# Application of biotechnology in plant disease management – Importance, production of pathogen free plants through tissue culture techniques

In modern terms "biotechnology' is defined as the manipulation, genetic modification and multiplication of living organisms through novel technologies, such as tissue culture and genetic engineering, resulting in the production of improved or new organisms and products that can be used in a variety of ways.

Genetic engineering is the technology by which it is possible to isolate particular gene from one organism, insert them into the genome of another organism and make them to express at right time. Cells of plants can be cultured in special nutrient medium and whole plants can be regenerated from cultured cells. This technique of growing plants in *vitro* is called "Tissue culture".

In calli derived from infected tissues not all cells uniformly carry the pathogen. Only 40% of the single cells mechanically separated from TMV - infected tobacco callus contained the virus. The two possible reasons for the escape of some cells of a systemically infected callus from virus infection are -. a) virus replication is unable to keep pace with cell proliferation, and b) some cells acquire resistance to virus infection through mutagenesis. Cells resistant to virus at back may even exist in the parent tissue together with susceptible ones. Several disease resistant plants have been evolved using somoclonal variation. Out of 370 tomato plants regenerated from calluses six showed resistant to TMV. Similarly, late blilght *(Phytophthora infestans)* - resistant potato plants and bacterial blight of rice resistant calli have been evolved.

The pathogen produced secondary metabolites can be used to screen calluses for evolving disease resistant plants. Toxins will kill the calluses, but the mutant toxin resistant calluses will survive. The regenerated toxin resistant calluses yielded disease resistant plants. Brown spot pathogen *(Helminthosporium oryzae)* produced a host specific toxin for which resistant plants have been successfully developed. Similariy, *Helminthosporium maydlis* - toxin resistant maize plants, *Phytophthora infestans* - resistant tobacco plants, *H. sacchari* resistant sugarcane plants have been evolved. Somaclonal variation refers to the tissue culture derived variation- Plants regenerated from somatic cells, using tissue culture. are not genetically uniform but display significant genetic variability. This variability is very high when compared to spontaneous mutation. Somacional variation has been deryionstrated in a large number of plant species

(wheat, rice, oats, maize, tobacco, potato, sugarcane, brassica, etc.) for various traits such as resistance to fungal, viral and bacterial diseases. The procedure involves growing of cell cultures for several cycles on nutrient medium without any selective agent, followed by regeneration of plants.

The regenerants and their progenies are screened for disease resistance. Embryo rescue and protoplast fusion techniques are important to obtain hybrids among incompatible species and introgression of alien genes for disease resistance. In number of cases, useful genetic variability in the cultivated germplasm for resistance to diseases is either limited or lacking. Under such situations, wild germsplasm seems to be a reservoir of useful genes for disease resistance. In the incorporation of alien genes, several crossability barriers are encountered. In many cases, the hybrid embryo aborts. However, the excised hybrid embryos when cultured on nutrient medium ran be grown to plantlets. To incorporate alien genes from divergent sources, embryo rescue appears to be promising.

Tissue culture in conduction with recombinant DNA technology is becoming increasingly important to insert foreign genes and produce transgenic plants. For successful infection of virus particles, the coat protein should be removed from viral RNA. If the host is made to synthesize coat proteins in large amount, naked viral RNA formation will be negligible. The host coat protein will encapsulate the RNA of the virus and prevent its multiplication. This will result in reduction and delay in symptom development. Eg. Transgenic tobacco plants expressing the tobacco mosaic virus coat protein protected the plants against this virus.

The expression of the viral genome in transgenic plants also conferred resistance to virus infection. These regions include portion of the viral replicas as well as, antisense RNA to coat protein. Transgenic tobacco plants transformed with a DNA copy of the satellite RNA of cucumber mosaic virus (CMV) were shown to produce large amounts of satellite RNA following inoculation with CMV and symptom development was greatly reduced.

Proteins with the ability to inhibit the growth of fungi *in vitro* are abundantly present in plants. Constitutive expression of these genes in transgenic plants may render these plants to fungus resistant. Transgenic tobacco plants constitutively expressing bean chitinase have been shown to display enhanced resistance to *Rhizoctonia solani*. Recently, tobacco plants expressing a ribosome inactivating protein (RIP) from barely showed resistance to *R. solani.* The RiPs do

not inactivate self ribosomes and show activity towards ribosomes of distantly related species including those from fungi.

The constitutive expression of the groundnut stilbene synthase gene in transgenic tobacco plants results in the synthesis of resveratrol (phytoalexin) and the transgenic plants show resistance to *Botrytis cinerea.*

Transgenic tobacco plants expressing acetyltransferase which detoxifies the tabtoxin, show resistance to *Pseudomonas syringae* pv. *tabaci.* More recently, chitinase gene from *Manduca sexta,* tobacco horn worm, has been cloned into *P.fluorescens* to increase their antagonistic potential against R.solani.

**Meristem or shoot tip culture**

Meristem and shoot tip culture are used to eliminate virus from infected germplasm. It has long been observed that the rapidly growing meristems of plants are usually free of viruses, or at least have much lower concentration of viruses than nonmeristem cells. This situation has been exploited forthe production of virus-free plants by meristem culture. It is commanly used in cassava, potato, sweet potato and ornamental plants.

"Virus-free" the term has been loosely used in literature. Plants infected with more than one type of virus and also may carry some unknown viruses. Thus, a plant can be claimed as free of only those viruses for which specifictests have givenne gative result sowever, the term "virus-free" is still retained by horticulturists for its commercial value.

Pathogen attack does not always lead to death of the plant. Many viruses may not even show visible symptoms. However, the presence of viruses in the plants can reduce the yield and quality of crops. It is well known that the distribution of viruses in plants is uneven. In infected plants, the apical meristems are generally either free or carry a very low concentration of the viruses. In the older tissues, the titre of the viruses increases with increasing distance from the meristem tips.

Five main possibilities have been suggested to explain the mechanisms underlying the 'resistance'of meristems to viruses.

(i) Exclusion of the viruses from the meristems by lack of suitable vascular or plasmodesmatal connections.

(ii) Competition for key metabolites by the rapidly dividing meristem cells.

(iii) The production of substances in meristem cells that result in breakdown of the virus.

(iv) Deficiency in some key components of the machinery of virus replication, and

(v) Presence of inhibitors of virus replication.

**Factors affecting virus eradication**

Factors such as culture medium explant size and culture storage influence the virus eradication. In addition, heat treatment before or during culture significantly influences the efficiency of this technique. The physiological stage of the explants also affects virus elimination by meristem tip culture.

(i) The success in obtaining complete plants can be considerably improved by the choice of the culture medium. The major features of the culture medium to be considered are its nutrients, growth regulators and physical mature.

(ii) The size of meristem tip is an important factor governing regeneration capacity of meristems and to obtain virus free plants. For example, in cassava, meristems exceeding 0.2 mm size regenerated to plantlets, but those less than 0.2 mm size developed either Gallus or callus with roots. In general, the larger the meristem, the greater is the number of regenerated plants, but the numberof virus free plant is inversely proportional to the size of meristem cultured.

(iii) For meristem - tip cultures light incubation has generally proved better than dark incubation. The optimum light intensity for initiating tip cultures of potato is 100 lux, which should be increased to 200 lux after 4 weeks. The cultures are generally stored understandard culture room temperatures (25 ± 2ºC).

(iv) Meristem tips should, preferably be taken from actively growing buds. Tips taken from terminal buds gave better results than those from axillary buds.

**Meristem tip culture to eliminate Cassava Mosaic Virus**

Rapidly growing vegetative buds are excised, rinsed with sterile distilled water and then disinfected by immersing them in mercuric chloride solution (0.1%) for 2-3 minutes. The buds are then rinsed with several changes of sterile distilled water. Under the microscope, 3-4 leaf primordia (0.3 to 0.6 mm in size) is removed from the bud with a sterile scalpel. The buds are then aseptically transferred to Murashige and Skoog (MS) medium in test tubes and incubated at 25 ± 2’C in light, for 45 days. The plantlets are then removed from the test tubes, washed in tab water and kept in Hoagland solution for 3-4 days for hardening. The plantlets are transferred to pots containing peat soil and vermiculite at 3:1 ratio and kept in mist chamber for 5-7 day. The plants are then transferred to glass house for further study.