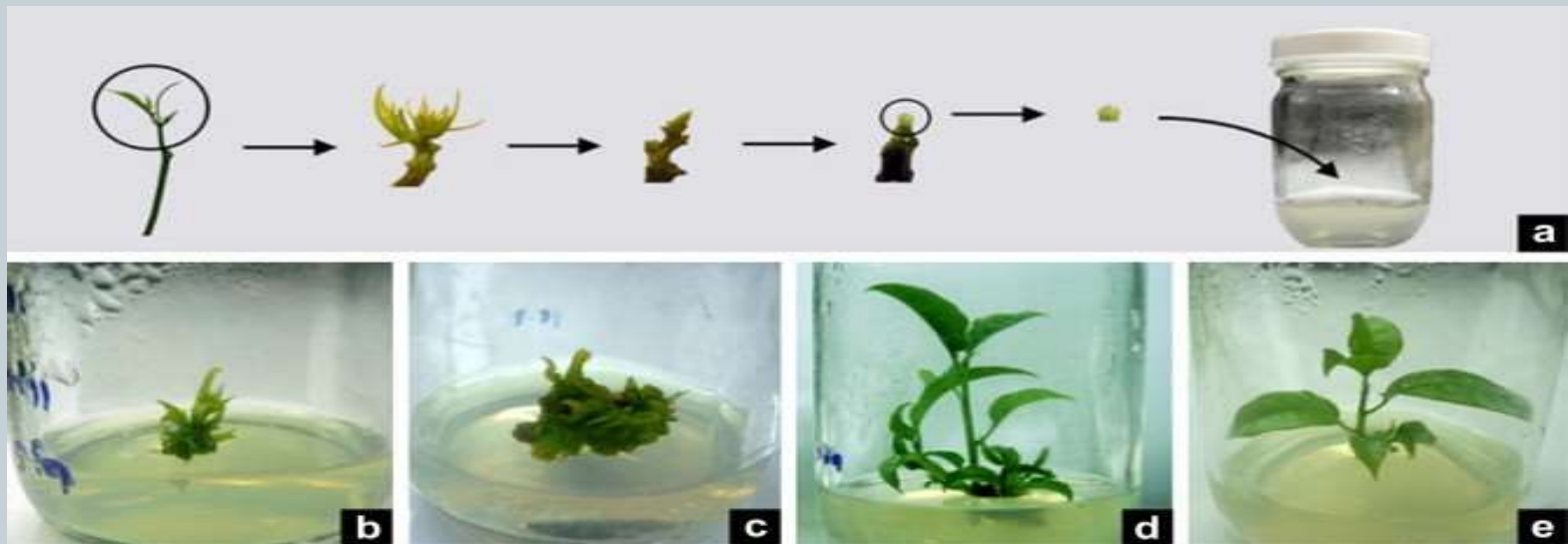


**TITLE: PRODUCTION OF THE VIRUS FREE PLANTS BY
MERISTEM TIP CULTURE
DR. NISHI MATHUR**

Objectives:

- a. To be acquainted with the principles, methods and procedures of the meristem tip culture**
- b. To be able to perform the meristem tip culture in the laboratory.**



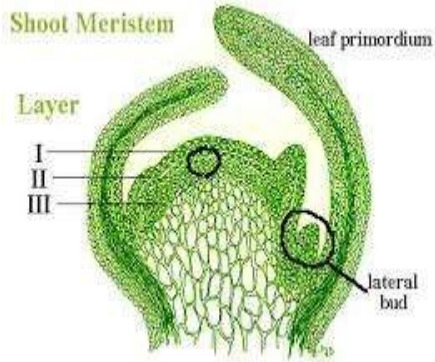
PRODUCTION OF THE VIRUS FREE PLANTS BY MERISTEM TIP CULTURE

CHEMICALS REQUIRED:

- Inorganic nutrients (N₂,P,Ca,Mg,S)
- Carbon source (sugar)
- Organic supplements including
- Vitamins (Thiamine, nicotinic acid, panthonic acid, pyridoxine)
- Amino acids (L-glutamine, L-asparagine, L-cysteine, Lglycine)
- Complex organics (casein hydrolysate, coconut milk, yeast extract, orange juice, tomato juice)

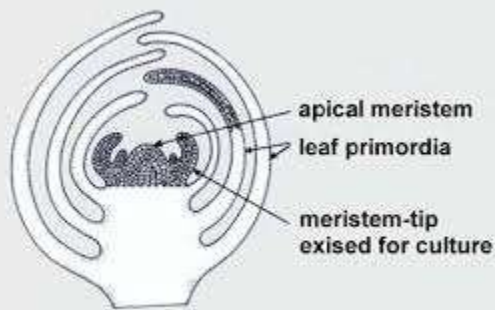


PRODUCTION OF THE VIRUS FREE PLANTS BY MERISTEM TIP CULTURE



In vitro culture techniques for virus elimination

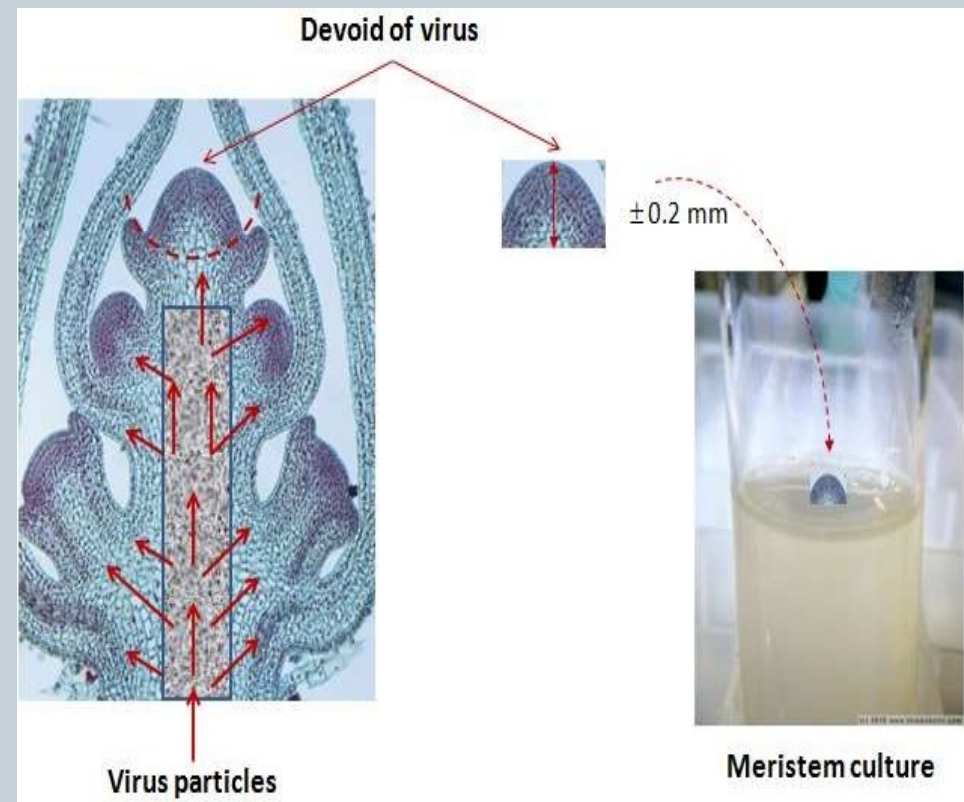
- Meristem tip culture
- Thermotherapy
- Cryotherapy
- Chemotherapy
- Electrotherapy
- Combination of these methods





Why meristem tip culture?

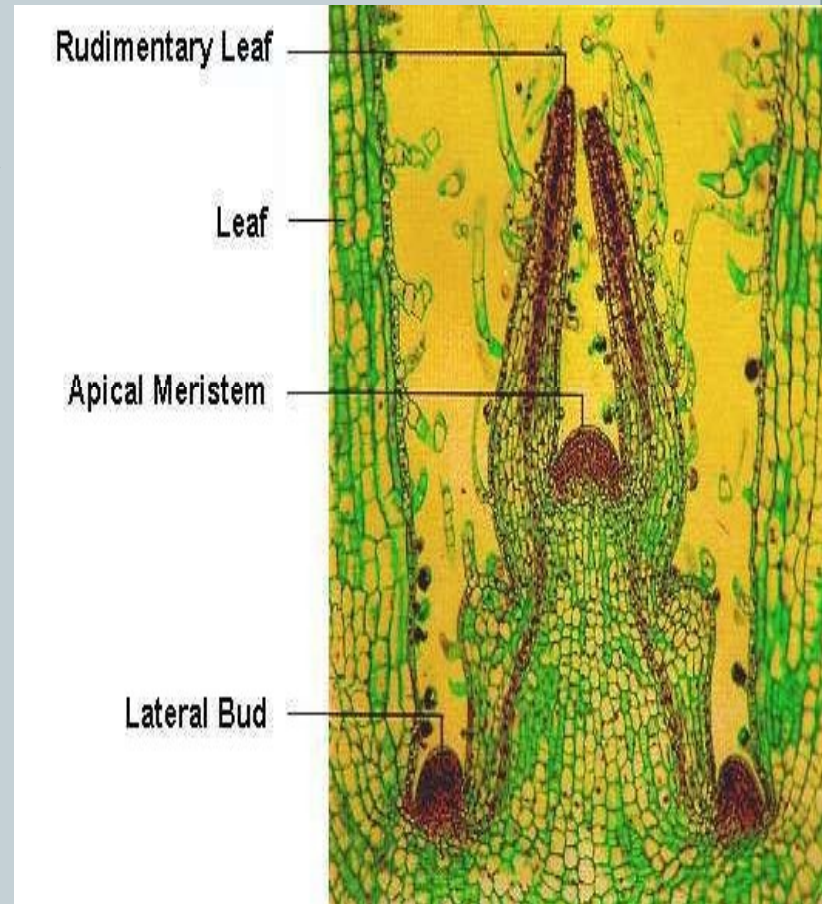
- ❖ Rate of cell division is high then virus multiplication
- ❖ High metabolic activity , no virus replication
- ❖ No vascular system , no movement of viruses
- ❖ Endogenous auxin level high , inhibit virus multiplication

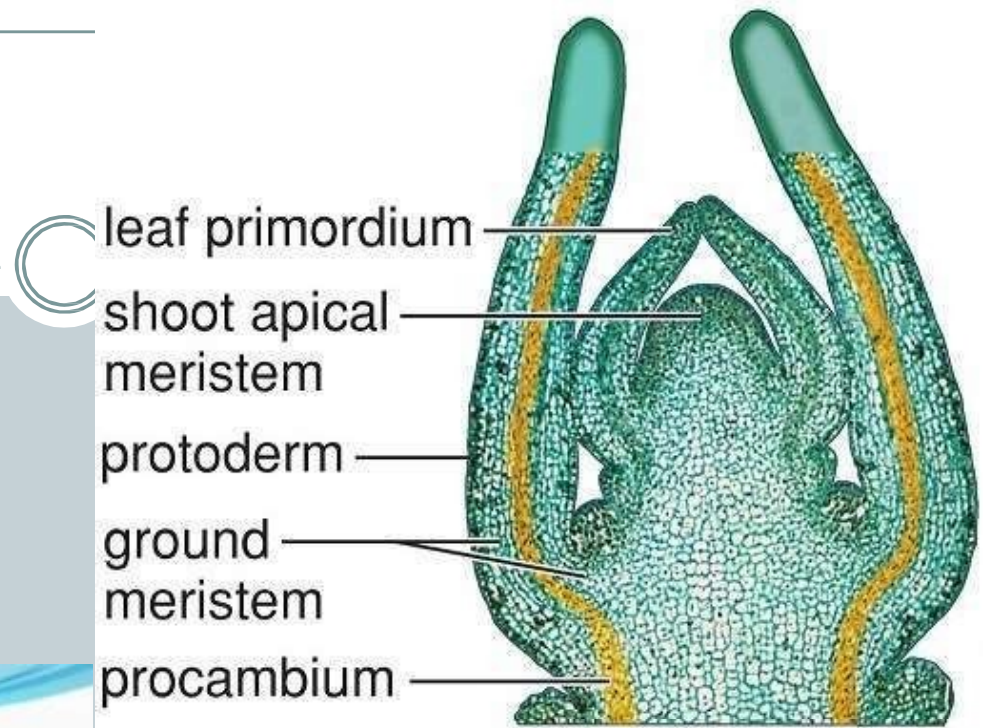


PRODUCTION OF THE VIRUS FREE PLANTS BY MERISTEM TIP CULTURE

Principle:

- ❖ **Morel and Martin (1952)** developed the technique of meristem culture for in vivo virus eradication of Dahlia.
- ❖ The cultivation of **axillary or apical meristems**, particularly of shoot apical meristem is known as meristem tip culture.
- ❖ The meristem or shoot apex is an active cell which is about **0.1 mm diameter and 0.25 mm long**.
- ❖ It is totipotent region that produces all the upper parts of plants and, when cultured, can be grown into a shoot that can be rooted to form a whole plant.
- ❖ It does not involve the regeneration of a new shoot meristem.
- ❖ Meristem culture involves the development of an **already existing shoot apical meristem** and the regeneration of adventitious roots.





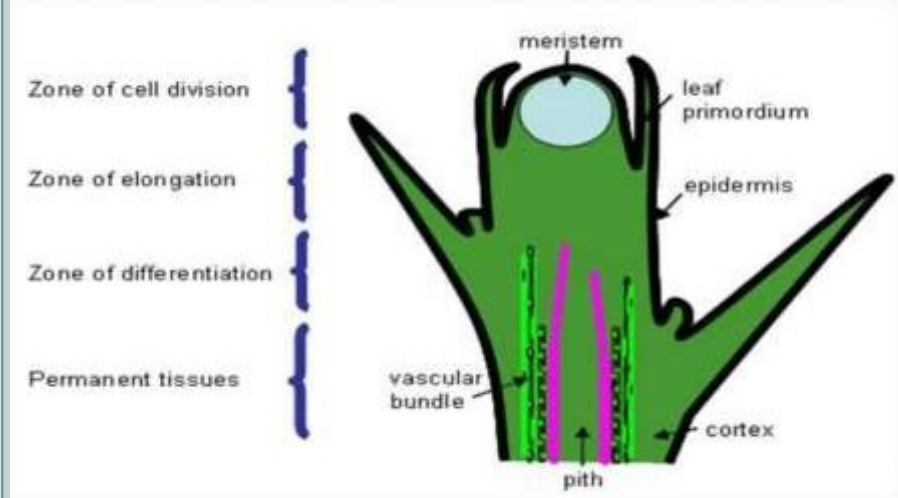
leaf primordium

shoot apical meristem

protoderm

ground meristem

procambium



Zone of cell division

Zone of elongation

Zone of differentiation

Permanent tissues

meristem
leaf primordium
epidermis
vascular bundle
pith
cortex

Apical Meristem

PRODUCTION OF THE VIRUS FREE PLANTS BY MERISTEM TIP CULTURE



Virus elimination depends on:

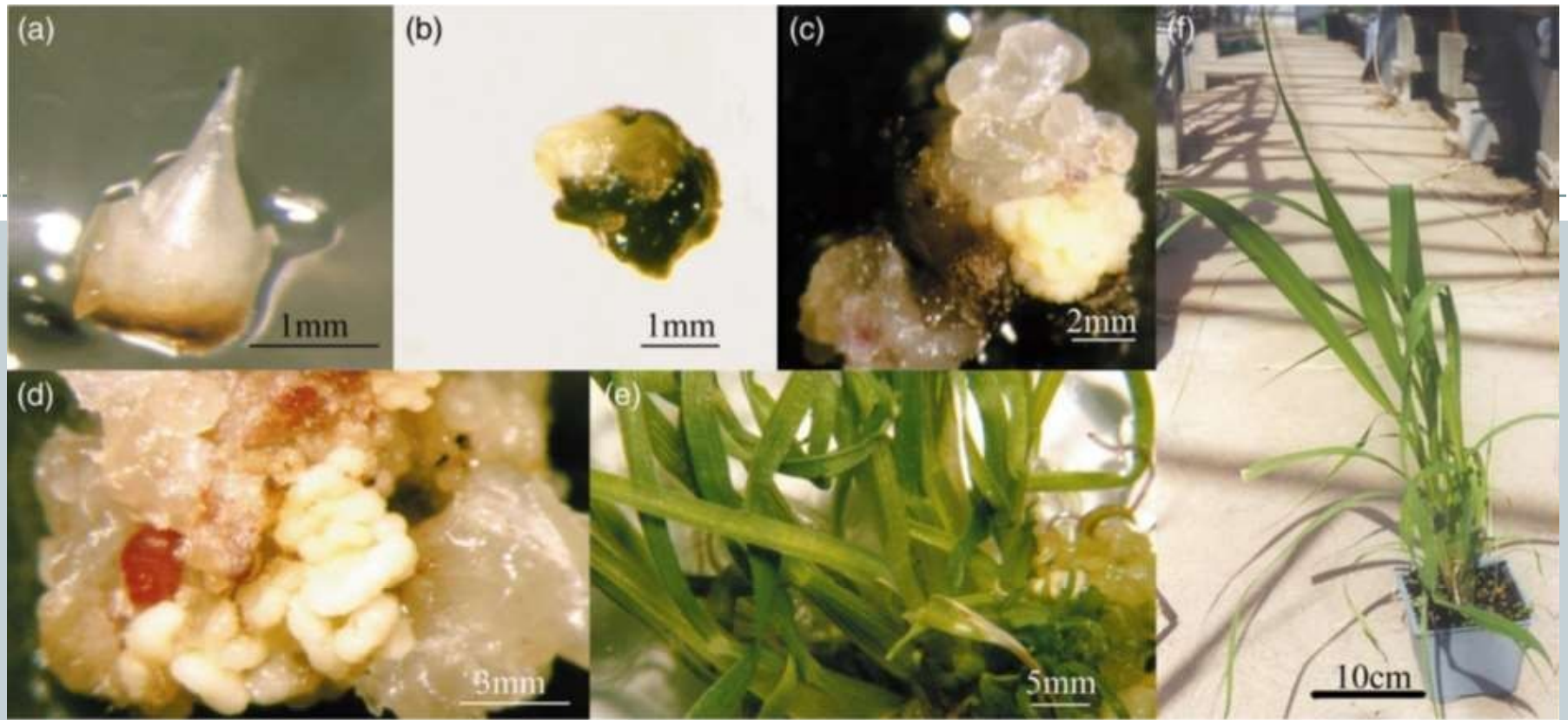
- **Meristem explant size**
- **Bud location** : terminal bud > have stronger growth potential than lateral.
- **Season**: early spring & early autumn > winter & summer; after dormancy of storage organs
- **Heat treatment** : in water or in air
- **Culture media** : high concentrations of growth hormones inhibit virus growth

Brief list of disease free plants

Sr#	Plant species	Virus eliminated	References
1	<i>Brasica oleracea</i> (cauliflower)	CbSvr TuMV,CIMV	Paludan (1971) Walkey et. al. (1974)
2	<i>Fragaria sp</i> (strawberry)	Crinkle Yellow virus complex	Kacharmozov and izovorsaka (1974) Miller and blekengren (1963)
3	<i>Malus sp</i> (apple)	Latent virus	Campbell (1962)
4	<i>Musa sp</i> (banana)	CMV, unidentified	Berg and bustamanate (1974)
5	<i>Nicotiana tobacum</i>	TMV Dark green island of TMV	White et. Al (1977) Murakishi and Carlson (1976)
6	<i>Rubus ideaus</i> (rusberry)	Mosaic	Putz (1971)
7	<i>Saccharum officinarum</i> (sugercane)	SCYLV Mosaic	Fitch et. Al (2001) Raj et. Al (1991)
8	<i>Solanum tuberosum</i> (Potato)	PaVM PSTV PVG	Dhingra et. al (1982) Lizarraga et. al (1980)
9	<i>Vitis vinifera</i>	GFLV AMV	Monette (1986) Monette (1986)
10	<i>Zingiber officinale</i>	Mosaic	Wang and Hu (1980)

Some of the works on the meristem tip culture

Species	Procedure	Virus	Reference
<i>Alstromeria</i> sp.	meristem culture	<i>Alstroemeria mosaic virus</i> (AlMV)	Chiari and Bridgen, 2002
<i>Chrysanthemum</i> sp.	meristem culture	<i>Cucumber mosaic virus</i> (CMV)	Verma et al., 2004
<i>Chrysanthemum morifolium</i> cv. Regol Time	meristem culture, chemotherapy and thermotherapy	<i>Chrysanthemum B Carla virus</i> (CVB)	Ram et al., 2005
<i>Chrysanthemum morifolium</i>	meristem culture	mixed infection by CMV and <i>Tomato aspermy virus</i> (TAV)	Kumar et al., 2009
<i>Chrysanthemum</i> sp.	meristem culture	<i>Tomato spotted wilt virus</i> (TSWV), <i>Impatiens necrotic spot virus</i> (INSV), <i>Iris yellow spot virus</i> (IYSV)	Balukiewicz and Kryczynski, 2005
<i>Dianthus gratianopolotanus</i>	meristem culture	<i>Carnation mottle virus</i> (CarMV), <i>Carnation latent virus</i> (CLV), potyviruses	Fraga et al, 2004a
<i>Lilium</i> sp.	meristem culture	<i>Lily symptomless virus</i> (LSV)	Allen, 1975
<i>L. x elegans</i>	meristem culture and thermotherapy	LSV	Nesi et al., 2009
New Guinea Impatiens (<i>I. hawkerii</i>)	meristem culture	mixed infection by TSWV and CMV	Gera and Dehan, 1992
<i>I. hawkerii</i>	meristem culture	TSWV	Milošević et al., 2011
<i>Phlox paniculata</i>	meristem culture and thermotherapy	CLV, CarMV, CMV, <i>Tobacco mosaic virus</i> (TMV), <i>Tospoviruses</i> (subgroups I, II and III), <i>Potyviruses</i>	Fraga et al., 2004b
<i>Viola odorata</i>	meristem culture	<i>Viola mottle virus</i> (VMV), CMV, <i>Bean yellow mosaic virus</i> (BYMV)	Van Caneghem et al., 1997



Photographs of sugarcane development from bud meristem explant to regenerated plant: (a) freshly excised bud meristem; (b) after 2 weeks, the meristem just emerging surrounded by leaf scales, which turned brownish; (c) after 6 weeks, with embryogenic calli in the middle and nonembryogenic calli at the sides; (d) embryogenic callus ready for regeneration; (e) after 2 months, regenerated plants in a Petri dish; (f) after 4 months, regenerated plants in soil

Fitch, M., Lehrer, A., Komor, E., & Moore, P. (2001). Elimination of Sugarcane yellow leaf virus from infected sugarcane plants by meristem tip culture visualized by tissue blot immunoassay. *Plant Pathology*, 50(6), 678.

PRODUCTION OF THE VIRUS FREE PLANTS BY MERISTEM TIP CULTURE



Chemotherapy and Meristem tip culture

1. Chemotherapy can be used with meristem-tip culture to increase the incidence of VF plants (Hollings, 1965; Walkey 1985).
2. Chemotherapy uses compounds such as **ribavirin (Virazole) or vidarabine (Vira A), Actinomycin-D, Cyclohexamide**, that affect the virus multiplication added into the culture medium for curing the shoot tips virus.
3. The principle of chemotherapy is similar to that of heat treatment in that the compound allows the virus to degrade and not allow it to multiply.

Host	Virus eliminated	Temperature
Chrysanthemum	Chrysanthemum B virus	35 – 38 °C
Carnation	Carnation ringspot virus 35 to 40°C Carnation vein mottle Viric virusus	35 – 40 °C
Banana	Cucumber mosa	35 – 43 °C
Goose Berry	Gooseberry vein banding virus	35 °C
Potato	Potato virus Y,S,X	35 – 38 °C

PRODUCTION OF THE VIRUS FREE PLANTS BY MERISTEM TIP CULTURE



Thermo-therapy and Meristem Culture

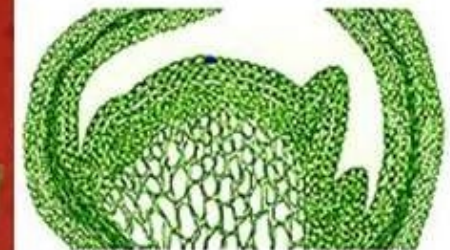
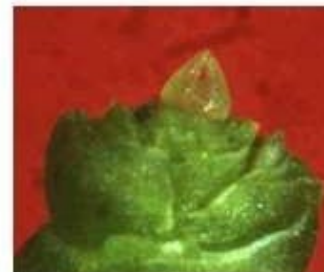
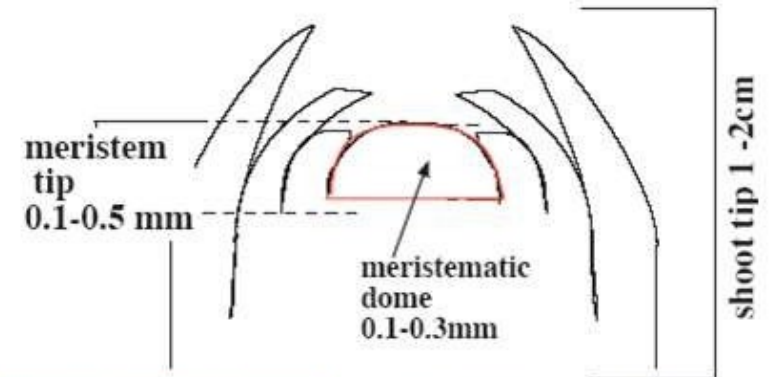
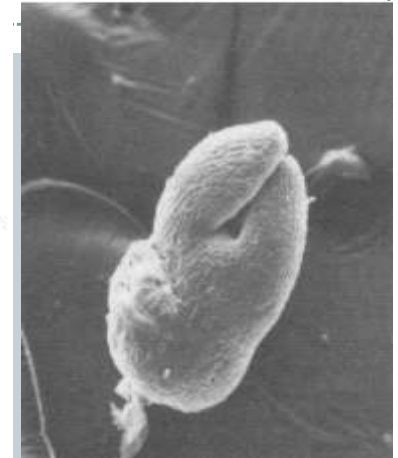
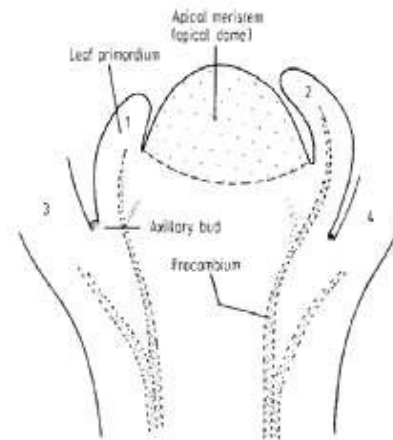
Ram *et al.*, 2005

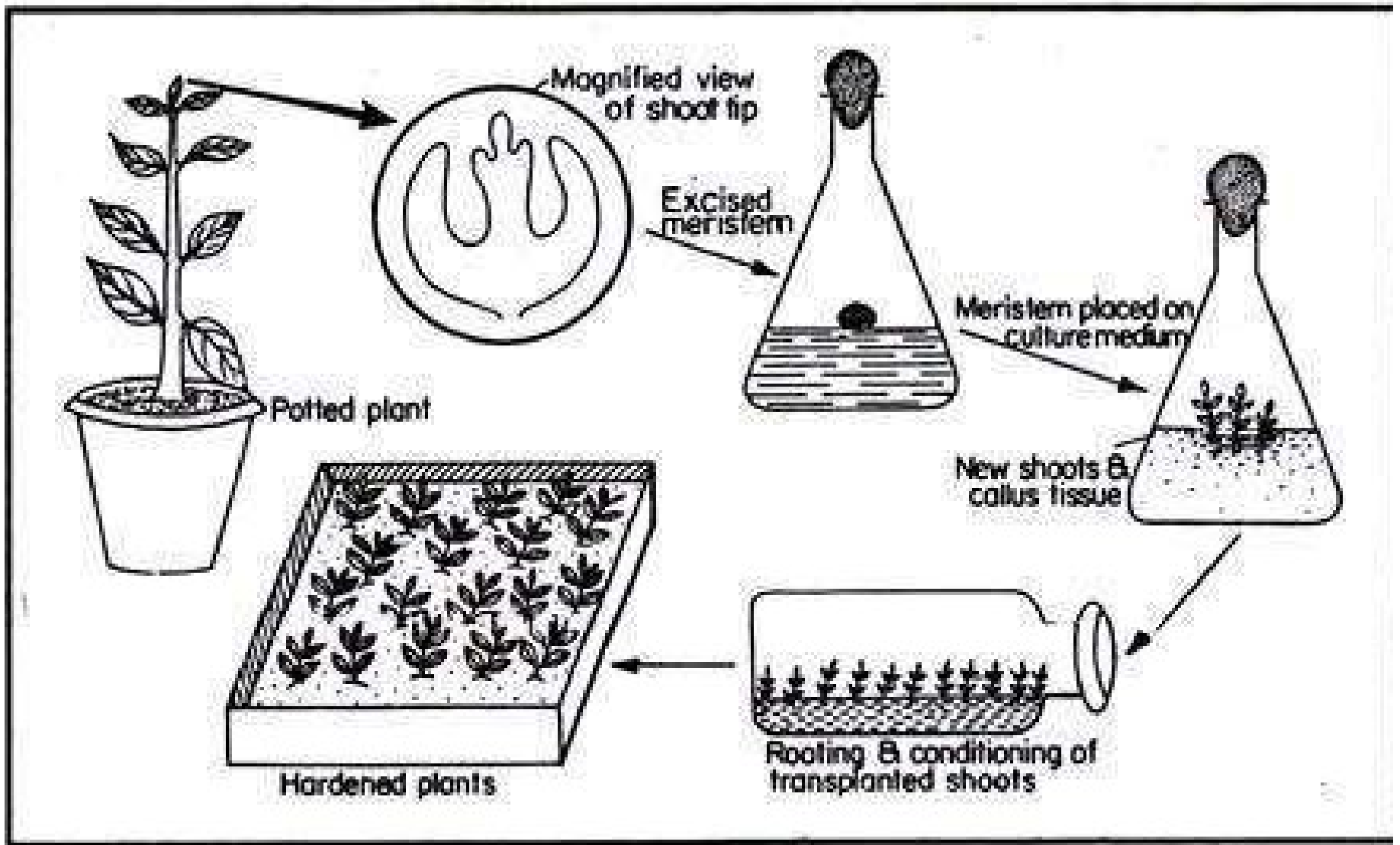
- Plants are exposed to temperature between **35 to 40° C**
- Growing host plants at higher temperatures significantly reduces replication of many plant viruses by **disrupting viral ssRNA and dsRNA synthesis.**
- Effective against **iso-metric** and **thread –like** viruses.
- Hot air treatment appears to be more successful in eliminating virus than the hot water method and causes less damage to the plant tissue.

PRODUCTION OF THE VIRUS FREE PLANTS BY MERISTEM TIP CULTURE

Protocol

- Apical meristem tips (domes of actively dividing cells with 1-2 leaf primordia) were excised in sterile conditions either from *in vivo* or *in vitro* plants or highly proliferating meristems
- Transferred to glass tubes on 10 ml of solid MS medium
- Tubes were maintained at $24 \pm 1^\circ \text{C}$ in dark conditions for 3 days, and then under standard illuminated conditions

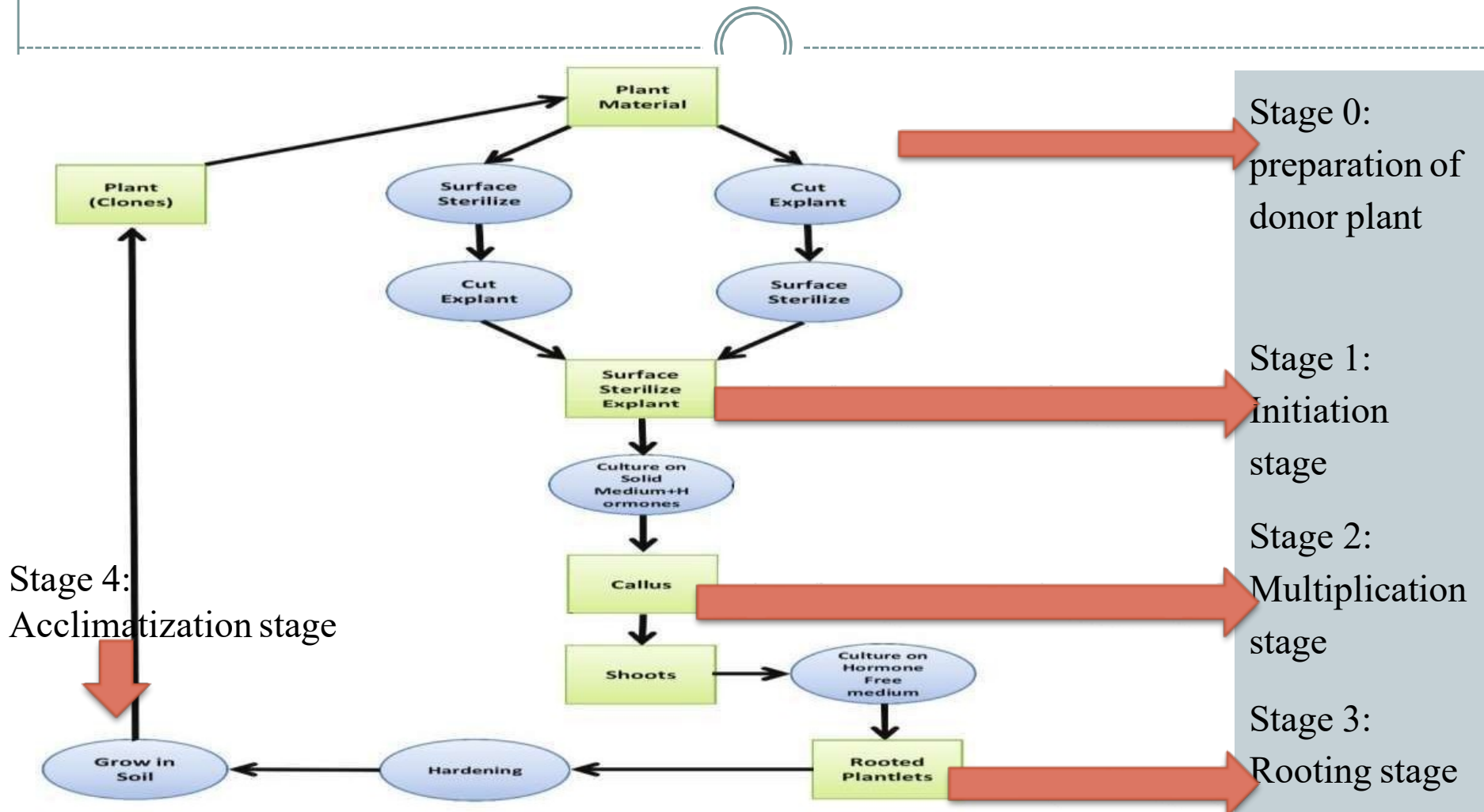




□ Fig 2.3

Flow diagram illustrating the technique of shoot tip or meristem culture

Steps of meristem tip culture



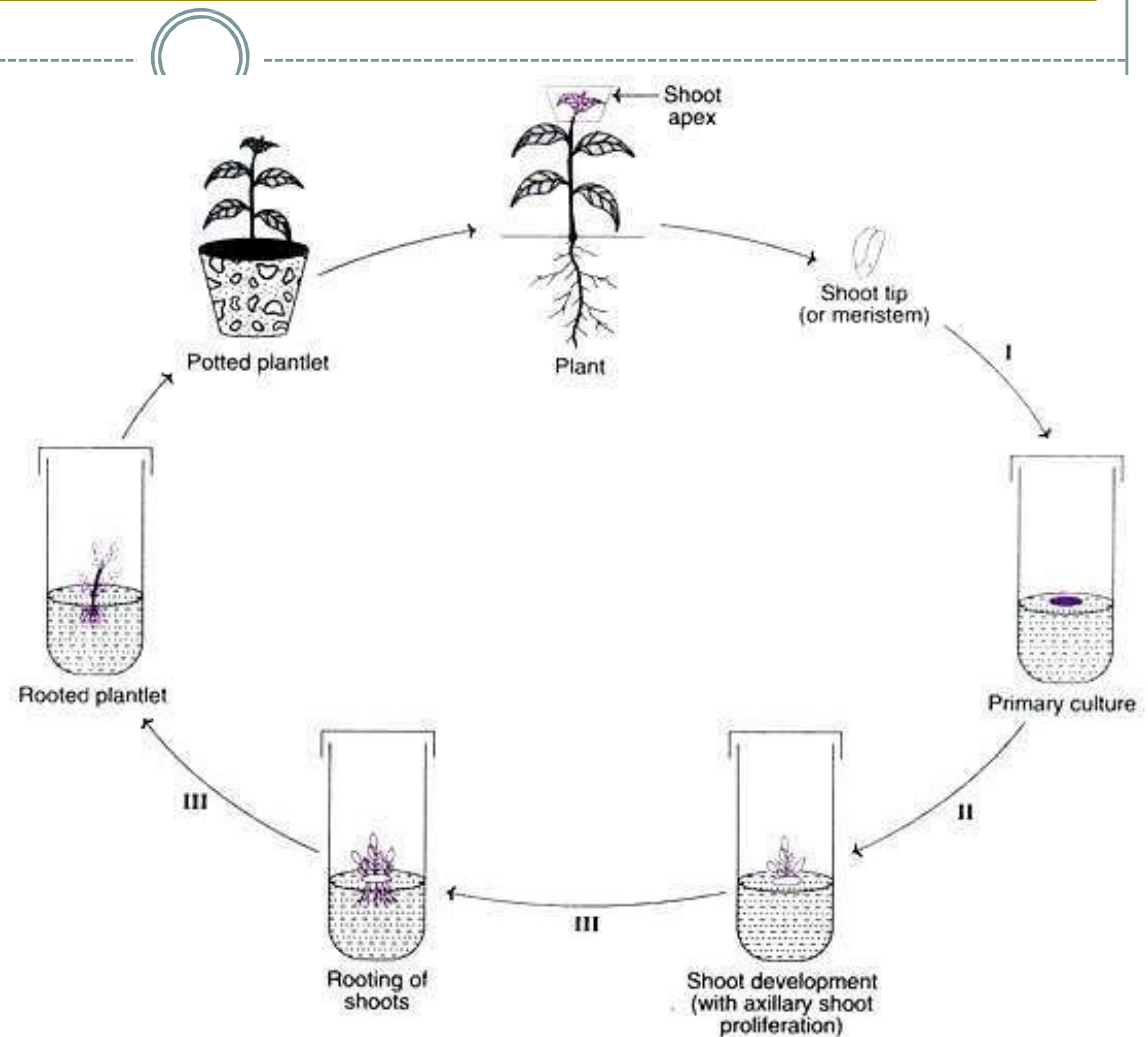
Hussain, A., Ahmed, I., Qarshi, Nazir, H. and Ullah, I. (2012). Plant Tissue Culture: Current Status and Opportunities. P 10.

PRODUCTION OF THE VIRUS FREE PLANTS BY MERISTEM TIP CULTURE

STAGES OF MERISTEM CULTURE

Stage 1: Culture establishment stage

- Stage when explant may develop into single shoot or multiple shoots.
- At this stage explant are supplemented with **cytokinin like BA, kinetin and 2iP.**

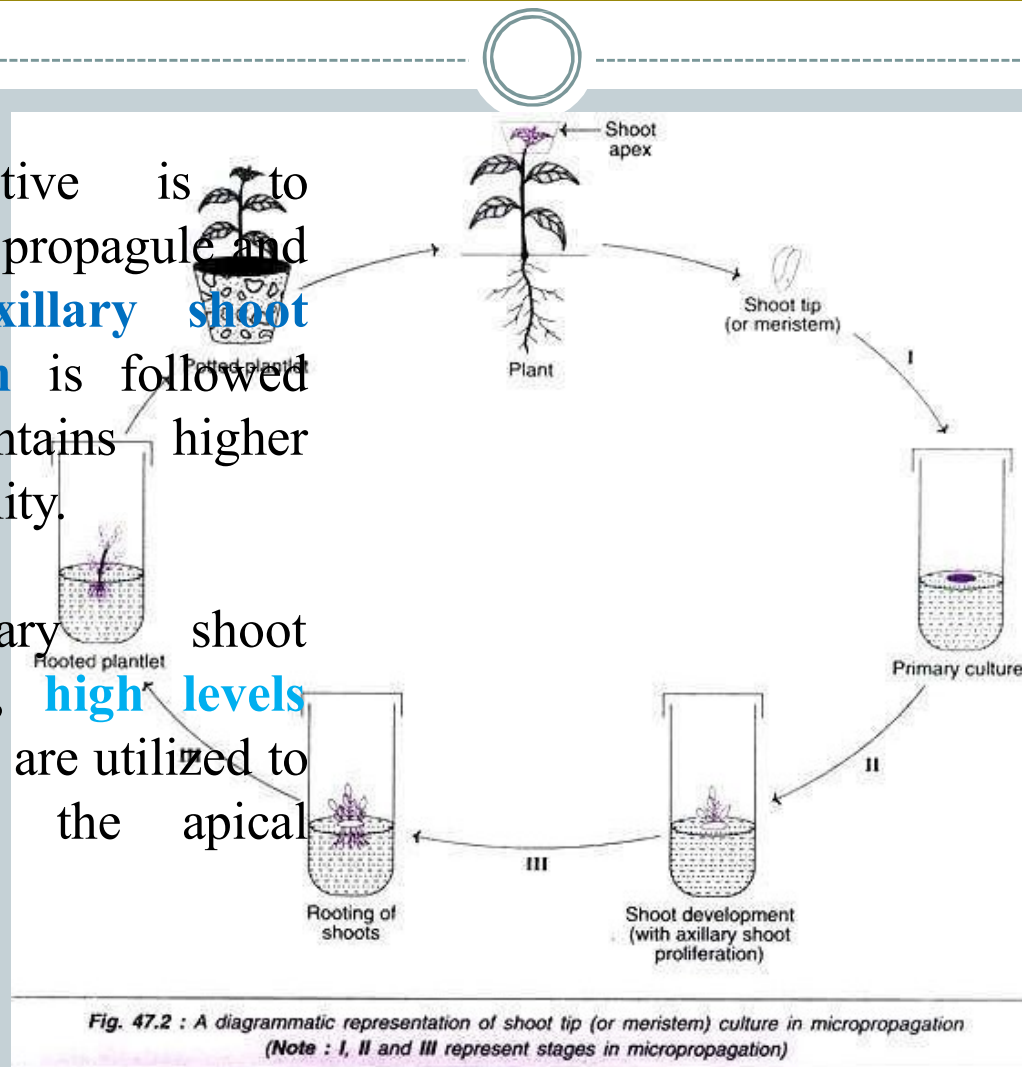


*Fig. 47.2 : A diagrammatic representation of shoot tip (or meristem) culture in micropropagation
(Note : I, II and III represent stages in micropropagation)*

PRODUCTION OF THE FREE PLANTS BY MERISTEM TIVIRUSP CULTURE

Stage 2:

- The objective is to multiply the propagule and for this **axillary shoot proliferation** is followed as it maintains higher genetic stability.
- In axillary shoot proliferation, **high levels of cytokinin** are utilized to overcome the apical dominance.



PRODUCTION OF THE VIRUS FREE PLANTS BY MERISTEM TIP CULTURE

Stage 3:

- purpose is **regeneration of adventitious roots** from the shoots obtain in stage 2
- Numerous studies have indicated that **NAA is followed by IBA, IAA, 2,4-D and other auxins** are used for induction of root generation.

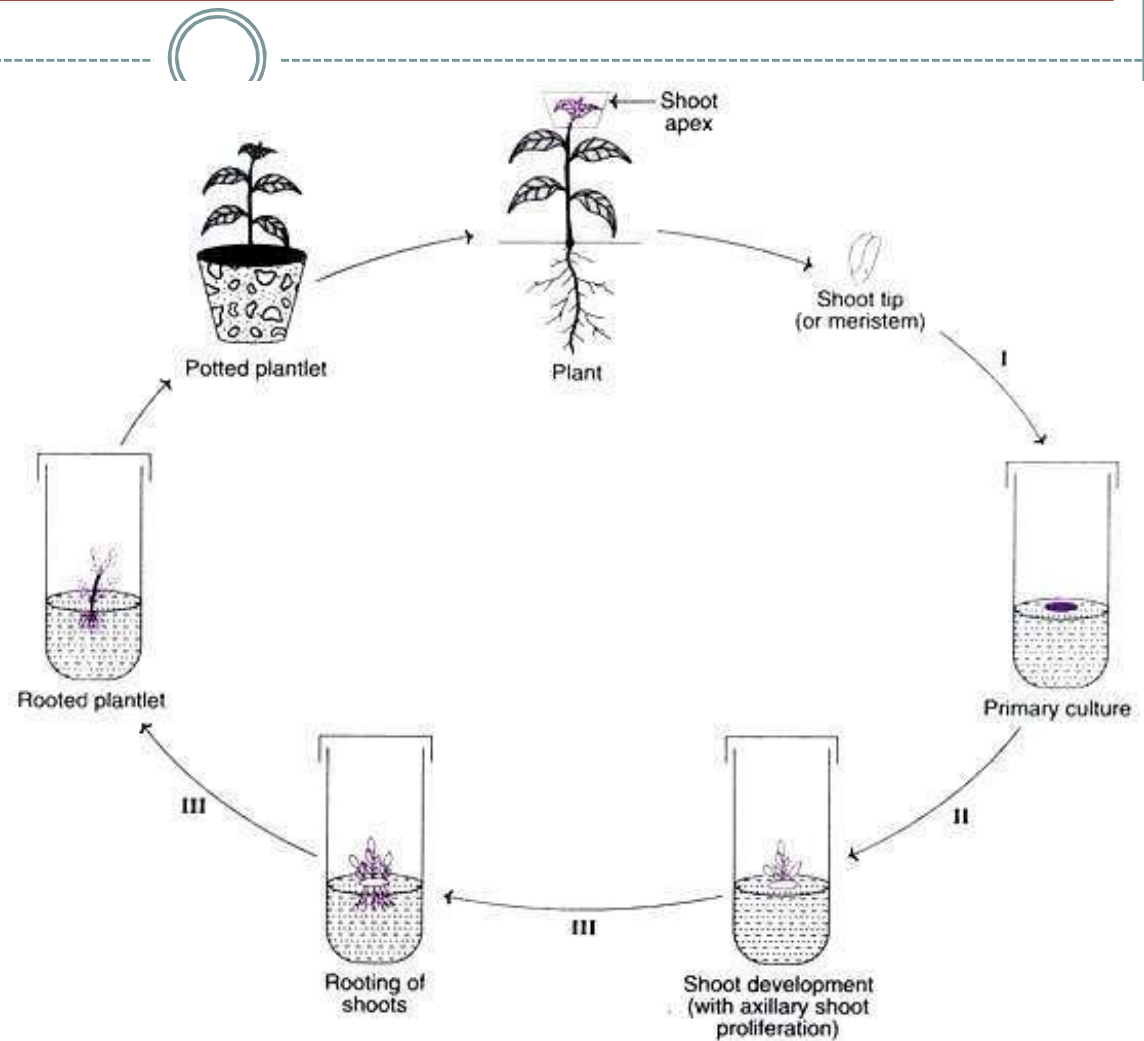


Fig. 47.2 : A diagrammatic representation of shoot tip (or meristem) culture in micropropagation
(Note : I, II and III represent stages in micropropagation)

PRODUCTION OF THE VIRUS FREE PLANTS BY MERISTEM TIP CULTURE



Conclusion:

- Meristem tip culture is very effective method of cloning of plant material and to develop virus free clean plant stock.
- Specific protocols, need to developed for different species/genotypes
- Combination of techniques for virus elimination is more effective

Literature cited



- Hussain, A., Ahmed, I., Nazir, H., & Ullah, I. (2012). Plant tissue culture: Current status and opportunities. Recent advances in plant in vitro culture, 1-28.
- Fitch, M., Lehrer, A., Komor, E., & Moore, P. (2001). Elimination of Sugarcane yellow leaf virus from infected sugarcane plants by meristem tip culture visualized by tissue blot immunoassay. Plant Pathology, 50(6), 676-680.
- http://en.wikipedia.org/wiki/plant_tissue_culture
- <http://en.wikipedia.org/wiki/micropropagation>
- http://en.wikipedia.org/wiki/meristem_tip_culture
- <http://agriinfo.in/default.aspx?page=topic&superid=3&topicid=1884>

THANK YOU

