTIAL ACTIVITIES IN PLANT TISSUES CULTURE LABORATORY

This is especially important in plant tissues culture laboratory before starting any experiment such as use of clean glassware (Burette, Culture tubes, Pipets Slides and Cover slip etc) since these activities play crucial roles to get optimal result. Therefore, the following points must be considered as mention below

1. Wash lab ware as quickly as possible after use.
2. New glassware items are slightly alkaline in reaction, so it should be soaked for several hours in acid water
3. Clean all glassware before use and dry in heat.
4. After cleaning, rinse the glassware with running tap water and then with distilled water.
5. After rinsing dry the glassware at 60°C to 65°C for 2 day inside the hot oven.
6. Culture tubes which have been used previously must be sterilized before cleaning.
7. Tubes filled with a media sterilized by autoclaving, do not plug until the media is added.
8. Do not blow into the pipets as it causes moisture to condense on the inside of the pipets.
9. Slides should be washed, placed in glacial acetic acid for 10 minutes, rinsed with distilled water, and wiped dry with clean paper towels or cloth.
10. If the slides are drying stored, wash them with alcohol before use.
11. For steam sterilization time should not exceed 15 minutes at 121 °C (250 °F).
12. The actual cavity of the autoclave should be checked to be sure the autoclave temperature does not exceed the recommended sterilization and drying temperature.
13. Used glass slide clean with 1-2% soft soap or detergent and dry it.

14. After sterilization of glassware label with markers.

DNA EXTRACTION BUFFER (DEB) COMPOSITION AND PREPARATION

1.1. Preparation of commonly used stock solution

Solution	Method of preparation
0.5 M EDTA (pH 8.0)	186.1 g of sodium salt of EDTA was dissolved in 800 ml of MQ water, pH was adjusted to 8.0 with NaOH pellets. The final volume was adjusted to one liter with MQ water and sterilized by autoclaving.
4 M NaCl	233.8 g of NaCl was dissolved in 800 ml of MQ water. The final volume was adjusted to one liter with MQ water and sterilized by autoclaving.
1M Tris-Cl	121.1 g of Tris-Cl salt was dissolved in 800 ml of sterile MQ water. pH was adjusted to 8.0with concentrated 1N HCl. The final volume was adjusted to one liter with MQ water and sterilized by autoclaving.
10% CTAB	100 gm of CTAB powder was dissolved in sterile MQ water and the volume was adjusted to one liter.
Phenol : Chloroform : isoamyl alcohol:	Buffer saturated phenol, chloroform and isoamyl alcohol were mixed in the ratio of 25 : 24 : 1. The equilibrated mixture was stored under a layer of 0.01 M Tris-HCl (pH 7.6) at 4^0C in dark glass bottle.
Chloroform : Isoamyl alcohol:	Buffer saturated chloroform and isoamyl alcohol were mixed in the ratio of 24 : 1.
3M Sodium acetate (pH 4.8):	408.1 g of $NaOAc.3H_2O$ was dissolved in 800 ml of MQ water. The pH was adjusted to 4.8 with glacial acetic acid. The final volume was adjusted to one litre with MQ water and sterilized by autoclaving.
Ethidium bromide (10 mg ml^{-1}):	1.0 g of ethidium bromide was added to 100 ml of distilled water and stirred on a magnetic stirrer for several hours to ensure that the dye has dissolved. The solution was transferred to a dark bottle and stored at room temperature.

I. DNA extraction buffer

Chemical	Concentration	Volume (ml)
1 M Tris-CL (pH 8.0)	100mM	5.0
0.5 M EDTA (pH 8.0)	20mM	2.0
4 M NaCl	1.4M	17.5
10% CTAB	2% (w/v)	10.0
B-mercaptoethanol	0.2% (W/v)	0.2
Milli-Q water	-	15.3
Total	**-**	**50**

II. DNA Electrophoresis reagents

TE-buffer	10 mM Tris-HCl (pH 8.0)	
	1 mM EDTA (pH 8.0)	
50X TAE	Tris base	242.0 g
	Glacial acetic acid	57.1 ml
	0.5 M EDTA (pH 8.0)	100 ml
	Distilled water to 1 Litre	
Loading dye	1% Bromophenol blue	200 µl
	Glycerol	200 µl
	10% SDS	60 µl
	0.5 M EDTA	50 µl
	10X TAE	60 µl
	Distilled water	30 µl
10X TBE buffer (pH 8.3)	89 mMTris-HCl	107.81 g
	89 mM Boric acid	55.02 g
	2.5 mM EDTA	9.36 g
	Adjust to pH 8.0 with acetic acid store buffer as TBE 10x stock solution.	
Tracking dye	Bromophenol blue	0.25%
	Xylene cyanol	0.25%

Sucrose 60% 10 ml 5x TBE
Dissolve the powdered dye in sucrose solution and filter by Whatman No. 1, divide in aliquots and store below 0^0C.

SOLUTION PREPARATION

The concentration of a particular substance in the media can be expressed in various units that are as follows:

Units in weight

It is represented as milligram per litre (mg/l)
10-6 = 1.0 mg/l or 1 part per million (ppm)
10-7 = 0.1 mg/l.
10-8 = 0.001 mg/l or 1 µg/l
Molar concentration
A molar solution (M) contains the same number of grams of substance as is given by molecular weight in total volume of one litre.
1 molar (M) = the molecular weight in g/l
1 mM = the molecular weight in mg/l or 10-3 M
1 µM = the molecular weight in µg/l or 10-6 M or 10-3 mM.

Conversion from milli molar (mM) to mg/l
For example, molecular weight of auxin 2,4-D = 221.0
1M 2,4-D solution consists of 221.0 g per litre
1 mM 2,4-D solution consists of 0.221 g per litre = 221.0 mg per litre
1 µM 2,4-D solution consists of 0.000221 g/l = 0.221 mg/l

Conversion from mg/l to mM
The molecular weight of CaCl2 - 2H2O
= 40.08 + 2 x 35.453 + 4 x 1.008 + 2 x 16 = 147.018
(the atomic weights of Ca, Cl, H and O being 40.08, 35.453, 1.008 and 16.0 respectively).

If, 440 mg/l of CaCl2 - 2H2O is to be converted into mM; then
The number of mM CaCl2 . 2H2O
= No. of mg CaCl2 - 2H2O/Molecular weight of CaCl2 . 2H2O =440/ 147.019 =2.99 mM Thus, 440 mg/l CaCl2 - 2H2O = 2.99 mM

Some important know facts about DNA, RNA and Protein

1. 1.0 kb DNA = Coding capacity for 333 amino acids = 37,000 dalton protein.
2. 1 pmol of 1000 bp DNA = 0.66 µg
3. 1 µg of 1000 bp DNA = 1,52 pmol = 9.1 x 10^{11} molecules
4. 1 A 260 unit of double-stranded DNA = 50 µg/ml
5.1 A 260 unit of single-stranded DNA = 33 µg/ml
6.1 A 260 unit of single-stranded RNA = 40 µg/ml

Gel percentage and size of DNA resolved

Gel percentage (%)	Approx size of DNA resolved in (bp)
1.0.4 to 0.5	10000 to 30000
2.0.6 to 0.7	800 to 12000
3.0.8 to 0.9	600 to 10000
4.1.0 to 1.2	300 to 7000
5.1.3 to 1.5	200 to 3000
6.1.6 to 2.0	100 to 2000
7. 3.0 to 4	10 to 300

GOOD PRACTICES IN MOLECULAR BIOLOGY LABORATORY

I. Safety procedures

A. Chemicals

1. A number of chemicals used in molecular biology laboratory are hazardous.
2. All manufacturers of hazardous materials are required by law to supply the user with pertinent information on any hazards associated with their chemicals.
3. This information is supplied in the form of Material Safety Data Sheets or MSDS and same should be kept in the lab.
4. Everyone is strongly urged to make use of this information prior to using a new chemical and certainly in the case of any accidental exposure or spill.
5. The following chemicals are particularly noteworthy:
    - Phenol: Can cause severe burns
    - Acrylamide: potential neurotoxin
    - Ethidium bromide: carcinogen

These chemicals are not harmful if used properly and safely: always wear gloves when using potentially hazardous chemicals and never mouth-pipette them. If you accidentally splash any of these chemicals on your skin, immediately rinse the area thoroughly with water and inform the instructor. Discard the waste in appropriate containers.

B. Ultraviolet Light

Exposure to ultraviolet light can cause acute eye irritation. Since the retina cannot detect UV light, you can have serious eye damage and not realize it until 30 min to 24 hours after exposure. Therefore, always wear appropriate eye protection when using UV lamps.

C. Electricity

The voltages used for electrophoresis are sufficient to cause electrocution. Cover the buffer reservoirs during electrophoresis. Always turn off the power supply and unplug the leads before removing a gel.

D. General Housekeeping

All common areas should be kept free of clutter and all dirty dishes, electrophoresis equipment, etc should be dealt with appropriately. All solutions and everything stored in an incubator, refrigerator, etc. must be labeled. In order to limit confusion, each person should use his initials or other unique designation for labeling plates, etc. Unlabeled material found in the refrigerators, incubators, or freezers may be destroyed. Always mark the backs of the plates with your initials, the date, and relevant experimental data, e.g. strain numbers.

PRECAUTIONS TO BE TAKEN WHEN WORKING WITH DNA

1. The chemical and physical properties of reagents and conditions are important considerations in processing and storing DNA and RNA.
2. Heavy metals promote phosphodiester breakage.
3. The EDTA is an excellent heavy metal chelator.
4. Free radicals are formed from chemical breakdown and radiation and they cause phosphodiester breakage.
5. UV light at 260 nm causes a variety of lesions, including thymine dimers and cross-link. Biological activity is rapidly lost. The irradiation at 320 nm can also cause cross-link, but less efficiently.
6. Ethidium bromide causes photo oxidation of DNA with visible light and molecular oxygen
7. Nucleases are found on human skin; therefore, avoid direct or indirect contact between nucleic acids and fingers. Most DNases are not very stable; however, many RNases are very stable and can adsorb to glass or plastic and remain active.
8. The 4ºC is one of the best and simplest conditions for storing DNA while -20ºC temperature causes extensive single and double strand breaks and -80ºC is excellent for long-term storage.

BASIC INFORMATION

Protoplasts are cells which have had their cell wall removed either manually or by digestion with enzymes.
Cellulase enzymes digest the cellulose in plant cell walls.
Pectinase enzymes break down the pectin holding cells together.
Process: Once the cell wall has been removed the resulting protoplast is spherical in shape. Digestion is usually carried out after incubation in an osmoticum (a solution of higher concentration than the cell contents which causes the cells to plasmolyse). This makes the cell walls easier to digest. Debris is filtered and/or centrifuged out of the suspension and the protoplasts are then centrifuged to form a pellet. On re-suspension, the protoplasts can be cultured on media which induce

cell division and differentiation. A large number of plants can be regenerated from a single experiment. For example, a gram of potato leaf tissue can produce more than a million protoplasts.

STEPS INVOLVED IN PROTOPLAST DNA ISOLATION

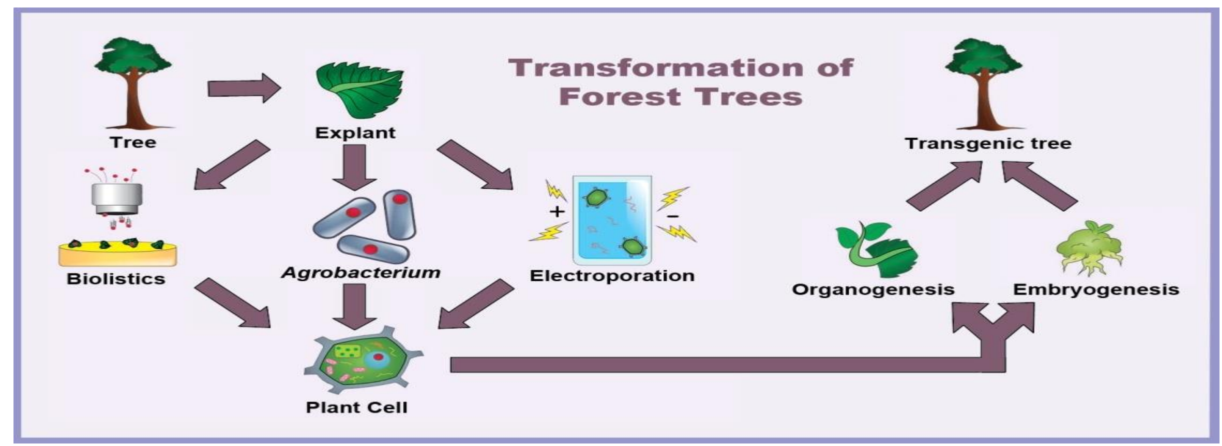
1. The young leaves are collected and washed in sterile distilled water thrice.
2. The leaves are cut into small bits.
3. Then the leaves are kept immersed in 13% Mannitol for 1 h for pre-plasmolysis.
4. Mannitol is removed after incubation and sterilized enzyme mixture (Cellulase + Macerozyme) is added and incubated at 25ºC in a shaker for 12 h
5. The filtrate iss centrifuged at 100g for 5 min to sediment the protoplast.
6. The supernatant is removed and the protoplast pellet is suspended in 10ml of CPW +21% sucrose solution.
7. The mixture is centrifuged at 100g for 5 min. The viable protoplast will float to the surface of the sucrose solution.
8. The supernatant is collected and viewed under microscope.
9. The protoplasts can be visualized under microscope.

Compositions of MS Basal Medium (Murashige & Skoog, 1962)

Components	**Quantity (mgl-1)**
Macronutrients	
NH4NO3	1650.0
KNO3	1900.0
CaCI22H2O	440.0
MgSO4.7H2O	370.0
KH2PO4	170.0
Micronutrients	
KI	0.83
H2BO3	6.20
MnSO4.4H2O	22.30
ZnSO47H2O	8.60
Na2MoO4.2H2O	0.25
CuSO45H2O	0.025
CoCI2.6H2O	0.025
Na2EDTA	37.30
FeSO4.7H2O	27.80
Vitamins and other supplements	
Inositol	100.0
Glycine	2.0
Thiamine	0.1
Pyridoxine HCI	0.5
Nicotinic acid	0.5
2. B5 Basal Medium (Gamborg et al., 1968)	
Macronutrients	
KNO_3	3000.0
$CaCl_2.2H_2O$	150.0
$MgSO_4.7H_2O$	500.0
$(NH4)_2SO_4$	134.0
$NaH_2PO_4H_2O$	150.0
Micronutrients	
KI	0.75
H3BO3	3.00
MnSO4.4H2O	10.00
$ZnSO_47H_2O$	2.00
$Na_2MoO_4.2H_2O$	0.25
$CuSO_45H_2O$	0.025

$CoCl_2.6H_2O$	0.025
Na_2EDTA	37.30
$FeSO_4.7H_2O$	27.80
Vitamins and other supplements	
Inositol	100.00
Thiamine HCl	10.0
Pyridoxine HCl	1.0
Nicotinic acid	1.0
3. White's Basal Medium (White. 1963)	
Macronutrients	
KNO_3	80.0
$MgSO_4.7H_2O$	720.0
NaH_2PO_4.	16.5
$Ca(NO3)_2.4H_2O$	300.0
Micronutrients	
KI	0.75
H_3BO_3	1.50
$MnSO_4$	7.00
$ZnSO_4.7H_2O$	2.60
Vitamins and other supplements	
Glycine	3.0
Thiamine HCl	0.1
Pyridoxine HCl	0.1
Nicotinic acid	0.5
Calcium pantathenate	1.0
Cysteine HCl	1.0
Woody Plant Medium (Lloyd and McCown, 1980)	
Macronutrients	
NH_4NO_3	400.0
KH_2PO_4	170.0
$MgSO_47H_2O$	370.O
K2SO4	980.0
Ca (NO3)24H2O	556.0
$CaCl_2$2H2O	96.00
Micronutrients	
H_3BO_3	6.20
$CuSO_4.5H_2O$	0.25
$MnSO_4.4H_2O$	22.30
$ZnSO_4.7H_2O$	8.80
$Na_2MoO_4.2H_2O$	0.25
Iron EDTA	
Na_2EDTA	37.30
$FeSO_47H_2O$	27.80
Organic nutrient	
Pyridoxine	0.50
Myoinositol	100.00
Glycine	2.00
Thiamine HCl	0.10
Nicotinic acid	0.50

Pictorial representations of different methods transformation with forest tree species



ESSENTIALS EQUIPMENT

1.Morter and Pestle **Use:** Crushing the biological samples	
2.Water bath **Use:** Heating the biological samples	
3.Centrifuge **Use:** separation of biological materials	
4.pH meter **Use:** Measures the pH of solutions	
5.Micro-pipette **Use:** measurement of small to volume of solution small	

6.Deep freezer racks with gloves **Use:** Keeping the biological materials for long uses	
7.Tip-box **Use:** Helps in measures the small to small volume of biological solutions	
8.Analytical Balance **Use:** Measurements of chemicals and the biological samples	
9.Conical flask **Use:** used calibration of solution	
10.Deep freezer **Use:** Storage of biological materials for long times	
11.Spectrophotometer **Use:** Quantification of biological samples based on specific absorption	
12.Autoclave **Use:** Autoclave the materials used in tissue culture lab	

13.Scizzers,forceps,scalpel ,needle **Use:** cutting and handling of tissue culture materials	
14.Tissue culture rack **Use:** Growing of plant tissue culture materials	
15.Laminar air –flow **Use:** Aseptic inoculation of tissue culture materials	
16. Bacterial filtration unit **Use:** Filtration of microbes	
17. Simple inoculation hood **Use:** Aseptic inoculation of tissue culture materials in small scale	
18. Inverted microscope **Use:** Visualization of plant tissues culture materials such as callus etc.	

19.Stereioscopic microscope **Use:** Visualization of three dimension plant tissues culture materials	
20. Heating block **Use:** Heating the small tube at desired temperature	
21. Blotting paper **Use:** Removing of excessive water and moistening the culture materials	
22. Tissue culture plate **Use:** Culture of plant tissue materials	
23 Sterilization unit **Use:** Heating the forceps, scissors and scalpels	
24. Shakes **Use:** Provide proper aeration of culturing materials	
25.Gene- gun **Use:** For plant transformation by direct method	
26.Helios **Use:** For plant transformation	

27.Micro-wave oven **Use:** Heating and melting of agarose	
28.Gel electrophoresis unit **Use:** To visualize the DNA /RNA after resolution of in gelling materials	
29.vortex shaker **Use:** Proper mixing of culturing materials	
30. Refrigerator **Use:** Store the plant materials for experimental purpouse	
31. Thermo -cycler **Use:** Amplify the target sequences with specific primers	
32. Gel document system **Use:** To visualize the DNA/RNA/Protein	

TERMINOLOGY

Anther culture: It is a biological culture technique in which generation of haploid plants from donor plants aseptically culture in nutrients medium.

Auxins: These are organic substances which at low concentration (less than 0.001 m) promote growth along the longitudinal axis, but auxins also influence a wide range of growth and development responses.

Abscisic acid (ABA): It is involved in the abscission of plant organs, retardation of vegetative buds, regulation of fruits repining and generally in reduction of growth.

Biotechnology: The production of useful products by the use of living organisms and basic scientific techniques in control environmental conditions

Cell: The smallest structural and functional unit of an organism, which is typically microscopic and consists of cytoplasm and a nucleus enclosed in a membrane

Central Dogma: It describes the two-step process, transcription and translation, by which the information in genes flows into proteins: DNA → RNA → protein.

DNA: It is an important biological macromolecule containing a ribose sugar that genetic information encoded transfer to RNA.

Cytokines: These hormones mainly stimulate cell divisions and prevent chlorophyll degradation.

Ethylene: It is the only gaseous hydrocarbon hormone which plays an important role in the ripening of fruits, inhibition of root growth, abscission and other growth processes.

Gene: It is polymer of nucleotides exhibit the functional unit of heredity consist of promoter, terminator and regulatory sequence with set of particular instructions or specific functions.

Gibberellins: The gibberellins are phytohormones which are active in regulating dormancy, flowering, fruit setting, and stimulating germination of seeds and extending growth of shoots.

Growth retardants: It is synthetic compound that retard the growth. e.g., Cycocel (CCC), Phosphon D and maleic hydrazide.

Growth inhibitor: A compounds that can inhibit the growth is called Growth inhibitor, e.g., Auxin inhibit growth of lateral buds (maintains apical dominance).

Phenotype: The physical appearance of the individual is known as the phenotype

Plant hormones (Phytohormones): It is organics signally molecules naturally produce in very small amount that regulates the many physiological processes.

Plant growth regulator: A growth regulator is a natural or synthetic organic compound, that modifies or controls the one or more specific physiological processes within a plant but the sites of action and production might be different.

Plant Tissue culture: It is aseptic culture of plant cells, tissues or organs under control environmental condition supplemented specific nutrients medium is called plant tissues culture.

Micropropagation: It is the technique of in-vitro production of the clones of plants from little segment of plant under controlled aseptic conditions in large scale.

Morphogenesis: It is the biological process that causes an organism to concerns the origin and development of the physical form and external structure of plants.

Pollen culture: The culture of microspore or the immature pollen directly as an explant for the production of haploid plants aseptically called pollen culture.

Ribonucleic acid (RNA): It is an important biological macromolecule of single stranded containing a ribose sugar that convert the genetic information encoded by DNA into proteins.

Somatic embryogenesis: It is an artificial process in which a plant or embryo is derived from a single somatic cell.

Test tube fertilization: It is a biological technique involves controlled artificial pollination of the female parent with pollen from the selected male parent performed outside the living system aseptically into a glass container is called test tube fertilization.

IMPORTANT QUESTIONS

1. Why the haploid plants are sterile?

In haploid plants, each chromosome is represented only once and this is the reason there is no zygotene pairing in first meiotic division. Thus, all the chromosomes appear as univalent. During anaphase I, each chromosome moves freely and forms generally more than two groups. Gametes with less than the haploid number are generally not viable; therefore, haploid plants are highly sterile.

2. Why colchicines treatment is important?

Colchicine has been utilized widely as a spindle inhibitor to induce chromosome duplication and to produce polyploid plants. This method has been employed for obtaining homozygous diploid plants from haploid culture. The young, plantlets while still enclosed within anther, are treated with 0.5% colchicine solution for 24-48 hrs. Treated plantlets are replanted in the medium after thorough washing. In case of mature haploid plantlets, 4% colchicine-lanoline paste may be applied to the axil of the leaves.

3. What is/are important of homozygous Plants?

Haploid plants derived from either anther culture or pollen cultures are sterile. These plants contain only one set of chromosomes. By doubling their chromosome number, the plants can be made fertile and the resultant plants will be homozygous diploid or isogenic diploid. These homozygous diploid plants show the normal meiotic segregation. The fertile homozygous diploid plants are more important than the sterile haploid plants. Homozygous diploid plants can be used as pure lines in breeding programme.

4. What is significance of haploid plants?

In a diploid cell the chromosomes exist in homologous pairs. The genes for specific characters are also formed in pairs which are known as allelic gene pairs. For an example, T gene (for tallness) is an allele of t gene (for dwarfness) and vice versa in heterozygous condition. Each allele is located on one of the pair of homologous chromosome at a particular gene locus.

5. How haploid culture used in molecular biology?

In genetic engineering, haploids can be successfully used to gene transfer. Haploid tissues of Arabidopsis and Lycopersicon have been used for the transfer and expression of three genes from Escherichia coli.