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Plant Propagation through Tissue Culture – A Biotechnological Intervention

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A B S T R A C T

Plant genetic transformation has become an important biotechnological tool for the improvement of many crops. A solid foundation for the fast development and implementation of biotechnology in agriculture has been provided by achievements in plant tissue culture. Plant tissue culture represents the most promising areas of application at present time and giving an out look into the future. The areas range from micropropagation of ornamental and forest trees, production of pharmaceutically interesting compounds, and plant breeding for improved nutritional value of staple crop plants, including trees to cryopreservation of valuable germplasm. It has broad applications in several areas but it is rather broadly used to include several variations, such as meristem culture for propagation of virus-free plants, protoplast culture and somatic cell hybridization for the introduction of new characteristics (salt tolerance, disease resistance, enhanced crop yield, etc.) into key species, anther/ pollen culture and ovule culture for producing haploid plants and embryo culture for embryo rescue in distant crosses. It also enables to select desirable traits directly from the culture setup, thereby decreasing the amount of space required for field trials. For species that have long generation time, or seeds that don't readily germinate, rapid propagation is possible by this method. A number of medicinally important alkaloids, anticancer drugs, recombinant proteins and food additives are produced in various cultures of plant cell and tissues. Thus, tissue culture is one of the most important part of applied biotechnology.

Introduction

Plant biotechnology is the technology which is used for getting modern product with high yield and at faster rate. Modern era of plant biotechnology started in the beginning of the 20th century and is associated with the ability to grow plant cells and tissues *in vitro*, to regenerate and clone new plants and later, to modify their genetic characteristics. A technology known asplant tissue culture is being widely used for producing large number of plants at a very fast rate, with improved genetic characteristics, under the controlled environmental conditions. Thus, Plant tissue culture is the technique of *in vitro* cultivation of plant cells and organs, which divide and regenerate into callus or particular plant

organs. The technique relies on (i) the totipotency - the inherent capacity of the individual cells of an organism to develop into a complete organism, (ii) the explants - which is a small tissue excised from any part of the plant, (iii) the aseptic environment -to avoid contamination from microorganisms and (iv) the nutrient media - that strongly govern the growth and morphogenesis of plant tissues (*Anonymous*, 2020).

Plant tissue culture can also be defined as a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. The plant material to be cultured may be cells, tissues or plant organs such as excised root tip, shoot tip, shoot bud, leaf petiole, inflorescence, anther, embryo, ovule or ovary. Thus, using the appropriate growing conditions for each explant type, plants can be induced to rapidly produce new shoots, and with the addition of suitable hormones, new roots. These plantlets or microplants can also be divided, usually at the shoot stage, to produce large numbers of new plantlets or microplants (*Sub-culturing*). The new plants can then be placed in soil and grown in the normal manner.

Organization of tissue culture laboratory

A sophisticated plant tissue culture laboratory should consist of the following areas;

Washing room; Inoculation room; Media preparation room; Culture/growth room

Plant Tissue Culture Media Composition

One of the most important factors governing the growth and morphogenesis of plant tissues in culture is the composition of the culture medium. Plant tissue culture media is generally composed of the following components;

Macronutrients

Macronutrients are those elements which are required in concentration > 0.5 mM/l. These include six major elements: Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg) and Sulphur (S), present as salts and constitute various media. Macronutrient stock solutions are generally made up at 10 times their final strength.

Micronutrients

Micronutrients are those elements which are required in concentration < 0.5 mM/l. Theseinclude eight minor elements: Iron (Fe), Manganese (Mn), Boron (B), Copper (Cu), Zinc (Zn), Iodine (I), Molybdenum (Mo), Cobalt (Co) and Nickel (Ni).Micronutrient stock solutions are generally made up at 100 times their final strength.

Carbon and energy source

In the cultured cells or tissues, photosynthesis is inhibited and thus carbon must be added in the form of carbohydrates for tissue growth in the medium. The commonly used carbon and energy source is sucrose. The sucrose in the medium is rapidly converted into glucose and sucrose. The glucose is then utilized first followed by fructose. Sucrose is generally used at a concentration of 2 – 3 %.

Organic supplements

Vitamins

Vitamins are required by plants as catalystes in various metabolic processes. The vitamins most frequently used in cell and tissue culture media include thiamine (B_1), nicotinic Acid (B_3), pyridoxine (B_6) and myo-inositol. The concentration are in the order of 0.1 to 10 $mgL^{-1.}$

Amino acids

The cultured cells are normally capable of synthesizing all of the required amino acids, however the addition of some amino acids may be used to further stimulate cell growth. The most common sources of organic nitrogen used in culture media are amino acid mixtures like Casein hydrolysate (0.05 – 0.1%), L – glutamine (8 mM L^{-1}), L- cysteine (10 mM L^{-1}).

Organic extracts

Addition of a wide variety of organic extracts such as coconut milk, yeast extract, malt extract, potato extract, protein hydrolysates, ground banana, orange juice and tomato juice, to the culture media results in favourable tissue responses. However, the success is achieved with the use of coconut milk (5 – 20 %) and protein hydrolysates (0.05 – 1%).

Growth regulators

Only 2 main classes of PGRs are of special importance in plant tissue culture i.e., Auxins and Cytokinins.

Auxins: Concerned with cell division, cell elongation, formation of meristems and maintenance of apical dominance.

E.g. *Natural*– IAA

***Synthetic* –** IBA, NAA, 2, 4-D, etc.

Cytokinins: Stimulates protein synthesis, stimulates cell division, induces shoot formation, induces axillary shoot proliferation, inhibits root formation and controls morphogenesis.
E.g. *Natural* – Kinetin, Zeatin, etc

***Synthetic* –** 6-BAP/ BA

Others viz., gibberellins, abscisic acid and ethylene are of minor importance.

Gelling agents

Those compounds which are capable of gelling the media. Gelling agents form clear gels at relatively lower concentrations of 1.25 – 2.5 g/l. These are the valuable aids for the detection of contamination and root formation during the culture. Commonly used gelling agents are agar, agarose, gellan gums, gelrite, etc.

Sterlization

It is the procedure used for the elimination of micro-organisms. Maintenance of aseptic (free from all micro-organisms) or sterile conditions is essential for successful tissue culture procedures. Need for asepsis requires that all culture vessel, media and instruments used in handling tissues, as well as explant itself be sterilized.

Sterilization procedures

Preparation of sterile media, containers and small instruments

Steam sterilization

It is performed either in an Autoclave or domestic pressure cooker.
The standard conditions for autoclaving are 121^0C with a pressure of 15 psi for 20 minutes.
It is used for sterilizing media, cotton plugs, plastic caps, water, pipettes, etc.
It is always recommended over dry sterilization.

Dry sterilization

It is performed in Hot Air oven.
It is a method of sterilizing glassware and metallic instruments in dry heat for 3 hours at 160 – 180^0C.

Dry goods can either be wrapped in Al foil,

brown paper or sealed metal containers to maintain sterility.

It has the disadvantage of poor circulation of air and slow penetration of heat.

Filter sterilization

This method is used for the sterilization of heat liable compounds (amino acids, vitamins, etc.) which get destroyed during autoclaving.

Ultra violet sterlization

This method is generally used for the sterilization of disposable plastic wares into which the autoclaved media is later on dispensed.
15 – 20 minutes exposure is adequate.

Maintenance of aseptic conditions

Alcohol sterilization

It is used for sterilizing hands, laminar air flow cabinets and various instruments.
It is done with help of 70% ethanol.

Flame sterilization

This method is used for the sterilization of instruments that continuously used during manipulation work.
Instruments are soaked in 70% ethanol followed by flaming on a burner in the laminar airflow hood.

Preparation of sterilized explant material

Chemical sterilization

It is the method of eradication of micro-organisms with the aid of chemicals.
The type and concentration of chemical sterilant to be used and exposure time varies with the type of explant used.

Techniques of Plant Tissue Culture

The various techniques of *in vitro* culture includes

Seed Culture

Growing seed aseptically *in vitro* on artificial media is called seed culture. It increases the efficiency of germination of seeds that are difficult to germinate or don't germinate well *in vivo*. It is used to raise the sterile or aseptic seedlings and to identify the plants which are resistant or tolerant to various stresses.

E.g., Orchids, Vanilla, Tomato, Chilli, etc

Case study 1 Studies on *in vitro* seed culture in vanilla(Kumar*et al.*, 2014).

In this study, an experiment was carried out to examine the effects of different treatment combinations of PGR's on the *in vitro* micro-propagation of vanilla. Seeds were cultured on standard MS media containing sucrose (2.5%) and agar (0.65%). Cultures were incubated in a growth chamber at a temperature of 26^0C, a 12h photoperiod and 2000 lux light intensity. After 4 weeks, the germinated seeds had produced young seedlings with 5 – 9 leaves with a survival rate of 70-90%. The seedlings after proper elongation were rooted on half-strength MS medium added with charcoal $2gl^{-1}$ and IBA $1mgl^{-1}$.

Meristem culture

It involves the culturing of apical meristems, especially of shoot meristem *in vitro* on artificial media. It is also known as *Meristemming*or *Mericlonning.* 3 – 5mm shoot apices having several leaf primordial are selected as explants. However, when the objective is virus free plant production, the size of explant should be < 1mm. It makes use of single nodes or axillary buds.

E.g., Capsicum, Tomato, Brinjal, Potato, etc

Applications

Plant propagation.
Production of virus free planting material.

Case study 2. Meristem Culture of Potato (*Solanumtuberosum*L.cv. Desiree) for Production of Virus-Free Plantlets (Zaman *et al.,* 2018).

This study was conducted to evaluate the effect of 3 different auxins NAA, IAA and IBA each at four levels (0, 0.1, 0.5 and 1 mg/l) on meristem culture of potato for the production of virus-free plantlets. Cultures were incubated in a growth chamber at a temperature of 22- 25 ^{0}C and 2500 lux light intensity. After 2-3 weeks, plantlets were studied for various parameters and transferred to greenhouse.

Bud culture

It is of 2 types;

Single Node Culture (SNC): Here, a nodal segment is isolated from the third and fourth nodes from the stem apex.The bud is then allowed to develop on a nutrient media, with the purpose of forming a shoot. Most commonly used method for propagating plants *in vitro.*

***Axillary Bud Culture*:** Here, an axillary shoot bud is isolated from a plant. The bud is then allowed to develop under the influence of a relatively high cytokinin concentration. High cytokinin concentration stops the apical dominance and allows axillary buds to develop.

E.g., Potato, Tomato, Chilli, Capsicum, etc

Applications

Simple and quick method of plant propagation
In most cases, organogenesis occurs directly i.e., without callus formation.
Favors high multiplication frequency coupled with genotypic uniformity of the plants produced.

Case study 3. *In vitro* Micropropagation of Potato cultivars (*Solanum tuberosum*L.) (Xhulaj*et al.,* 2019).

This study was conducted to standardize the protocol for *in vitro* micropropagation of potato (*Solanum tuberosum*L.) cultivars by using sprouts as explant. Explants were cultured on standard MS media containing sucrose (3%), agar (0.6%), Calcium D pantothenate (2 ppm) and GA_3 (0.25ppm). Cultures were incubated in a growth chamber at a temperature of 25 $\pm$ 1^{0}C, a photoperiod of 16/8 hourlight/ dark and 2000 lux light intensity. Young seedlings were obtained after 3-4 weeks of inoculation with a survival rate of 80-90%.

Callus culture

Callus is an undifferentiated, tumor-like mass of cells. *In vitro* culturing of callus tissue aseptically on artificial media is known as *Callus culture*. Regeneration *via* callus culture involves 2 important processes;
De-differentiation – the non-dividing quiescent cells of explant are reverted to meristematic state by placing on nutrient media. It results in the formation of undifferentiated mass of cells (Callus).
Re-differentiation – the de-differentiated of cells or callus undergo differentiation i.e., shoot & root formation and develops capacity to regenerate into the complete plant.

E.g. Potato, Tomato, Chilli, Capsicum, Brinjal, etc.

Case study 4. *In vitro* micropropagation of *Capsicum chinense*Jacq. (Gayathri*et al.,* 2015).

The study was conducted to studythe effect of plant growth regulators in different concentration with combination for the regeneration of multiple shoot proliferation and callus induction in *Capsicum chinense* Jacq. by using Shoot tip, axillary buds, leaves, nodal and inter-nodal parts as the explants. Explants were cultured on MS basal medium containing sucrose (3%), phytagel (0.4%) and different combinations of BAP, NAA and 2,4-D. Cultures were incubated in a growth chamber at a temperature of 24 $\pm$ 2 ^{0}C, a 16/8h light/dark cycle and 3000 lux light intensity. Callus formation was observed after 2 weeks of inoculation. Among all the explants, leaves showed 90% capacity for the formation of callus.

Cell culture

It is also called *Cell Suspension Culture.* It consists of single isolated cells or cell aggregates dispersed and growing in moving liquid media. It is normally initiated by transferring pieces of explant/ un-differentiated and friable calluses to a liquid medium which is continuously agitated by a rotary shaker to provide aeration and dispersion of cells.

E.g., *Capsicum frutescens*(Capsaicin-Pungency), Saffron (crocin&picrocrocin – medicinal importance), *Dioscorea* spp. (Diosgenin), *Vanilla* spp. (vanillin- flavouring chemical), 3-N-Butyl-pthalide in Celery (Effective against hypertension), etc.

Applications

Large scale clonal propagation through embryogenic cell suspension.
Somatic embryos from cell suspensions can prove useful for long-term storage in germplasm banks.
Somatic embryos from cell suspensions produce the same flavour compounds or secondary metabolites as present in the mature plant.

Organ culture

In organ culture, two in vitro methods have been used;

Ovule culture – it refers to the culture of excised ovaries and ovules.
Anther culture – It refers to the culture of excised anthers and pollens.

Anther culture

Anther culture is the aseptic excision and culturing of developing anthers from unopened flower buds in a nutrient medium, where pollen grains are induced to produce callus or embryoids and finally to haploid plantlets. The process by which haploid plant develops from male gametophyte is called *androgenesis.* It has been observed that uninucleate microspores midway between the tetrad release and the first pollen mitosis are the most responsive.

Applications

Simple, quick and efficient technique of haploid production.
Reduction of time in developing variety of cross-pollinated crop.
Fixation of heterosis through dihaploid production.
Induction of genetic variability.

Case study 5. Studies on Anther Culture in tomato *(Solanum lycopersicum*L.) (Shere*et al.,* 2009)

The present investigation has been undertaken using 3 varieties of tomato; Vaishali, Wild cultivar and Pusa ruby. Unopened flower buds of different sizes viz., 2-4mm, 5-6mm and 8-10mm of each of the 3 cultivars were selected. Anthers were excised from flower buds and inoculated in petri-dish containing the suitable media. Dishes were exposed to cold treatment at 8 ^{0}C for 2, 4 and 10 days. After cold treatment incubation was done in dark at 23$\pm$ 1 ^{0}C.

Results and Discussion

Flower bud size of 2-4mm was significantly superior over other 2 sizes.
Earlier callus initiation was observed in Vaishali (26 days) followed by Pusa ruby (27 days) and Wild (29 days).
Plant regeneration was observed on MS media supplemented with BAP (2 mg/l) and NAA (1 mg/l).

Microspore culture

Microspore or the immature pollen can be used as the explant to get the haploid plants directly. For pollen or microspore culture, the flower buds are collected, surface sterilized and the anther lobes are dissected out from the flower buds. Then the anther lobes are squeezed with the help of a scalpel within a tube or small beaker to collect the microspore or pollen in nutrient media. Then the anther tissue debris is removed by filtering the suspension through a nylon sieve with a diameter slightly larger than the pollen size (40µ-100µ) allowing the microspore only to pass through it.

Then, the microspore-suspension is washed and concentrated to a plating density. The microspores obtained are then mixed with an appropriate culture medium at a density of 10^3- 10^4 microspore ml^{-1}, and plated in small petriplate. To ensure good aeration, the layer of liquid in the dish should be as thin as possible, and sealed with 'parafilm' to avoid dehydration. The responsive pollen will divide and form embryos or calli which directly or indirectly will form the haploid plantlet. By following the method of sub-culturing the whole plant suitable for soil transfer can be obtained.

Applications

The explants i.e., microspores or pollens are all haploid cells.
The sequence of androgenesis can be observed starting from a single cell.
The microspores are ideal for uptake, transformation and mutagenic studies, and the microspores are evenly exposed to chemicals and physical mutagens.
Higher yields of plants/anther could be obtained.

Double haploidy

Haploid plants obtained either from anther or ovule culture may grow normally under *in vitro* conditions up to the flowering stage but viable gametes are not formed. Also, there is no seed set due to the absence of one set of homologous chromosomes.
The only mechanism for perpetuating the haploids is by duplicating the chromosome no. in order to obtain homozygous diploids. Diploidization is achieved by immersing very young haploids in a filter sterilized solution of colchicine (0.4%) for 2-4 days, followed by their transfer to the culture medium for further growth. In this procedure, chromosome or gene instabilities are minimal compared to other methods of chemical treatment.

Embryo culture

It consists of isolation of immature or mature embryos under aseptic conditions and culturing it on nutrient media.

E.g., Legumes (Green gram, Black gram,

French bean, Soybean, etc.), Tomato, Brinjal, Potato, Turnip, etc.

Applications

Embryo rescue in case of F_1 hybrids obtained through wide/ distant hybridization.
Propagation of seeds having short viability i.e., low to negligible amount of endosperm.
Shortening of breeding cycle.

Somatic hybridization

It isalso known as *Parasexual Hybridization* as the procedure eliminates gametes in hybridization procedure. It is also referred as *Protoplast Fusion*, as it involves fusion of protoplast of 2 species. It is a technique in which the protoplast belonging to different species, genera or families are fused together to form hybrid product *(Heterokaryon)* under *in vitro* conditions. Protoplasts are naked plant cells i.e., without cell wall. They are produced by subjecting the plasmolysed cells to the treatment of mixture of enzymes (cellulose & pectinases).Culture medium of protoplasts is similar to PTC but devoid of ammonium and increased concentration of Ca.

Methods of Protoplast fusion

Polyethylene Glycol Method

Suspend the protoplasts in 1ml solution of *Polyethylene Glycol.*
Shake the culture tubes for 5 seconds and left undisturbed for 10 – 15 minutes.
Wash the protoplast material several times to remove Polyethylene Glycol and then resuspend it in culture medium.

Treatment with sodium nitrate

Suspend the isolated protoplasts in 10% *Sucrose* solution.
Incubate the solution containing protoplasts in a water bath at 35^0C for 5 min.
Centrifuge the sample at 200xg for 5 minutes.
Decant the supernatant and transfer the protoplast pellet to a water bath at 30^0C for 30 min.
Decant the aggregating mixture and replace it with the culture medium containing 0.1% $NaNO_3$.
Left the protoplasts undisturbed for sometime and wash twice with culture medium and plate.

Electrofusion

In this technique, protoplasts are placed in a small culture cell containing electrodes and an extremely short wave electric shock is applied, which induces the fusion of protoplasts.

Table.1 History of Plant Tissue Culture

S.No.	Year	Scientist	Contribution
1.	**1902**	**G. Haberlandt**	Proposed concept of *in vitro* cell culture.
2.	**1904**	**Hanning**	Cultured embryos from several cruciferous species.
3.	**1922**	**Kolte& Robbins**	Successfully cultured root and stem tips.
4.	**1926**	**Went**	Discovered first plant growth hormone – IAA.
5.	**1934**	**White**	Introduced vitamin B as growth supplement in tissue culture media for tomato root tip.
6.	**1939**	**R.J Gautheret**	Successfully cultured cells of carrot on synthetic media and reported that growth regulators and vitamins, if added to media enhance the growth of

			forming callus.
7.	**1941**	**Van Overbeck**	Demonstrated for the first time the stimulatory effect of coconut milk on embryo development and callus formation in Datura.
8.	**1946**	**Ball**	Raised whole plants of *Lupinus*by shoot tip culture.
9.	**1954**	**Muir**	Breaks callus tissues into single cells.
10.	**1955**	**Skoog & Miller**	Discovered kinetin as cell division hormone.
11.	**1957**	**Skoog & Miller**	Proposed *Auxin – Cytokinin Hypothesis* i.e., by changing the relative concentrations of the two substances (Auxins and Cytokinins) in the medium could regulate the organ differentiation.
12.	**1959**	**Reinert& Steward**	Demonstrated regeneration of embryos from callus clumps and cell suspensions of *Daucus carota.*
13.	**1962**	**Murashige& Skoog**	Develops a nutrient medium called Murashige& Skoog Medium (MS media).
14.	**1964**	**Guha&Maheshwari**	Produced first haploid embryo from the pollen grains of *Daturainnoxia.*
15.	**1967**	**Bourgin&Nitsch**	Produced androgenic haploid plants of *Nicotiana.*
16.	**1970**	**Power *et al.***	Successfully achieved the regeneration of plants from protoplast fusion.
17.	**1972**	**Carlson *et al.***	Produced the first somatic hybrid between *Nicotianagluca* and *N. langschorffii* by fusing their protoplasts.
18.	**1974**	**Reinhard**	Introduced biotransformation in plant tissue cultures.
19.	**1977**	**Chilton *et al.***	Successfully integrated Ti plasmid DNA from *Agrobacterium tumefaciens*in plants.
20.	**1978**	**Melchers *et al.***	Did somatic hybridization of tomato and potato to form Pomato.
21.	**1981**	**Larkin &Scowcroft**	Introduced the term *Somaclonal variation*.
22.	**1983**	**Pelletier *et al.***	Conducted inter-generic cytoplasmic hybridization in Radish & Grape.
23.	**1984**	**Horsh*et al.***	Developed transgenic tobacco by transformation with *Agrobacterium.*
24.	**1987**	**Klien*et al.***	Developed biolistic gene transfer method for plant transformation.

Razdan, 2019

Table.2 Basic tissue culture laboratory equipments

Media preparation room	**Inoculation room**	**Culture/Growth room**	**Acclimatization room**
Water purification system	Laminar Air Flow	Air conditioner	Polyhouse
Precision balances	Microscope	Lights with timer	Glasshouse
Autoclave	Balance	Humidifier	Shading nets
Hot plate/ stirrer	UV light	Dehumidifiers	Humidifier
Water filtration unit pH meter		**Temperature controller**	**Dehumidifiers**

Razdan, 2019

Table.3 Stages involved in plant tissue culture

Stage	Methodology involved
Stage 0	Selection of mother plant and its maintenance
Stage I	Preparation of nutrient medium
Stage II	Sterilization of nutrient media and other auto-clavable items
Stage III	Sterilization of explant
Stage IV	Inoculation
Stage V	Development of plant in growth room
Stage VI	Sub-culturing of shoots
Stage VII	Hardening of micro plants

Razdan, 2019

Table.4 Composition of Macronutrients in Different Tissue Culture Media

Macronutrients(mgl^{1-})	MS	G_5	W	LM	VW	KM	M	NN
$Ca_3(PO4)_2$					200.0			
NH_4NO_3	1650.0			400.0				720.0
KNO_3	1900.0	2500.0	80.0		525.0	180.0	180.0	950.0
$CaCl_2.2H_2O$	440.0	150.0		96.0				166.0
$MgSO_4.7H_2O$	370.0	250.0	720.0	370.0	250.0	250.0	250.0	185.0
KH_2PO_4	170.0			170.0	250.0	150.0	150.0	68.0
$(NH4)_2SO4$		134.0			500.0	100.0	100.0	
$NaH_2PO_4.H_2O$		150.0	16.5					
$CaNO_3.4H_2O$			300.0	556.0		200.0	200.0	
Na_2SO_4			200.0					
KCl			65.0					
K_2SO_4				990.0				
$Ca_3(PO4)_2$					200.0			

*MS = Murashige and Skoog Medium;G5= Gamborg B_5 Medium;W = White's Medium; LM= Linsmaier and Skoog Medium;VW = Vacin and Went Medium;KM = Kao and Michayluck Medium;M= Medium 199; NN = Nitsch and Nitsch Medium. Razdan, 2019

Table.5 Composition of Micronutrients in Different Tissue Culture Media

Micronutrients (mgl^{1-})	MS	G_5	W	LM	VW	KM	M	NN
KI	0.83	0.75	0.75			80.0	0.03	
H_3BO_3	6.20	3.0	1.5	6.2		6.2	0.6	10.0
$MnSO_4.4H_2O$	22.30		7.0		0.75	0.075		25.0
$MnSO_4.H_2O$		10.0		29.43				
$ZnSO_4.7H_2O$	8.6	2.0	2.6	8.6			0.05	10.0
$Na_2MoO_4.2H_2O$	0.25	0.25		0.25		0.25	0.05	0.25
$CuSO_4.5H_2O$	0.025	0.025		0.25		0.025		0.025
$CoCl_2.6H_2O$	0.025	0.025				0.025		
$Co(NO_3)_2.6H_2O$							0.05	
Na_2EDTA	37.3	37.3		37.3		74.6	37.3	37.3
$FeSO_4.7H_2O$	27.8	27.8		27.8		25.0	27.8	27.8
$MnCl_2$						3.9	0.4	
$Fe(C_4H_4O_6)_3.2H_2O$					28.0			

*MS = Murashige and Skoog Medium; G5= Gamborg B_5 Medium; W = White's Medium; LM= Linsmaier and Skoog Medium; VW = Vacin and Went Medium; KM = Kao and Michayluck Medium; M= Medium 199; NN = Nitsch and Nitsch Medium. Razdan, 2019

Table.6 Composition of Vitamins and Other Organic Supplements in Different Tissue Culture Media

Vitamins and other supplements(mgl^{1-})	MS	G_5	W	LM	VW	KM	M	NN
Inositol	**100.0**	**100.0**		**100.0**				**100.0**
Glycine	**2.0**	**2.0**	**3.0**	**2.0**				**2.0**
Thiamine HCl	**0.1**	**10.0**	**0.1**	**1.0**		**0.3**	**0.3**	**0.5**
Pyridoxine HCl	**0.5**		**0.1**	**0.5**		**0.3**	**0.3**	**0.5**
Nicotinic acid	**0.5**		**0.5**	**0.5**			**1.25**	**5.0**
Ca-D-panthothenate			**1.0**					
Cysteine HCl			**1.0**					
Riboflavin						**0.3**	**0.05**	
Biotin							**0.05**	**0.05**
Folic acid							**0.3**	**0.5**

*MS = Murashige and Skoog Medium; G5= Gamborg B_5 Medium; W = White's Medium; LM= Linsmaier and Skoog Medium; VW = Vacin and Went Medium; KM = Kao and Michayluck Medium; M= Medium 199; NN = Nitsch and Nitsch Medium.
Razdan, 2019

Table.7: Concentration and time of exposure of various sterilizing agents

Sterilizing agent	Concentrations % (w/v)	Time of exposure (min)
Sodium hypochlorite (NaOCl)	0.1 - 3	1 - 20
Calcium hypochlorite $Ca(ClO)_2$	1 - 5	5 - 30
Sodium dicholoroisocyanurate (DICA)	1 - 2	10 - 20
Mercuric (II) chloride ($HgCl_2$)	0.1 - 1	2 - 10
Silver nitrate ($AgNO_3$)	1	5 - 20
Hydrogen peroxide (H_2O_2)	10 - 30	5 - 15

Chawla, 2019

Table.8 Effect of media and growth regulators on vanilla seed germination

S.No.	Treatment	Media Composition	Days taken for Germination
1.	T_1	½ MS + BA (1ppm) + NAA (0.5ppm)	41 ± 2.55
2.	T_2	½ MS + BA (0.5ppm) + NAA (0.5ppm)	42 ± 2.18
3.	T_3	½ MS + BA (1ppm) + NAA (1ppm)	39 ± 2.04
4.	T_4	½ MS + BA (0.5ppm) + IAA (0.5ppm)	41 ± 2.17
5.	T_5	½ MS + BA (0.5ppm) + IBA (0.5ppm)	46.5 ± 3.47
6.	**T_6**	**½ MS + NAA (0.5ppm) + Kinetin (0.5ppm)**	**35.5 ± 1.97**
7.	T_7	½ MS + NAA (1ppm) + Kinetin (1ppm)	43.0 ± 4.63

Kumar *et al.*, 2014

Table.9 Effect of different levels of auxins on meristem culture of potato

PGR	NAA				IAA				IBA			
Level (mgl^{-1})	**0**	**0.1**	**0.5**	**1.0**	**0**	**0.1**	**0.5**	**1.0**	**0**	**0.1**	**0.5**	**1.0**
Plantlet height (cm)	5.0	7.9	**8.3**	7.3	5.1	6.2	6.8	7.2	5.3	6.5	7.2	7.7
No. of nodes $plantlet^{-1}$	5.5	6.6	**7.3**	6.4	4.5	5.6	6.0	7.1	5.2	6.7	6.1	7.3
No. of leaves $plantlet^{-1}$	6.6	7.7	**8.9**	8.3	4.6	5.7	7.0	7.6	5.0	7.9	7.1	7.3
Root length (cm)	1.7	2.3	3.2	3.8	2.4	3.3	3.3	**4.2**	2.3	2.7	1.8	3.0
No. of roots $plantlet^{-1}$	10.	12.0	12.6	20.1	9.2	12.0	15.0	13.6	10.0	16.3	15.8	**23.7**
No. of days to micro-tuber formation	34.0	21.3	26.0	22.0	30.0	31.0	28.0	25.3	33.0	27.0	21.3	**17.0**

Zaman *et al.*, 2018

Table.10 Response of callus to different combination of hormones (µM)

Combination of hormones(µM)			Proliferation	Percentage of response	Colour and texture
BAP	**2,4-D**	**NAA**			
8.87	-	-	Moderate	60	White friable
11.09	-	-	Moderate	67	Soft, White friable
13.31	-	-	Moderate	70	Soft, White friable
4.44	4.52	-	High	80	Soft, White friable
4.44	6.78	-	High	83	Soft, White friable
6.66	9.05	-	High	**87**	Soft, White friable
4.44	-	5.37	High	83	Pale green
4.44	-	6.71	High	84	Pale green, Hard & Off-white
6.66	-	8.06	High	**87**	Pale green, Hard & Off-white

Gayathri*et al.*, 2015

Table.11 Resistant Traits transferred to hybrid species through embryo rescue technique

Crossing species	Resistance trait (S)
Lycopersiconesculentum x L. peruvianum	Virus, fungi & nematodes
Solanum melongena x S. khasianum	Brinjal shoot and fruit borer
Solanum tuberosum x S. etuberosum	Potato leaf roll virus
Brassica napus x Raphanobrassica	Shattering resistance
Brassica oleracea x B. napus	Triazine resistance
B. Napus x Brassica oleracea	Cabbage aphid

Chawla, 2019

Table.12 Genetic traits transferred via Somatic Hybridization

CROP	CROSSING SPECIES	TRAITS
Tomato	***Lycopersiconesculentu.m x L. peruvianum***	TMV, Spotted wilt virus & Cold tolerance
	Solanum lycopersicoides x L. esculentum	CMS (*Cybrids*)
Brinjal	***Solanum melongena x S. sysimbrifolium***	Nematode resistance
Potato	***Solanum tuberosum x S. chacoense***	Late blight & Potato virus X
	S. Circalifolium x Solanum tuberosum	Frost resistance
Cabbage	***Brassica oleracea var. capitata x B. oleracea***	Cold tolerance
Watermelon	***Citulluslanatus x Cucumismelo***	Club rot resistance
Radish	***Raphanussativus x Brassica napus***	Club rot resistance
Carrot	***Hordeumvulgare x Daucus carota***	Frost & salt tolerance

Razdan, 2019

Applications

To produce novel interspecific and intergeneric crosses between plants that are difficult or impossible to hybridize conventionally.

To produce fertile diploids and polyploids from the protoplasts of sexually sterile plants.
In vitro fusion of protoplast opens a way of developing unique hybrid plants by overcoming the barriers of sexual incompatibility.
To produce transgenic plants through genetic transformation of protoplasts.

Cybrids

These are the genotypes having nucleus from one of the parents but the cytoplasms of both the parents.This process of protoplast fusion which results in the development of cybrids is known as *Cybridization.* This type of hybridization is obtained by inactivating the nucleus of one of the protoplasts.The inactivation is achieved by either the application or treatment with *Iodoacetate.*Herbicide resistance and CMS have been transferred by this method in Tobacco and Tomato.

Advantages of plant tissue culture technique

Mass multiplication of elite clones.
Beneficial when conventional propagation is difficult.
Plants can be produced or multiplied in large numbers in a shorter period of time from small vegetative parts

Micropropagation is not season dependent because the controlled conditions in the Tissue Culture Laboratory permits the year round production of tissue culture plants in "season-controlled" growth rooms, where environmental conditions are set for optimal regeneration and growth.

Plants produced through micropropagation may have increased branching and flowering, greater vigour and higher yield, mainly due to the possibility of elimination of diseases.
Plant cultures in approved media are easier to export than the soil- grown plants.

Disadvantages of plant tissue culture technique

During the course of micro propagation, several slow-growing microorganisms (e.g.*Eswinia* sp., *Bacillus* sp.) contaminate and grow in cultures that will adversely influence propagation of plants.

Micro propagation of certain plants is often associated with accumulation of growth inhibitory substances in the medium. Chemically, these substances are phenolic compounds, which are toxic, turns the medium into dark colour and can inhibit the growth of tissues (*Brewing of media*).

During the course of repeated *in vitro* shoot multiplication, the cultures exhibit water soaked or almost translucent leaves. Such shoots do not grow and even may die.

In conclusion the total, it has been estimated that in India more than Three hundred and fifty million tissue cultured plants are being produced annually through tissue culture method. In India, tissue culture is rapidly becoming a commercial method for propagating new and rare species, difficult-to-propagate plants, healthy, virus free and true-to-type plants.Plant production can be carried out throughout the year, irrespective of season and weather, which solves farmers' climatic plantation problems. Permits germplasm exchange and distribution throughout the world. Tissue culture activity is taking a shape of an industry as many farmers are planting tissue culture grown plantlets; agro-traders are buying and selling tissue culture grown plantlets while some are exporting either the plantlets or the produces of plants grown by tissue culture, especially varieties of potato like *K. jyoti*, *K. Giriraj*, *etc.* are enjoying high profits in Punjab. Plant cell and tissue cultures provides a way for controlled production of myriad of useful flavor compounds and secondary metabolites. E.g. Capsaicin*(Capsicum frutescens), etc.*

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