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# Advanced Applications of Biotechnology in Plant Disease Management

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Biotechnology is defined as genetic modification and manipulation of living organisms through the novel technologies such as tissue culture and genetic engineering resulting in production of improved or new organisms that can be used in variety of ways.

## Application of Biotechnology in Plant Disease Management

**1. Diagnosis of plant diseases**

a) Diagnostic kits helps in identification of plant diseases, viz., bacterial canker of tomato, soybean root rot, viral diseases of potato, etc., at an early stage of development and helps in devising suitable management practices.

b) Polymerase Chain Reaction (PCR): Detection of very small amount of pathogen in a sample by amplifying the pathogen sequences to a detectable level. PCR is especially used in plant quarantine.

**2. Strain improvement of biocontrol agents**: It has the following advantages

a) Expanding the range of target species
b) Restricting the range of non-target species
c) To improve the survival ability or rhizosphere competence
d) Expanding the bio-agents environmental range beyond its congenial habitat
e) Development of fungicide tolerant strains

**3. Transgenics for plant disease management**

a) Coat protein mediated resistance for papaya ring spot virus in Hawaii islands

b) Cloning of resistance genes, viz., Xa 21, bacterial blight resistance gene isolated from African rice, Oryza longistaminata was introduced into cultivable rice, Oryza sativa

**4. Determination of biochemical nature** and the signals involved in plants reaction to pathogen invasion and disease development. Ex: Host-pathogen interaction has been studied in rice blast disease incited by Magnaporthe grisea.

**5. Manipulation of resistance of host** by expression of PR-proteins, antifungal peptides, etc. Ex: Expression of multiple PR-proteins (Chitinases and β-1,3 glucanases) in rice enhanced disease resistance to rice sheath blight pathogen, Rhizoctonia *solani*.

## Plant Tissue Culture

*In vitro* culture of plant cells, tissues as well as organs. **Totipotency** is the ability of a plant cell to perform all the functions of development which are characteristic of zygote, i.e., its ability to develop in to a complete plant.

## Important Tissue Culture Techniques of Importance to Plant Pathology

1. Meristem tip culture
2. Protoplast culture

**A. Production of virus free plants through plant tissue culture: Meristem tip culture**: Cultivation of axillary or apical meristems, particularly of shoot apical meristem, is known as meristem culture.

1. **Explant**: the explant must consist of the meristematic dome of cells together with atleast one leaf primordial. Meristem tips varying in size from 0.1 to 2.0 mm in diameter (usually 0.3-1.5 mm) can be used for meristem tip culture. The infected parent plant or organ of the plant from which explant is excised is generally subjected to thermotherapy in a temperature controlled cabinet at $30^0$C to $40^0$C for six to twelve weeks to inactivate the virus.
2. **Culture initiation on suitable medium**: In general Murashige and Skoog medium has been found satisfactory for most plant species. But for some species, a much lower salt concentration may be adequate or even necessary since the high salt concentration of MS medium may be deleterious or even toxic. Culture initiation consists of surface sterilization of explants and establishing them in vitro on culture medium. Culture
3. initiation often involves anti-metabolite chemicals such as ribavirin (virazole) in the tissue culture medium.
4. **Shoot multiplication**: After 2-3 weeks, the cultures are transferred to a shoot multiplication medium designed to promote axillary branching. This medium generally contains cytokinins, either alone or in combination with an auxin. Higher concentration of cytokinins induces adventitious buds. During culture initiation and shoot multiplication phases, the cultures are generally kept at $25^0$C.
5. **Rooting of shoots**: In general, the rooting medium has low salt (1/2 or even ¼ salts of MS medium) and reduced sugar levels. But in most species, 0.1-1 mg/l Naphthalene Acetic Acid (NAA) or Indole-3-Butyric acid (IBA) is required for rooting. Rooting takes about 10-15 days depending on species.
6. **Transfer of plantlets to soil**: Rooted shoots are removed from the medium, agar sticking to roots is washed with tap water, and they are transplanted into plastic cups containing a suitable potting mix. Plants are kept in high (>90%) humidity and initially low light intensities. The humidity is generally decreased to the ambient level after about 7-15 days, and the light intensity is increased. The plants are finally exposed to greenhouse conditions (**hardening**).
7. **Indexing, clone selection and stock maintenance**: Virus indexing is done several times during first year and the virus free plantlet is used as a nuclear stock material for commercial multiplication. Virus indexing is generally made by Enzyme Linked Immuno-Sorbent Assay (ELISA) or Immuno Sorbent Electron Microscopy (ISEM).

**B. Protoplast culture**: Fungal protoplasts are important tools in physiological and genetic research. Interspecific, intraspecific and intrageneric hybridization could be done by this technique for strain improvement of biocontrol agents to enhance the biocontrol potential for the management of pathogenic fungi. Isolation and self-fusion of protoplasts were achieved in *Trichoderma harzianum* and *T. viride.*

**Steps in protoplast fusion**

1. Isolation of protoplasts is achieved by treating cells with a suitable mixture of cell wall degrading enzymes.
2. The pH of enzyme solution is adjusted between 4.7 and 6.0 and temperature is kept around 25-$30^0$C. The osmotic concentration of enzyme mixture and of subsequent media is elevated to stabilize the protoplasts and to prevent them from bursting. Usually, 50-100 m mol/l $CaCl_2$ is added to the osmoticum as it improves plasma membrane stability.
3. The protoplasts of different strains are treated with 28-50% **Poly Ethylene Glycol** (**fusogen**) for 15-30 min followed by gradual washing of the protoplasts to remove PEG.

The washing medium may be alkaline and contain high calcium ion concentration (50 m mol/l). Protoplast fusion occurs during washing step.

4. Selection of hybrid cells and culturing on suitable medium.

## Gene cloning/ Recombinant DNA technology / Genetic engineering

Integration of specific fragment of foreign DNA into a cell through a suitable vector in such a way that the inserted DNA replicate independently and transferred to progenies as a result of cell division. Recombinant DNA molecule is a vector into which the desired DNA fragment has been inserted to enable its cloning in an appropriate host. Recombinant DNA molecule is produced by joining together two or more DNA segments usually originated from different organisms.

**Steps in gene cloning**

1. Identification and isolation of the desired gene or DNA fragment to be cloned (Restriction digestion and electrophoresis)
2. Insertion of the isolated gene in a suitable vector (ligation)
3. Introduction of this vector into a suitable organism or cell called host (transformation)
4. Selection of transformed host cells (selectable markers)
5. Multiplication / integration followed by expression of the introduced gene in the host