PRODUCTION OF VIRUS FREE PLANTS

Mersitem culture is often useful in recovering virus free plants from virus infected plants or clones

Eg. (potato, sugarcane)

What is a meristem?

A localized group of actively dividing cells from which permenant tissue system, i.e., root, shoot, leaf , and flower are derived. Thye contain only one pair of subtending leaves

Apical meristem?

The apical meristem is a group of cells , situated at the extreme tip of the shoot or root in the shape of dome. This shoot meristem measures approximately 0.1 mm in diameter and 2.5 to 3 mm in length covered by developing leaf primordial. The apical meristem remain in an active state during the vegetative growth phase and they are in a permanent embryonic stage.

How meristem tip culture useful for micropropagation ?

The cells constituting the meristems are highly conservative and hence genetically stable so the plants regenerated from the plants are genetically identical.

Why plants derived from meristem tip culture are free from virus?

Since, the rate of cell division is higher the virus is not able to cope up with the same and get eliminated. Further the absence of plasmodesmata and vascular elements in the meristem greatly hinders the transport of virus particles leading to very low concentration in meristem.

Meristem culture

In vitro culture of a generally shiny, domelike structure measuring less than 0.1 mm in length when excised , most often excised from the shoot apex

First application of meristem was to obtain virus free plants of Dhalias. Considerable expertise is required to dissect out the shoot apical meristem with only one or two leaf primordia.

Meristem tips are carefully dissected away from the apical or lateral shoot buds under a steremicroscope under aseptic condition. After this though most mersitems can be directly on suitable culture medium without surface sterilization . Meristem can be directly inoculated on a suitable culture medium without surface sterilization . Meristems are usually cultured on solid medium occasionally on liquid media supplemented with a low concentration of cytokinins and a moderate level of auxins. Further, transferred to root development media for improving root growth. After a period of 2-6 months the plants are transferred to green house and sample plants are tested for the presence of virus by ELISA and PAGE techniques

Sometimes mersitem tip cuture have failed to eliminate virus infection because the explant contains shoot apices with vascular tissue instead of true meristem. Meristem tip culture combined with heat treatment (thermo therapy) or chemical treatment (chemotherapy) has proved to be very effective in virus eradication.

Thermo therapy

Explants are exposed to the incidence of higher temperature which are not lethal for plant cells, but they are lethal for viruses. Mostly used temperature range is 50 – 52 ° C with exposition about 10 30 minutes. In case of whole plant, lower temperature has to be used (32 – 40 °with exposure for 4 to 30 days

Chemotherapy

By using some of the chemicals viz., malchite green, ribavirin or 2 thiouracil when added to the medium it kills the virus in the explants

Shoot tip grafting *in vitro* and micrografting

Shoot tip grafting (STG) in vitro, also known as micrografting is extremely beneficial with woody species . This method involves micrografting of aseptically isolated, very small shoot tips consisting of meristems with 2 – 3 leaf primordia to in vitro grown virus free seedling (root stock) . This method is employed in citurs, apple, prunus plants.

Virus indexing

It may be emphasized that all the plants obtained through meristem culture with or without chemo or thermotherapy are not virus free. Therefore, such plants have to be tested for the presence of the concerned virus. This is called virus indexing.

* The simplest method for virus indexing is to score the plants for the presence of specific symptoms produced by the relevant virus
* The saps from test plant may be used to inoculate highly sensitive and healthy indicator plants
* A highly sensitive and precise technique for virus indexing embryos is ELISA test (Enzyme Linked Immuno Sorbant Assay) It is more convenient, rapid and efficient when a large number of plants are handled
* DNA and RNA probes can also be used for virus detection

Virus have eliminated form a number of economically important plant species including the following:

* + Mottle virus free from cassava
  + Cauliflower mosaic virus from cauliflower
  + Other crop spp include garlic, pineapple, dahlia, cymbidium, orchid, carnation, straw berry, iris, lily, apple, cassava, banana, raspberry, sugarcane, grape, potato, ginger