Callus Culture

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HISTORY

- 1924-Calluscultureofcarrots (Daucus carota)-R.BlumenthatandP.Meyer-Pathologicalimplications,comparedcallustumourgrowth.
1927-L.Rehwald-Cultivationofcallusfromcarrotslices.P.Boysen-Jensengrowthpromotingsubstancesfoundinplantshoottipwoulddiffuseacrossawoundcoveredwithgelatin.1928
- FritsWentcollectedthisgrowthsubstanceformcoleptiletipsintinyblocksofAgar.

- 1934 F.Kogl,A.J.HagenSmithandH.Erxleben isolationandchemicalanalysesofthesubstance-planthormoneorauxin-indole-3-aceticacid(IAA)1937-R.Gautheret undifferentiatedcarrottissues1939
- R.J.GautheretandNobecourt,France,growthofcallusfromcarrotcambiumwhenusingauxininthenutrientmedium.P.R.White(USA)
- Excisedroottipsoftomatoes(Lycopersicon)incontinuousculture.1939
- Whitereportedsuccessfulcultureoftobbaco(Nicotiana) callus.

- 1941 J.van Overbeek, M.E. Conklin and AlbertF. Blakeslee-Coconut milk stimulatedcallus formation in cultures of excisedembryos of jimson weed(Daturastramonium).1943
- White A Handbook of Plant Tissue Culture, accumulated knowledge of PTC.

CALLUS CULTURE

- Callus : An unorganised mass of loosely arrangedparenchymatouscells which develop from parenttissues due to proliferation of cells.
- Angiosperms , gymnosperms , pteridophytesandbryophytes.
- Has the potentiality to produce normal roots andembryoids-plantlets
- Callus culture: development of an unorganised mass of cellsfrom an explanton an artificial medium supplemented withsuitable PGR when provided with appropriate environment(i.e. Proper incubation conditions)

Physical Appearance Of A Callus

- HARDNESS : Hard (due to lignificationof cell walls),brittle or sometimes soft
- COLOUR : Dirty or off white , creamishto brown orlight green to dark green.Degree of darker pattern varies from plant to plantand mainly depends upon the quantity of polyphenolspresent in the plant species.Higher the polyphenolcontent darker the callusappear, i.e. Brown coloration on the culture.

Development of Aseptic Callus Cultures

- EXPLANTS : Juvenile tissues, seedlings , young shoots, buds , root tips, developing embryos: fruits, floralparts, tubers and bulbs.
- CULTURE MEDIUM :
- Media: MS
- Hormonal balance: Auxin: Cytokinin= 1
- Carbon source :sucrose (3% w/v)
- ph = 5.6 -6.0, optimum 5.8
- Gelling agent

- **Surface Sterilization**
- Wash throughlytween-20under running tap --
- Antifungal (Bavistin;0.04%) water Antibacterial agents(Streptomycin sulfate;0.0 4 %)__UNDER_ROOM
  ↓
- 15-20 minDouble Distilled Water
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- washing70 % Ethanol treatment
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- 1 min / dip Under Laminar Air Flow HgCl2 treatment(0.04 or 0.02 %)
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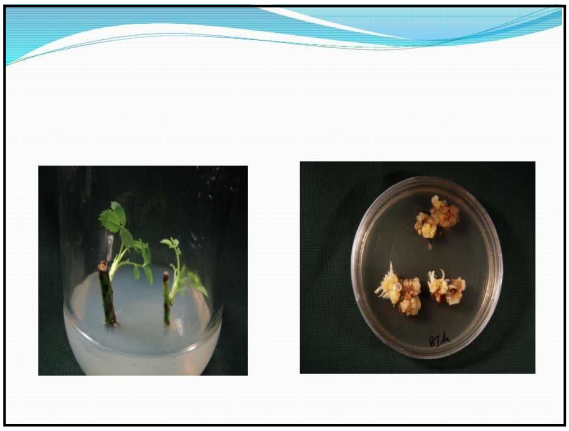
- 4-5 min Washing with Autoclaved Water

Inoculation and Incubation

- **Nodal explant**
- Cutting of the nodal endsthat comes in contact withthe surface sterilantwith thesterile surgical blade
- Place the explantin vertical position on mediumsupplemented withappropriate PGR with thehelp of forceps.

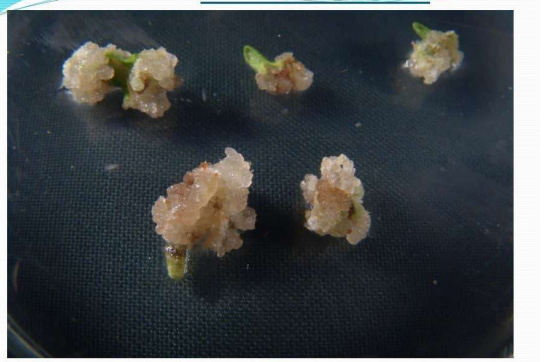
- **Leaf segments, buds, root tipsetc.**
- Segmentation of the explantinto2-3 parts i.e. Basalmedium and tip with sterilesurgical blade
- Place the explantwith theirabaxialsurface in contact with the medium with thehelp of sterile inoculatingforceps.



Incubation :

- Incubation conditions : The Environment Temperature :25 ±2ºC
- Light : 5000-10,000 luxm Duration of incubation: 16 hr light08 hr dark

- **How a piece of explant gets converted into the callus ?**
- Produced from the outer layers of cortical cells in a stem explant by repetitive division of cells. These dividing cells create pressure on the epidermis --- rupturing of the epidermis exposing newly formed callus Separation of callus and then sub culturing it.2-3 weeks to grow (10-15 days) but sometimes 4 weeks.



- **SUBCULTURING OF THE CALLUS CULTURE**
- 3-4 weeks
- 250-500mg approx. pieces --------transferred to thefresh media.NEED:
- 1)Nutrition depletion
- 2) Accumulation of toxic substances
- 3)Drying of media ADVANTAGES :1)Maintains the state of viability of cells2)Provides fresh instalment of media for further growth

- **DISADVANTAGES**
- 1) Cells lose the power to regenerate to a plantlet.
- 2) Chromosomal abbrations: polyploidy and aneuploidy. Polyploidcells appear to originate through endoreduplication (additional rounds of DNA replication without intervening cell division) ;aneuploid cells-anaphase irregularities
- 3) Non chromosomal changes : changes in metabolic pathways and alteration in composition of media.
- 4)Selection of explant size highly dependent upon the type of glassware is being used for culture purpose.

- **Flow sheet (sub culturing process)**
- Callus ⟶ work station(glass plate , sterile)cut ting of the callus into small pieces (250-500 mg approx)fresh media having appropriate composition and hormonal balance OR Agitation can be done (25-150 rpm)-------fresh media