Chapter 5

PLANT TISSUE CULTURE

INTRODUCTION

In developing countries the most important challenges are to produce sufficient food, fibre and fuel for the continously increasing population from inelastic land area. Plant tissue culture offers excellent opportunities of mass propagation of plants in test tubes. The idea of totipotency has been the foundation for tissue culture techniques. Tissue culture is the process whereby small pieces of living tissue (explants) are isolated from plant and grown aseptically for indefinite periods on a semi-defined or defined nutrient medium. Explants range from large seedlings and organs (as in ovule and embryo culture) to small single cells and protoplasts. **In short, tissue culture is *in vitro* cultivation of plant cell or tissue under aseptic and controlled environmental conditions in a defined nutrient medium for the production of primary and secondary metabolites or to regenerate the plant.**

HISTORY OF PLANT TISSUE CULTURE

The technique of plant tissue culture is about 100 years old but its importance have been realised in the last two decades in various fields including pharmacy also. The principles of plant tissue culture can be traced in the cell theory proposed by *Schleiden* and *Schwann* in 1839. They proposed that each living cell of an organism, if provided with proper environment is capable of independent development. This theory gave birth to the concept of totipotency predicted by Haberblandt. In 1902, the German botanist G. Haberblandt reported culture of isolated single palisade cells from leaves in Knop's salt solution enriched with sucrose. The cells remained alive up to one month, increased in size, accumulated starch but failed to divide. Efforts to demonstrate totipotency led to the development of techniques for cultivation of plant cells under defined conditions. This was made possible by brilliant contribution from R.J Gautheret in France and P.R. White in U.S.A and by others.

The first **Embryo culture,** although crude was carried out by Hanning in 1904. He cultured nearly mature embryos of certain Crucifers (*Cochleria donica, Raphanus landra, R. sativus and R. caudatus*) and grew them up to maturity. This became an important area of investigation using an *in vitro* technique. In 1908, *Simon* achieved success in regenerating the bulky callus, buds and roots from popular stem segments and thus he succeeded in establishing the basis for callus culture and to some extent also micropropagation. *Kotte* in 1922, cultured small excised root tips of pea and grew it for two weeks by using a variety of media containing salts of Knop's solution, glucose and various nitrogenous compounds.

By this, Kotte achieved a new approach to tissue culture and he reported that true *in vitro* cultures can be made easier by using meristematic cells (root tips or buds).

A successful establishment of callus cultures depended on the discovery during mid thirties of IAA, the endogenous auxin and of the role of B vitamins in plant growth and in root cultures. The first continously growing callus cultures were established from cambium tissue independently by Gautheret and White. **Gautheret** in 1934, successfully cultured cambium cells of various tree species (*Robinia pseudoacacia, Acer pseudoplatanus, Ulmus campestre* etc) on the surface of the media solidified with agar and observed that after six months proliferation of callus was ceased but on addition of auxin, it enhanced the proliferation of cambial culture and was possible to prepare subculture. **White** in 1934 carried out *in vitro* technique by changing the nature of media. He replaced the yeast extract in a medium containing inorganic salts and sucrose with three vitamins viz thiamine, pyridoxine and nicotinic acid and was able to maintain the cultures of tomato roots. Later on **White's** synthetic media proved to be one of the basic media for cell and tissue culture . In 1941, **Van Overbeek et al** used the coconut milk for development of embryo and callus formation in *Datura*. This became the turning point in the development of embryo culture and later on proved to be helpful in development of various hybrids.

In 1945 **Loo**, succeeded in developing the whole plant from stem tip culture by using the stem tips of *Asparagus* and *Dodder*. In 1946, **Ball** identified the exact part of the shoot meristem, which give rise to whole plant. This method is used now a days for plant propagation at industrial level. Later in 1955 **Skoog** postulated that adenine derived from nucleic acids enhances cell proliferation and bud formation in callus cultures. **Skoog** and **Miller** in 1957 proposed the roles of auxin and cytokinin on shoot and root induction in tobacco callus cultures. High proportion of auxin promoted rooting whereas proportionately more cytokinin initiated shoot or bud formation. In 1960, **Bergmann** developed the plating technique for cloning a large number of isolated single cells by using callus cultures of *Phaseolus vulgaris* and *Nicotiana tobacum*. In the same year **Cocking** introduced the protoplasmic plant tissue culture. He isolated the protoplasts of plant tissue by using cell wall enzymes like cellulase, hemicellulase, protease and pectinase. **Steward** and co-workers in 1966 raised large number of plantlets from carrot root suspension culture via somatic embryogenesis. In 1968, **Reinert** introduced the somatic embryogenesis in callus, cultured on a semi solid medium. All the above discoveries contributed to the establishment of concept of totipotency as laid by the Haberblandt.

Power et al in 1970, demonstrated the intra and interspecific fusion between the protoplasts of different plant roots. **Carlson** and **co-workers** in 1972 produced the first somatic hybrid plant by fusing the protoplasts of *Nicotiana glauca and N. langsdorfii*. **Vilnken** in 1981 succeeded in the electrical fusion of protoplasts. Since then many divergent somatic hybrids have been produced. In the following years the technique of plant tissue culture was refined and various new developments were made.

Thus within a brief period, the tissue culture technique have made a great progress. From the sole objective of demonstrating the totipotency of differentiated plant cells, the technique now finds a variety of applications in both basic and applied researches in a number of fields.

LABORATORY REQUIREMENTS FOR PLANT TISSUE CULTURE

A. Laboratory space- The organization of tissue culture laboratory depends mainly on the nature and the scale of activity. In general space for the following is needed :

1-Washing, drying and storage of vessels

2-Preparation, sterilization and storage of media

3-Aseptic handling of explant and cultures

4-Maintenance of cultures, and

5-Observation of cultures

In the modern laboratories the activities 1 and 2 are done in **Media room** whereas the remaining activities 3 to 5 are performed in **Culture room.** In such a situation the following precautions should be taken :

The working area should be physically separated even by a temporary partition from that used for medium preparations.

The weighing balances should be kept in a separate space.

Refrigerator, Deep freeze, Incubators and Autoclave may be kept in corridor.

For aseptic manipulations, laminar flow hoods are commonly used which can be housed in culture room. A small table having a stereoscopic microscope may be adequate for culture observation.

B. Culture room- The culture room should have the following facilities-

1. Controlled temperature (usally at $25^0 \pm 2$ degree C with the help of airconditioners and room heaters; higher or lower temperature may be desirable in some cases)
2. Culture racks fitted with light (generally 1000 lux or lower light generated by fluorescent tubes)
3. A shaker for agitation of liquid cultures. It is desirable to have a generator set for providing electricity to the culture room when there is electricity failure or cuts.

C. Culture vessels and their washing- Generally glass culture vessels are used as they are cheaper, reusable and autoclavable. It is desirable to use only borosilicate or pyrex glass ware as ordinary soda glass may be toxic to some tissues. Culture vessels of plastic are available for a variety of purpose; these vessels are generally presterilized and disposable, but certain types are autoclavable and therefore reusable. In general , plastic vessels in the long run are costly than glass vessels.

Tissues are cultured in culture tubes (rimless tubes of 25 X 150 mm or larger), flasks (long neck or even ordinary conical flasks) and petriplates; but mainly especially designed dishes are also used. Wide mouth bottles, including milk bottles are often employed especially for micropropagation work. Suspension cultures of necessity are maintained in long neck culture flasks.

Culture tubes and flasks are usually stoppered with cotton plugs, which are often wrapped

in cheese cloth, but preparing such plugs on large scale may be time consuming and inconvenient. Many workers use caps made up of either polypropylene or a metal for this purpose. These caps are effective but their design may affect the performance.

Culture vessel and other labware are generally soaked in a suitable nontoxic detergent solution overnight, washed with a suitable brush, thoroughly rinsed, clean with tap water, followed by rinse with distilled water and dried in hot air oven (75 to 80°C). Culture vessels having contamination are first autoclaved. Washed culture vessels should be stored preferably in a dust proof cabinet.

D. Sterilization- All the materials like instruments, vessels, plant materials, medium etc. used in culture work should be free from microorganisms. This is achieved by one of the following method-

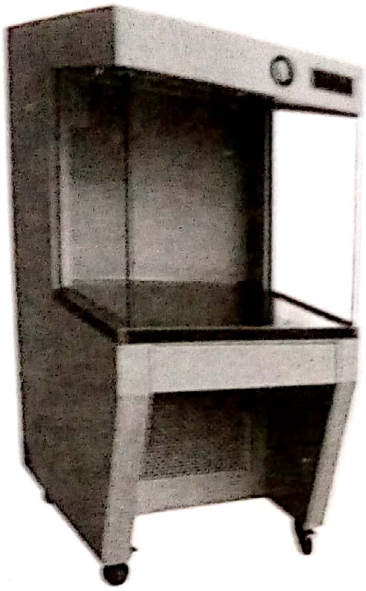
(i) Dry Heat- Glassware and Teflon plastic ware (empty vessels) and instruments can be sterilized by dry heat in hot air oven at 160-180°C for 3 hours. But most of the workers prefer to autoclave glass ware and plastic ware and flame sterilize instruments like forceps etc. More recently glass bead sterilizers (300°C) are being employed for the sterilization of forceps, scalpels etc.

(ii) Flame Sterilization- The instruments like scalpels, needles, forceps etc. are ordinarily flame sterilized by dipping them in 95% alcohol followed by flaming. These instruments are repeatedly sterilized during the operation to avoid contamination. The mouths of culture vessels are flammed prior to inoculation / subculture.

(iii) Autoclaving- Culture vessels etc. (both empty and containing media) are generally sterilized by heating in an autoclave to 121°C at 15 p.s.i (Pounds per square inch) for 15 (20-50 ml of medium) to 40 (2 L medium) minutes, the time being longer for larger medium volumes. Sterilization during autoclaving depends mainly on temperature. Certain types of plasticware and instruments like micropipettes etc are also autoclavable. All the vessels should be stoppered during autoclaving.

(iv) Filter Sterilization- Certain vitamins, enzymes and growth regulators like Zeatin, GA3, Abscisic acid (ABA) and Urea are heat liable, so these compounds are filter sterilized by passing their solution through a membrane filter of 0.45 u or lower pore size. The membrane filter is held in a suitable assembly; the assembly together with the filter is sterilized by autoclaving before use.

Laminar air flow cabinets are used to create an aseptic working area by blowing filter-sterilized air through an enclosed (on all sides except one) space. The air is first filtered through a coarser prefilter to remove larger particles; it is then passed through High efficiency particulate air (HEPA) filter, which filters out all particles larger than 0.3um. This sterilized air blows through the cabinet at 1.8 km/hr which is sufficient to keep the enclosed working area aseptic. Inside the cabinet there is an arrangement of bunsen burner and UV tube fitted on the ceiling of the cabinet which helps the area to be free from live contamination.



Laminar Air Flow

(v) Wiping with 70% Ethanol- The surfaces that cannot be sterilized by other techniques e.g. platform of the laminar flow cabinet, hands of the operator etc. are sterilized by wiping them thoroughly with 70% ethyl alcohol and alcohol is allowed to dry.

(vi) Surface Sterilization- In this method all plant materials used for culture is treated with an appropriate sterilizing agent to inactivate the microbes present on their surface. The sterilizing agents used for surface disinfection are sodium hypochlorite (2%), calcium hypochlorite (9-10%), mercuric chloride (0.1-1%), H_2O_2 (10-12%), bromine water(1-2%) and antibiotics (4-50mg/litre). Among these sodium or calcium hypochlorite gives very good results and mercuric chloride gives satisfactory results and these are most commonly used. The duration of treatment varies from 15-30 minutes. As these agents are toxic to plant tissues the duration and concentration used should be such as to cause minimum tissue death.

Surface sterilization protocol depends mainly on the source and the type of tissue of the explant, which determines the contamination load and tolerance to the sterilizing agent. An explant is the excised piece of tissue or organ used for culture. Explant can be taken from any part of the plant like root, stem, leaf, meristematic tissue like cambium and floral parts like stamens, anthers etc. **A general protocol for disinfection of respective explant is mentioned below :**

Seeds- Dip the seeds into 70% ethyl alcohol for 30 seconds and then treat with 0.2% mercuric chloride (acidified with few drops of 1N Hcl) for 10-15 minutes. Again rinse with 70% ethyl alcohol and finally rinse 4-6 times with sterilized distilled water. The entire protocol should be carried out in an aseptic area generally created by laminar air flow.

Leaves- Wash the explant thoroughly by purified water to remove the dirt and rub the surface with ethyl alcohol. Dip the explant in 0.1% mercuric cholride solution, wash with sterile water and finally dry the surface with sterilize tissue paper.

Fruits- Rinse the fruit with absolute alcohol and then dip into 2% sodium hypochlorite solution for 10 minutes. Finally wash thoroughly with sterile water and remove seeds of interior tissue.

Stem- Wash the explant thoroughly with running tap water and rinse with pure alcohol. Dip into 2% sodium hypochlorite solution for 15-20 minutes and wash 2-3 times with sterile water.

PRODUCTION OF CALLUS FROM EXPLANT

The sterilized explant is transferred aseptically to a defined medium in the flasks. These flasks are incubated in BOD incubator for maintenance of culture at the temperature of 25 ± 2°C. Little amount of light is also essential for the production of callus (unorganized mass of cells). After 3 to 8 days of incubation, sufficient amount of callus is produced.

Proliferation of callus

When callus is well developed it should be cut into pieces and transferred to another fresh medium. This medium contains an altered composition of hormones which supports the growth. The medium used for production of more amount of callus is known as proliferation medium.

Subculturing of callus

After a period of time, it becomes neccessary to transfer the callus to fresh media chiefly due to nutrient depletion and medium drying. In general, callus cultures are subcultured every 4 to 6 weeks.

Suspension culture

Tissue and cells cultured in a liquid medium produce a suspension of single cells called as suspension cultures. For the preparation of suspension culture, callus is transferred to the liquid medium, which is constantly agiated by a rotary shaker at 50-150 rpm. This facilitates aeration and keeps the cells separate. After the production of sufficient number of cells subculturing can be done. In general, suspension cultures are subcultured every 3 to 14 days.

CULTURE MEDIA

The plant tissues or organs growing *in vitro* have different nutritional requirements for their satisfactory growth. But there is no single medium which is entirely sufficient for the satisfactory growth of all types of plant tissues and organs. Hence details of culture medium need to be worked out by hit and trial method for each plant material separately. The various culture media developed during last few decades are Gautheret(1942), White(1943), Haller(1953), Murashige and Skoog (MS) (1962), Erikson(ER) (1965) and Gamberg et al (B5) (1968). Out of these MS and B5 are most commonly used. The pH of the medium is usually adjusted between 5.0 to 6.0 with 1N Hcl or 1N NaOH. The composition of some plant tissue culture media is listed in Table no:-1

TABLE NO. 1

Ingredient	White's medium	Haller's medium	MS medium	ER medium	B5 medium
Micronutrients					
NH_4NO_3	-	-	1650	1200	-
KNO_3	80	-	1900	1900	2527.5
$NaNO_3$	-	600	-	-	-
$Ca(NO_3)_2.4H_2O$	300	-	-	-	-
$MgSO_4.7H_2O$	750	250	370	370	246.5
KH_2PO_4	-	-	170	340	-
$CaCl_2.2H_2O$	-	75	440	440	150
$NaH_2PO_4.H_2O$	19	125	-	-	150
$(NH_4)_2SO_4$	-	-	-	-	134
Micronutrients					
$MnSO_4.H_2O$	-	-	-	-	10
$MnSO_4.4H_2O$	5	0.1	22.3	2.23	-
$CuSO_4.5H_2O$	0.01	0.03	0.025	-	0.025
$CoCl_2.6H_2O$	-	-	0.025	0.0025	0.025
$ZnSO_4.7H_2O$	3	1	8.6	-	2
$Fe_2(SO_4)_3$	2.5	-	-	-	-
$FeSO_4.7H_2O$	-	-	27.8	27.8	-
$NaMoO_4.2H_2O$	-	-	0.25	0.025	0.25
KI	0.75	0.01	0.83	-	0.75
KCl	65	750	-	-	-
MoO_3	0.001	-	-	-	-
$FeCl_3.6H_2O$	-	1	-	-	-
$AlCl_3$	-	0.03	-	-	-
H_3BO_3	1.5	1.0	6.2	0.63	3
$NiCl_2.6H_2O$	-	0.03	-	-	-
EDTA					
$Zn.Na_2EDTA$	-	-	-	15	-
$Na_2EDTA.2H_2O$	-	-	37.3	37.3	-
Organic nutrients					
Vitamins					
Pyridoxine HCl	0.01	-	0.5	0.5	1
Nicotinic acid	0.05	-	0.5	0.5	1
Thiamine HCl	0.01	-	0.1	0.5	10

Inositol	-	-	100	-	100
Amino acids					
Glycine	3.0	-	2.0	2.0	-
Carbon Source					
Sucrose	2%	-	3%	4%	2%
Growth regulators					
IAA	-	-	1.0	30	-
2-4 Dichlorophe-noxyacetic acid	-	-	0.1	1.0	-
NAA	-	-	-	1.0	-
Kinetin	-	-	0.04	10.0	0.02
Ph	5.5	-	5.7	5.8	5.5

MS-Murashige & skoog

ER- Erikson

B5- Gamberg et al

Media constituents- The major constituents of medium that are essential to maintain the vital functions of culture are-

1- Inorganic nutrients

2-Organic nutrients

3-Growth regulators (Hormones)

4-Gelling agent (Agar)

1-Inorganic nutrients- In addition to C, H and O all culture media requires 12 elements for the growth of plant tissues. Out of these, six elements viz Nitrogen(N), Phosphorous(P), Potassium(K), Calcium(Ca), Sulphur(S) and Magnesium(Mg) are needed in the concentration greater than 0.5 m mol l^{-1} and are known as **macronutrients.** The remaining six elements viz Iron(Fe), Zinc(Zn), Manganese(Mn), Copper(Cu), Boron(B) and Molybdenum(Mo) are required in the concentration less than 0.5 m mol l^{-1} and are known as **micronutrients.**

The active factor in the culture medium is the ions of different types rather than salt. A single ion can be contributed by more than one salt. For e.g in Murashige and Skoog's medium K^+ ions are contributed by KNO_3 and KH_2PO_4 whereas NO^{3-} ions are contributed by NH_4NO_3 and KNO_3. The various culture media differ mainly in quantity rather than in quality of these elements. Therefore the various culture media provide different concentration of the inorganic nutrients for e.g. in White's medium the concentration of K and N is very less as compared to MS and B5 medium. The White's medium though widely used earlier was later found inadequate by various investigators because the inorganic nutrients which provides the good callus growth were very less in quantity. Hence most of the plant tissue culture media that are now being used widely (Table no-1) are richer in mineral salts as compared to White's medium.

In most of the medium, iron is now used as FeEDTA and in this form iron remains available at higher pH (>5.8). FeEDTA may be prepared by using $Na_2EDTA.2H_2O$ and $Fe_2(SO_4)_3 7H_2O$.

The inorganic nitrogen is supplied in the medium in the forms of nitrates and ammonium compounds. When nitrate is used alone, the pH of the medium shifts towards alkalinity. So, to check this drift small amount of ammonium compound is added along with nitrate.

In addition to the above mentioned elements , the various media are also enriched with sodium (Na), Cobalt (Co) and Iodine (I) but their necessity has not been established.

2-Organic nutrients- The organic nutrients can be broadly calssified into nitrogen sources and carbon sources.

Nitrogen sources- For the optimum callus growth it is necessary to supplement the tissue culture media with one or more vitamins and amino acids. The vitamins required are pyridoxine, thiamine, nicotinic acid and inositol. Of these thiamine is essential and the rest are promotory. Pantothenic acid is also known to be promotory but is not included in most of the recipes.

Other complex nutrients of undefined composition such as casein hydrolysate, coconut milk, corn milk, malt extract, tomato juice and yeast extract have also been used to promote the growth of tissue culture. However it is recommended to avoid their use and replace each by a single amino acid, as these substances may affect the reproducibility of results because of variation in the quantity and quality of growth promoting constituents in these substances.

Carbon sources- The most commonly used carbon source for all cultured plant materials including even green shoots is sucrose. It is used in the concentration of 2-5%. **Ball** demonstrated that autoclaved sucrose is better than filtered sterilized sucrose because autoclaving causes the hydrolysis of sucrose which enhances its availability to plant cells. Generally, monocots grow better with glucose whereas dicotyledonous roots do best with sucrose. Plant tissues can utilize other sugars also like galactose, lactose, mannose and even starch, but these are rarely used.

3-Growth regulators(Hormones) - The growth hormones included in culture media are auxins, cytokinins and gibberellins.

Auxins- Auxins are mainly used to facilitate cell division and root differentiation. Commonly used auxins are IAA (indole-3-acetic acid), IBA (indole-3-butyric acid), NAA (naphthalene acetic acid), NOA (naphthoxy acetic acid), p-CPA (Para-chlorophenoxyacetic acid), 2, 4-D (2,4 dichlorophenoxy acetic acid) and 2,4, 5-T (trichlorophenoxyacetic acid). IBA and NAA are widely used for rooting and (in combination with cytokinin) for shoot proliferation. 2,4-D and 2,4,5-T are very effective for the induction and growth of callus. Auxin generally dissolve in ethanol or dil NaoH.

Cytokinins- Chemically, cytokinins are adenine derivatives and are employed to promote cell division, regeneration of shoots, often somatic embryo induction, to enhance proliferation and growth of auxillary buds. Commonly used cytokinins are Kinetin (furfurylamino purine), BAP (6-benzylamino purine), 2-ip (isopentenyl adenine), Zeatin and TDZ (thiadiazuron). 2-ip and Zeatin are naturally occuring cytokinins while Kinetin and BAP are

derived synthetically. Cytokinins generally dissolve in dil Hcl or NaoH.

Gibberellins- Of the over 120 gibberellins known, GA_3 is almost exclusively used as it promotes shoot elongation and somatic embryo germination. Gibberellins is soluble in cold water up to 1000 mg^{-1}.

4. Gelling agent- Another component of culture medium is the gelling agent which makes the medium solid because in liquid medium the tissue submerges and die due to lack of availability of oxygen.In solid medium there is improved oxygen supply and provides the support to the culture growth (agar is not a nutrient) as compared to liquid medium.For this purpose the most commonly used gelling agent is agar-agar obtained from red algae like *Gracilaria*. Agar is used at a concentration of 0.8-1.0%. If the concentration is increased than it makes the medium very hard and than diffusion of nutrients into the tissue medium is not possible. Agar(Agarose) has the resistance to enzymatic hydrolysis at incubation temperature and due to this characteristic it is commonly used in culture medium. Moreover it is neutral to the media constituents and thus do not react with them.

However, agar is not an essential constituent of the nutrient medium.Single cell and cell aggregates can also be grown in suspension culture,devoid of agar,but such cultures should be aerated regularly either by bubbling sterile air or by gentle agitation. Another gelling agents used to solidify liquid media are like(alginate, carrageenan, starch, gelatin, polyacrylamide, silica gel and hydroxyethylcellulose. HEC

MEDIA PREPARATION

Glass distilled water and chemicals of highest purity should be used.A convenient approach to prepare a medium is to have stock solutions of all the nutrients (macronutrients, micronutrients, iron solution and organic components) and store them in refrigerator. The preparation of **Murashige** and **Skoog's** medium is discussed below-

TABLE NO. 2

Ingredients	Amount (mg/litre)
Group 1	
NH_4NO_3	1650
KNO_3	1900
$MgSO_4.7H_2O$	370
KH2PO4	170
$CaCl_2.2H_2O$	440
Group 2	
$MnSO_4.4H_2O$	22.3
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
$ZnSO_4.7H_2O$	8.6
$Na_2MoO_4.2H_2O$	0.25
KI	0.83
H_3BO_3	6.2
Group 3	
$FeSO_4.7H_2O$	27.8
$Na_2EDTA.2H_2O$	37.3
Group 4	
Pyridoxine HCl	0.5
Nicotinic acid	0.5
Thiamine HCl	0.1
Inositol	100
Glycine	2.0

All the ingredients of Murashige & Skoog's medium as listed in table no-2 is categorised into four groups-

(1) Concentration of ingredients- The stock solution of group I is prepared 20x concentrated solution and of Group 2, 200x concentrated solution. Group 3 , iron salts is prepared 200x concentrated and group 4 organic ingredients(except sucrose) 200x concentrated.

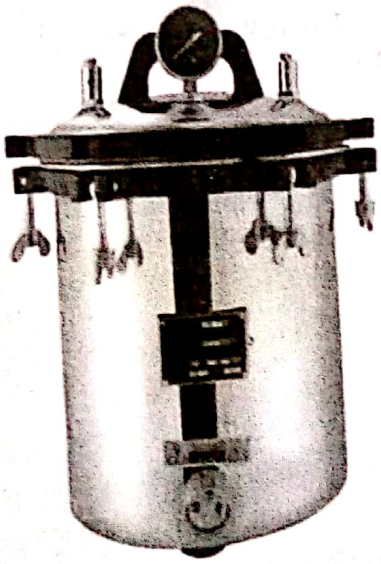
(2) Solution preparation- Stock solutions are prepared in the strength of 1m mol l^{-1} or 10m mol l^{-1}. In the preparation of stock solution each component should be weighed and dissolved separately in glass distilled water and than mix them together.

IAA, 2,4-D and similar compounds are dissolved in small amount of ethanol and made to desired volume with water. The cytokinins are dissolved in a small amount of 0.5 NHcl with slight heat and then made to volume with water. The iron solution is prepared by dissolving $Na_2EDTA.2H_2O$ and $FeSO_4.7H_2O$ separately in 450 ml of distilled water by gentle heating and continous stirring . Mix the two solutions and make up the volume to 1L with ditilled water.

(3) Semisolid media preparation- Agar and sucrose are weighed as per requirement and dissolved in 3/4 th volume of the distilled water by heating on water bath. The adequate quantities of stock solution (for 1L media, 50ml of stock solution of Group I and 5 ml of stock solution of Group 2,3 and 4) are added . Other desired supplements are also added and final volume is made up with distilled water. The pH of the medium is adjusted to 5.7 using 1N Hcl or 1N NaoH and medium is poured in the culture vessels.

(Note- A large variety of prepared media are now available in the market in the powdered form from Sigma and Himedia companies.The powdered media is disolved in 3/4 th volume of distilled water and after adding sucrose, agar and other desired chemicals, final volume is made up with distilled water. pH is adjusted and finally sterilized by autoclave. However, media prepared in the laboratory cost less as compared to ready- made media purchased from market.)

(4) Sterilization of media- All the culture vessels containing media are plugged with non-absorbent cotton, covered with aluminium foil and are sterilized by autoclaving at 121°C for 15-40 minutes (time depends on the volume of liquid to be sterilized). These vessels may be stored at 4°C and used whenever needed .



Autoclave

TYPES OF PLANT TISSUE CULTURE

The present knowledge permits the use of any plant part as a source of material to initiate cultures. The plant part used for this purpose is known as an explant. Nodal and