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A REVIEW OF PLANT TISSUE AND ORGAN CULTURE

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Abstract: **Plant cell, tissue and organ culture basically involve isolating a cell, tissue or organ and aseptically placing it on a nutrient medium under controlled environmental conditions, so as to obtain plant cells or whole plants from it. It was in 1902 that a German plant physiologist Habertlandt, first demonstrated totipotency of cells and tried to cultivate plant tissue and organs *in vitro*. He was not very successful, but his work was soon followed by other workers. The first true plants to be established via tissue culture were those of carrot and tobacco in 1939 (White, 1939, 1943).**

Keywords: **Plant Tissue, Organ Culture, German plant physiologist Habertlandt, carrot and tobacco.**

1. TYPES OF CULTURES

1. Callus culture

Plant tissues typically cultured to form callus are vascular cambium, storage parenchyma, pericycle or root, cotyledons, leaf mesophyll and perivascular tissue. The tissue is excised from the living plant under aseptic conditions, a piece of suitable size is cut from it, sterilized and placed in suitable culture nutritive medium in aseptic conditions. The cultured tissue forms an amorphous mass of loosely arranged parenchyma cells arising from proliferation of the cells of parent tissue. This mass is called callus.

First successful prolonged callus culture was obtained by White Nobecourt and Gautheret in 1939. Since then such work has been done in many angiosperms, gymnosperms, ferns, mosses and liverworts.

Primary explants of different origins have different nutritional requirements for initiation of callus. Majority of excised tissues require addition of one or more growth regulators in the medium to stimulate callus development. Explants may be grouped according to their need of growth factors in the medium into auxin-, cytokinin-, auxin and cytokinin- and complex natural extract requiring explants.

After callus has grown in association with original explant tissue for some time, its sub culturing becomes necessary to maintain its continued growth which stops otherwise due to depletion of nutrients in the medium, desiccation of agar due to water loss and secretion of metabolites by callus which reach toxic levels in the medium. Successive subcultures are made usually every 28 days in culture tubes having 30 cm^3 of medium.

Callus does not have a predictable pattern of organization. Extent of cellular differentiation varies considerably in it. Some treachery elements, sieve elements, suberized cells, secretory cells and trichomes may be differentiated. Small localized nests of meristematic cells originate the form ‘meristemoids’ or vascular nodules and these become centers for formation of shoot apices, root primordial or incipient embryos if callus is changed into suitable culture medium.

Callus growths may be yellowish, white, green or coloured variously with anthocyanins. Colour may be uniform in the callus or in patches.

Some calluses are heavily lignified, hard in texture and difficult to break while some are quite fragile breaking easily into fragments (friable cultures).

Pattern of callus growth varies considerably in tissues of different species, different plant parts, and age of plant at the time of taking out the explant, nutritional and environmental conditions of the culture.

2. Cell suspension culture

Such cultures are generally initiated by transferring fragments of undifferentiated callus to a liquid medium, which is then agitated during the period of culture. They can also be started by inoculating liquid medium with an explant of differentiated plant material e.g. fragments of hypocotyls or cotyledon but such cultures take longer times. These cultures consist of single cells, cellular aggregates or varying sizes, residual pieces of inoculums and the remains of dead cells. No such culture is ever composed entirely of single cells. A good suspension culture is obtained from a friable callus and has high percentage of single cells and small cell clumps. Sometimes increased auxin: cytokinin ratio produces a more friable culture giving better suspension culture. Successful suspension culture also requires periodical sub culturing. Sub culturing is always done at the time of maximum cell density in the medium.

Growth of the culture shows an initial lag phase soon after inoculation prior to any sign of cell division. It is followed by an exponential phase in which cell number rises at every increasing rate, i.e. exponentially. Soon after this is linear phase in which rate of growth of cell population shows continuous decrease. Finally, cell division stops and cell density becomes constant at a maximum. This is stationary phase. To maintain the viability of suspension culture, it should be sub cultured early during the stationary phase.

Suspension culture grown in a fixed volume of culture medium is called batch culture. If all the cells of the culture are retained within the system, it is termed a closed culture. If such a culture involves continuous influx of fresh nutritive medium and a continuous withdrawal of spent medium but all the cells are retained within the system, it is termed closed continuous culture. If cell density is kept constant in the culture, it is termed open culture. If nutritive medium is periodically or continuously replaced and excess cells are flushed out with spent medium, it is termed open continuous culture.

3. Organ culture

It is defined as potentially unlimited growth of an isolated organ in a suitable culture medium. Important organs cultured in this way are root-tips, shoot-tips, anthers, pollens, ovules, nucellus, embryos, seeds and ovary. The consideration, techniques and uses of these different types of organ cultures are slightly different from each other and, therefore, they cannot be described as a group.

Excised roots were to be the first organs to be cultured. Root tips approximately 10 mm long are taken from young seedlings produced in axenic conditions and transferred to aqueous culture medium where they produce laterals. Main explant is now subdivided, each sector having portion of main root with some laterals. Each sector is transferred to fresh medium and the process repeated periodically maintained a continuous supply of roots. Medium requirements are met by essential substances only but some cultures respond favorably to addition of auxin and other growth regulators. The capability of secondary vascular tissue formation is gradually lost in cultured roots though basic anatomy and metabolism remains intact**.**

Shoot tip cultures may be of two types: shoot apex culture in which a portion of shoot tip of varying length but always having apical meristem, few leaf primordial and subjacent stem tissue is cultured. Bud may also be used as explant. These require exogenous gibberellin and cytokinin for growth. Medium lacking exogenous growth regulators is needed to promote stem elongation. Finally culture needs third medium containing auxin for root initiation. The explant may consist of excised and isolated apical meristematic dome only devoid of any leaf primordial and stem tissue. Then may be cultured on agar media or on filter paper immersed in liquid medium. These cultures are called apical meristem culture. These cultures require exogenous hormones in medium. High potassium ion concentration in medium is found essential.

Anthers cultured on basal nutrient medium produce haploid plants. Excised anthers from buds selected at uninucleate microspore stage when microspore mother cells are in early meiosis are sterilized and placed on suitable culture medium. Pollens in anthers may directly form haploid plants or first a callus may be formed from which plants arise. Development

of haploid plants depends on medium composition, age of anther at culture, age of plant and temperature. Medium generally requires supplement of coconut milk, auxins and cytokinins, either alone or in various combinations. Percentage of plants per anther drops rapidly with age of the plant. 20-25^{o}C is optimum temperature for most of the anther cultures. The pollens in an anther are genetically heterogeneous so the plants arising are also so. Further, plants are often a chimera in case a callus is first formed as many pollens to get her form the callus. Isolated pollens are also cultured to obtain pure clones. Anthers are placed on a semi-solid medium. Isolated pollens are taken in a liquid medium and about 10 pollens from this are placed on a filter paper kept over the anthers on agar medium.

First embryo cultures were made by Hannig in 1904. Embryo is excised from the seed and cultured on a medium. Media requirements vary with species, stage of the excised embryo and purpose of the experiment. Young heterotrophic embryos, globular embryos and undifferentiated preglobular embryos vary in the richness and composition of required media.

Nucellar tissue excised from post-pollinated or pre-pollinated ovules cultured on suitable medium first form a callus from which many tumor-like pseudo bulbils arise which develop into embryos and ultimately into seedlings. Nucellar tissue from post-fertilized ovule needs supplementation of casein hydrolase in medium while embryogenesis in Nucellar tissue of pre-fertilized ovule occurs only if medium is supplemented with malt extract and adenine. Embryos developed on the medium can germinate only if they are excised and planted on medium supplemented with GA_3.

Raising mature seeds by culturing ovaries at globular or a later stage of embryo development is comparatively easy. This needs quite simple nutritive medium. Seeds of plants with reduced embryos and seed of parasites have also been cultured for various purposes.

Ovary culture for improvement of fruit quality or for study of fruit physiology is quite common. Ovaries are cultured after pollination. Only a simple medium with mineral salts and sugar sometimes supplemented with vitamins, glycine and yeast extract is sufficient. The maximum size of fruits obtained by ovary culture is usually smaller than natural fruit. In culture of un pollinated ovaries, auxin in medium may act to replace pollination signal. Such cultures give parthenocarpic fruit.

4. Protoplast culture

Isolated protoplasts from cells of plant are also cultured to give rise to plantlets. Cells of the tissue are first separated by treating them with pectinase and then cell walls are digested with cellulase. Concentration of two enzymes and duration of treatment varies with different types of tissues. The protoplast is always isolated from the cell in a suitable osmotic stabilizer to maintain their osmatic fragility. Mostly a metabolically inert sugar e.g. sorbitol or mannitol solution is used in appropriate concentration in which work of isolation is carried out. The isolated protoplast on suitable culture medium, first forms a new cell wall around it and reconstitutes a cell which undergoes sustained divisions to form a callus. Though cell wall synthesis occurs readily, sometimes addition of $CaCl_2$ and NH_4NO_3 are required in the medium for it (in *Phaseolus*). General composition of medium is not much different from that required for cell or callus cultures. From the callus obtained from protoplasm culture, plant may be obtained *in vitro* by same method as in callus culture. Protoplast culture has been used to culture of hybrids of two such plant species that normally do not hybridize. For their first isolated protoplasts of two types of cells are fused together and the fused protoplast is cultured to give hybrid plant. Inter- and intra-specific fusion of higher angiospermic protoplasts has been obtained under influence to sodium nitrate. Freshly isolated protoplasts are suspended in sodium nitrate or a mixture of sodium nitrate and osmoticum of proper osmolarity and kept for about 5 minutes in a water bath at 37^{o}C. Protoplasts are then compacted at the base of tubes by centrifuging them at low speed and left undisturbed for about 30 minutes at 37^{o}C in dark. Polyethylene glycol is now another common fusing agent which gives higher frequency of protoplast fusion than sodium nitrate. Microspore protoplasts and meiocyte protoplasts can fuse in the absence of a fusogen. Fused protoplasts of two species exhibit obligate requirement of growth regulators in the medium otherwise only hybrid colonies of cells are found to grow.

In case of all the types of tissue, organ or protoplast culture works, variety of techniques have been developed suiting to different types of experimental studies, agricultural or horticultural applications. Study of many physiological, biochemical and developmental phenomena; study of morphogenesis, differentiation, organogenesis nutritional needs at various stages in the life of plants, development of pathogen free economically important plants are some of the new developments associate with plant tissue culture.

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2. USES OF PLANT TISSUE CULTURE TECHNIQUES

All types of callus, cell suspension, organ and protoplast culture techniques have found wide applications in different fields of biological research, agriculture and horticulture.

USE IN RESEARCH:

1. Study of nutritional requirements of various tissues: By culturing different types of tissues on artificial media and changing its composition, knowledge about requirements of various substances in the life of the tissue may be found out.

2. Study of organogenesis: By inducing formation of roots, shoot tip and plant from callus successfully on artificial medium, conditions needed for their initiation, differentiation and growth may be studied.

3. Study of differentiation: By inducing formation of different types of tissues e.g. xylem elements, sieve elements, sclereids, fibers etc. hormonal and other requirements for their differentiation and the changes occurring during this cytodifferentiation may be studied.

4. Physiological and biochemical studies: Study of different physiological and biochemical processes can be made in cultures without complications of all the factors being involved together as in case of living plant.

5. Genetical studies: In haploid plants produced from anther or pollen cultures, recessive characters can be observed, such lines can be maintained and by diplodisation of such plants, homozygous recessive diploids may be obtained. Mutations induced in haploids can be easily detected due to absence of interference from other alleles. In cell suspension cultures of haploid cells, genetical researches on higher plants may be carried out by techniques similar to micro-organisms. By techniques of protoplast fusion and protoplast culture, hybrids of normally incompatible plant species can be made.

6. Embryological studies: The nutritional requirements, requirements of changing hormones, interrelationships among various parts of ovule, and embryo have been studies thoroughly by ovule culture, embryo culture, endosperm culture and nucellus culture. Potentiality of each type of tissue have been worked out.

7. Studies of the role of hormones and mineral nutrients: By changing hormonal levels and their combinations and various mineral nutrients in the medium, exact roles of different hormones in different processes of differentiation and roles of different minerals in physiology of different tissues has been investigated.

USE IN AGRICULTURE, HORTICULTURE AND FORESTRY:

1. Improvement of crops: For using hybrid vigour in crop improvement, homozygous, true-breeding cultivates are extremely important. Obtaining such cultivars by plant breeding techniques, especially in out-breeding crops involves selfing for many generations which requires work of many years and may also be impossible due to self-incompatibility. Such work may be made much easy by use of the techniques of anther or pollen culture, raising haploid plants and then diplodisation of these haploids by use of colchicine thus giving homozygous diploids in one generation.

2. Shortening of breeding time: Sometimes breeding work on horticultural plants may be delayed due to long dormancy periods of seeds. By embryo culture techniques, this period can be considerably shortened.

3. Rapid seed-viability test: As embryo culture can be used to break seed dormancy, this technique can be used as a rapid test for seed-viability. Germination of excised embryos is reliable and capable of more exact interpretation than the commonly used staining tests for seed viability.

4. Production of rare hybrids: In plant breeding, all desirable crosses are not successful due to pre-fertilization barriers, death of embryo at early stage or endosperm-embryo incompatibility. In such cases, complete viable plants can be raised by embryo culture or by somatic hybridization and culture of hybrid line.

5. Maintenance of pure desirable lines of plants: In horticulture and crops also, sometimes it is desirable to maintain the variety in pure form which is quite difficult in natural field conditions. By tissue culture techniques, genetically pure clones can be maintained indefinitely and loss of desirable character may be averted.

6. Rapid multiplication of plants: In forestry, plants may be required in very large numbers for plantation of forest. Multiplication of plants by natural methods takes much time and labor. By tissue culture techniques, plants can be made in very large numbers in very short time in the small space and then these plants can be used for plantation.

The plant tissue culture techniques have already found wide applications in various fields and are hoped to find newer applications in future.

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