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Review Article

TISSUE CULTURE TECNIQUES AND TYPES IN RELATION TO *EMBELIA RIBES IN VITRO* PROCESS

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Tissue culture is the growth of tissues and/or cells separate from the organism. This is typically facilitated via use of a liquid, semi-solid, or solid growth medium, such as broth or agar. Tissue culture commonly refers to the culture of animal cells and tissues, while the more specific term plant tissue culture is being named for the plants. These are grown in a sterile medium containing the nutrients the cells require. The technique can yield information about the nutritional requirements of individual cells, and whole plants can often be grown from tissue samples or even from single cells. In gene banks, tissue culture methods may be used to store plants, in a non-bulky growth phase, if their seeds do not remain viable for long periods of time. It is also possible to save crop plant varieties from extinction through an attack by pathogens by isolating and culturing meristematic tissue (MERISTEM), which is rarely attacked. Crosses can also be made between otherwise incompatible plants by removing embryos before they are aborted and growing them by this method. The maintaining or growing of tissue, **organ primordial**, or the whole or part of an organ in vitro so as to preserve its architecture and function. Used loosely to refer to monolayer cell cultures.

Tissue culture technology includes plant transformation, molecular biology, genetically modified crops, important patents and events pertaining to our interest one deals with use of biotechnology in production of parthenocarpic fruits (seedless) in non-parthenocarpic species. As many as six genes have been identified and cloned which hold a great promise for inducing seedless fruit without fertilization and contribute to full development of fruits in varieties where seedless fruits are misshapen. The second article is about embryo development without fertilization.

It is most widely used technique for rapid asexual invitro prorogation which is economical in time & space affords greater output & provides diseases free varieties and produces virus free planting material using meristem culture where as invitro germplasm also assures the exchange of pest & disease free material and helps in better quarantine.

Culture

1. The propagation of microorganisms or of living tissue cells in media conductive to their growth.
2. To induce such propagation.
3. The product of such propagation.

Cell culture a growth of cells in vitro; although the cells proliferate they do not organize into tissue.

Continuous flow culture the cultivation of cells in a continuous flow of fresh medium to maintain cells growth in logarithmic phase.

Hanging-drop culture a culture in which the material to be cultivated is inoculated into a drop of fluid attached to a cover glass inverted over a hollow slide.

Plate culture one grown on a medium, usually agar or gelatin, on a Petri dish.

Pure culture a culture of a single cell species, without presence of any contaminants.

Slant culture
One made on the surface of solidified medium in a tube which has been tilted when the agar was solidifying to provide a greater surface area for growth.

Stab culture
A culture into which the organisms are introduced by thrusting a needle deep into the medium Stab culture one in which the medium is inoculated by thrusting a needle deep into its substance.

Serial dilution plate method (viable plate count method)
Pour Plate: mixed culture is diluted in tubes of liquid/agar medium
(a).loop dilution technique
(b).serial dilution technique

Roll Tube Method.: Screw capped bottle in Suspension culture**.**

Micromanipulator method: Device as Micropipette with Microscope which picks up a single microbial cell from hanging drop preparation.

Streak culture[2]
One in which the medium is inoculated by drawing an infected Platinum or Nichrome wire loop across it.

Type culture a culture of a species of microorganism usually maintained in a central reference collection of type or standard cultures.

Anaerobic culture
One carried out in the absence of air.

Explant culture[5]
A small piece of tissue maintained in culture.
Immobilized cell culture[5] [(241)]: Beads/Beats require surface coating for adhesion of sufficient number of cells and supported by physiological and biochemical conditions from growth medium eg: Digitalis lanata. Catharanthus Roseus.

Protoplast culture[5(293-294)] (Cell minus cell wall/naked plant cell)

(a) Intraspecific: Croos between same species in an individual eg; Fusarium & aspergillus species.
(b) Interspecific crosses between two species eg; p.chrysogenium X P.notatum.
(c) Anther & pollen culture Eg; (production of Haploid/Diploid plants invitro. Eg: Datura innoxia.

Embryo culture[5(302-303)]: Recovery of plants from distinct crosses useful where embryo fails to develop due to degeneration of embryonic tissues Eg Hordeum valgare/Bulbosum.

Embryo rescue: Growing immature embryo which have greater resistance to pests & pathogens & produce grains of bitter quality Eg; Cow pea, Pigeon pea.

Primary culture a cell or tissue culture started from material taken directly from an **organism,** as opposed to that from an explant from an **organism.** Subsequent passages of cells are referred to as secondary cultures.

Secondary culture
A subculture derived from a primary culture.

Suspension culture[5]
a culture in which cells multiply while suspended in a suitable liquid medium(Biopharmaceuticals) which is the most ideal medium for carrying out an exhaustive studies which regard to metabolism and also the underlying drug biosynthetic pathway (biogenesis[5(24)]) yielding specifically the secondary metabolites.

Regeneration in Tissue Culture - Regeneration refers to the development of organized structures like roots, shoots, flower buds, somatic embryos (SEs), etc. from cultured cells/tissues;

Micropropagation[5] - Regeneration of a whole plant through tissue culture is popularly called micropropagation. Within a very short time and space, a large number of plantlets can be produced from callus tissue. Again, it is possible to make a large number of callus pieces from the original stock culture during sub culturing. Then it is possible to produce hundreds of plantlets that develop on each of these callus pieces. Therefore, the most obvious advantage of micropropagation is numerical as numerous cell aggregates can be

produced relatively rapidly, generally growing faster than callus tissues. The number of plantlets produced depends on the number of shoot primordia that can be induced to form within these cell aggregates. Alternatively, if the cell suspension culture happens to be embryogenic, this asexual propagation potential depends on the rate at which embryoids are formed by cell aggregates and the rate at which new embryogenic aggregates are formed in culture.

Again, the shoot tip (Apical Meristem) and nodes (terminal) of the regenerated plantlets derived from callus culture and cell suspension culture can be multiplied further following the organ culture method. As a result, large numbers of a plant species or variety can be propagated all year around. The plant breeder or grower is no longer restricted by season in the production of large numbers of plants.

Organogenesis[5] (Development of organs) is also used to describe these events. Root regeneration occurs quite frequently, but it is useful only in conjunction with shoots and embryo germination. Only shoot and SE regenerations give rise to complete plants, which is essential for applications of tissue culture technology in agriculture and horticulture. Often differentiation is used as synonym for regeneration. But differentiation describes the development of different cell types, e.g., vascular elements, etc., as well (cyto-differentiation). Therefore, it is more appropriate to use phrases like shoot differentiation, SE differentiation, etc. than using the term differentiation alone. Regeneration may occur either directly from the explant or may follow an intervening callus phase. The capability to induce the formation of adventitious roots & shoots invitro is the utmost importance in plant tissue culture methodology, Studies involving the transformation of protoplasts would be of little value unless the genetically altered plant material could be regenerated into a plantlet, Plant regeneration by tissue culture techniques can be achieved by zygotic embryo culture, somatic embryogenesis or organogenesis, The latter approach is employed in micropropogation from bud & shoot material & in organ production from callus & suspension culture, Embryos are not classified as organs because these structures have an independent existence I.e. embryos do not have vascular connections with the parent plant body, Organogenesis is regulated by a balance between cytokinin ratio induced root formation, where as a low ratio of the same compounds favored shoot production.

Somatic embryogenesis[5]: is an asexual form of plant propagations in nature that mimics many of the events of sexual reproduction also this process may be reproduced artificially by the manipulation of tissues & cells in vitro some of the most important factors for successful plant regeneration are the culture medium & environmental incubation conditions.
Invitro somatic embryogenesis is an important prerequisite for the use of many biotechnological tools for genetic improvement as well as for mass propagation.

(a) Zygotic embryo: formed by the zygote eg; Atropa belladonna, Nicotiana tobacum.
(b) Non zygotic embryo(from cells)
1. Somatic embryo (embryoids/seed embryos): formed from somatic cells in vitro
2. Parthenogenesis embryo: formes by unfertilized egg.
3. Androgenic embryo[5]: formed by pollen grains/anther (production of haploid/diploid plants invitro) eg Datura innoxia

Growth Regulators and Other Factors for Somatic Embryogenesis
In most species an auxin (generally 2,4-D, at 0.5-5 mg/l) is essential for somatic embryogenesis. The auxin causes dedifferentiation of a proportion of cells of the explant, which begin to divide. Eg In carrot, these small, compact cells divide asymmetrically, and their daughter cells stick together to produce cell masses called pro-embryogenic masses or embryogenic clumps (ECs). In the presence of auxin, the ECs grow and break up into smaller cell masses, which again produce ECs. But when the auxin is either removed or reduced (0.01-0.1 mg/l) and cell density is lowered, each EC gives rise to few to several SEs; each SE is believed to develop from a single superficial cell.
The ability to regenerate SEs, i.e., totipotency, is acquired by cells during dedifferentiation in response to high auxin treatment but the mechanism is not well known. Some glycoprotein's produced by totipotent cell masses are secreted into the medium; when these proteins are added into the culture medium they speed up the process of

acquisition of totipotency. A class of proteins, called arabinogalactan proteins, induces SE regeneration in undifferentiated Eg In carrot cells, indicating their role in the process.

Auxins promote hypermethylation of DNA which may have a role in totipotency acquisition. In many species like Eg***Embelia ribes,*** In carrot, coffee; alfalfa etc., somatic embryogenesis is a two step process:

(I) SE induction on high auxin (up to 40-60 mg/l,Eg 2, 4 D)

(ii) SE development on a low auxin or OR-free medium.

In the SE induction phase, explant cells dedifferentiate, became totipotent and, in many species, form embryogenic clumps (ECs). Cells can be maintained in embryogenic stage on the induction medium for prolonged periods (over 10 years in carrot). When ECs are transferred from induction medium to an appropriate medium, SE differentiation proceeds from globular, heart-shaped, torpedo to cotyledonary stages; this is called SE development phase. Clearly in species like Eg In carrot, etc., OR requirements for the two phases are drastically different.

In most cases, SEs begin to germinate immediately after the cotyeledonary stage; this is called SE conversion. But often the plantlets so obtained are rather weak. It is, therefore, desirable to subject SEs to a maturation phase, following their development; in this phase the SEs usually do not grow but undergo biochemical changes to become more sturdy and hardy.SE maturation is achieved by culturing than on a high sucrose (up to 6% or even 40%) medium or in presence of a suitable concentration (0.2-0.4 mg/l) of ABA, or by subjecting them to partial desiccation. In most species, SE maturation improves their conversion, often by several-fold. In some species, e.g., embelia ribes etc., SE induction and development may take place on the same high auxin medium, although the frequency of mature embryos is rather low. In some species, SEs are produced in response to a cytokinin, e.g., BAP induces SEs in hypocotyls of young zygotic embryos of Eg In Trifolium sp., pea, etc. But SEs are produced on immature cotyledons of these explants when 2,4-D is used in the medium. It seems that cytokinins are effective in SE regeneration from embryogenic cells of young zygotic embryos, while auxins are effective on differentiated cells of both embryos and somatic tissues. Many workers have used combinations of auxins and cytokinins for SE regeneration in different species, but the role of cytokinin in these systems is not known.

Other Factors Certain other factors are reported to affect SE regeneration. For example, high K+ levels and low dissolved O2 levels promote SE regeneration in some species. In some other species, e.g. Eg***Embelia ribes,*** Citrus medica, some volatile compounds like ethanol inhibit SE regeneration. In Eg***Embelia ribes,*** soybean, low sucrose concentrations (5 and 10 g/l) promote SE regeneration as compared to high concentrations (20 and 30 g/l). In alfalfa, use of maltose as carbon source improves both SE induction and maturation (including germination) as compared to those on sucrose.

Tissue culture Types for *Embelia ribes*

Ingridients	**Authour**	**Raghu[6] & geetha 2006**	**Annapurna[11]Rathore. 2009**	**Shankarmurthy[7],Krishna. 2006**	**Krishna[8], Shankarmurthy-2004**	**Shankarmurthy, rahim[9]. 2004**	**Shankarmurthy, Nagaraja[10] 2004**
	Method/Type	Direct shoot organogenesis	Direct adventitious shoot induction	Micropropagation	Somatic embryogenesis	Rapid adventitious organogenesis	High frequency plant regeneration
	specimen	leaf explants	Seeds/explants	Inflorescence (immature ovaries)	leaf callus(primary)	Leaf segment	leaf callus
Surface Sterilization agent	Surface sterilization	-	Mercuric chloride 0.075%for 3.5min	-	-	-	-
	(MS) medium	(MS)	(MS)	(MS)	(MS)	(MS)	(MS)
Gelling agent	Agar	-	0.6%	-	-	-	-
Organic salts/acids	Ascorbic acid	-	283.85 1m	-	-	-	-
	Citric acid	-	118.96 1m	-	-	-	-
	Cysteine	-	142.33 1m	-	-	-	-
Growth regulator/Cytokinins	2,4-D	-	-	-	2 micro M		2.0 mg/liter)

	FAP(furfuryl amino purine)	-	-	-	-	3 mg/liter	-
	GA_3	-	5.78 1m	-	-	-	-
	Glutamine	-	684.22 1m	-	-	-	-
	IBA	-	-	3.5 mg/liter	-	-	-
Inorganic salts	Kn*	-	-	0.5 mg/liter +0.3 mg/liter	a- 2000-4T mg/lt, b-3800 mg/lt, c-1900 mg/lt.	-	-
	Kinetin	-	-	-	0.5 micro M	-	0.5 micro M
	NAA*	-	-	0.4 mg/liter +0.4 mg/liter	0.1-0.5 microM +0.3 micro M	0.4mg/litre	0.2 -0.6 mg/litre
	Thidiazuron	0.272 micro M	1.13 1m	-	-	-	-
	Indole-3-butyric acid (IBA)	4.90 micro M	0.49 1m	-	-	-	-
	Benzyl amino purine [benzyl adenine] (BAP)	-	-	-	-	-	-
Organic additives	Coconut milk	-	-	-	10%	-	-
Carbon source	Sucrose	3% (w/v)	-	-	-	-	-
	Period	2-3weeks	4+*(3x2)+4+6weeks	1+1 weeks	1+2+6 weeks	1 weeks	1 weeks
	Soilrite mix	Sufficient to pots	Sufficient to pots	Sufficient to pots	Sufficient to pots	Sufficient to pots	Sufficient to pots

NOTE: **In Somatic embryogenesis** Kn* & **Micropropagation** NAA*added in stages a,b,c. & Period +* =Subsequent stages.
Potting= Soilrite mix (sand: soil: compost: charcoal: small brick pieces) (30:10:50:5:5) page no=798*[12].
Abbreviation=(T=thousand)

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