CHAPTER 1

PLANT TISSUE CULTURE: AN OVERVIEW

Plant tissue culture is the technique of growing plant cells, tissues and organs in an artificial prepared nutrient medium static or liquid, under aseptic conditions. It has advanced the knowledge of fundamental botany, especially in the field of agriculture, horticulture, plant breeding, forestry, somatic cell hybridization, phytopathology and industrial production of plant metabolites, etc.

The term tissue culture is actually a misnomer borrowed from the field of animal tissue culture. It is a misnomer because plant micropropagation is concerned with the whole plantlet and not just isolated tissues, though the explant may be a particular tissue. The term's *plantlets culture* or *micropropagation*, therefore, are more accurate. However, whether we call it cloning, tissue culture, micropropagation, or growing *in vitro*, the process remains the same; it is a vegetative method for multiplying plants. The nursery trade commonly accepts this method of propagation and it has had a significant impact on commercial horticulture.

Tissue culture is the ever-ready tool for specialists who hybridize plants by either sexual or asexual means. It is a clean and rapid way for genetic engineers to grow material for identifying and manipulating genes or to transfer individual characteristics from one plant to another. It plays a role in a wide array of fields, such as botany, chemistry, physics, genetic engineering, molecular biology, hybrid development, pesticide testing, and food science.

CALLUS TISSUE AND ORGANOGENESIS

Callus (pl. calli) on a wounded plant parts or on a culture medium is made of an amorphous aggregate of loose parenchyma cells which proliferate from the mother cells. Callus is either homogenous parenchymatous mass or treachery elements or sieve elements or summarized cells or secretory cells or the trichomes. Callus formation has been found in angiosperms, gymnosperms, pteridophytes, and bryophytes. Callus contains no organized meristems. Callus

Cytokinin = cell division
Auxin = cell enlargation

is somewhat an abnormal tissue which has the potentiality to produce normal roots and embryoids and in turn it develops into plantlets. Callus may be hard (due to lignifications of cell walls) or brittle and sometimes soft.

Organogenesis is the development of adventitious organs or primordia (embryoid) from undifferentiated cell mass (callus) in tissue culture. It is controlled mostly by a balance between cytokinin and auxin. A relatively high ratio of auxin: cytokinin induces root formation in callus tissues whereas, a low ratio induces shoot formation. **Caulogenesis** is a type of organogenesis by which only adventitious shoot bud initiation takes place in the callus tissue. When it is applicable for root, it is known as **rhizogenesis.** Anomalous structures when develop during organogenesis is called **organoids.** The localized meristematic cells on a callus which give rise to shoots and/or roots is termed as **meristemoids.**

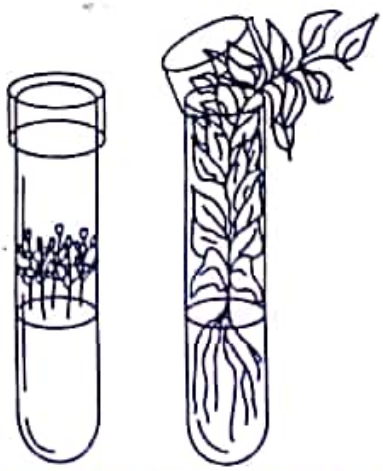

FIG. 1.1: If tissue cultures plants did not revert to a juvenile state, most material would never fir into a test tube or jar and they would be too unwieldy to micropropagate.

When plants are multiplied vegetatively-as distinguished from those grown from seeds-whether by tissue culture or by cuttings, all the offspring from a single plant can be classified as a *clone.* This means that the genetic make-up of each offspring is identical to that of all the other offspring and to that of the single parent. On the other hand, plants propagated by seed, resulting from sexual reproduction, are not clones because each seed (and the resultant plant) has a unique genetic make-up-a mixture from two parents, different from either parent and different from one seed to another. The term **cloning**, with respect to tissue culture, refers to the process of propagating in culture large numbers of selected plants with the same *genotype* (the same genes or hereditary factors) as their respective parent plant.

PRINCIPLES OF GROWTH

Growth is the self-multiplication of living material, the protoplasm it self. Growth is an increase in size (volume or length) due to cell divisions and subsequent enlargement. It is an increase in dry weight of bulk of an organism

associated with development. Development is defined as *an ordered change or progress, often towards a higher, more ordered or more complex state.* Development may take place with growth and growth may take place with development. However, these are often quite integrated processes.

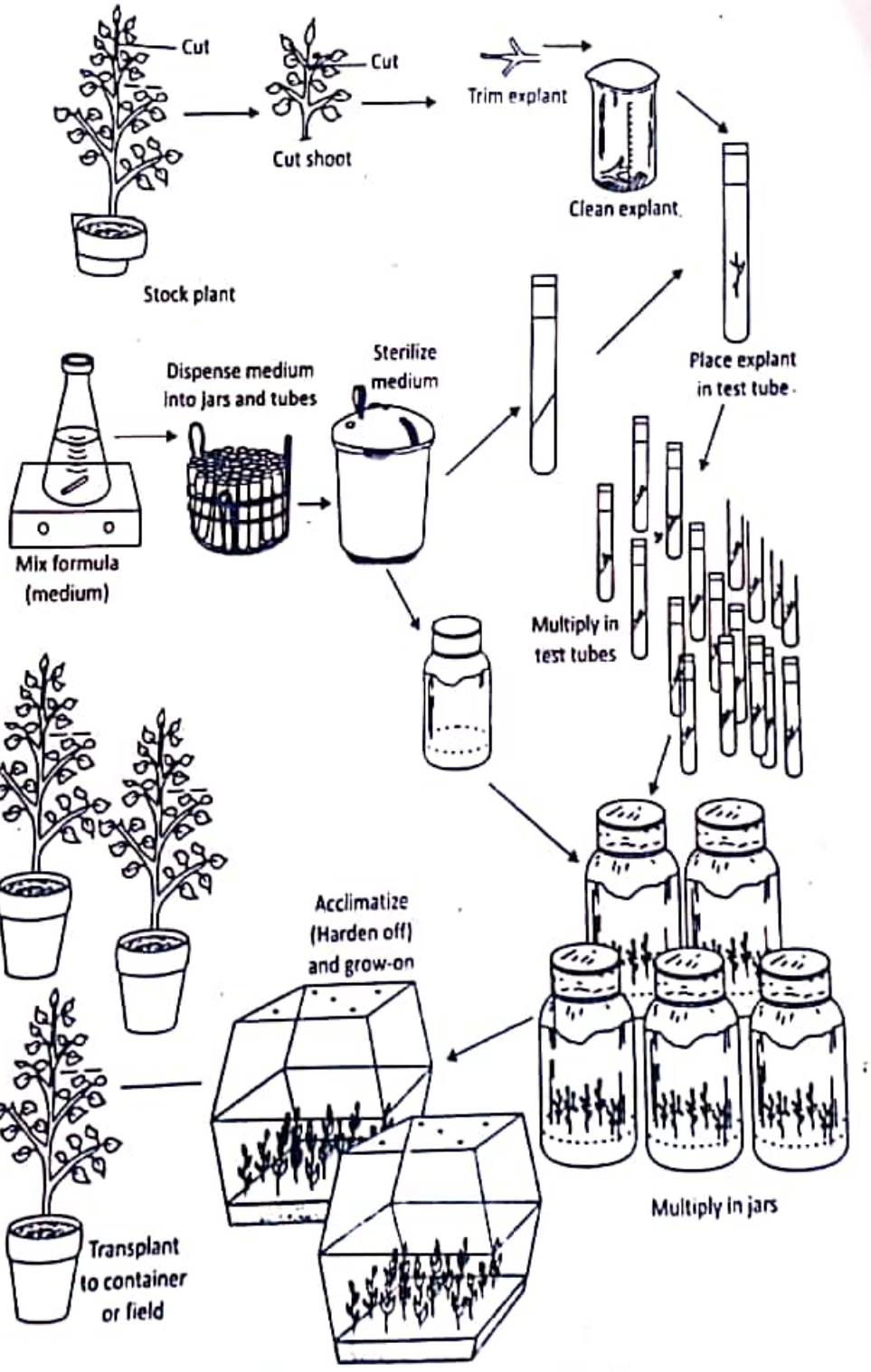

FIG. 1.2: Sequence of shoot tip micropropagation.

In most organisms, growth involves **cell division, expansion, differentiation** and **morphogenesis**. Increases in height and dry weight are

the most obvious manifestation of growth, e.g., in forage and pasture crops. In grain crops, dry weight increases in non-grain components are important only in producing a plant capable of high grain yield. Growth is also expressed as the advance of a plant from one development stage to another. A seed germinates, the seedling develops roots, leaves, and stem, and the plant grows to maturity. The plant flowers and produces seed to complete its life cycle.

Morphological and chemical differentiation takes place during the development. These processes of cells are complicated and involve synthesis of many organic compounds like protein, cellulose, and nucleic acids and requiring physical forces that cause cell enlargement. The process becomes more complicated because it involves division and expansion of many different cell types. Growth includes assimilation and the formation of new protoplasm, permanent change in size, and increase in weight, either of the plant as a whole or of some organ or tissue. The growth pattern and division of the cells in root types, leaf buds, and developing seeds occur in the same way, but they serve vastly different functions for the plant. Several abiotic and biotic factors influence growth of an organ (leaf, fruit, root, etc.) or whole plant at the cellular level.

WHY GROWTH OCCURS?

Growth is expressed as the division of a cell to form two cells and the enlargement of the newly divided cells. When we say that cells double in all their constituents and then divide in half, we are obviously describing the average case. When we observe individual cells, however, we find deviations from the average. In some instances, the growth rate is constant rather than accelerating. In others, the growth rate is high at first and then decreases. A mass of cells doubles and halves only in an average way and not in an exact way. Cells divide when they are ready, and they are ready only when they have completed certain preparations for division.

Speculation and experimentation have now focused on other cellular factors as possible cell cycle controls. These include changes in the concentration of cyclic nucleotides, CAMP and cGMP.

Kinetics of Growth or Measurement and Pattern of Growth

Growth of a plant is usually measured in terms of increase in dry weight, length or height and such measurement indicates that growth-rate varies with the age of the organism. The growth of most plants follows a similar pattern, generally S-shaped or sigmoid shape curve. The size versus time plot is the most direct way of handling growth data. One can also plot the logarithm of the size as a function of time. In the size plot, the units are length, volume or weight, but in the rate curve, the units are length, volume or weight per unit time. A relatively slow growth rate characterizes early or seedling growth. The rate of growth increases as the plant becomes larger, is greatest just before or in early stages of flowering and then decreases as the plant matures

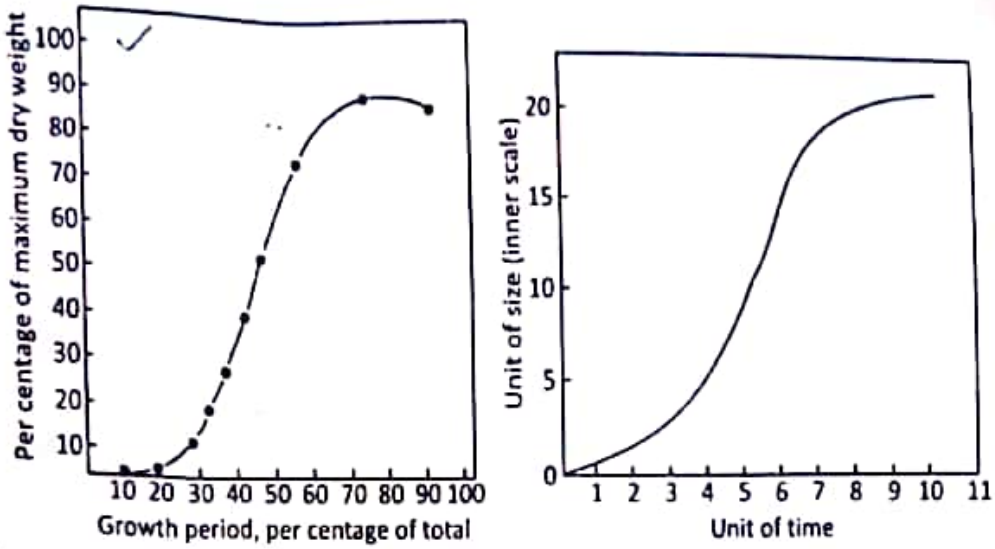

FIG. 1.3: (A) Sigmoid shaped growth curve, (B) Phases a= exponential phase, b= linear growth phase and c= senescence phase when the rate of growth decreases.

The plotted growth curves have following phases:

- **First rapid phase:** During this phase the rate curve as well as size curve increases. It is also known as logarithmatic or exponential phase
- **Maximum growth rate phase:** Growth curves of many plants also show another evident phase called maximum growth rate or the linear phase. Sachs called it the grand period of growth, and it lies between the logarithmatic phase and the senescence phase.
- **Last phase:** During this phase, the size continues to increase but more slowly so that rate decreases. This phase is known as senescence or decreasing phase.

What Happens, When Growth Occurs?

Growth of a plant is initiated in the meristems and the cellular changes in these tissues. The three main changes in the complete development of a cell are **cell division, cell elongation** and **cell differentiation.**

After cell division, daughter cell enlarges to the size of the mother cell before they again divide. During cell growth, uptake of water takes place, which produce turgor pressure sufficient to overcome the force of attraction between the cell particles in cell wall. Consequently, wall stretches and become thin and the osmotic potential increases because of water uptake, which dilutes cell contents. The consequent increase in the cell's water potential brings water uptake (and therefore growth) to a stop. Besides water and solute absorption, new cell wall material is also added and cell wall became thick due to opposition (accumulation of new material on the inner surface of the wall). This happens during cell maturation.

Biochemical Changes

The important biochemical changes that occur during growth are: synthesis of protein, nucleic acid, phospholipids, multiplication of organelles and utilization of energy in the form of ATP, etc.

Dry Matter Accumulation

Dry weight accumulation pattern of plants in the early stages is like the accumulation of compound interest in bank account. The weight of the plant at any given time is likened to the size of the bank account and the increase in weight equated with the interest. In the early stages when plants are very small, the actual dry weight increase per day (interest) in small (first 4 weeks), but at the plant becomes larger, its weight gain per day increases (first 10 weeks).)

THE BOTANICAL BASIS FOR TISSUE CULTURE

The diversity of naturally occurring vegetative reproduction is the landmark and reflects the amazing capability and potential of plants for multiplication. The same factors involved in multiplication and growth initiation in nature are involved in the greenhouse and in tissue culture. The natural capability of plants to multiply by asexual means is the basis for multiplication *in vitro*. Vegetative reproduction, whether occurring naturally or through human intervention, is initiated in stems, buds, roots, or leaves. We take cuttings, we make divisions, we layer, we make grafts, and we tissue culture-in short, we promote vegetative reproduction, a natural phenomenon.

Power of Regeneration in Plants

The multiplication of plants *in vitro* does not establish any new processes within the plants. Tissue culture simply directs and assists the natural potential within the plant to put forth new growth and to multiply in a highly efficient and predictable way. In contrast to plants produced from seeds as a result of sexual reproduction, new plants produced through vegetative reproduction are basically severed extensions of the original plants.

Plant stems have tremendous potential for regeneration because they can grow in various forms and habits long Of short, slender or stout, round, flat, or square, above ground or underground, trailing or upright One of the methods of vegetative propagation used most often by growers is that of growing new plants from stem cuttings. Stem cuttings are shoots or sections of stems that root when they are inserted into a growing medium (potting mix), which may be a mixture of peat or bark with sand or perlite, or simply sand and perlite.

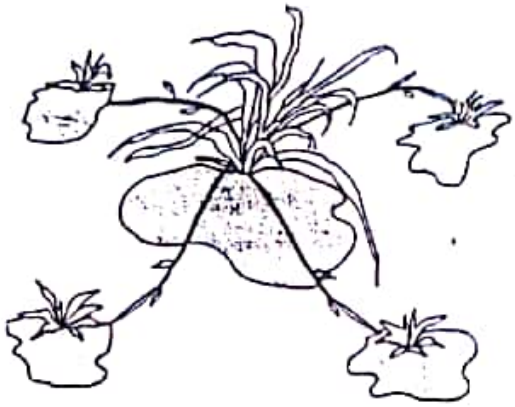

FIG. 1.4: A natural clone. Nature has been cloning. Any time a plant reproduces itself vegetatively it produces a clone.

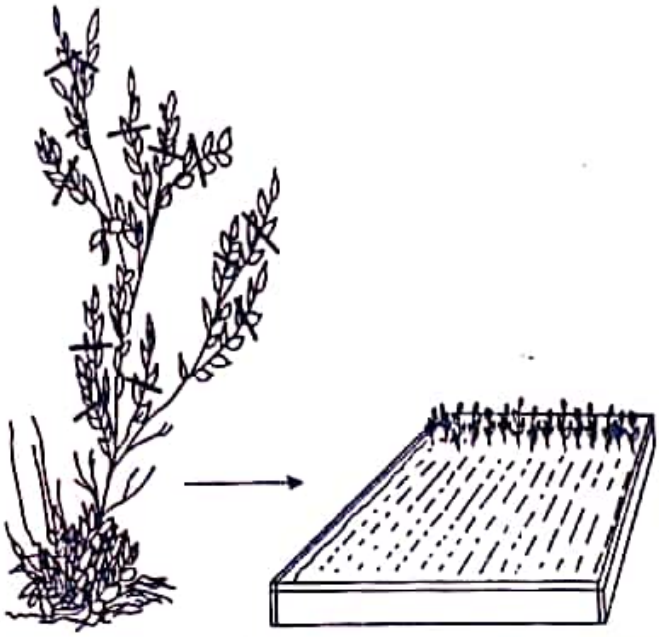

FIG. 1.5: A grower assisted clone by use of stem cuttings. Growers have been cloning by cuttings for centuries.

Roots grow from the nodes (the part of the stem from which buds or leaves originate) when placed below the surface of the medium. Frequently cuttings are encouraged to root by wounding, and often an auxin (a growth regulator) is applied to the base of the shoot. To wound a cutting, a thin slice of epidermis (outer layer of tissue) is peeled away with a knife on 1 or 2 sides of the lower part of the stem. This will often cause the stem to grow callus, which can help root initiation.

Small **stem cuttings** are frequently used as starting material for tissue cultures, and microcuttings, tiny cuttings taken from tissue cultured material, are a product of tissue culture that can be planted and grow on to mature plants. The size of microcuttings can be critical to the success of growth; 1 to 2 in (2.5 to 5 cm) is usually best.

Layering is another form of vegetative reproduction that occurs frequently

in nature and is widely used by growers as well. A branch is said to layer when it comes in contact with the soil, forms roots, and grows on to become a new plant. Wild blackberries *(Rubus)* rapidly expand their territory by layering. Layering is used commercially to propagate filberts *(Corylus)*, grapes *(Vitis)*, black raspberries and trailing blackberries *(Rubus)*, currants (Ribes), apple *(Malus)* rootstocks, and some ornamentals. Air layering is a method whereby growers induce a stem to root without it being in contact with the soil.

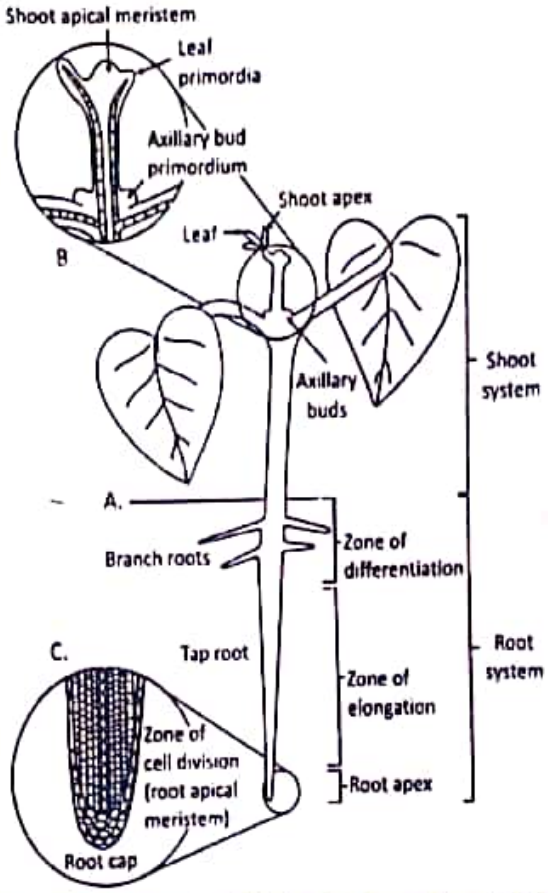

FIG. 1.6: Primary organization and growth of dicot plants. (A) Overall morphology of a small bean seedling. (B) Longitudinal section of the shoot apex. (C) Longitudinal section of the root apex.

In **grafting** growers attach a scion (a shoot or bud) of one plant onto the understock or rootstock (rooted stem) of another plant to obtain the desired traits of both. Various methods can be employed in grafting, but whatever method is used, it is important that the cambial layers match-the cambium is the thin layer of tissue between the wood and the bark of a stem. Micrografting involves the grafting of tissue cultured material in aseptic conditions, a craft that requires great skill.

True **bulbs**, such as those of lilies *(Lilium)*, tulips *(Tulipa)*, and daffodils (Narcissus), are underground storage organs that function as modified stems. A bulb consists of scales (modified leaves) attached to a basal plate (a flat modified stem at the base of the bulb and the source of roots).

Corms are swollen underground stems, complete with nodes, internodes (the section of stem between 2 nodes) and lateral buds; they do not have scales like true bulbs. Plants that produce corms, such as Gladiolus and Crocus, multiply naturally by producing cormets (miniature corms).

Rhizomes, horizontal underground stems, and tubers, the swollen fleshy part of a rhizome. Potatoes *(Solarium tuberosum)* Caladium, and gloxinias *(Sinningia)* produce stem tubers. A piece of tuber or fleshy rhizome generates a new plant if it contains an eye, or bud.

Stolons or runners are long, prostrate, aboveground modified stems. Examples of stoloniferous plants are creeping dogwood *(Corpus canadensis)*, strawberry *(Fragaria)*, Bermuda grass *(Cynodon dactylon)* and white clover *(Trifolium repens)*. The sole purpose of stolons or runners is the production of new plants.

Root cuttings are also used for propagation of gooseberries *(Ribes)*, raspberries *(Rubus)*, horseradish *(Armoracia rusticana*, syn. *Cochlearia armoracia)*, apples *(Malus)*, flowering quince *(Chaenomeles)*, bayberries *(Myrica)*, aspens *(Populus)*, and roses *(Rosa)*, to name a few.

Leaves can occasionally produce new plants. Leaf cuttings are made from such plants as *Bryophyllum, Begonia rex, Sedum*, African violets *(Saintpaulia)*, and *Sansevieria*, among others.

WHY PLANTS REGENERATE?

Meristematic cells are located at the tips of stems and roots, in leaf axils, in stems as cambium, on leaf margins, and in callus tissue. Under the influence of genetic make-up, location, light, temperature, nutrients, hormones, and probably many other factors, meristematic cells differentiate into leaves, stems, roots, and other organs and tissues in a organized fashion. Meristematic tissue is the basis of plant growth and development. Parenchyma cells, the most common type of plant cell, are thin -walled cells that have the capacity to regenerate and differentiate, to initiate the growth of new and varied tissues or organs for specialized functions

Meiotic division, or reduction division, is the process of forming sexually reproductive cells. In meiosis, each chromosome of a 2n cell splits in 2. The chromosomes segregate such that one chromosome from each set of 2 goes to each of the 2 new sex cells, or gametes, each of which has, thus, only one set of chromosomes (n).

Whenever cells divide there is the possibility of genetic variability. If a mutation (a change in genetic make-up) occurs during cell division, and assuming the cell survives the mutation, the mutation is carried in all future divisions. A mutation is a sudden abnormal change in genetic order that will alter some characteristic, or it can be a change in chromosome number. The effects of mutations are not always noticeable. Most mutations will cause a plant to die or to produce undesirable qualities, such as misshapen fruit or

abnormal shoots. Growers expect a small percentage of plants to undergo mutation, and they will discard them if they see them.

PLANT TISSUE CULTURE: PRINCIPLES

The principles of tissue culture are all around us-in nature, in the field, and in the greenhouse.

The technique has developed around the concept that a cell is totipotent that is has the capacity and ability to develop into whole organism. The principles involved in plant tissue culture are very simple and primarily an attempt, whereby an explant can be to some extent freed from inter-organ, inter-tissue and inter-cellular interactions and subjected to direct experimental control.

Cell culture is the cultivation of cells on a solid gel medium or in a liquid medium, the latter commonly known as cell suspension culture. ***Callus culture*** is the multiplication of ***callus*** (a mass of disorganized, mostly undifferentiated or undeveloped cells), usually on a **solid medium.**

The apical meristem is the new, undifferentiated tissue at the microscopic tip of a shoot. It is often virus free even in diseased plants because these meristematic cells are not yet joined to the plant's vascular system, and perhaps they grow faster than the viruses. Thus, if the few virus-free cells that make up the microscopic dome of apical meristem are removed from the plant and placed in a culture, they can grow and produce healthy, disease free plants. This technique is known as ***meristem culture***, a term sometimes wrongly used to broadly denote micropropagation or tissue culture, but which should be limited to indicate cultures started from an apical meristem.

Callus Culture

For raising the callus tissues, a tissue culturist must have clear understanding of some basic principles. A cell from any part of the plant like shoot apex, bud, leaf, mesophyll cells, epidermis, cambium, anthers, pollen, fruit etc., when inoculated in a suitable medium under aseptic laboratory conditions can able to differentiate and multiply. This results into the formation of an amorphous mass of cells known as callus, which can induced to re-differentiate on appropriate medium to develop embryoids which directly develop into the plantlets, eventually giving rise to a whole viable plant.

The term **clone** (from the Greek *klon*, meaning: a slip or twig suitable for plant propagation) was suggested by Webber (USA) in 1903 to explain those plants which were obtained by a sexual reproduction; it is even applied to DNA multiplication (cloning of genes in bacteria). In strict scientific sense. cloning means *an organism obtained from a single cell through mitotic divisions.*

2) Meristem Culture

When a meristem is cultured *in vitro*, then it produces a small plant bearing 5 or 6 leaves. This could be obtained within a few weeks. Then the stem is cut into 5-6 small micro cuttings, which under favourable conditions, become fully-grown plants.

3) Organ Culture

A body of higher plants has complex inter-relationships between different organs like root, shoot, apical meristem, leaf primordia, floral buds, ovary, ovule, anther lobs, pollen grains, fruit, seed, etc. In this method a particular organ is isolated and cultured under laboratory conditions in a chemically defined medium where they retain their characteristic structures and other features and continue to grow as usual. In organ culture, organs are not induced to form callus, therefore, it differs from the callus culture where the organization of the intact tissues is lost.

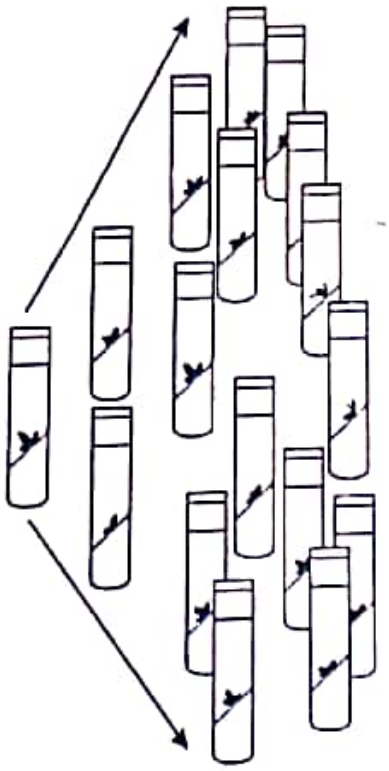

FIG. 1.7: Tissue cultured clone. Potentially, one explant can produce an infinite number of plants.

This technique provides an experimental system to define the nutrients and growth factors that are usually received by the organ from other organs of the plant body and from surrounding environment. It also helps us in understanding the inter-dependence of organs with respect to various physical and chemical growth factors including growth hormones. Organ culture technique also provides the knowledge about the various problems of morphogenesis and the sites of biosynthesis of specific metabolites and growth

compounds. It may be used as a tool for improvement of various economically important crops.

Embryo culture, cell culture, and callus culture are usually designated as such, but they may also fall under the broad term of tissue culture. *Embryo culture* can mean the *rescue* of an embryo from a seed and fostering its plantlet development and multiplication in a culture, or it can refer to *somatic embryogenesis*, whereby embryos are induced to form from *somatic* (vegetative, or asexual) cells.

Organ culture may be grouped into two major categories: **vegetative organs** (root culture, leaf culture, and shoot tip culture) and **reproductive organs** (complete flower culture, isolated ovary culture, isolated ovule and embryo culture, pollen mother cell culture, seed and fruit culture).

Anther and Microspore Culture (Production and Uses of Haploids)

Haploids are the sporophyte with gametophytic chromosome number. They are produced in a variety of plant species using varies methods. Haploids have their significance in genetics and plant breeding. Currently, haploids are the important tools for biotechnological programmes. They are being produced either by ovule/anther/microspore culture on artificial culture medium or by chromosome elimination.

Anther and Microspore Culture: In *Datura*, Guha and Maheshwari (1966, 1967) successfully produced haploids from anther culture. Use of microspores as the basic source material is very common, specially in plants like *Datura inoxia, Nicotiana sylvestris, N. tabacum, Oryza sativa*, etc. Various conditions are important of such culture, however, the important are: genotype of donor plant, the placement, growth and development stage of donor plant and type of culture medium used. Though, the culture includes various steps, the important are:

1. The flower buds from selected plant species are collected and brought to a laminar flow chamber. They are sterilized by appropriate chemical treatment, before dissection.
2. Avoid injury while removing anthers from flower buds. The injury will effect growth and development of callus and may produce variations in chromosome number. Injury may produce diploids. haploids or aneuploids.
3. Culture the anthers on solid agar medium. This will directly gives embryoids or may directly develops in callus, before differentiation.
4. Embryoids develop into plantlets.
5. Colchicine treatment gives rise to diploid homozygous plantlets, which may be directly used for field-tested for selection.
6. Microspore culture is more preferred than anther culture.

Collection of Microspore

The anthers are first dissected and then transferred to beaker containing liquid medium. Press them with a glass rod or syringe piston for squeezing out the microspore. Use nylon sieve to filter the suspensions with anther which allows only the microspores to pass through. Centrifuge the filtrate for three times at 600-800 rpm. The pellets get resuspended in a fresh medium every time. Then the microspores are inoculated on a solid or in a liquid medium. The cultures are maintained at 16/8 hr photoperiod and 25° C. In about 15 days, the microspores may directly develop into embryoids. In anther culture spontaneous doubled haploids (SDH) are also obtained. This does not requires colchicine treatment. SDH are used directly for field test selection.

Ovule Culture

Female gametophyte cells ate also a source for haploid production. First success was obtained from gymnosperms plants e.g., some cycads, *Ephedra*, *Zamia*, etc. Similar haploids have also been obtained from crop species like barley, wheat, tobacco etc. For haploid production from female gametophyte it is necessary to know: (1) the events related to the induction of haploidy in these tissues, (2) factors that control *in vitro* development of proembryo into the fully organized plants, and (3) major differences in the growth patterns of *in vitro* development of unfertilizes ovule cells (female gametophyte) and in pollen cells (male gametophyte.

THE CONCEPT OF TOTIPOTENCY OF CELLS

Some concepts in science become inherently acceptable long before their practicality is demonstrable. This was so in the concept of the totipotency of cells of higher plants. Even in the mid-twenties, one encountered the tacit view that, apart from inherent practical difficulties, there was no theoretical reason why one should not rear a begonia plant from a single leaf hair cell. This view was traceable first to the then well recognized principle that as cells divide mitotically, they do so equationally to produce daughter cells in facsimile.

Secondly, the concept was due to G. Haberlandt' s historic but then unsupported claim, expressed in 1902, that one day it should be possible to rear plants from isolated surviving cells of flowering plants. Haberlandt' s insight was, in fact, the more remarkable because he even stated that out of surviving somatic cells artificial embryos would be reared asexually. In higher plants, embryos do develop in situ from appropriately stimulated somatic cells but without any prior sexual act. At the outset, the special significance of the fertilized egg in plants should be seen as restricted to its role as the genetically unique product of a fusion of male and female gametes. Having established the genetic constitution of a given individual, the zygote (or fertilized egg) really behaves developmentally as a very general kind of living cell- a cell with a

built-in capacity to grow in an organ, the ovule, that fosters that growth. Moreover, as will be shown, and in a satisfying number of cases, isolated somatic cells may return to a simulated zygotic state and grow into embryos and to plants under the appropriately applied conditions which furnish the requisite nutrients and stimuli.

The Autonomous Organelles: Their Behaviour during Growth Induction and Morphogenesis

The stimuli that cause quiescent cells of carrot to proliferate and grow also affect all their organelles (mitochondria, plastids, dictyosomes, reticulum and polysomal aggregates of ribosomes in the ground cytoplasm) in various ways. The same stimuli affect the metabolism of the tissue and its ability to form residual or secondary biochemical products.

GENETIC APPROACH OF PLANT TISSUE CULTURE

1. Somatic embryogenesis is studied because it is an interesting process, liable to produce results of economic interest.
2. The field of plant embryogenesis is not well developed and somatic embryogenesis looks a promising model system.
3. Somatic embryos provide an interesting possibility as it is possible to mutagenize a cell population, make it to embryogenize and look for mutants of conditional type, e.g. temperature-sensitive mutants.
4. The seed embryos, being wrapped in various coats, are not amenable to microsurgery as in case of animal embryos. Moreover, for biochemistry, a large number of synchronized embryos are needed and this is not so easy to obtain, particularly if one is interested in early embryonic stages.

IMPORTANCE OF TISSUE CULTURE TECHNIQUE

All the cells in an organism carry the same genetic information, yet show variations in expression. Our knowledge of cell and tissue cultures has been developing with full swing, specially in biotransformation, forestry, genetic engineering, morphogenesis, somatic hybridization, secondary metabolite production, hybridization, variety development and their conservation, maintaining pathogen free plants and rapid clonal propagation, totipotency, differentiation, cell division, cell nutrition, metabolism, radio biology, cell preservation, etc.

It is now possible to cultivate cells in quantity, or as clones from single cells; to grow whole plant from isolated meristems and to induce callus or even single cell to develop into complete plant either by organogenesis or directly by embryogenesis *in vitro*.

The production of haploid through tissue culture from anthers or isolated microspores and of protoplasts from higher plant cells has served as the basic tools for genetic engineering and somatic hybridization. Tissue culture

technique helps to propagate plants of economic importance such as orchids and other ornamental plants in large numbers by their meristem culture or by other *in vitro* methods. This provides them virus-free plantlets. Propagation of valuable economic plants through tissue culture based on the principle of totipotency *(every cell within the plant has potential to regenerate into a whole plant)*.

In plant breeding, embryo, ovary and ovule culture as well as *in vitro* pollination have been employed to overcome morphological and physiological sterility and incompatibility. In recent years, plant tissue culture technique is in increasing use for producing haploids from anthers or isolated microspores, and of protoplasts from higher plant cells and the recognition of the potential of these materials in genetics and plant breeding. One of the most significant developments in the field of plant tissues culture during recent years are the isolation, culture and fusion techniques which have their special importance in studies of plant improvement by cell modification and somatic hybridization.

Plant tissue culture technique is a boon in the studies of the biosynthesis of secondary metabolites and provides an efficient means of producing economically important plant products (fine chemicals).

Tissue culture can serve a number of purposes, and growers have started their own commercial production laboratories for a variety of reasons. Growing plants from seeds or cuttings can be unacceptable or impractical due to some of the following (and other) factors:

1. Seed-grown products lack uniformity
2. Seed-grown products are not true to type
3. Seeds take too long to grow to mature plants
4. Seeds are difficult to handle
5. Seeds are not available
6. Cuttings grow too slowly
7. Cuttings have poor survival rate
8. Cuttings require too much care
9. Cuttings are too vulnerable to disease
10. There is a shortage of stock plants from which to take cuttings because there is:
    a) Only one hybrid
    b) Only one virus-free plant
    c) Only one desirable mutant
    d) There is insufficient room for stock plants

The simplest tissue culture hobby is the multiplication of easy, fast-growing plant material, such as *Kalanchoe*, Boston ferns *(Nephrolepis)*, African violets *(Saintpaulia)*, or *Begonia*; next in order of complexity are carnations *(Dianthus)*, strawberries *(Fragaria)*, or *Syngonium*. The chemist looking for a tissue culture hobby may be challenged to explore the field of plant by products: dyes, flavorings, medicinals, and oils are just some of the by-