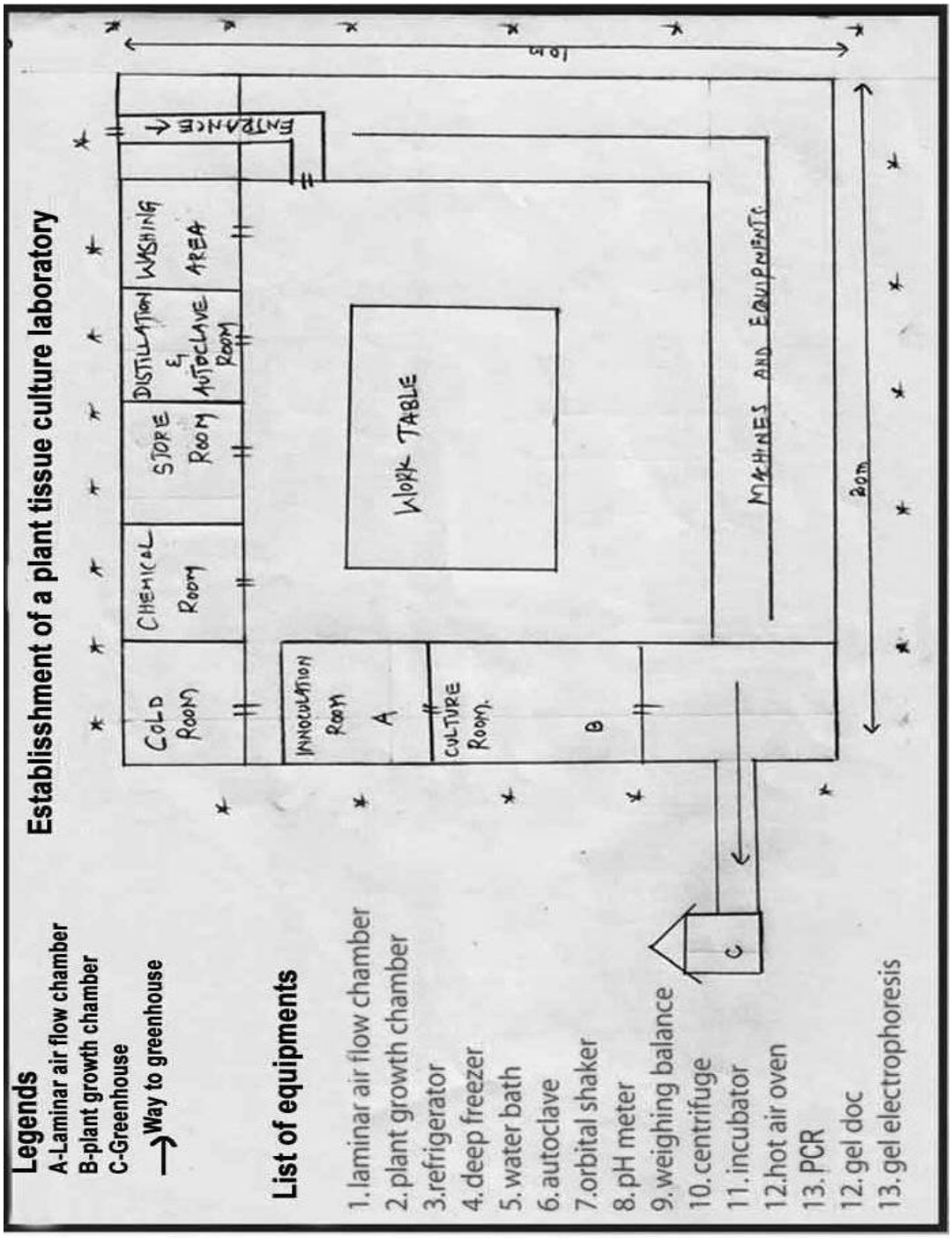
OPC SUPPLEMENTARY MATERIALS

Ex. No. 1 Layout of a laboratory



Ex.no. 2. See the material

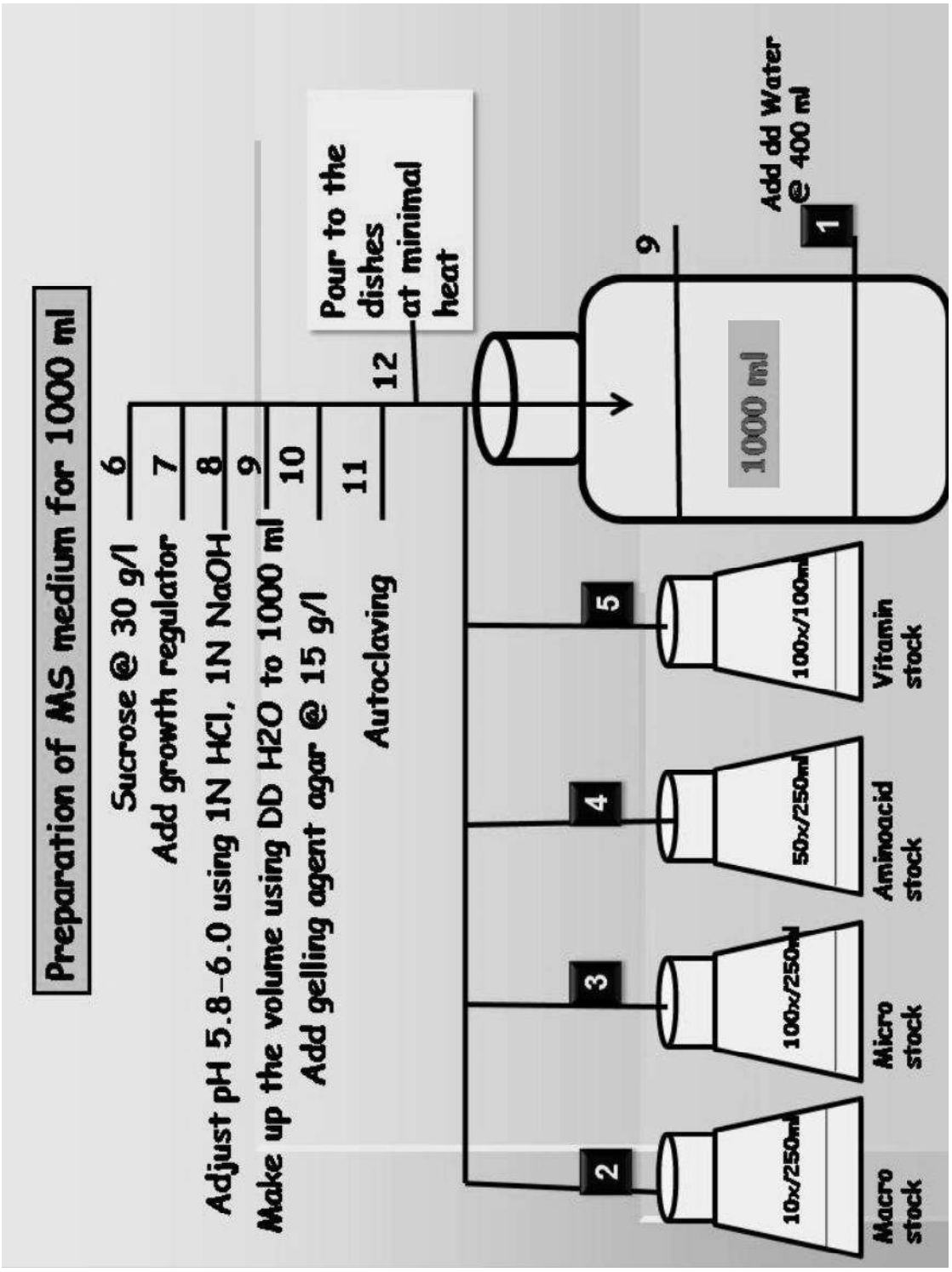
Ex. NO. 3 Aseptic techniques

Technique	Material	Materials sterilized
Steam sterilization/Autoclaving	Autoclave (121°C at 15 psi for 20-40 min)	Nutrient media, culture vessels, glassware and solvents such as water etc
Dry heat/hot air sterilization	Hot air oven (160-180°C for 3h)	Only glassware
Red hot/Flame sterilization	Spirit lamb	scalpel, forceps, needles etc, mouth of culture vessel
Filter sterilization	(membrane filter made of cellulose nitrate or cellulose acetate of 0.45- 0.22µm pore size)	Thermolabile substances like growth factors, amino acids, vitamins and enzymes.
Alcohol sterilization	70% ethanol	Worker's hands, laminar flow cabinet
Surface sterilization	(70%ethanol, Sodium hypochlorite, hydrogen peroxide, mercuric chloride etc)	Explants
Radiations	Ionizing UV lights	Sterilize the laminar inner cabinet
Fumigations	Fumigants-HCHO and KMNO4	Sterilize the entire laboratory

Ex. NO. 4 a.Composistion of MS Medium

Ingredients	Final composition in medium (mg/l)	Stock solution (W/V)	Volume of the stock to be taken per litre of medium
Macro Nutrients (10X)			
NH_4NO_3	1650	16.50 g	
KNO_3	1900	19.00 g	
$CaCl_2$ $2H_2O$	440	4.40 g	
$MgSO_4$. $7H_2O$	370	3.70 g	
KH_2PO_4	170	1.70 g in 250 ml	25 ml
Minor Nutrients (100X)			
$MnSO_4$ $4H_2O$	22.3	2.23 g	
$ZnSO_4$ $4H_2O$	8.6	0.86 g	
H_3BO_3	6.2	0.62 g in 250 ml	2.5 ml
Micro Nutrients (100X)			1.0 ml
Na_2MoO_4. $2H_2O$	0.250	25.0 mg	
$CuSO_4$ $5\ H_2O$	0.025	2.5 mg	
$CoCl_2$ $6\ H_2O$	0.025	2.5 mg in 100 ml	
KI (100X) KI	0.83	0.083 g in 250 ml	2.5 ml
Iron Stock (50X)			5.0 ml
Na_2EDTA	37.25	1.863 g	
$FeSO_4$ $7H_2O$	27.85	1.393 g in 250 ml	
MS Vitamins (100X)			1.0 ml
Nicotinic Acid	0.5	50 mg	
Pyridoxine.HCl	0.5	50 mg	
Thiamine. HCl	0.1	10 mg	
Glycine	2.0	20 mg in 100 ml	
Carbon source-source	30g/l	Added as solid	
Solidifying agent-agar	15g/l	Added as solid	
pH	5.8	Adjust with 0.1 KOH and 0.1 HCl	

(B) Preparation of MS medium for 1000 ml



EX. NO. 5 Preparation of PGR stock solutions

Characteristics of plant growth regulators

Name	Chemical formula	Molecular weight	Solubility
p-Chlorophenoxy acetic acid	$C_8H_7O_3Cl$	186.6	96% ethanol
2,4-Dichlorophenoxy acetic acid	$C_8H_6O_3Cl$	221.0	96% ethanol, heated lightly
Indole-3 acetic acid	$C_{10}H_9NO_2$	175.2	1N NaOH/96% ethanol
Indole-3 butyric acid	$C_{12}H_{13}NO_2$	203.2	1N NaOH/96% ethanol
α-Naphthalene acetic acid	$C_{12}H_{10}O_2$	186.2	1N NaOH/96% ethanol
β-Naphthoxy acetic acid	$C_{12}H_{10}O_3$	202.3	1N NaOH
Adenine	$C_5H_5N_5.3H_2O$	189.1	H_2O
Adenine sulphate	$(C_5H_5N_5)_2.H_2SO_4.2H_2O$	404.4	H_2O
Benzyl adenine 6 benzyl amino purine	$C_{12}H_{11}N_5$	225.2	1N NaOH
N-isopentenyladenine (2 iP)	$C_{10}H_{13}N_5$	203.3	1N NaOH
Kinetic	$C_{10}H_9N_5O$	215.2	1N NaOH
Zeatin	$C_{10}H_{13}N_5O$	219.2	1N NaOH/1N HCl, heated lightly
Gibberellic acid	$C_{19}H_{22}O_6$	346.4	Ethanol
Abscisic acid	$C_{15}H_20O_4$	264.3	1N NaOH
Colchicine	$C_{22}H_{25}NO_6$	399.4	H_2O

Formula

$$V1N1 = V2N2$$

Therefore

$$V1 = \frac{V2N2}{N1}$$

V1 = Volume to be pipetted from stock

N1 = Concentration of stock prepared

V2 = volume of media prepared

N2 = Required stock concentration

$$\text{Volume of stock solution Required} = \frac{\text{Desired hormone concentration} \times \text{Volume of media required}}{\text{Stock solution concentration}}$$

Calculations in plant growth regulators

1. Calculate the volume of stock solution to be pipetted out for the preparation of 500 ml of papaya callus induction medium comprised of 2, 4-D @ 2 mg/l and the given stock concentration of 1 mg/ml.

Solutions

Given V1 =? V2= 500 ml

N1= 1 mg/ml N2= 2 mg/l = 2 mg/1000 ml

$$V1 = \frac{V2 \ X \ N2}{N1}$$

$$= 500 \text{ ml } X \ \frac{\frac{2 \text{ mg}}{1000 \text{ ml}}}{\frac{1 \text{ mg}}{\text{ml}}}$$

$$= 2.0 \text{ ml}$$

RESULT: The volume of stock solution to be pipetted out the given stock concentration of 1 mg/l is 2.0 ml.

2. Calculate the volume of stocks solution to be pipetted out from the given stock concentration of BAP (1mg/ml) and NAA (0. 2 mg/ml) for the preparation of 500 ml of rice regeneration medium comprised of BAP (1 mg/l) and NAA (0.1 mg/l).

Solutions

Given BAP	**Given NAA**
V1 =?	**V1 =?**
V2= 500 ml	**V2= 500 ml**
N1= 1 mg/ml	**N1= 0.2 mg/ml**
N2= 1 mg/l=1 mg/1000 ml	**N2= 0.1 mg/l=0.1mg/1000 ml**
$V1 = \frac{V2 \times N2}{N1}$	$V1 = \frac{V2 \times N2}{N1}$
$= 500 \text{ ml} \times \frac{1 \text{ mg}}{\frac{1000 \text{ ml}}{\frac{1 \text{ mg}}{\text{ml}}}}$	$= 500 \text{ ml} \times \frac{0.1 \text{ mg}}{\frac{1000 \text{ ml}}{\frac{0.2 \text{ mg}}{\text{ml}}}}$
$= 0.5 \text{ ml}$	$= 0.25 \text{ ml}$
$= 500 \ \mu\text{l}$	$= 250 \ \mu\text{l}$

RESULT: The volume of stock solution to be pipetted out the given stock concentrations of BAP is 500 µl and NAA is 250 µl

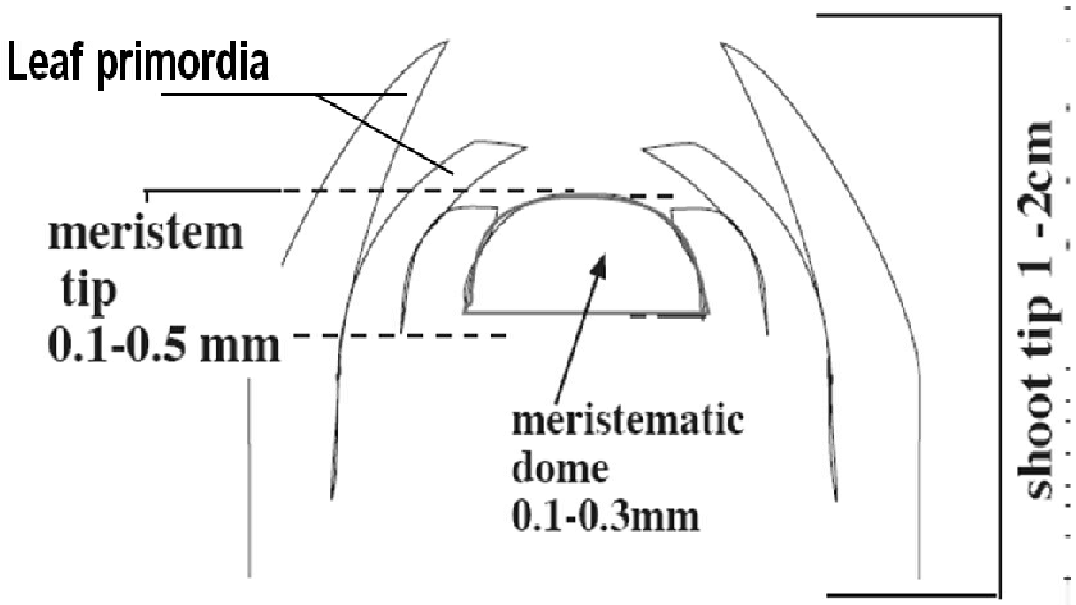
3. The sesame regeneration medium consisted of MS supplemented with TDZ (1.0 mg/l) and IBA (0.5 mg/l). Calculate the volume of stocks to be pipetted out from the given stock concentration of TDZ (0.1 mg/ml) and IBA (0.01 mg/ml) for preparation of 250 ml medium

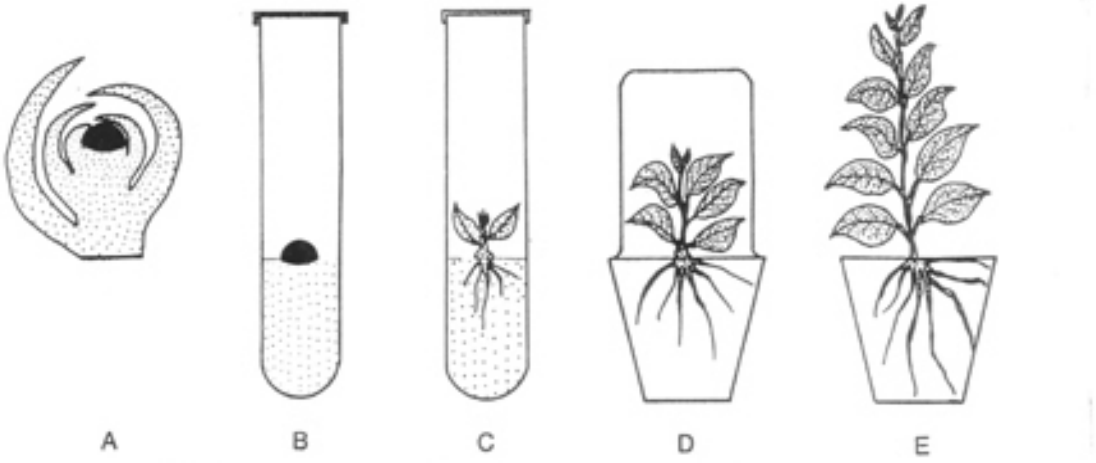
Solutions

Given TDZ	**Given IBA**
V1 =? **V2= 250 ml** **N1= 0.1 mg/ml** **N2= 1 mg/l=1 mg/1000 ml** $V1 = \frac{V2 \times N2}{N1}$ $= 250 \text{ ml} \times \frac{1 \text{ mg}}{\frac{1000 \text{ ml}}{\frac{0.1 \text{ mg}}{\text{ml}}}}$ $= 2.5 \text{ ml}$	**V1 =?** **V2= 250 ml** **N1= 0.2 mg/ml** **N2= 0.5 mg/l=0.5 mg/1000 ml** $V1 = \frac{V2 \times N2}{N1}$ $= 250 \text{ ml} \times \frac{0.5 \text{ mg}}{\frac{1000 \text{ ml}}{\frac{0.2 \text{ mg}}{\text{ml}}}}$ $= 0.625 \text{ ml}$ $= 625 \ \mu l$

RESULT: The volume of stock solution to be pipetted out the given stock concentrations of TDZ is 2.5 ml and IBA is 625 µl

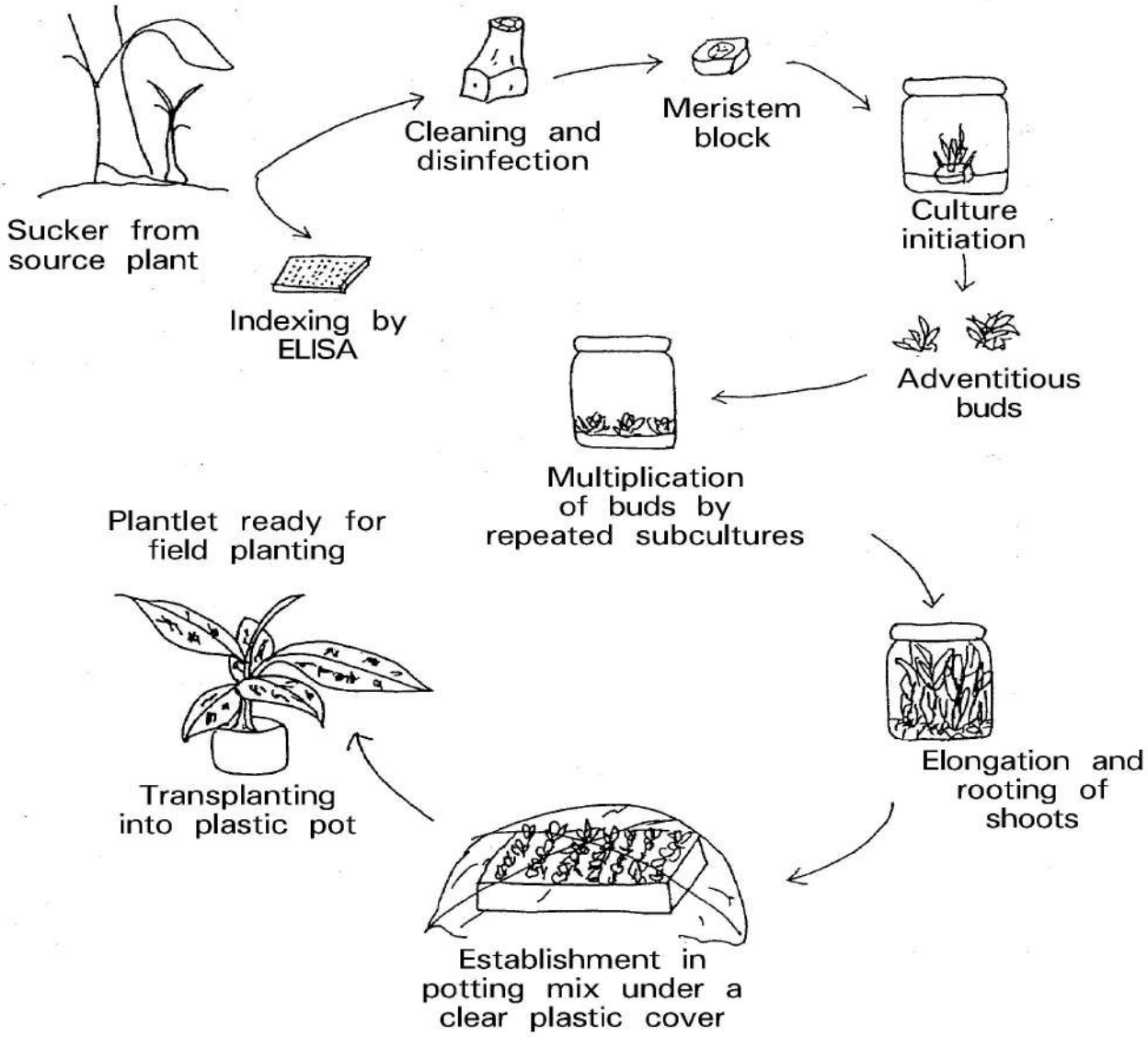
Ex. No. 6 Meristem tip culture



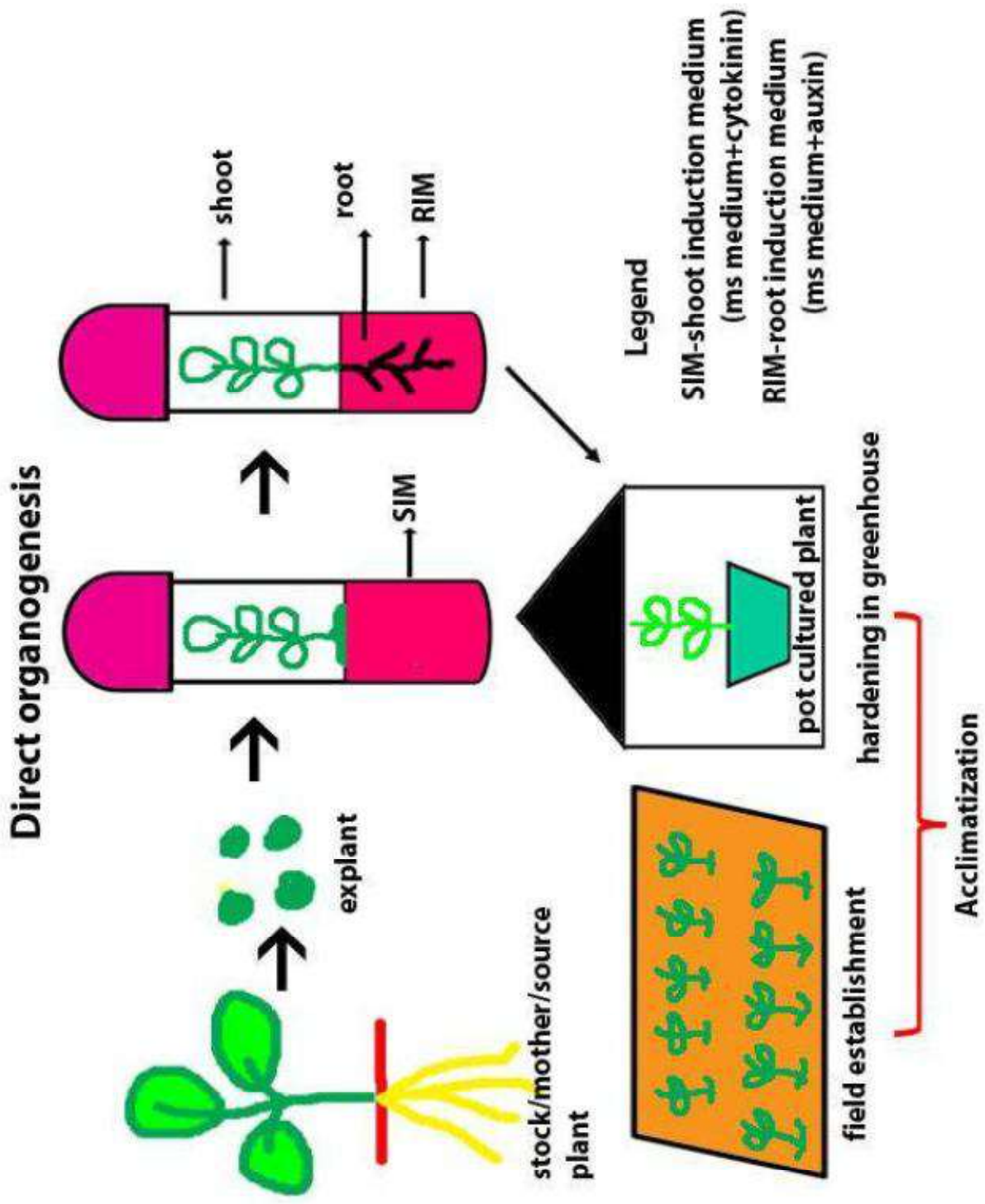


A) Apical meristem showing section to be excised. B) Excised meristem tip cultured on agar medium. C) plantlet regenerated from excised meristem tip. d) plantlet transferred to sterile soil. E) virus-free plant growing in soil.

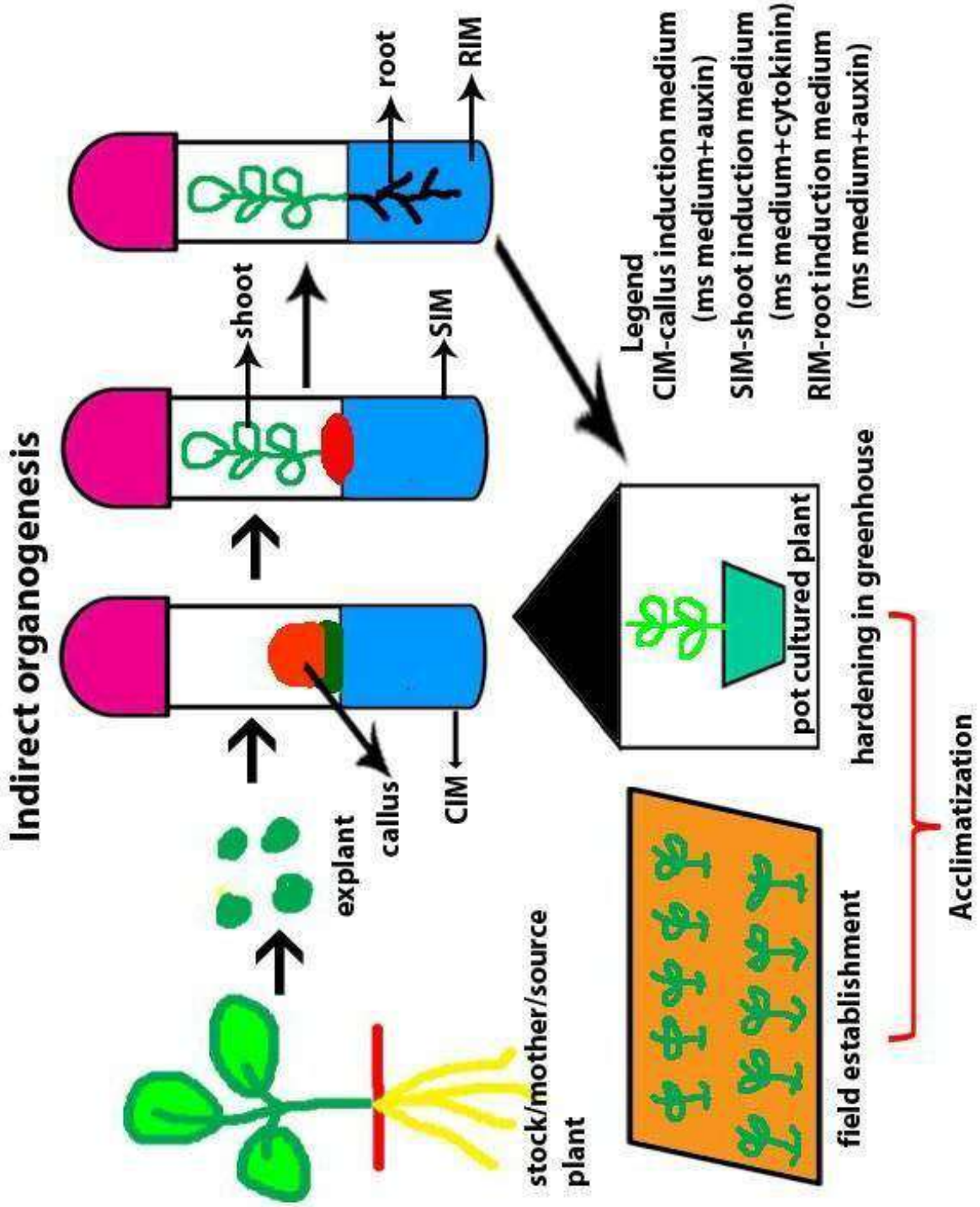
Ex. No. 7. Micropropagation in Banana via Rhizome bud culture



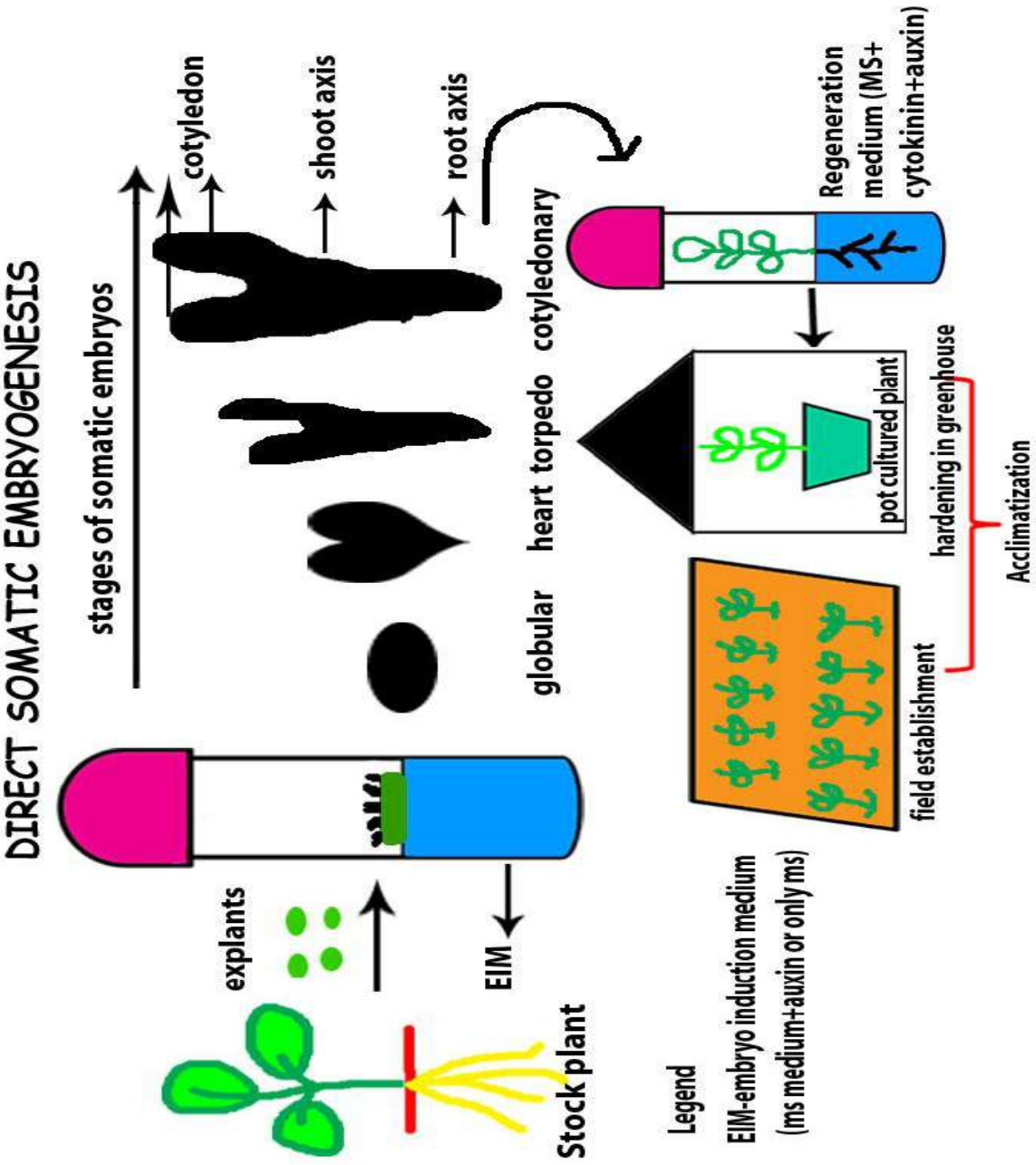
E. No. 8; Regeneration via direct organogenesis pathway



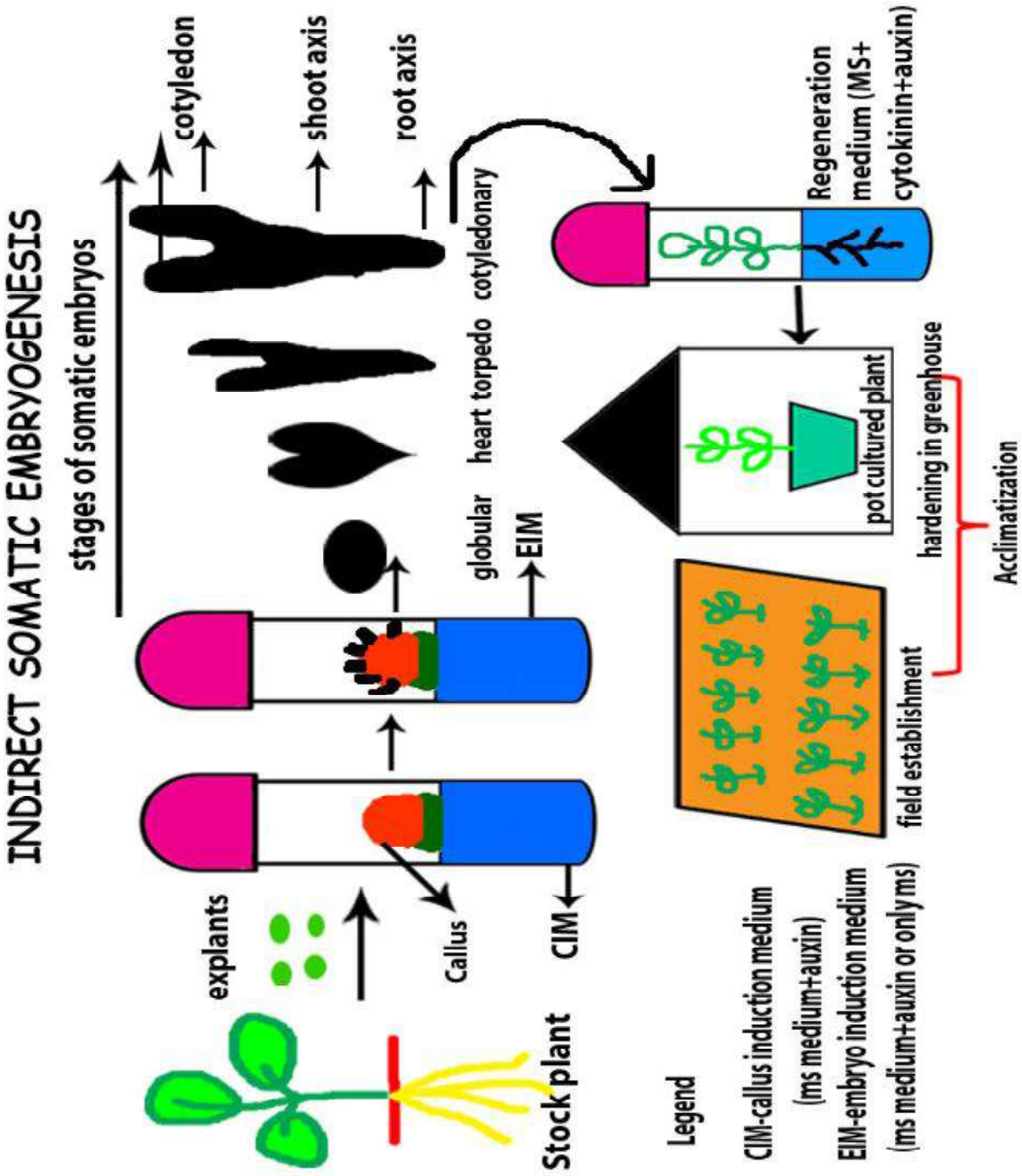
E. No. 9; Regeneration via indirect organogenesis pathway



E. No.10; Regeneration via direct somatic embryogenesis pathway



E. No.11; Regeneration via indirect somatic embryogenesis pathway



E. No.12, 13, 14 see the record material