MARUMEGH

Kisaan E- Patrika

Available online at www.marumegh.com

ISSN: 2456-2904

ROLE OF TISSUE CULTURE IN CROP IMPROVEMENT

[1]Kana Ram Kumawat, [2]M.L. Jakhar and [2]Ravi Kumar

[1]Department of Plant Breeding and Genetics

College of Agriculture, SKRAU, Bikaner -334006

[2]Department of Plant Breeding and Genetics

Sri Karan Narendra College of Agriculture, SKNAU, Jobner -303329

Email: kanaramkumawat8@gmail.com

What Is Plant Tissue Culture?

The *in vitro* cultivation of plants, seeds, plant parts i.e. tissues, organs, embryos, cells, protoplasts etc on nutrient media under aseptic conditions is termed as plant tissue culture. In another way, tissue culture is the term used for the process of growing cells artificially in the laboratory.

History of Plant Tissue Culture

The German botanist Haberlandt (1902) first attempt to plant tissue culture and considered as father of plant tissue culture. Carrel (1910) was first scientist who demonstrated the culture of living cells outside the body of an organism. Some other scientists and their work are described in following table 1:

Table 1 History of plant tissue culture

Worker	Year	Advancement
Kotte	1922	Cultivated small root tips of pea and maize in various nutrients
Robbins	1922	Cultivated excised root tips and stem tips of maize under sterile conditions
Skoog	1944	Started his work on organogenesis in tobacco callus
Guha and Maheshwari	1964	Cultured mature anthers of *Datura innoxia* and produced haploid plants
Steward	1970	Pointed out, the plant tissue culture technique is another "Silent Revolution in Agriculture" having very good potentials to supplement conventional breeding approaches.

Needs of Plant Tissue Culture

There are three basic needs for plant tissue culture viz.:

1. Appropriate tissue or Explant having totipotency
2. A suitable growth medium containing energy sources and inorganic salts for proper growth of explant.
3. Maintaining aseptic (sterile) conditions because microorganisms grow much more quickly than plant tissues and can over run a culture

Why Do Plant Tissue Culture?

There are following reasons to adopt plant tissue culture:

- A single explant can be multiplied into several thousand plants in less than a year which allows fast commercial propagation of new cultivars.
- Taking an explant does not usually destroy the mother plant, so rare and endangered plants can be cloned safely.

- Once established, a plant tissue culture line can give a continuous supply of young plants throughout the year.
- In plants prone to viral diseases, virus free explants (new meristem tissue is usually virus free) can be cultivated to provide virus free plants.
- Plant tissues can be frozen in 'tissue banks' then regenerated through tissue culture after a long time.
- Plant cultures in approved media are easier to export than soil grown plants, as they are pathogen free and take up little space.
- Tissue culture allows fast selection for crop improvement i.e. explants are chosen from superior plants, then cloned.
- Tissue culture clones are 'true to type' as compared with seedlings, which show greater variability.

Basic Steps of Plant Tissue Culture

There are some basic steps of plant tissue culture which are representing here with a Figure:

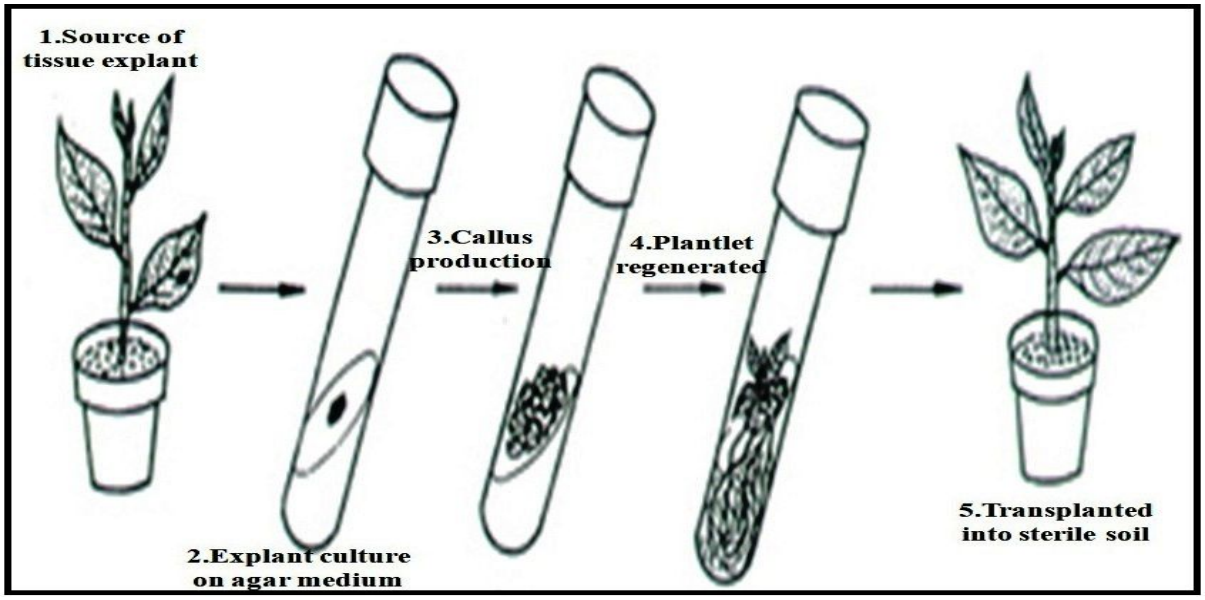

Figure 1 Basic steps of plant tissue culture

Types of Plant Tissue Culture

There are several types of plant tissue cultures viz. (Figure 2):

- Embryo culture
- Seed culture
- Organ culture
- Callus culture
- Cell culture
- Protoplast culture
- Anther or pollen culture
- Ovule culture

The last two are types of organ culture.

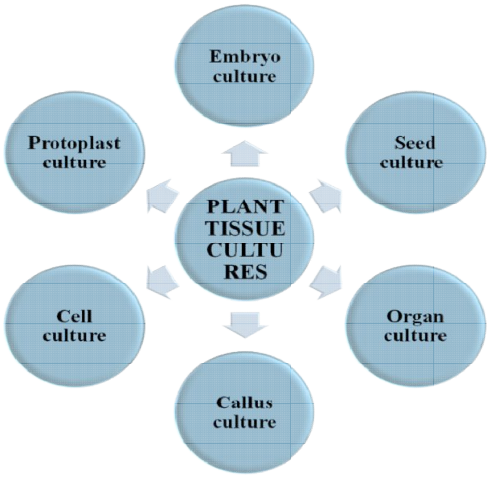

Figure 2 Types of plant tissue cultures

Embryo culture

The first attempt to grow the embryos of angiosperms was made by Hannig (1904). In this culture the mature or immature embryos are isolated from young seeds and placed on a medium containing nutrients and vitamins. Embryos are cultured at 25°C, first in dark until seedlings are about 2 cm long and root formation has started, and then in light until the seedlings can be planted in soil.

Applications of embryo culture in crop improvement

There are several following applications of embryo culture in crop improvement:

- Prevention of embryo abortion in wide crosses/ embryo rescue
- Haploid production
- Overcoming seed dormancy in seeds of vegetatively propagated crops like bananas and colocasia
- Shortening of breeding cycle
- *In vitro* clonal propagation
- Germination of seeds of obligatory parasites
- Prevention of embryo abortion in early ripening stone fruits

Callus culture

When an excised and isolated piece of tissue is cultured on a nutrient medium, an unorganized mass of cell appears, is called callus. This callus is transferred into different media to regenerate plants and this technique is called callus culture.

Protoplast culture

It is one of the most significant and recent developments in the field of plant tissue culture. The protoplasts are usually isolated from cultured cell or leaf mesophyll cell by treating them with enzyme solutions and then isolated protoplast may be used to regenerate the plants directly, or for the production of somatic hybrids through fusion.

Cell suspension culture

It is the culture of isolated cells or very small cell aggregates dispersed in liquid medium. The cell suspension is obtained by agitating pieces of callus in liquid medium on gyrating shaker.

Ovule culture

Ovule culture technique is an important technique in modern plant breeding. It is much easier to culture whole ovule than to isolate a single embryo, especially in small seeded plants like tobacco.

Applications of Plant Tissue Culture

There are following applications of plant tissue culture

- Haploid production
- Micro propagation
- Somaclonal variation
- Protoplast fusion
- Germplasm preservation
- Secondary metabolites production
- Genetic engineering

Haploid production

There are following methods of haploid production representing in Figure 3:

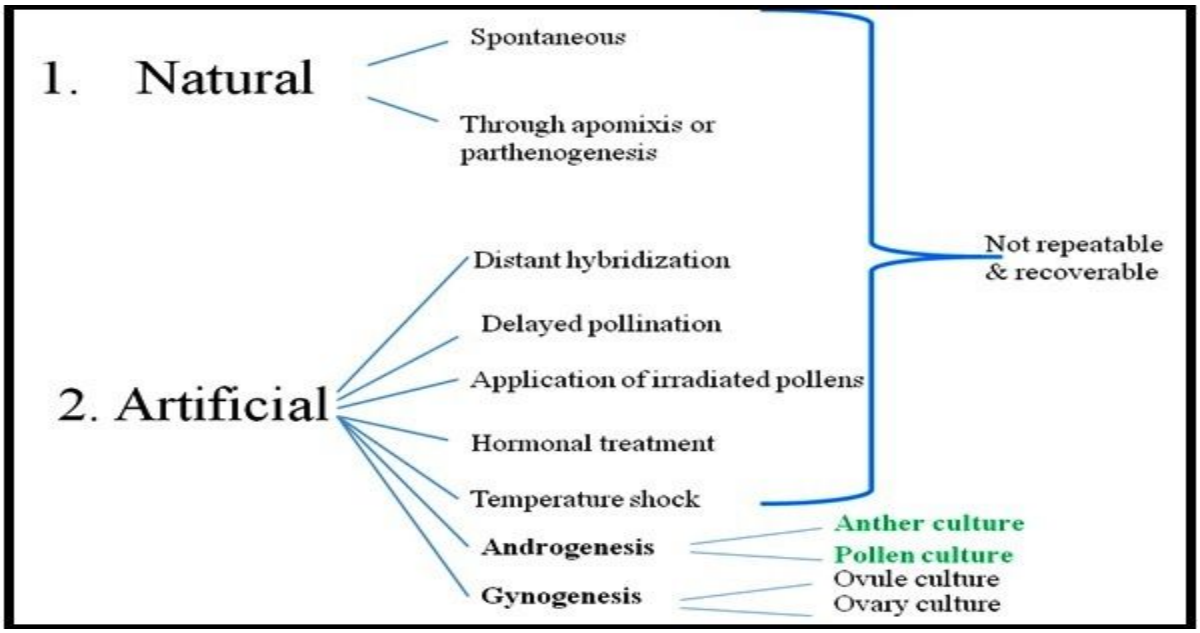

Figure 3 Methods of haploid production

Anther culture

Anther culture is a mean to produce plants with a gametic number of chromosomes by aseptic culture of anthers. The technique gives rise to haploid plants either directly or through formation of a haploid callus. The basic steps for androgenesis or anther culture are representing in Figure 4. The anther and pollen cultures are mostly used to produce haploids.

Pollen culture

Pollen culture is a mean to produce plants with a gametic number of chromosomes (haploid) by aseptic culture of pollen grains. The technique gives rise to haploid plants directly through formation of a microspore embryo.

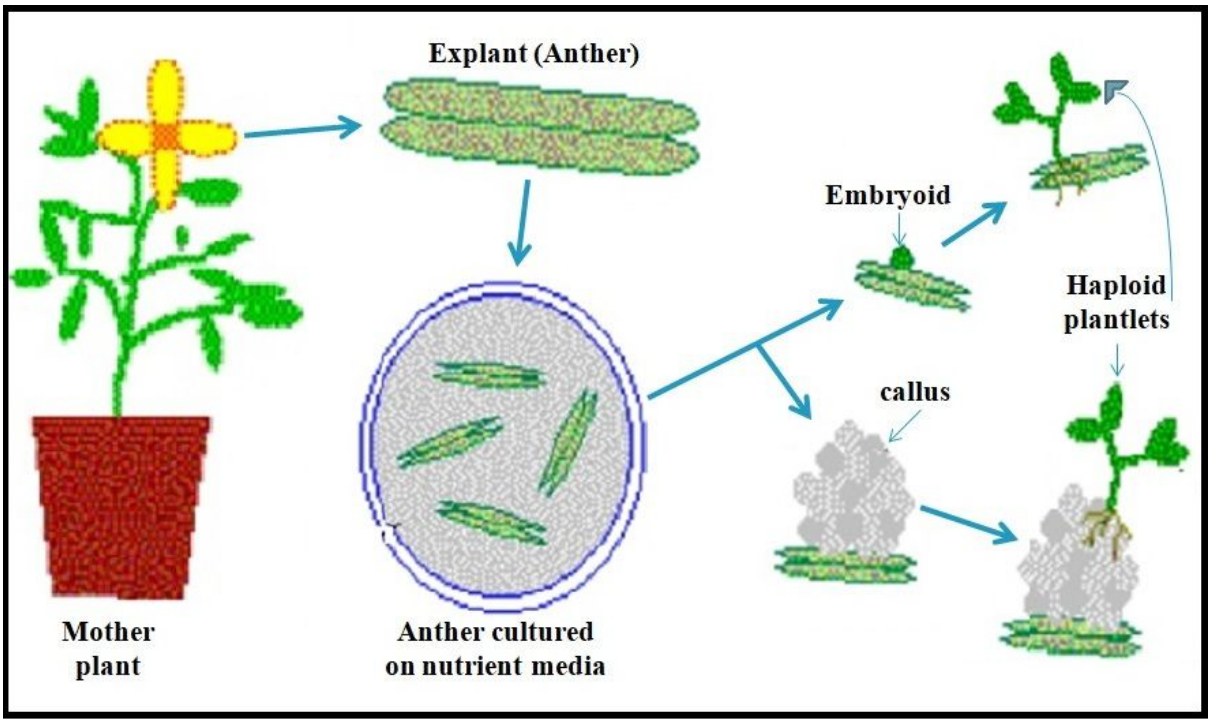

Figure 4 Basic steps of anther culture

Applications of haploids in crop improvement

There are several applications of haploids in crop improvement which are following:

- Production of homozygous lines
- Reducing breeding cycle
- Use as parents in hybridization programme
- Induction of genetic variability
- Inductions of mutations
- Cytological studies
- Production of lines for biotic and abiotic stress resistance

Micropropagation

Clonal propagation *in vitro* is called micro propagation. The micro propagation can be performed using various methods like:

1. Proliferation of axillary buds	2. Organogenesis
a. Bud culture	a. Direct organogenesis
b. Shoot tip/meristem	b. Indirect organogenesis
c. Single node culture	3. Somatic embryogenesis

Here is an example of basic steps in meristem culture (micro propagation) representing in Figure 5:

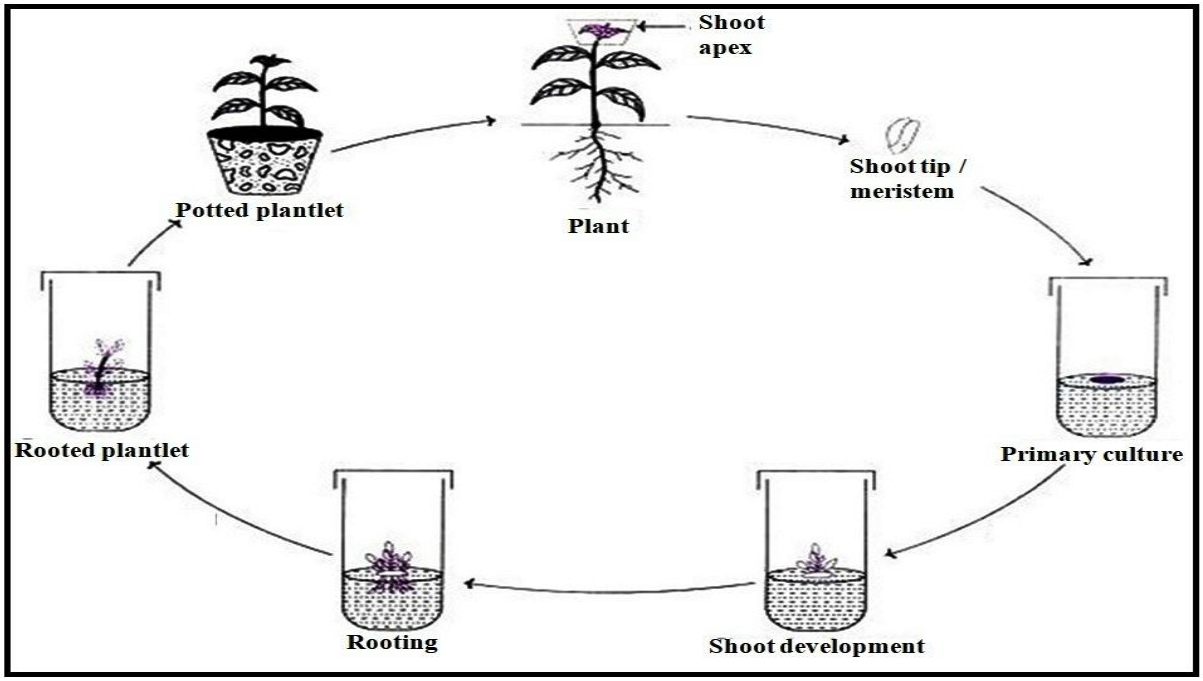

Figure 5 Basic steps of meristem culture

Advantages or importance of micro propagation

- A small amount of tissue is needed to produce millions of clonal plants in one year
- Helps bulking up rapidly new cultivars
- Provide a method of speedy international exchange of plant materials
- *In vitro* stocks can be proliferated at any time of the year
- Production of disease free plants
- Germplasm storage
- Production of artificial seeds using somatic embryogenesis

Somaclonal variation

Somaclonal variations are the variations seen in plants that have been produced by plant tissue culture. Chromosomal rearrangements are important source of somaclonal variations. These can be induced using two schemes viz. with or without *in vitro* selection.

Application/Achievement of somaclonal variation

- **Novel variants:** 'Velvet rose' in geranium, 'Pusa Jai Kisan' in mustard and 'CIMAP/Bio 13' in citronella have been released for cultivation.
- **Disease resistant:** Reported in sugarcane for eye spot, downy mildew and fiji virus diseases.
- **Salt tolerance:** Mandal *et al.* (1999) developed somaclone 'BTS24' from rice cultivar pokalli.
- **Drought tolerance:** Wang *et al.* (1997) reported sorghum somaclone 'R111'.
- **Insect resistance:** Zemetra *et al.* (1993) reported a somaclone 'Stephens' which was Russian wheat aphid tolerant.
- **Seed quality**: Mehta and Santha reported 'Bio L 212' of lathyrus has low ODAP, a neurotoxin.

Protoplast fusion

A plant, bacterial or fungal cell that had its cell wall completely or partially removed using either mechanical or enzymatic means is called protoplast and fusion of two protoplasts is termed as protoplast fusion.

Methods of protoplast fusion

1. Spontaneous fusion
2. Chemical fusion
   a. $NaNO_3$ treatment
   b. High pH and high Ca^{2+} treatment
   c. Polyethylene glycol (PEG) treatment
3. Electrofusion

A figure of basic steps representing somatic hybridization is given hare:

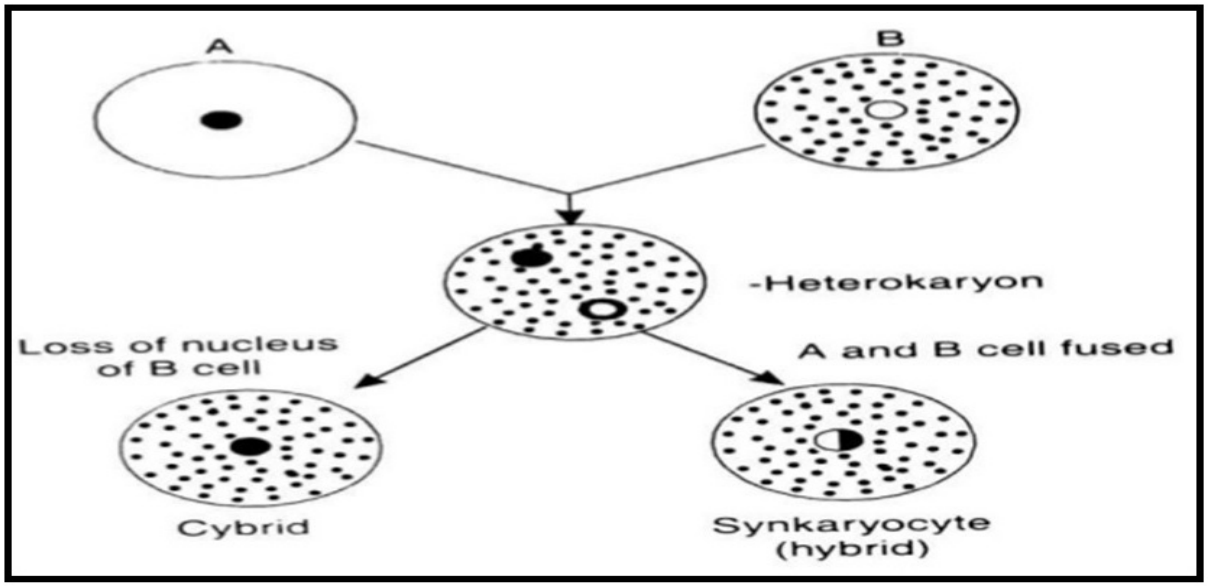

Figure 6 Basic steps in somatic hybridization

Application or potential of somatic hybridization

- Production of interspecific and intergenic crosses: Eg. Pometo by Melchers *et al.* in 1978.
- Gene transfer: For disease, abiotic stress, quality characters and CMS. Eg. Potato leaf roll virus, leaf blight, *phytophthora* etc
- Hybridization becomes possible b/w plants that are still in juvenile phase
- Production of polyploids
- Production of heterozygous lines within a single species could only be propagated by vegetative means
- Study of cytoplasmic genes and their activities
- Production of unique nuclear-cytoplasmic combinations

Cryopreservation

Cryopreservation or cryoconservation is a process where organelles, cells, tissues, extracellular matrix, organs or any other biological constructs susceptible to damage caused by unregulated chemical kinetics are preserved by cooling at very low temperatures (typically -80 °C using solid carbon dioxide or -196 °C using liquid nitrogen). At low enough temperatures, any enzymatic or chemical activity which might cause damage to the biological material in question is effectively stopped.

Applications of cryopreservation

- Conservation of genetic material: like cells, embryos, tissues or proplasts
- Freeze storage of cell cultures or to cease cell division.
- Maintenance of disease free stocks
- Cold acclimation and frost resistance

References

Chawla, H.S. 2002 Introduction to Plant Biotechnology. 2th Edition, Science Publishers, U.S.

Brief History of Plant Tissue Culture, 2018. Online accessed on March 7, at http://agriinfo.in/?page=topic&superid=3&topicid=1881.

Tissue Culture- An Introduction 2018 Online accessed on March 8, at http://agritech.tnau.ac.in/bio-tech/biotech_tc_notes.html.

Anther Culture. 2018 Online accessed on March 8, at https://www.slideshare.net/ramchandra026/anther-culture-30969136.

Tissue Culture and Micropropagation. 2018. Online accessed on March 8, at https://isaaa.org/kc/inforesources/publications/biotechinagriculture/Tissue_Culture_and_Micropropagation_.htm.