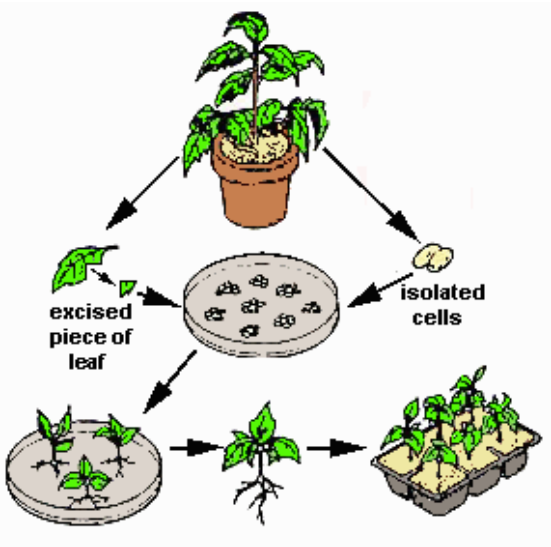
Tissue culture and its history

Plant tissue culture broadly refers to the cultivation *in vitro* of all plant parts, whether a single cell, a tissue or organ under aseptic conditions. Recent progress in the field of plant tissue culture made this area as one of the most dynamic and promising experimental biology.



Overview of tissue culture process

This new technique has enabled us to increase the knowledge in the following field of studies.

- Totipotency, nutrition, metabolism, division, differentiation and preservation of plant cells.
- Morphogenesis and plant regeneration from individual cells or tissues through the process namely organogenesis or somatic embryogenesis.
- Variations generated through *in vitro* culture.
- Evolution of haploids through anther and pollen culture including ovule culture.
- Wide hybridization programmes through ovule, ovary and embryo cultures to overcome both pre zygotic and post zygotic sterility mechanisms
- Micropropagation of plant materials
- *In vitro* selection of mutants tolerant to biotic and abiotic stresses.
- *In vitro* culture and secondary metabolite biosynthesis.
- Plant genetic engineering through *in vitro* culture methods and DNA transfer technique.

Thus plant cell, tissue and organ culture permeates plant biotechnology and cements together its various aspects like Physiology, Biochemistry, Genetics and Cell Biology.

Like other subjects, plant cell and tissue culture has its own origin and development. The chronology of major events in this field is presented for the benefit of the new entrants into this field.

1756- Duharmel du Monceau H. L discovered callus formation from the decorticated elm tree. This very old work was foreword for the discovery of plant tissue culture.

1839- Schwann, T.H expressed the view that each living cell of a multicellular organism would be capable of developing independently if provided with proper external conditions.

1853- Trecul. A performed experiment on callus formation by decorticated trees such as *Robinia, Pawlonia* and *Ulmus*.

1878- Vochting. H obtained very luxuriant callus from *Brassica rapa* and proposed the polarity in development of buds from the upper portion and roots or callus and from the lower portion of a stem piece.

1885- Roux, W.Z made the first experimental step in tissue culture when he removed a fragment of the neural plate of a chick embryo and cultivated in warm salt solution.

1893- Rechinger, C described callus formation on isolated stem fragments and root slices.

1901- Morgan, T.H coined the term totipotency to describe the capability of a cell to form an individual plant.

1902- Haberlandt, G – Father of plant tissue culture published a paper on "Experiments on the culture of isolated plant cells: In that he says "I should like to point out the fact that, in my cultures, despite the conspicuous growth of the cells which frequently occurred, cell division was never observed. It will be the problem of future culture experiments to discover the condition under which isolated cells undergo division". He clearly set forth the purposes and potentialities of cell culture after having attempted and failed in the culture of isolated plant cells. The reasons for his failure may be (i) use of three monocotyledonous genera for much of his work, (ii) culture of mature differentiated green mesophyll and pallisade tissues, (iii) contamination during culture growth.

Haberlandt, G – Father of plant tissue culture

1907- Harrison, R.G devised methods of cultivating fragments of living nerve.

1910- Carrel, A was the first scientist who demonstrated the culture of living cells outside the body of an organism.

1922- Kotte, W succeeded in cultivating small root tips of pea and maize in various nutrients. The roots developed well and their growth was maintained for long periods but no subculture was attempted.

1922- Robbins, W.J started cultivation of excised root tips and stem tips of maize under sterile conditions; however, the cultures did not survive independently.

1925- Liabach, F demonstrated the most important application of the embryo culture by crossing *Linum perenae* with *L. austriacum* to get hybrid plants from shriveled seeds.

1934- Gautheret, R.J made preliminary attempts with liquid medium for cultivating plant tissues but failed completely. Later he cultured the explants on medium solidified with agar, and got healthy calli from the explants.

1934- White, P.R obtained indefinite survival of cultured tomato roots on sub culturing in liquid medium.

1939- White, P.R., Gautheret, R.J. and Nobecourt, P simultaneously announced the possibility of cultivating plant tissues for unlimited periods.

194l- Van Overbeek, J., Conklin, **M.E. and Blackeslee, A.F** established importance of coconut milk for growth and development of very young *Datura* embryos.

1942- White, P.R. and Braun, A.C initiated studies on crown gall and tumour formation in plants.

1944- Skoog, F started his work on organogenesis in tobacco callus.

1946- Ball, E.A showed development of plantlets from sterile cultures of stem tips in *Tropaeolum* and *Lupinus.* He is considered as father of micropropagation.

1947- La Rue C.D initiated endosperm cultures of *Zea mays* and obtained subcultures.

1948- Skoog, F. and Tsui, C studied the chemical control of growth and bud formation in tobacco stem segments and callus cultured *in vitro* and suggested that callus induction and shoot initiation can be regulated by making manipulations in the culture medium.

1949- Street, H.E. and Dormer, K.J initiated work on root culture and its nutrient requirements.

1951- Morel, G. and Wetmore, R.H got successful culture from monocots, once considered as recalcitrants to the cultural conditions.

1952- Steward, F.C., Caplin, S.M. and Miller, F. K discovered the synergistic action of 2, 4-D and coconut milk in a culture of potato tissue.

1952- Morel, G. and Martin, C were the first to demonstrate that virus free plants can be recovered from infected plants through shoot meristem culture.

1953- Muir, W.H found out the cultural conditions favouring the isolation and growth of single cells from higher plants *in vitro* and established nurse culture technique.

1954- Muir, W.H. Hildebrandt A.C. and Riker. A.J obtained the first suspension cultures by transferring callus fragments to agitated liquid medium

1955- Miller, C.O., Skoog, F., Von Saltza, M. and Strong, F.M identified a cell division factor *viz*., 6-furfualamino purine commonly called kinetin.

1957- Skoog, F. and Miller, C.O advanced the hypothesis that shoot and root initiation in cultured callus can be regulated by particular ratios of auxins and cytokinin.

1957- Skoog, F. and Miller, C.O discovered and introduced the idea of synergistic effects of auxins and cytokinins in promoting cell division in tobacco.

1958- Steward, F.C., Mapes, M.O. and Mears, K observed the phenomenon of somatic embryogenesis in suspension culture of carrot. They also reported that cells in suspension derived from explanted roots of cultivated carrots were capable of forming unorganised cell clusters, which in turn could yield first roots, then shoots and ultimately whole plants.

1959- Reinert, J observed the somatic embryo formation from callus cultures of carrot grown on an agarified medium.

1959- Melchers, G. and Bergmann L were first to culture haploid tissues other than pollen.

1960- Cocking, E.C discovered the technique of isolation and culture of protoplasts after digesting the cell walls enzymatically and demonstrated new cell wall regeneration on protoplasts from tomato fruit locule tissue.

1960- Bergmann L was first to obtain callus by plating cells from suspension cultures on to solid medium. This plating involved mixing cells with warm sugar medium just prior to gelation in petridish (Bergmann plating technique)

1960- Morel, G discovered a technique to produce virus free progenies by meristem culture in *Cymbidium.*

1964- Guha, S. and Maheshwari, S.C cultured mature anthers of *Datura innoxia* to study the physiology of meiosis and accidentally noticed the development of embryoids from the anthers plated on basal medium supplemented with kinetin and coconut milk.

1965- Vasil, V. and Hildebrandt, A.C described rearing of a mature tobacco plant from a single cell grown initially in microculture.

1966- Torrey, J.G advanced the hypothesis that organogenesis in callus is initiated with the formation of clusters of meristematic cells called meristemoids.

1966- Stroun, M. Anker, P., Charles, P. and Le Doux L made DNA transfer in tomato under *in vitro* conditions.

1970- Kasha, K. J and Kao, K.N produced haploid plants of *Hordeum vulgare* by *in vitro* culturing of embryos obtained by cross *Hordeum vulgare* with *Hordeum bulbosum* in which elimination of *bulbosum* chromosome occurred.

1971- Takebe, I., Labib, G. and Melchers, G regenerated whole plants from isolated mesophyll protoplasts of tobacco.

1971- Bendich, A.J. and Filner, P used the cells and tissues in culture for transformation studies.

1972- Withers, L. and Cocking, E.C laid foundation for the protoplast fusion technique.

1973- Potrykus, I made the first attempt on chloroplast and nucleus transfer from *Petunia hybrida* into albino protoplasts of the same species.

1974- Melchers, G. and Labib, G proposed hybrids resembling the sexual hybrids by fusing protoplasts of two varieties of *Nicotiana tabacum*

1974- Murashige, T developed the concept of developmental stages in cultures *in vitro*: Stage I: Explant establishment; Stage II: Multiplication of propagule and Stage III: Rooting and hardening for planting into soil.

1975- Morel, G established cold storage of regenerated plants for a year.

1976- Mullin, R.H. and Schlegal, D.E successfully employed cold storage to maintain *in vitro* virus free plantlets of strawberry.

1976- Seibert, M established shoot initiation from carnation shoot apices frozen to -196xC.

1978- Zelcer, A., Aviv, D. and Galun E developed a protoplast fusion procedure called Donor - Recipient protoplast fusion to favour organelle transfer among plants.

1979- Polacco, J.C. Sparks, R.B. and Havir, E.A described the cloning of soyabean urease structural gene by the vector mediated transfer system.

1980- Gleba Y. Y. and Hoffmann F synthesized a new plant "*Arabidobrassica* by fusing the protoplasts *Arabidopsis* and *Brassica*.

1981- Larkin, P.J. and Scowcroft, W.R developed the concept of somaclonal variation: A noval source of variability from cell cultures for plant improvement.

1981- Patnaik, G., Wilson, D. and Cocking, E.C regenerated a whole plant from a single free cultured tobacco protoplast.

1982- Krens, F.A., Molondijk, L. Williams G. J. and Schilperoort, R.A developed poly ethylene glycol method for the direct delivery of DNA into protoplasts.

1983- Zambryski, P. Joos, H., Genetello, C., Leemans, J. Van Montagu M. and Schell Constructed Ti plasmid vector for the introduction of DNA into plant cells without alteration of their normal regeneration capacity.

l984- Watts , J. W. and King, J. M developed a simple method for large scale electrofusion of protoplasts.

l984- Brisson, N. Paszkowski, J. Penswick, J. R. Gronenborn, B. Potrykus, I. and Hohn, T Achieved transformation in which part of the cauliflower mosaic virus genome was replaced by selectable marker.

1985- Gheysen, G. Dahese, P., Van Montaque, M. and Schell, J developed very efficient gene transfer system using natural gene transfer mechanism of *Agrobacterium tumifaciens.*

1985- Cocking E. C exposed plasma membrane in the tips of root hairs of wide range of crop plants. The procedure enabled whole seedlings to have the plasma membrane at the tips of their root hairs exposed to foreign DNA and other microorganisms.

1985- Tabata, M. and Fujita, Y developed the technique of elucidation of the physical and chemical factors controlling the biosynthesis of the red napthoquinone pigments by *Lythospermum erythrorhizon*.

1986- Crossway, A. Oakes, J.V., Irvine , J.M., Ward B. Knauf, V.C. and Shewmaker, C.K developed a direct way of transferring cloned genes into protoplasts by microinjection of DNA directly into the nucleus of tobacco mesophyll protoplasts.

1986-Hamill, J. D. Parr, A. J., Robins, R. J. and Rhodes, M.J.C established hairy root cultures of *Beta vulgaris* and *Nicotinna rustica* following infection with *Agrobacterium rhizogenes* and the transformed cultures synthesized their characteristic secondary products at levels comparable with those of *in vitro* roots from the same variety.

1986- Abdullah, R., Cocking, E.C., and Thompson, J.A demonstrated that normal green rice plants can be regenerated efficiently and reproducibly from rice protoplasts *via* Somatic embryogenesis.

1986- Pirrie, A. and Power, J.B produced fertile, interspecific gametosomatic triploid hybrids of tobacco by fusing protoplasts of leaf (2n) and pollen tetrad (n).

1986- Kinsara A., Patnaik, S.N., Cocking, E.C. and Power, J.B produced somatic hybrids between *Lycopersicon esculentum* and *L. peruvianum*.

1987- Terada, R., Kyozuka, J., Nishibayashi, S., and Shimamoto, K regenerated plantlets form somatic hybrid cells of *Oryza sativa*, and *Echinochloa oryzicola*.

1987- Ethlenfelt, N.K. and Helgeson, J.P produced tetroploid and hexaploid somatic hybrids from protoplast fusions between *Solanum bravidens* (2x, non tuber bearing species) and 2x and 4x *S. tuberosum*.

1987- Neuhaus, G., Spangenberg, G. Mittelsten Sheid, O and Schweiger, H.G effected gene transfer by microinjecting the DNA into the cells of microspore derived proembryos.

1987- De la Pe$a, A., Lornz, H., Schell, J developed transgenic rye plants obtained by injecting DNA into young floral tillers.

1988- Nomoru, K. and Komamine, A used single cells of carrot from a cell suspension instead of protoplasts, for microinjection and the microinjected carrot cells could divide and differentiated to embryos at a frequency of about 50 percent.

1988- Rhodes, C.A., Pierce, D.A., Mettler, I. J.. Mascarenhas, D. and Detmer J.J produced transgenic maize plant by electroporation.

1988- Toriyama, K., Arimoto, Y. Uchimiya, H, and Hinata K produced transgenic rice plant by electroporation.

1989- Shimamoto, K., Terada, R., Izawa, T. and Fujimoto, H produced fertile transgenic rice plants regenerated from transformed protoplasts.

1989- Prioli, L. M. and Sondahl, M. R recovered fertile plants from protoplasts of maize.

1990- Milanova, V. and Zagorska, N. A succeeded in overcoming hybrid incompatibility between *Nicotiana africana* and *N. tabacum* and produced cytoplasmic male sterile plants by embryo culture.

1990- Iida, A., Seki, M. Kamada, M. YHamada Y. and Morikawa delivered genes into cultured plant cells by DNA-coated gold particles accelerated by a pneumatic particle gun.

1991- Kyozuka, J. Fujimoto, H., Izawa, T. and Shimamoto- K succeeded in getting tissue specific expression of maize alcohol dehydrogenase l gene in transgenie rice plants and their progenies.

1991- Spangenberg, G., Fredyl, E., Osusky, M. Nagel, J. and Potrykus, I developed a method for the predictable transfer of partial genomes predictable transfer of partial genomes by using sub protoplasts (cytoplasts and karyoplasts).

1991- Sautter, C., Waldner, H. Neuhaus, G., Galli, A. Neuhaus, G. and Potrykus developed a novel method for the acceleration of micro projectiles. The method is called as micro targeting.

The history of plant tissue culture had its real beginning in 1934 when Gautheret tried to cultivate isolated cells and root tips on organised medium. The momentum from this pioneering work, a new turn in this ongoing research occurred, because of World War II. After the Second World War, American plant pathologists became interested in plant tissue culture.

As **Steward (1970)** pointed out, the plant tissue culture technique is another "**Silent Revolution in Agriculture**" having very good potentials to supplement conventional breeding approaches. Its potentials and prospects are discussed in subsequent chapters. The techniques' theoretical aspects and their applicabilities are simplified and presented.

Genetic engineering

Manipulation of genes is called genetic engineering or recombinant DNA technology. It removes gene(s) from one organism and either

- Transfers them to another
- Puts them back in the original with a different combination

Current interest in genetic engineering centres on its various applications, such as:

- Isolation of a particular gene, part of a gene, or region of a genome
- Production of particular RNA and protein molecules in quantities formerly thought to be unobtainable
- Improvement in the production of biochemicals (such as enzymes and drugs) and commercially important organic chemicals
- Production of varieties of plants having particular desirable characteristics (for example, requiring less fertilizer or being resistant to disease)
- Correction of genetic defects in higher organisms
- Creation of organisms with economically important features (for example, plants capable of maturing faster or having greater yield).

The basic requirements for successful genetic engineering are

- Restriction enzymes
- Cloning vehicles (vectors) to carry the genes of interest
- Detection and selection of cloned genes.

Various gene transfer techniques used in genetic engineering includes

- ***Agrobacterium* mediated gene transfer**: Desired trait is isolated from DNA of original organism, inserted into *Agrobacterium*, target plant is infected. Cells that accept the DNA are grown into plants with the new trait.
- **Gene gun**: DNA that codes for the desired trait is coated onto tiny particles of tungsten and fired into a group of plant cells. Cells that accept the DNA are grown into plants with the desired trait.

A model genetic engineering of a plant comprises the following general steps:

- Selection of a plant gene whose introduction in other plants would be of positive agricultural value;
- Identification and isolation of such genes;
- Transference of isolated genes to the plant cell;
- Regeneration of complete plants from transferred cells or tissues.

Some of the goals of plant genetic engineers include production of plants that are

- Resistant to herbicide, insect, fungal and viral pathogens
- Improved protein quality and amino acid composition
- Improved photosynthetic efficiency,
- Improved post harvest handling.

This technology could provide an additional tool for the plant breeder who is trying to improve crops by traditional methods. In addition, plants can be viewed as a genetic resource, genes being cloned into, and expressed in bacteria. These bacteria may then be used to produce desirable plant products on an industrial scale using fermenter. The first transgenic plants expressing engineered foreign genes were recovered in 1984.

Dramatic progress has been made in the last few years in the development of a gene transfer system for higher plants. About 20 crops can be genetically engineered at present. Rapid progress is being made in the genetic manipulation of many species and almost every month another successful plant transformation is reported.

Questions

1. Callus formation in the decorticated elm tree was discovered by ……..

a) Duharmel du Monceau b) Morgan, T.H

c) Haberlandt d) Rechinger

2. Who's work was foreword for the discovery of plant tissue culture?.

a) Duharmel du Monceau b) Morgan, T.H

c) Haberlandt d) Rechinger

3. Name the scientist who expressed the view that each living cell of a multicellular organism would be capable of developing independently if provided with proper external conditions.

a) Duharmel du Monceau **b) Schwann**

c) Haberlandt d) Rechinger

4. Name the scientist who proposed the polarity in development of buds from the upper portion and roots or callus and from the lower portion of a stem piece.

a) Duharmel du Monceau b) Morgan, T.H

c) Haberlandt **d) Vochting**

5. Callus formation on isolated stem fragments and root slices is described by …….

a) Duharmel du Monceau b) Morgan, T.H

c) Haberlandt **d) Rechinger**

6. The term totipotency was coined by ……..

a) Duharmel du Monceau **b) Morgan, T.H**

c) Haberlandt d) Rechinger

7. The term totipotency means ……..

a) the capability of a cell to form an individual plant **b)** the capability of a cell to form an individual cell

c) both a & b d) None of the above

8. Father of plant tissue culture is ……..

a) Duharmel du Monceau
b) Morgan, T.H
c) Haberlandt
d) Rechinger

9. The embryo culture was first demonstrated by ……..

a) Robbins
b) Harrison
c) Liabach
d) Kotte

10. Father of micropropagation is ……..

a) Ball
b) Harrison
c) Robbins
d) Kotte

11. The work on organogenesis in tobacco callus is first started by……..

a) Ball
b) Skoog
c) Robbins
d) White

12. ……. initiated studies on crown gall and tumour formation in plants

a) White
b) Braun
c) Both a & b
d) None of the above

13. Who demonstrated that virus free plants can be recovered from infected plants through shoot meristem culture

a) Morel
b) Martin
c) Both a & b
d) None of the above

14. The nurse culture technique was established by …….

a) White
b) Braun
c) Muir
d) None of the above

15. The first suspension culture by transferring callus fragments to agitated liquid medium was obtained by …….

a) Hildebrandt
b) Riker
c) Muir
d) All the above

16. Kinetin was identified by …….

a) Miller
b) Skoog
c) Von Saltza and Strong
d) All the above

17. The phenomenon of somatic embryogenesis in suspension culture of carrot was observed by …….

a) Steward
b) Mapes
c) Mears
d) All the above

18. The first suspension culture by transferring callus fragments to agitated liquid medium was obtained by ……..

a) Hildebrandt
b) Riker
c) Muir
d) All the above

19. The somatic embryo formation from callus cultures of carrot grown on an agarified medium was observed by ……..

a) Hildebrandt
b) Reinert
c) Muir
d) All the above

20. the technique of isolation and culture of protoplasts after digesting the cell walls enzymatically was discovered by …….

a) Hildebrandt
b) Cocking
c) Muir
d) All the above

21. The plating technique was invented by …….

a) Bergmann
b) Riker
c) Muir
d) All the above

22. Meristem culture was discovered by …….

a) Bergmann
b) Riker
c) Morel
d) All the above

23. Anther culture was discovered by …….

a) Guha
b) Maheshwari
c) Both a & b
d) None of the above

24. The concept of developmental stages in cultures *in vitro* culture was developed by …….

a) Murashige
b) Skoog
c) Both a & b
d) None of the above

25. The concept of developmental stages in cultures *in vitro* culture was developed by …….

a) Murashige
b) Skoog
c) Both a & b
d) None of the above

26. The poly ethylene glycol method for the direct delivery of DNA into protoplasts was developed ……..

a) Krens
b) Molondijk
c) Williams
d) All the above

27. A simple method for large scale electrofusion of protoplasts was developed by …….

a) Watts
b) King
c) Both a & b
d) None of the above

28. The basic requirements for successful genetic engineering are ……

a) Restriction enzymes
b) Cloning vehicles (vectors)
c) Detection and selection of cloned genes
d) All the above