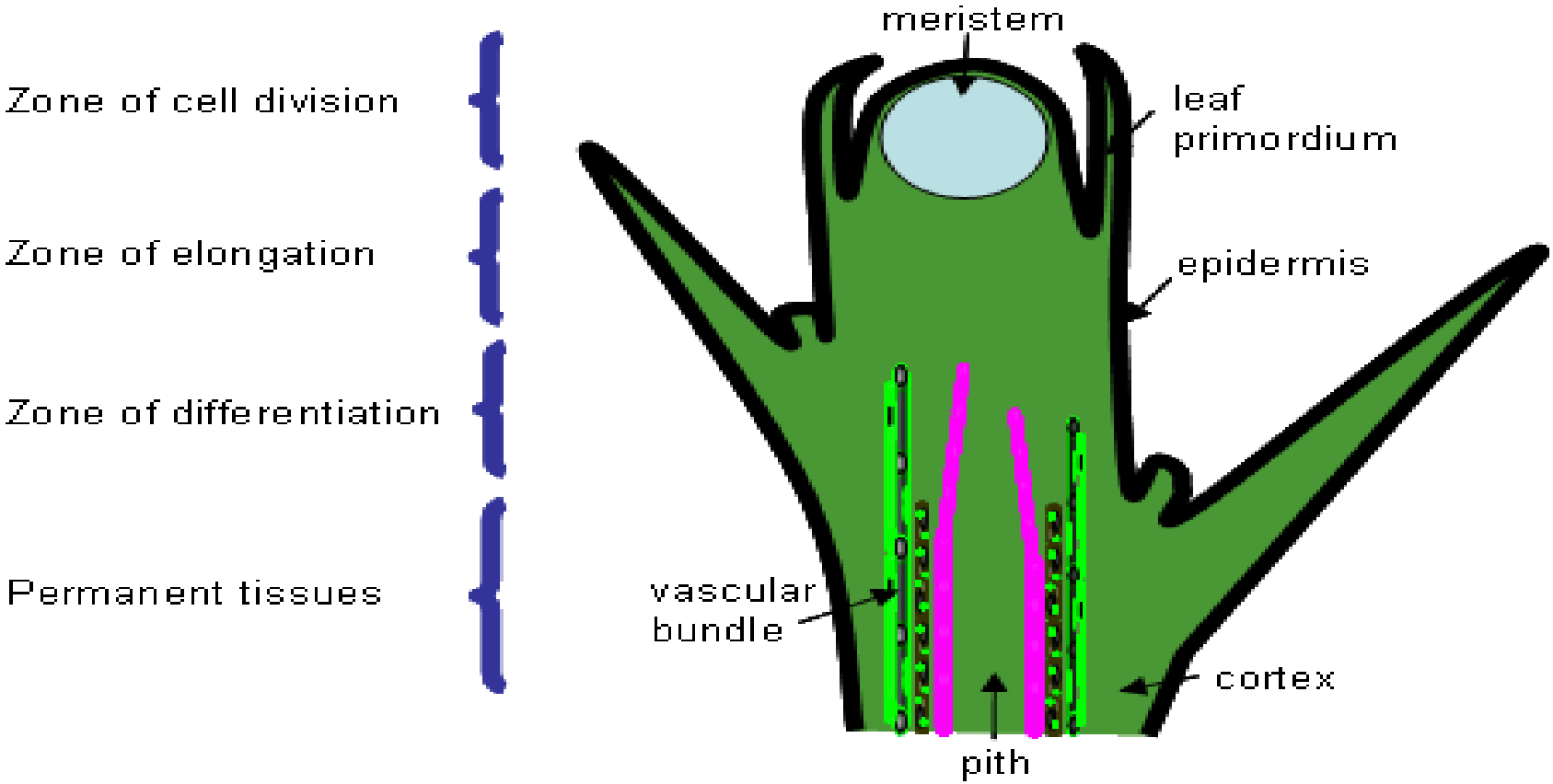
MERISTEM CULTURE

أ.م.د. هديل مكي المؤمن

محاضرة ٥

https://youtu.be/cD9CFtpLL2s



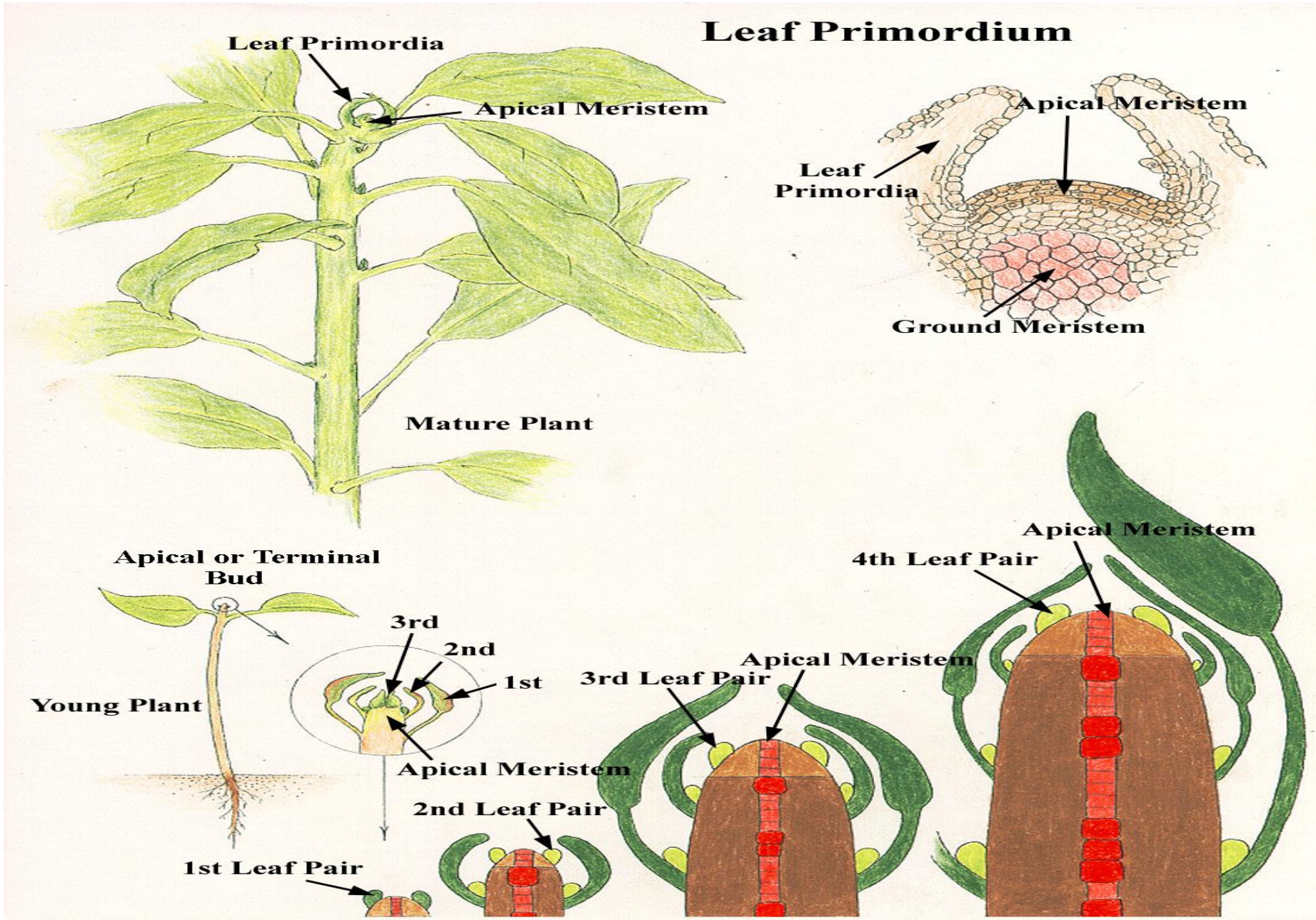
Apical Meristem

Introduction

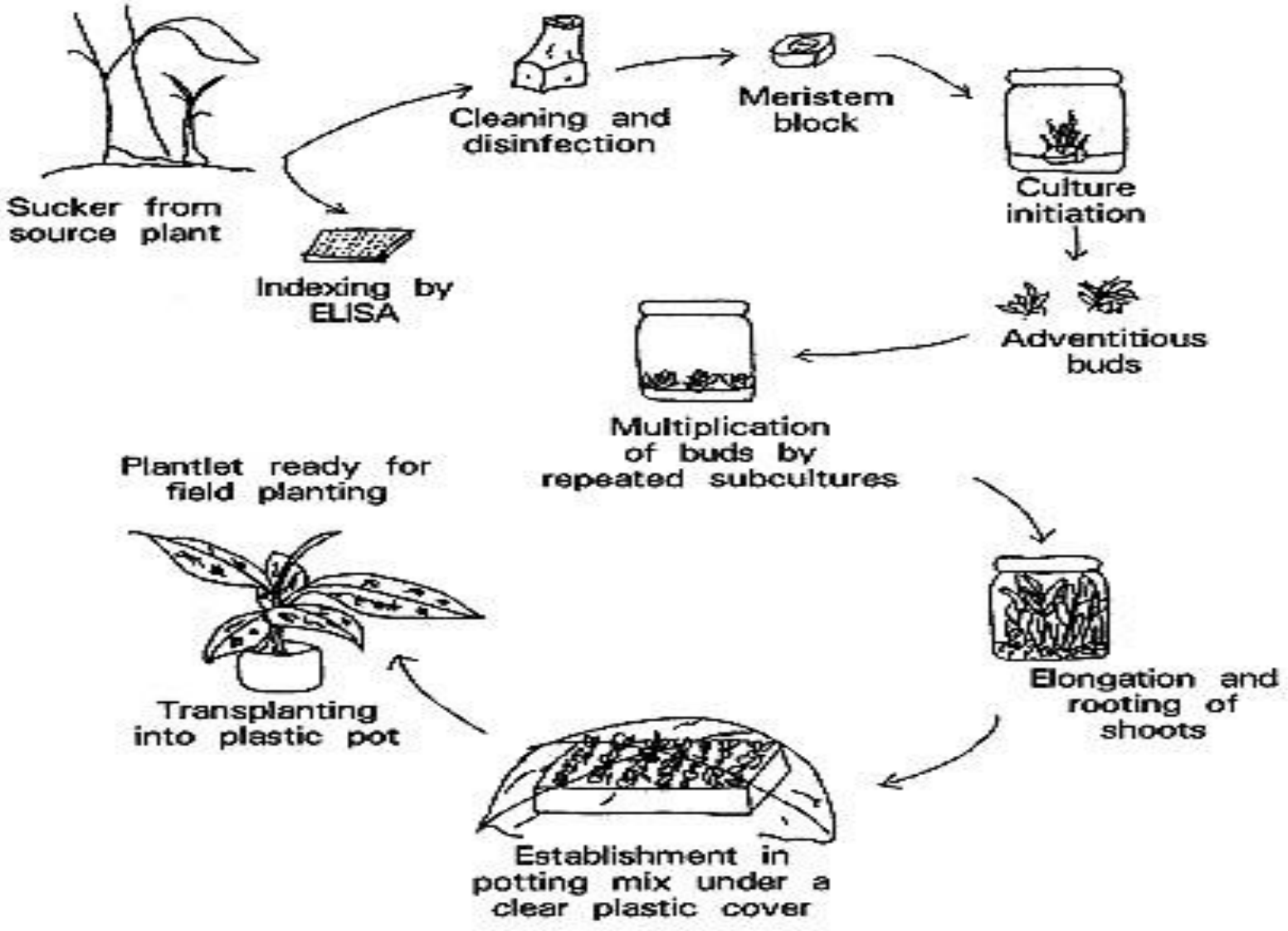
- A **meristem** is the tissue in all plants consisting of undifferentiated cells (**meristematic cells**) and found in zones of the plant where growth can take place.
- The term “meristem” was first used by Karl Wilhelm von Nägeli (1817-1891) from his book “Beiträge zur Wissenschaftlichen Botanik” in 1858. It is derived from the Greek word “merizein”, meaning to divide in recognition of its inherent function.
- Meristematic cells are analogous in function to stem cells in animals, are incompletely or not at all differentiated, and are capable of continued cellular division (youthful).
- meristem is a dome of actively dividing cells, on average *c*. 0.1 mm in diameter and *c*. 0.25 mm long
- The **apical meristem**, or growing tip, is a completely undifferentiated meristematic tissue found in the buds and growing tips of roots in plants. Its main function is to begin growth of new cells in young seedlings at the tips of roots and shoots (forming buds, among other things).size -0.25-0.30mm(length)and 0.1mm(dia).

- The Apical meristem can be shoot,root or of floral origin
- **Shoot Meristem Culture** - The first application of meristem culture was to obtain virus free plants of dahalias. In 1952, Morel and Martin isolated 100 µm long shoot meristems and cultured them to obtain virus free shoots.

  Since then the technique of meristem culture has been greatly refined and used for obtaining plants free from viruses, viroids, mycoplasma and even fungi and bacteria in a range of crops.
- In India, some valuable clones of potato, sugarcane, etc. have been freed from virus infections through meristem culture. Care must be taken to remove the apical meristem with as little surrounding tissue as possible to minimize the chances of virus particles being present in the explant.


Leaf Primordium
Leaf Primordia
Apical Meristem
Mature Plant
Apical Meristem
Leaf Primordia
Ground Meristem
Apical or Terminal Bud
Young Plant
3rd
2nd
1st
Apical Meristem
2nd Leaf Pair
1st Leaf Pair
Apical Meristem
4th Leaf Pair
Apical Meristem
3rd Leaf Pair

Meristem culture –an overview



MERISTEM CULTURE

- **Micropropagation**
- **Storage of Genetic Resources:**Many plants produce seeds that are highly heterozygous in nature or that is recalcitrant. Such seeds are not accepted for storing genetic resources. So, the meristem from such plants can be stored in vitro. Besides the above-mentioned uses of shoot tip or meristem culture, it can also be utilized in various important fields of plant science such
- **Propagation of Haploid Plants:**
- Haploid plants derived from anther or pollen culture always remain sterile unless and until they are made homozygous diploid. Meristem or shoot tip culture of haploid plants can be used for their propagation from which detailed genetic analysis can be done on the basis of morphological characters and biochemical assay
- **Meristem Culture for Virus-Free Plants**

Factors affecting meristem culture:

- Size of explant(inversely proportional).
- Physiological condition of explant(should be taken from actively growing region).
- Season of culture(imp. for plants which display periodic growth).
- Culture medium(auxin &cytokinin are used,NAA most effective).
- Storage condition(light incubation).

Methods of virus elemination

- Thermotherapy- hot water/ hot air(temp.-30˚C to 40˚C)
- Cryotherapy-low temp.(5˚C) .
- Chemotherapy-(malachite green,thiouracil,acetylsalicyclic acid).

Virizole,vidarbine,cyclohexamide,actinomycin-D-more effective.

- Physical methods (
- Other methods like somatic cell hybridization,somaclonal variation also used.

Production of virus free crysanthemums

- *Crysanthemum marifolium* plants were rescued from CVB by using meristem culture aided with thermo & chemotherapy .

Meristem tip (0.3-1.0) along with 2-3leaf primordia of virus infected plant

↓

Sterilization &establishment in MS media

↓

Maintained at a photoperiod of 16h,temp-20±2˚C,humidity-70-80%

↓

shoot development after 7-8 weeks

↓

subculturing in1/2 MS media +6gm Agar+IBA(0.05gm/l)for rooting

↓

Incubation at 38°C,16h photoperiod in a thermotherapy chamber

↓

Transferred to potting mixture

↓

kept in hardening chamber(temp.-25±2°C,16h photoperiod,80% humidity

↓

virus indexing

- Addition of 5-Bromouracil , 2- thiouracil,Acyclovir to the rooting media was done at diff. conc. to test their activity against CVB.
- 2- thiouracil gave max. no. of virus free plants at 40 gdm^3 conc.
- Least effective was 5-Bromouracil (gave only 10%virus free plants)

Combined effect of chemotherapy and meristem culture on sugarcane

(sugarcane breeding institute ,ICAR,coimbatore-M.Balamuralikrishnan

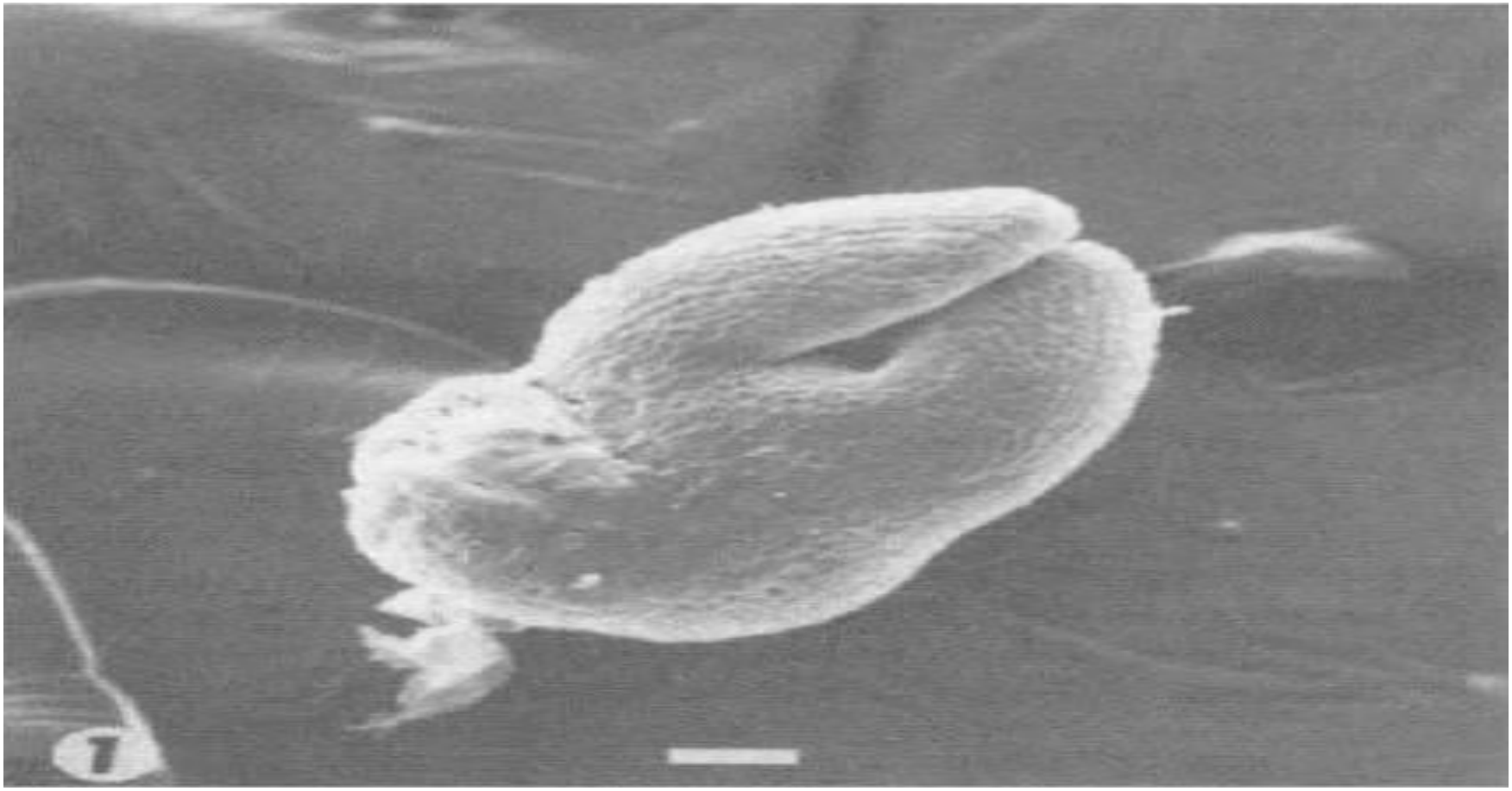
- Meristem tip culture in combination with antiviral chemo therapy was significant in elemination of SCMV.
- 2 antiviral chemotherapeutants viz.,ribavirin& 8-azaguanine were used with MS Medium in diff. conc.and culture was done.
- Assesed by DAC-ELISA technique.
- Observation- ribavirin at 50 ppm eleminated 95%of virus without causing any phytotoxicity to sugarcane.
- 8-azaguanine reduced plant regeneration &exhibited phytotoxicity.

Exploitation of meristem culture

- Meristem culture has been used in eradication of banana virus(BMV,BBTV) from infected banana plants and production of certified banana plant.
- Elimination of viruses(PPV,PNRSV)through themotherapy and meristem tip culture in nectarine.
- Production of virus free crysanthemums.
- Pathogens other than virus like fungi,bacteria,mycoplasmacould also be got rid off.
- Baker &Philips(1962)obtained carnation plants free from *fusarium roseum f.cerialis*.
- Tramier (1965)obtained gladiolus plants free from *oxysporium f.gladioli*.

Virus indexing

- Involves testing plants for +nce/-nce of virus before using it as mother plant to produce virus free stock.
- Methods used- sap transmisson test,serological test (ELISA and PCR)and microscopical studies.
- Sap transmission includes the use of indicator plants like *Chenopodium amaranticolor & Nicotiana tabacum.*



A freshly excised meristem tip from an axillary bud of the potato *Solanum tuberosum*. The two smallest emergent leaf primordia are present. Scale bar represents 50 µ*M*.

Pros n cons of meristem culture

ADVANTAGES;

- ***Lack of vascular tissue.***
- ***Meristem have high metabolic activity.***
- ***High auxin conc.***

DISADVANTAGES;

- ***Isolation is difficult.***
- ***Low survival rate $ regeneration time for explants may be long(about 8 months for potato explant).***
- ***Removal of explant causes a setback in the growth of mother plant.***

Table 15.1 Plants from which viruses have been eliminated by tissue culture technique[a]

Family	Species	Virus eliminated[b]
Amaryllidaceae	*Hippeastrum* sp.	Mosaic
	Nerine	Nerine latent, unidentified
	Narcissus tazetta	AMV, NDV
Araceae	*Caladium hortulanum*	Dasheen Mosaic
	Calocasia esculenta	Dasheen Mosaic
	Xanthemonas brasiliensis	Unidentified
Bromeliaceae	*Ananas sativus*	Unspecified
Caryophyllaceae	*Dianthus barbatus*	Latent, Mottle, Ringspot
	D. caryophyllus	Vein Mottle
Compositae	*Chrysanthemum* sp.	Chlorotic Mottle, Complex of viruses, Green Flower Stunt
		Vein Mottle, Virus B
	Dahlia sp.	Complex of viruses
		Dahlia Mosaic
		Tomato Aspermy
		Vein Mottle, Virus B
Convolvulaceae	*Ipomoea batatas*	Feathery Mottle, Hanmon Mosaic
		Internal Cork, Rugosa Mosaic
		Synkuyo Mosaic
Cruciferae	*Armoracia lapathifolia*	CLMV, TuMV
	A. rusticana	TuMV
	Brassica oleracea	CbBRSV
	Nasturtium officinale	CMV, CLMV, TuMV
Daphneceae	*Daphne* sp.	AMV, CMV, RbRSV
	D. odora	Daphne Virus S
Euphorbiaceae	*Manihot* sp.	African Cassava, Mosaic, Cassava Brown Streak, Mosaic
Geraniaceae	*Pelargonium* sp.	CMV, Tomato Black, Ringspot,Tomato Ringspot
Gramineae	*Lolium multiflorum*	RgMV
	Saccharum officinarum	Mosaic
Hydrangeaceae	*Hydrangea macrophylla*	Hydrangea Ringspot
Iridaceae	*Freesia* sp.	FrMV
		Freesia Virus I
		Phaseolus Virus 2
	Iris sp.	IMV
	Gladiolus sp.	Unidentified virus
Labiatae	*Lavendula* sp.	Dieback
Leguminosae	*Glycine max*	SMV
	Trifolium pratense	WCMV
Liliaceae	*Allium sativum*	GMV, OYDV, GYSV

Cont

CONCLUSION

Meristem culture thus plays a useful role in eradication of systemic diseases in plants.Its the most reliable method for pathogen elemination.The only requisite is the knowledge about various pathogens and methods of their elemination.It is also essential to have a good knowledge of greenhouse maintenance to control the reinfection of disease-free plants.

THANK YOU