fruit characteristics. For better contact between cambial surfaces to assure grafting surface, rootstock and scion should be of the same vigour and thickness, and of suitable age.

- Grafted seedlings in pots or nursery rows should be carefully tended. They should also be protected from desiccating winds, direct sunlight, rain, and frost injury.
- The material used as bandages and for wrapping graft union should be removed once the bud sprouts have made some growth (Childers 1969; Garner and Chaudhri 1976; Singh and Krishnamurthi 1967).

6.3 Plant tissue culture

Plant tissue culture commonly refers to *in vitro* and aseptic cultivation of any plant part (cells or tissues) on a nutrient medium in an artificial environment. Research efforts in plant-cell and tissue culture have increased dramatically worldwide in recent years, including efforts in the developing nations. Plant propagation using shoot-tip culture is the most widely used area in plant-cell and tissue culture and is currently being used to propagate elite genotypes of several plant species in a number of countries (Garner and Chaudhri 1976; Hartman and Kester 1968; Yusuf 1989).

Clonal propagation. True-to-type plants can be raised by shoot-tip/meristem culture. The terminal 2–3 mm portion of plants (meristem) is almost free from viruses, for the reason that cell divisions in such parts are very active. Virus-infected cells, on the other hand, divide comparatively slowly and lag behind. On nutritive media under controlled environmental conditions, such meristems can be made to grow into complete healthy shoots.

6.3.1 Commercial considerations

Some important commercial considerations in tissue culture are, briefly:

Capital investment. The initial cost of a tissue culture laboratory is higher than the cost of using conventional methods.

Expertise. Well-trained personnel are required for this technique. They should be conversant with standard microbiological techniques and have sufficient botanical knowledge of the material and of its *in vitro* behaviour.

Range of applicability. Although in theory it would seem that any plant can be cultured by supplying the appropriate metabolites in suitable *in vitro* conditions, so far only a small proportion of plants can be cultured well enough to be effectively propagated. The time and expense involved would

probably be worthwhile for the major crop plants, but not necessarily for the minor crops.

6.3.2 Technical problems

Phenolics and other inhibiting factors. A number of plants produce excessive amounts of phenolic substances whose oxidation products not only darken both the tissues and the medium but often inhibit growth. Where the problem is confined to the reaction of the initial explant it may be prevented by treatment with ascorbic or citric acid.

Vitrification. An occasional problem with cultured plants is the development of swollen, distorted leaves which become irreversibly translucent and eventually necrotic, a condition that may lead to the death of the shoot cluster. This has been referred to as **watersoaking** (Khan et al. 1987; Murashige 1977) and is more recently described as **vitrification** (loc. cit.). The phenomenon occurs in shoots cultured on media containing cytokinin and can generally be prevented by making sure that the concentrations are not higher than necessary.

Bacterial contamination. A persistent difficulty in tissue culture laboratories is the occurrence of contamination by slowly-growing and often macroscopically invisible microorganisms, particularly bacteria.

Quality of plants propagated by *in vitro* techniques. It has been observed that when proper culture media and conditions are not provided to the explants certain changes may occur in plants raised through tissue culture. These changes are due to epigenetic effects and genetic differences.

Supply of electricity. A continuous supply of electricity is very essential for tissue culture techniques. Since there is frequent load-shedding in this country, an alternate power supply through generators is required for tissue culture laboratories.

6.3.3 Techniques

There are three main steps or stages in the tissue culture multiplication technique.

1. Establishment stage. The explant or tissue fragment is sterilized in a disinfectant and placed in a carefully selected and formulated nutrient medium for a period ranging from four to eight weeks. The purpose is to confirm sterility and to establish growth of the explant.

2. Multiplication stage. Plant material resulting from the establishment stage is divided and each part replanted in separate culture tubes. As multiplication occurs, the fragments are again divided and replanted in separate fresh containers. This procedure may be repeated several times to rapidly propagate shoots. Since each transplanted shoot has the ability to produce

a complete new plant, it will produce new shoots of its own, each of which also has the potential to produce shoots and roots.

3. Pre–transplant stage. Before transplantation, the prepared plants are generally hardened by deleting the hormones from the medium and by increasing the light intensity. They are then transferred to soil in the greenhouse. About two to four weeks are usually required to condition or harden a plant before potting it. Special greenhouse procedures are required for about four to eight weeks until a complete transition from laboratory to greenhouse is completed.

6.3.4 Preparation for tissue culture

In preparation for the above stages, the following procedures and precautions are followed.

1. Selection of tissue. For maximum success in using the tissue culture method, the explant must be chosen systematically. The success of this method is directly related to the organ source—its age, the season in which explant is taken, size of the explant, and overall quality of the plant from which the explants are obtained. Shoot tips have been satisfactory for many plants, whereas sections of root, leaf, inflorescence and other organs may be better for other plants. Tissue obtained from young plants tends to reproduce better than that from older source plants. Tissue taken from the growing point has the power of rapid cell division and is generally free from viruses and virus–like disorders. The explants should be free from visible attack of pests and diseases.

2. Sterilization. The primary objectives of this step of *in vitro* propagation are the establishment of a microbe–free explant culture, and the initiation of new growth. The outer surfaces of plants growing under natural conditions are normally infested with spores and other microbial cells. Mature inner tissue may harbour fungi and bacteria without showing visible symptoms. Plants grown in the greenhouse or growth room are somewhat free of these maladies. Before culturing the explants, it is essential to disinfect the material and then wash it four to five times with autoclaved water to remove the residual toxic effect of the chemical. Some disinfectants commonly used for this purpose, and their concentrations, are listed below.

Disinfectant	Concentration	Dipping time
Sodium hypochlorite	0.5–5%	5–20 min
Ethyl alcohol	75–80%	Several sec
Benzalkonium chloride	0.01–0.1%	Several min
Hydrogen peroxide	3%	15–30 min
Mercuric chloride	0.01–0.1%	10–20 min

Each plant behaves differently with these disinfectants.

Since all plants have a waxy outer surface, it is usually necessary to add a small amount of detergent to the disinfectant to allow better penetration. Tween is a detergent being widely used for this purpose (Khan et al. 1987; Murashige 1977; Qureshi et al. 1991; Street 1977).

Inoculation area. For this purpose a special room is prepared which should be free from dust and microorganisms; it should have ultraviolet lights and be air–conditioned to maintain the desired room temperature. It should be equipped with a ventilation unit with laminar flow and positive pressure. A laminar flow cabinet is a special piece of equipment with a 0.3 UM HEPA filter of 99.97–99.99 percent efficiency. It should be fitted in such a way that the air is forced into the cabinet through a dust filter and is then directed over the working area at a uniform rate. Before the cabinet is used, it should be thoroughly cleaned and sterilized with 70–80% ethyl alcohol.

Culture facility. Plant tissue to be cultured on any nutritive medium should be incubated under proper conditions of well–controlled temperature and light for photoperiod and humidity control and air circulation. All these factors are directly related to the success of this technique.

Media for plant tissue culture. It is very important that tap water never be used for dissolving the chemicals because it contains many impurities such as cations, anions, mineral salts, microorganisms, and gases. Double distilled water should always be used for this purpose.

Four different media—MS, B5, White, and Heller—are used in the propagation of horticultural plants by this technique. Of these, the medium named after Murashige and Skoog (ms) is more common (Khan et al. 1987; Murashige 1977; Qureshi et al. 1991; Street 1977).

Culturing of tissues. After samples of explants are collected from healthy young plants at the juvenile stage, and before proceeding further, the worker should disinfect his hands and tools with 70-80% ethyl alcohol to avoid any contamination. The samples are disinfected with a proper disinfectant along with a few drops of detergent under a laminar flow cabinet, and then rinsed thoroughly with autoclaved water to remove the residual toxic effect of the disinfectant. The terminal and lateral buds are removed from the sample with a scalpel and are placed in autoclaved test tubes or jars containing solidified or liquid nutritive medium. Then these test tubes/jars are covered with autoclaved polythene sheeting and closed with rubber bands.

The tubes/jars are labelled with a marker, indicating the treatment, variety, date, and any other essential information. The trays of culture tubes/jars should be placed in the culture room for growth.