**TISSUE CULTURE**

During the latter half of the 20th century many new techniques were developed in the field of biology. The technique of the maintenance and growth of plant cells, tissues and organs, in vitro within a suitable culture medium is ‘Tissue Culture.’

 The technique of the plant tissue culture was developed by ‘G. Haberlandt’ of Germany in 1902.It was demonstrated successfully by F.C.Steward in 1964 by using phloem cells of carrot, under controlled laboratory conditions. The idea of cell culture has originated from the concept of totipotency. The potency of an individual plant cell to develop into a complete plant is called *totipotency.* The cell, tissue or organ of the plant to be used in plant tissue culture is called an ‘*explant.’*

 Some promising results are obtained in the field of tissue culture by the work of several scientists. A scientist by name ‘white’ was able to culture tomato roots. ‘Skoog and Miller’(1957)induced rooting and shooting with the help of auxins and cytokinins**. ‘**Muir and his co-workers’ produced a complete tobacco plant froma single isolated cell of this plant and thus proved that the cells are totipotent.

 **Tissue Culture Techniques**

For tissue culture experiment, certain requirements are needed.

* **Selection of Culturing material**

A suitable part of a tissue is needed as material to be cultured. Before conducting the experiment should ascertain then the selected tissue should grow quickly in the medium.

* **Selection of nutrient medium**

For the proper growth of the tissue a balanced nutrient medium is necessary. The nutrient medium adopted for every tissue should be ascertained because different tissues need different kinds of media. But generally the medium should certain inorganicsalts of elements both macro and micro types together with various vitamins a sucrose. This medium is referred to as ‘basal medium.’ Later, growth hormones such as auxins, gibbererellins and cytokinins are also added to the basal medium. Sometimes in order to facilitate the growth of the particular tissues, natural plant extracts like fruit juice, coconut milk, extracts of yeast etc. are also added.

 All these above nutrients are

Dissolved in distilled water and the pH of this medium are kept at 5.8. This medium may be in the liquid form, semisolid form or even solid form. Solid medium is prepared by the addition of one percent agar-agar or gelatin in the medium. The amount of the sucrose in the medium is adjusted to about 2 to 4%.

Next, this medium is transferred to culture vials, which consist of glass tubes or flasks. These vials are plugged with non-absorbent cotton wrapped in cheesecloths. This cotton lid allows the gaseous exchange but does not allow the contamination of the medium by micro-organisms.

* **Sterilization**

The nutrient medium may contain a large number of micro organisms like bacteria and fungi, which may interfere in the culture or even kill the tissue to be cultured. Hence, the elimination of all types of microorganisms from culture vials and tissues is being transferred to the medium. In order to eliminate the micro organisms from the medium and the plugged cotton the vials containing the medium should be autoclaved. During this process the vials are heated under pressure. If the medium is subjected to a high temperature of 120 c for about 20minutes all the germs are killed.

 In order to kill the micro organisms seen on the tissue, the plant material from which the culture tissue is taken is to be surface-sterilized. First the plant material is washed with detergents and after this the surface of the plant organ is surface-sterilized with con.chlorine water or sodium hypochlorite. This material is washed in distilled water to remove all the traces of the chlorine. If the plant material is very hard the surface sterilization is done by alcohol.

* **Transference of excised tissue in to vial**

Tissue culture is transferred in to the culture vial without contamination by germs of microorganisms. For this, inoculation chamber is used. In this chamber, ultra-violet is provided which kills all the microorganisms. Again, this chamber has a device to get filter-sterilized air. This also kills the entire organism. This chamber has a table and this is made aseptic by alcohol treatement.During inoculation the neck of the vial is sterilized, by flaming. The removal of cotton plug, the transfer of tissue in to the medium and closure of vial by the plug is all done very quickly. So that, there is no time for the microorganisms to enter the culture solution. All this instruments used for this purpose are also tube sterilized with alcohol treatment.

* **Aeration**

Aeration to the culturing tissue is very important especially if the culture is maintained in liquid media. In solid media, the tissue gets enough air as it is above the surface. However, in liquid media, the tissue may be immersed. In this condition, aeration of the medium is very important. In some cases, a filter paper bridge is used.

 

 **Applications**

* Rapid Clonal Propagation: In several cases the viable seeds are not formed. In such cases, a large number of genetically identical plants can be produced. In several ornamental and useful plants shoot tip clonal propagation is practiced as an easy method of getting large number of plants

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* Study of Cellular Genetics: The method of culturing single cell enables scientists to study the genetics of cells and thereby the organism developed from it. In cell culture variability can be induced by the fusion of two cells, by the use of mutagens or by the absorption of DNA from out side medium.
* Shortening of Breeding Cycle: In certain cases, the seeds have along dormancy period. If their tissue is cultured, the total time required to raise generations can be shortened.
* To Get Rare Hybrids: In certain plants the embryos abort after their formation due to lack of food. In others there is lack of compatibility between the embryo and endosperm. In such cases the embryonic tissue is cultured by tissue culture methods so that viable hybrids can be produced.
* Elimination of Diseases: Some of the cultivated plants are affected by pathogens like bacteria or viruses. Their meristamatic tips, free of pathogens, can be cultured so that disease free plants can be maintained. In tapioca, sugarcane and in potato, the virus disease called mosaicism is prevented by tissue culture methods

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* Conservation of Germplasm: The germplasm can be preserved in invitro condition by keeping tissue at low temperature. Sometimes growth retardants are used to preserve the tissue. Thus tissue can be preserved for future use just like seeds of plants.

**GRAFTING**

 Vegetative propagation of plant is form of asexual reproduction which involves the regeneration of new plants from portions of vegetative organ such as stem, leaf and root. Vegetative propagation can be classified in to natural and artificial. Grafting is an artificial vegetative propagation. It enables the rapid production of new plants, preserving all desirable traits.

 It is an asexual method in which parts of two different plants are joined together. So that they continue their growth as one plant. In grafting, a stem cutting from a donor plant is incorporated with a rooted recipient plant. The donor and the recipient usually belong to related species or varieties. The stem cutting from the donor plant is ‘scion/graft’. The rooted recipient plant to which the scion is attached is called ‘root stock’. The scion must always be superior to the stock in desirable traits or qualities and the stock must be highly resistant to pest and diseases and must be highly efficient in absorbing and conducting water and minerals.

The scion grows on the stock and becomes the shoot system of the new plant and the stock forms its root system. The two new plants, formed by the union of scion and stock through grafting are called `*composite plant’.* In grafting the cambia of scion and stock are brought into close contact with each other. This establishes a vascular continuity between scion and stock. Afterwards, scion grows further retaining its desirable features. During this, it supplies the stock organic food, which it has made by photosynthesis. In return, the stock supplies the scion water and nutrients, which it has absorbed from soil.

 Grafting is successful if the healing is complete and the vascular transport between scion and stock is continuous. It is critical that the issues in the two parts are correctly aligned. Healing starts with the production of callus, and it occurs in the cambium region of the two parts.

**Advantages of Grafting**

* Enables the propagation of those plants whose cuttings will not produced roots.
* Provides disease-resistance to plants but desirable cultivars; In this case, a desirable cultivar is grafted into a cultivar that is resistant to diseases and pests.
* Rapidly increases the number of desirable cultivars
* Reduces the dependence on seed propagation.

**Methods of Grafting**

 Different methods are now in practice. They include whip grafting, cleft grafting, approach grafting and bud grafting. There are different forms of grafting and the method adopted varies according to the kind of plant and the season of operation. The commonest type is ***approach grafting.***

**Approach Grafting**

 This is one of the best methods of grafting for species, which are difficult to propagate by other methods. Healing in approach grafting is rapid especially when both components are in an active state of growth. Once the healing is complete, the top of the stock above the graft joint and the base of the scion below the graft joint are severed gradually in stages. The scion grows on the stock as a new plant. The two common methods of approach grafting are `***splice approach grafting and tongue approach grafting’.***

**Splice Approach Grafting:**

In this method both scion and stock must be of equal thickness (10-12mm in diameter).The pot containing root stock (usually seedlings) is placed near the scion. A thin slice of bark and wood about 6-7cm long is removed from the stock at a height about 25-30cm from the soil surface. A similar cut is made on the scion shoot also. The cuts, exposing the cambium layer, should be smooth and even so that they may fit closely with each other without any gap between them. In this position, they are tied firmly with jute fiber, twine, waxed tape or adhesive rubber tape. Grafting wax is applied at graft joint to prevent the wilting of tissue. Strips of polythene films are now used to protect the graft union from the sun and rain. The pot is regularly watered to encourage growth.

After 45-60 days, when the union is incomplete a v-shaped cut is made on the root stock half way through the wood just above the graft joint. A similar cut is made on the scion also about 1-2cm below the graft joint. After some 15-30 days, the cuts are depending to 2/3 or ¾ of the shoot. After another 15days, cuts are completed and the top of the stock and the base of the scion are completely severed from the respective mother plants. This results in a new plant consisting of root stock and grafted top.

 **Tongue Grafting:**

 This is similar to splice approach grafting except that the tongue is provided on the cut surface of both the stock and the scion to provide rigidity to the graft union. For this purpose, after the first cut, a second downward cut is made on the stock and an upward cut on the scion. The scion is then interlocked with the stock. Tying and waxing are done in the same way as in splice approach grafting.