المحاضرة الثانية

Other lines of work can be used for the future to serve the science and technology :

1- Plant growth regulators play an important roles (auxin , Gibberellins , Cytokinins ……etc) in the tissue culture technique for research in Agriculture and biological work .

2- Also for changing the behaviors of any parts of plant under Different type of condition like temperature ,light , humidity And other .

3- The modern technique of tissue culture were use the protoplast fusion especially under breeding programs and Genetics .

4- Also single cell culture one of the most important parts in Tissue culture technique were be used for research and industries .

Setup of a Tissue Culture Laboratory

Work Areas

1- Bench
2- Gas outlet
3- Hot plate and magnetic stirrer
4- Analysis and top-loading balance
5- pH meter
6- Refrigerator ,freezer
7- Water purification and storage system
8- Dish –wishing area
9-Storage facilities – glassware , chemicals
10- Autoclave
11- Low bench with inverted light and dissecting microscopes

12- Fume hood
13- Desk and file cabinets
14- Centrifuge , spectrophotometer , microwave ,

Aseptic Transfer Area

1- Laminar air flow transfer hood and comfortable chair

2- Dissecting microscope

3- Gas outlet

4- Vacuum lines

5-Forcepes spatulas , scalpel , and disposable blades

Environmentally Controlled Culture Area

1- Shelves with lighting on a timer and controlled temperature.

2-Incubators – with controlled temperature and light.

3- Orbital shakers .

High humidity in the culture room should be avoided because

It increase contamination .Most cultures can be incubated in a temperature range of 25-27 C under a 16: 8 h light : dark photoperiod controlled by clock timers .

Avoiding contamination: تجنب التلوث

The important factors prevent contamination of culture are :

1-Removing air borne contamination in the sterile area .

2- Keep all windows and doors closed to avoid drafts .

3- A minimum number of person should person in the area.

4- Avoid breathing into the transfer chamber.

5- Never pass the hand or arm over a sterile material surface

(e.g. water or an open agar media plate)

6- At the conclusion of each step of the procedure remove

All unnecessary glassware , instruments , aluminum foil

,and other materials have been used

Plant Tissue Culture Media :-

Plant Tissue Culture refers to the technique of growing plant cells, tissues, organs, seeds or other plant parts in a sterile environment on a nutrient medium. Culture media used for in vitro cultivation of plant cells are composed of following basic components:

Complex Mixture of Salts:

Essential elements, or mineral ions Organic Supplement: vitamins and/or amino acids Carbon Source: usually sugar sucrose Gelling Agents Plant Growth Regulators Antibiotics

Complex Mixture of Salts:-

Macro elements (or macronutrients)

Microelements (or micronutrients)

Iron source

Organic Supplements :

These include vitamins and amino acids. Two vitamins, i.e., thiamine (vitamin B1) and myoinositol (a vitamin B) are essential for the culture of plant cells in vitro. However, other vitamins are often added to for historical reasons. The most commonly used amino acid is glycine. However, arginine, asparagine, aspartic acid, alanine, glutamic acid, glutamine and proline are also used. Amino acids provide a source of reduced nitrogen and, like ammonium ions, uptake causes acidification of the medium. Casein hydrolysate can be used as a source of a mixture of amino acids.

Carbon Source

The most commonly used carbon source is sucrose. It is readily assimilated and relatively stable. Other carbohydrates like glucose, maltose, galactose and sorbitol can also be used and may prove better than sucrose in specialized circumstances.

Gelling Agents

Plant tissue culture media can be used in either liquid or 'solid' forms, depending on the type of culture being grown. Agar, produced from seaweed, is the most common type of gelling agent, and is ideal for routine applications. For more demanding applications, a range of purer gelling agents are available. Purified agar or agarose can be used, as can a variety of gellan gums.

Plant Growth Regulators

Specific media manipulations can be used to direct the development of plant cells in culture due to plasticity and totipotency. Plant growth regulators are the critical media components in determining the developmental pathway of the plant cells. There are five main classes of plant growth regulator used in plant cell culture, namely:

Auxins

Cytokinins

Gibberellins

Abscisic Acid

Ethylene

Auxins:-

Auxins promote both cell division and cell growth. IAA (indole-3-acetic acid) is the most important naturally occurring auxin but its use in plant tissue culture media is limited because it is unstable to both heat and light. 2,4-Dichlorophenoxyacetic acid (2,4-D) is the most commonly used auxin and is extremely effective in most circumstances.

Cytokinins:-

Cytokinins promote cell division. Of the naturally occurring cytokinins, only zeatin and 2iP (2-isopentyl adenine have some use in plant tissue culture media. The synthetic analogues, kinetin and BAP (benzylaminopurine), are used more frequently. Non-purine-based chemicals, such as substituted phenylureas, are also used as cytokinins in plant tissue culture media.

Gibberellins :-

Gibberellins are involved in regulating cell elongation, in determining plant height and fruit-set. Only a few of the gibberellins like GA3 are used in plant tissue culture media.

Abscisic Acid:-

It is used in plant tissue culture to promote distinct developmental pathways such as somatic embryogenesis. Abscisic acid (ABA) inhibits cell division.

Ethylene:-

Ethylene is associated with controlling fruit ripening in climacteric fruits, and its use inplant

tissue culture is not widespread. Some plant cell cultures produce ethylene, which, if it builds up sufficiently, can inhibit the growth and development of the culture.

Antibiotics

Antibiotics are substances produced by certain microorganisms that suppress the growth of other microorganisms and eventually destroy them. Their applications include:

A. Suppresses bacterial infections in plant cell and tissue culture.

B. Suppresses mould and yeast infections in cell cultures.

C. Eliminates Agrobacterium species after the transformation of plant tissue.

These antibiotics can be divided into different classes on the basis of chemical structure and their mechanism of action:

Inhibitors of Bacterial Cell Wall Synthesis

e.g. β-lactam antibiotics, Penicillins and Cephalosporins.

Antibiotics that affect Cell Membrane permeability.

Antibacterial e.g. Colistin Sulphate,

Polymixin B Sulphate, Gramicidin

•Antifungal e.g. Amphotericin B, Nystatin, Pimaricin

Bacteriostatic Inhibitorsof Protein

Antibiotics that affect the function of 30 S or 50 S ribosomal subunits to cause a reversible inhibition of protein synthesis. e.g. Chloramphenicol, Chlortetracycline HCl, Clindamycin HCl, Doxycycline HCl, Erythromycin, LincomycinHCl,

Oxytetracycline HCl, Spectinomycin sulphate, Tetracycline HCl, Tylosin tartrate, Lincomycin HCl

Bactericide Inhibitors of Protein Synthesis

Antibiotics that bind to the 30 S ribosomal subunit and alter protein synthesis which eventually leads to cell death. This group includes:

- Amino glycosides: Apramycin, Butirosine, Gentamicin, Kanamycin, Neomycin, Streptomycin, Tobramycin.
- Inhibitors of Nucleic Acid Metabolism: e.g. Rifampicin, Plant Cell and Tissue Mitomycin C and Nalidixic acid
- Antimetabolites: Antibiotics, which block specific metabolic steps that are essential to microorganismse.g. Metronidazole, Miconazole, Nitrofurantoin, Trimethoprim and Sulphomethoxazole.

- Nucleic Acid Analogs, which inhibit enzymes essential for DNA synthesis. e.g. 5-Fluorouracil, Mercaptopurine.

Preparation of Plant Tissue Culture Medium

Measure approximately 90% of the required volume of the deionized-distilled water in a flask/container of double the size of the required volume.

Add the dehydrated medium into the water and stir to dissolve the medium completely. Gentle heating of the solution may be required to bring powder into solution.

Add desired heat stable supplements to the medium solution. Add additional deionized-distilled water to the medium solution to obtain the final required volume.

Set the desired pH with NaOH or HCl.Dispense the medium into culture vessels. Sterilize the medium by autoclaving at 15 psi (121oC) for appropriate time period. Higher temperature may result in poor cell growth.

Add heat labile supplements after autoclaving

Callus culture

A callus culture of an amorphous mass of loosely arranged thin –walled parenchyma cells arising from the proliferating cells of the parent tissue .

Frequently , as a result of wounding , a callus is formed at the cut end of a stem or root .

Using tissue culture techniques , callus formation can be induced in numerous plant tissue and organ that do not usually develop callus in response to an injury .

The most important characteristic of callus , form a functional viewpoint , is that abnormal growth has the potential to develop normal roots , shoots and embryoids that can form plants .

The general growth characteristics of a callus involve a complex relationship between the plant material used to initiate the callus , the composition of the medium and the environmental conditions during the incubation period .

Establishment of callus from the explant can be divided roughly into three developmental stages :

1- Induction . 2- Cell division . 3- Differentiation .

During the initial induction phase metabolism is stimulated as the cells prepare for division .

The length of this phase depends mainly on the physiological status of the explants as wellas the cultural conditions .

Subsequently , there is a phase of active cell division as the explant cells revert to meristematic or dedifferentiated state .

A third phase involved the appearance of cellular differentiation and the expression of certain metabolic pathway that lead to the formation of secondary productions .

Some callus growths are heavily lignified and hard in texture , whereas others break easily into small fragments . Fragile growths that readily separate are termed friable cultures. Callus may appear yellowish , white ,green , or pigmented with anthocyanin .

One serious problem associated with the use of callus , as well as other cell culture systems , is genetic instability resulting in variations in phenotype within the cell population . Phenotypic variations arising during culture may have either a development (epigenetic) or a genetic basis .

Callus subculture :

After the callus has been grown for a period of time in association with the original tissue , it becomes necessary to subculture the callus to a fresh medium . Grown on the same medium for an extended period will lead to a depletion of essential nutrients and to a gradual desiccation of the agar because of water loss . Metabolites secreted by the growing callus may also accumulate to toxic levels in the medium . The

transferred fragment of callus must be of a sufficient size to assure renewed growth on the fresh medium .

Factors affect callus culture

1) The level of plant growth regulators (auxin , cytokinins , gibberellin , ethylene ,etc) is a major factor that controls callus formation in the culture medium .

2) Culture conditions (temperature , solid media , light , etc) are also important in callus formation and development .

Reference (Plant tissue cult. John H. Dodds & L.W.Roberts)

The major stages of *in vitro* clonal propagation

In vitro clonal propagation is a complicated process requiring steps or stages . Murashige(1978) proposed four distinct stage that can be adopted for overall production technology of clones commercially . Stages 1,2,3, are followed under in vitro conditions , whereas 4 stage is accomplished in greenhouse environment . Debergh and Maene (1981)suggested an additional stage 0 stage for various micropropagating Systems.

Stage 0 :

This is initial step of micropropagation in which stock plant used for culture initiation are grown for at least 3 months under carefully monitored glasshouse conditions . Stock plants are grown at a relatively low humidity and water either with irrigation tubes or by capillary sand beds or mats . This stock plant preconditioning stage also includes measures to be adopted for reduction of surface and systemic microbial contaminations .

Stage 1 :

Murashige (1974) defined this stage as the initiation and establishment of aseptic cultures . The main steps involved are preparation of the explants from stock plants (stage 0) fowolled by this establishment on suitable culture medium . Cultures are initiated from explants of several organs but shoot tips and axillary buds are most often used for commercial micropropagation . Procedures to surface – sterilize the explant and

induce a healthy growth in the culture medium . It may also be advisable to control microbial contamination within explant tissues in case such efforts at stage 0 were not successful .

Factors influencing stage 1 : Include the choice of a suitable explant , the composition of the medium , and the environ mental conditions .

The larger size of the tip explant , the more rapid growth and the greater rates of survival . The basal medium is supplemented with vitamins , sucrose , and the appropriate hormones .

LIGHT , necessary for photomorphogenesis and chlorophyll biosynthesis , is provided with Gro- Lux and White florescent tube . Murashige (1974) found that many cultures grew best with 1000 Lux for stages 1 and 2 , with light intensity increased to 30000 – 10000 Lux for stage 4 photoperiods of 16 hours were optimum for several species .

Stage 2 :

This stage takes up the bulk of micropropagation activity using a defined culture medium that stimulates maximum proliferation of regenerated shoots . Various approaches followed for micropropagation include :

1) Multiplication through the growth and proliferations of meristems excised form apical and axillary shoots of the parent plant .

2) Induction and multiplication of adventitious meristems through processes of organogenesis or somatic embryogenesis directly on explants .

3) Multiplication of calli derived from organs , tissue , cells , or protoplasts and their subsequent expression of either organogenesis or somatic embryogenesis in serial subcultures . Shoots obtained from these calli can be further multiplied following procedures 1 and 2 .

This stage need medium supplemented with cytokinin like BA , Kin , 2iP, Zeatin ……others .

Stage 3:

Shoots proliferated during stage 2 are transferred to a rooting (storage) medium . Sometimes shoots are directly established in soil as microcuttings to develop roots . Since such a possibility depends on the particular species and , at present , a large number of species cannot be handled in this manner , the shoots are generally rooted in vitro . When the shoots or plantlets are prepared for soil , it may be necessary to evaluate several factors such as (i) dividing the shoots and rootingthem individually , (ii) hardening the shoots to increase their resistance to moisture stress and disease , (iii) rendering plants capable of autotrophic development in contrast to the hetero –trophic state induced by culture and (iv) fulfilling requirements of breaking dormancy , especially of bulb crops . stage 3 requires 1-6 weeks .This stage need medium supplemented with AUXIN like IAA , IBA , NAA , ….OTHER .

Stage 4 :

Steps taken to ensure successful transfer of the plantlets of stages 3 from the aseptic environment of the laboratory to the environment of the greenhouse comprise stage 4 . Unrooted stage 2 shoots are also acclimatized in suitable compost mixture or soil in pots under controlled condition of light , temperature ,and humidity inside the greenhouse . Supplying bottom heat-aids to pots with plantlets or cutting and maintenance of a dense fine –particle fog system within the greenhouse enhance the rooting process .

Factors affecting shoot multiplication

1- Physiological status of plant material .
2-Culture medium .
3- Culture environment .
4- Genotype .

(Reference ,introduction to plant tissue cul. M.K. Razdan)
Page 234 .

Phenolic compounds

Tissue containing relatively high concentrations of phenolic compounds are difficult to culture . Polyphenolases stimulated by tissue injury will oxidize these phenolic substance to growth –inhibiting , dark –colored compounds . Techniques used to suppress this metabolic sequence include :

(1) adding antioxidants to the medium ,

(2) presoaking the explants in antioxidant solutions prior to culture ,

(3) subculturing to fresh medium on signs of enzymatic browning ,

(4) providing little or no light during the initial period of culture .

Also we can presoaked the explant in sterile distilled water for 3 hours prior culture , and we can used the filter –paper bridge technique

Methods of sterilization classified as follows :

1- Dry heat

2- Wet heat

3- Ultrafilteration

4- Chemical sterilization

1- Dry heat :

This methods is used for glassware , metal instruments ,or other materials that are not charred by high temperatures . Objects containing cotton , paper , or plastic cannot be sterilized with dry heat . The moderate temperature (160 c) for 4 hours . The material to be sterilized are carefully wrapped in a heavy aluminum foil before placed in the oven .

2- Wet heat :

This procedure employ an autoclave operated with steam under pressure . For the sterilization of paper products , glassware , instruments , a steam pressure of 15 1b/in² temperature of 121°c for 20 min. (Do not start timing until temperature and pressure reached the proper teprature) .

3- Ultra filtration :

Some media components are unstable at high temperature and must be sterilized by ultra filtration at room temperature . Usually a small volume is sterilized by passage through a membrane filtration unit attached to a

4- Chemical sterilization :

The working area is generally surface sterilized with ethanol (70%) . A slightly higher concentration of ethanol (80%) a considerably more inflammable mixture , is employed for periodically sterilizing the instruments . The surface sterilization of plant material may be accomplished with an aqueous solution of either hypochlorite (NaOCl)or calisium hypochlorite ($Ca(OCl)^2$).

Shoot tip Culture :

1- Any plants want to be used for shoot tips culture should be kept in green house or lath house .
2- The plants should be supplied with a general liquid or solid nutrient feed a week or two weeks .
3- one cm shoot tips were collected in small beaker with distilled water , then rinsed with D. W. three times before transferred to the sterile bench .

4-Shoot tips sterilized with 5% (NaOCl) for 13-15 mints . Then washed three times in sterile D. W. for 8-10 mints .

5- The shoot tips placed on sterile filter paper to remove excess water .

6- The lower leaves were removed .

7- One or two of youngest leaf primordial (the shoot tips at this stage were 1.5 to 2 mm in length) with the apical meristematic dome remained .

8- The explants were transferred to the culture medium (MS) supplemented with different hormones , 3% sucrose and 0.6% agar at PH 5.7 .

9- Ten ml in tubes or 50 ml of agar culture in a 100 ml flasks , and one shoot tips was placed in each flask .

10- The mouth of the culture was flamed before and after transplanting the shoot tips to the medium closed by a cotton wool plug covered with foil or other covers .

11- All the cultures were incubated in growth room under a different day length at 25±1 c° temp. .

Leaf disc culture :

1- Leaf pieces taken from young healthy and mature leaves of any plants .

2- The surface sterilized as in shoot tips .

3- One cm leaf disc prepared by means of sterilized cork borer .
4- Three pieces of leaf disc were cultured on any media with different hormones treatments in each Petri dish or flask , those sealed with Para film and kept them in growth room as the shoot tips culture .

Callus culture :

Callus culture could be established from:

1- Hypocotyls .
2- Roots.
3- Internodes .
4- Shoot tips .
5- Other organ or tissue of any plants .

The initial callus was further subculture on fresh medium with auxin and cytokinin to establish healthy callus culture.
For subculture callus , pieces (0.5 – 0.7)cm were used .
The undifferentiated callus cells varied in color and texture from creamy , light green , yellowish or green callus , friable or soft .

Reference (Plant tissue cult. John H. Dodds & L.W.Roberts)

Embryo culture :

Zygotic embryo culture is the aseptic isolation and growth of sexually produced embryo in vitro with the objective of obtaining viable plants .

In 1904 , Hannig published a paper describing the first systemic attempt to culture isolated mature embryos of angiosperms *Cochleria* and *Raphanus* (family :Cruciferae) aseptically . He successfully raised transplantable seedlings from the embryos cultured on semi-solid medium containing mineral salts and sugar . Subsequently , many workers obtained plants by culturing exised embryos from mature seeds especially of species which failed to germinate due to embryo abortion .

Types of Embryo Culture :

According to Pierik (1989) ,there are in principle two types of embryo culture :

(1) Culture of Immature Embryos

This type of embryo culture is mainly used to grow immature embryos originating from unripe or hybrid seeds which fail to germinate . Excising such embryos is arduous and generally a complex nutrient medium is required to raise them to produce plants .The chances of success in this type of culture depend largely on the developmental stage of the excised embryo .

(2)Culture of Mature Embryos

Mature embryos are excised from ripe seeds and cultured mainly to avoid inhibition in the seed for germination . This type of culture is relatively easy as the

embryos require a simple nutrient medium containing mineral salts sugar and agar for growthand development.

Surface Disinfecttion :

Embryos of seed plants normally develop inside the ovules which , in turn , are covered by ovaries . Since they already exit in a sterile environment , disinfection of the embryo surface is unnecessary unless the seed coats are injured or a systemic infection is present . Instead, mature seeds , entire ovules , or fruits are surface-sterilized and the embryos removed aseptically from the surrounding tissues .

Excision of Embryo :

The embryo –excision operation is performed aseptically in a laminar airflow hood .

Nutritional Requirements :

The nutritional requirements of an embryo during its development in vivo constitute two phases :

(a) *heterotrophic- phase* – an early phase wherein the embryo is dependent and draws upon the endosperm and maternal tissues .

(b) Autotrophic phase – a later phase in which the embryo is metabolically capable of synthesising

substances required for its growth , thus becoming fairly independent for nutrition .
The critical stage at which the embryo passes from the heterotrophic phase into the autotrophic varies with the species .

Mineral Salts :
Inorganic nutrients of MS , B5 , and White s media with certain degrees of modifications are most widely used basal media for embryo culture work . Monnier (1978) modified the MS medium and formulated a new mineral solution which promoted higher survival rate of cultured immature embryos of *Capsella* .

Carbohydrates
Sucrose is the must commonly used source of energy for embryo culture . Addition of maltose , lactose , or manitol may be require in the embryo culture of some species (Zea mays) .

Nitrogen and Vitamins
Embryos which have an excellent enzyme system can reduce nitrates to ammonium . Ammonium nitratesis significantly superior to KNO3 , NaNO3 and (NH4)2HPO4 .Especially the present of NH4 in the medium has been found essential for proper growth and differentiation o embryos .
Various amino acids and their amides have been tested for embryo culture . Hannig (1904) reported that

asparagine enhanced embry growth but some workers found glutamine to be a superior source of nitrogen for the embryos of some species (*Capsella bursa –pastoris* , *Arabidopsis thaliana, Reseda odorata)* .

Growth regulators

Auxin and cytokinins are not generally used in embryo cultures since they induce callus formation . At low levels (0.01 mgL) GA promotes embryogenesis of young barly embryos without inducing precocious germination.

Methods of Virus Elimination :-

Plants are often infected with more than one type of virus , including some not even know . A plant can be claimed as free of only those viruses for which specific tests have given negative results and accordingly labeled as free of a specific virus or pathogen .

1) Heat Treatment :-

At temperature higher than optimum manyviruses in plant tissues are partially or completely inactivated with little or no injury to the host tissues. Heat treatment is given through hot water or hot air . The hot-water treatment effectively eliminates virus in dormant buds , whereas hot-air treatment is recommended for elimination of virus from actively growing shoots . Moreover , the survival rate of the host tissue is better in hot-air treatment . For hot-air treatment , actively growing plants are pleased in a thermotherapy chamber adjusted at a temperature of 35-40 C° for apical varying from a few minutes. to several months . The temperature of the air should be gradually raised during the first few days until the desired temperature is reached.

2) Meristem –tip culture :-

Meristem –tip explants used for virus elimination are too small to be isolated with unaided eyes . A stereoscopic microscope (8-40 magnification) with a suitable (preferably cool fiber) light sources is required to excise active moistens . Care must be taken to prevent desiccation of shoot tips due to constant flow of air through the laminar air-flow cabinet and the heat generated bythe light source attached to the microscope used for dissection .

3) Chemical Treatment :-

In the absence of effective therapeutic chemicalscapable of eradicating virus from infected plants , there are reports of some attempts to suppress viruses in plant tissue and protoplast by the addition of some chemicals in the media . In some instances virus multiplication was suppressed by the addition of cytokinins and other growth substances , while in others it was actually stimulated . Moreover of these substances thiouracil, acetylsalicylic acid , Cyclohexamide and actinmycin –Dare also inhibit virus in plants.

4) Other in vitro Methods :-

Callus culture may also prove useful in raising plants free of virus . Based on the fact that distribution of viruses in plants is uneven , the callus derived from infected tissues doesnot carry the pathogen uniformly in all cells .The possible reasons for the escape of some cells of a systematically infected callus from virus are : (a) virus replication is unable to keep pace with cell proliferation and (b) some cells acquire resistance to virus infection through mutagenesis . Other in vitro methods that can be followed for regeneration of plants for disease resistance are somatic cell hybridization , gene transformation and somaclonal variation

Anther and Pollen Culture

More than forty years ago Larue 1945 from the university of Michigan's reports the idea of culturing pollen of higher plants in order to explore their growth and in vitro conditions , then he carried out several investigation with male and female gametophytes of higher plants .

Young flower buds at the appropriate stage of pollen development anthers are surface-sterilized uninjured anther cultured on nutrient media for most species .

Cultured medium solidified with agar has been used to induce pollen in Nicotina species , the yield of pollen plantlets could be increased considerably by using liquid medium .

Success in obtaining division of pollen grains outside the anther has been reported in a number of plants .

Mature pollen grains of Brassica could be induced to form cell clusters in hanging drop cultures.

Plantlets could formed from anther culture after several fresh subculture .

In species with a determinate number of anther per flower , a series of buds must be examined in order to give all the stages of development . Tow basic methods are used :

1) Excised anthers are cultured on an agar or liquid medium , and embryogenesis occurs within the anther ; or

2) the pollen is removed from the anther , either by mechanical means or by natural dehiscence of the anther , and the isolated pollen is cultured on a liquid medium .

It may take 3-8 weeks for haploid plantlets to emerge from the cultured anthers .

Factors affecting plantlets formation from anther and pollen culture :

1) Pollen development stage .

2) Anther wall factor .

3) Type of plant and environmental factors .

Purpose of anther and pollen culture ;

The cell of haploid plant contain a single complete set of chromosomes and these plants are useful in plant-breeding programs for the selection of desirable characteristics .

The purpose of anther and pollen culture is to produce haploid plants by the induction of embryogenesis from repeated divisions of monoploid spores , either microspores or immature pollen grains .

The chromosome complement of these haploids can be doubled by colchicine or by regeneration techniques to yield fertile homozygous diploids .

This technique has resulted in several improved varieties of crup plants .

(J. H. Dodds 1985)

Somatic embryogenesis

The capacity of flowering plants to produced embryos is not restricted to the development of the fertilized egg ; embrys (embryoids) can be induced to form in cultured plant tissues . . This phenomenon was first observed in suspension cultures of carrot (Daucus carota) by Steward and co-workers (1958) and in carrot callus grown on an agar medium by Reinert (1959) .This is a general phenomenon in higher plants , and experimental somatic embryogenesis has been reported in tissue cultured from more than 30 plant families (Raghavan,1976). Somatic embryoids may arise in vitro from three sources of cultured diploid cells :

(1) vegetative cells of mature plants ,

(2) reproductive tissues other than zygote ,and

(3) hypocotyls of embryos and young plantlet without any intervening callus development (Kohlenbach,1978).

According to Sharp and his colleagues (1980) , somatic embryogenesis may be initiated in two different ways . In some cultures embryogenesis occurs directly in the absence of any callus production from"

preembryonic determined cells" that are programmed for embryonic differentiation . The second type of development requires some prior callus proliferation , and embryos originate from "inducd
The embryoid-forming cells ج embryogenic cells" within the callus . are characterized by dense cytoplasmic contents , large starch grains ,and a relatively large nucleus with a darkly stained nucleolus . Staining reagents indicated that these embryogenic cels have high concentrations of protein and RNA .

These cells also exhibited high dehydrogenase activity with tetrazolium staining . . Each developing embryoid passes through the

sequential stages of embryo formation (i.g., globular , heart shape , and torpedo shape) as in fig.

. Although a given culture may differentiate these embryogenic cells , their further development may be blocked by an imbalance of chemicals in the culture medium .

Abnormalities know as embryonal budding and embryogenic clump formation may occur , if relatively high levels of auxin

In other words , two distinctly different types of media may be required , one medium for initiation of the embryonic cells and another for the subsequent development of these cells into embryoids .

The first (induction) medium must contain auxin .

The second medium generally consists of a mixture either lacking auxin , with a lower concentration of the same auxin, or with reduced levels of a different auxin .

With some plants , however , both embryo initiation and subsequent maturation occurs on the first medium , and a second medium is employed for plantlet development .

The most important chemical factors involved in the induction medium are auxin and reduced nitrogen . Substantial amounts of reduced nitrogen are required in both the first and second media .

Practical Application of Somatic Embryogenesis

1. Clonal propagation
2. Synthesis of synthetic \Artificial seed
3. Source of regenerable protoplast system.
4. Genetic transformation.
5. Conservation of genetic resources