TOPIC : SOMATIC HYBRIDIZATION
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Somatic Hybridization

Somatic hybridization is the technique that allows manipulation of the cellular genome by a process called protoplast fusion. It is a type of genetic modification in plants by which two distinct species of plants are fused together to form a new hybrid plant with the characteristics of both. It may be intraspecific, interspecific, intrageneric, and intergeneric. Somatic hybridization was first introduced by Carlson et al. in *Nicotiana glauca*.

Sexual hybridization has been the conventional method to improve the characteristics of cultivated plants, for years. The major limitation of sexual hybridization is that it can be performed within a plant species or very closely related species. This restricts the improvements that can be done in plants. The incompatibility barriers in plant improvement during sexual hybridization can be overcome by somatic cell fusion resulting in viable hybrids. Hybrids have been produced either between different varieties of the same species (e.g. between non-flowering potato plants and flowering potato plants) or between two different species (e.g. between wheat *Triticum* and rye *Secale* to produce *Triticale*). The resulting hybrid has the chromosomes of both plants and is thus similar to polyploid plants. Somatic hybridization broadly involves *in vitro* fusion of isolated protoplasts to form a hybrid cell and its subsequent development to form a hybrid plant. Somatic hybridization involves three aspects:

(A) Fusion of Protoplasts
(B) Selection of Hybrid Cells and
(C) Identification of Hybrid Plants

Plant protoplasts are of immense utility in somatic plant cell genetic manipulation and crop improvement. Protoplasts of sexually sterile (haploid, triploid, and aneuploid) plants can be fused to produce fertile diploids and polyploids. Thus, protoplasts provide a novel opportunity to create cells with new genetic traits. And protoplast fusion is a wonderful approach to overcome sexual incompatibility between different species of plants. These plants show an application in industries and agriculture.

A. Fusion of Protoplasts:

As the isolated protoplasts are devoid of cell wall, *in vitro* fusion becomes relatively easy. There are no barriers of incompatibility (at interspecific, inter-generic, or even at inter-kingdom levels) for the protoplast fusion. Somatic cell fusion appears to be the only means through which two different parental genomes can be recombined among plants that cannot reproduce sexually (asexual or sterile). Protoplast fusion involves the

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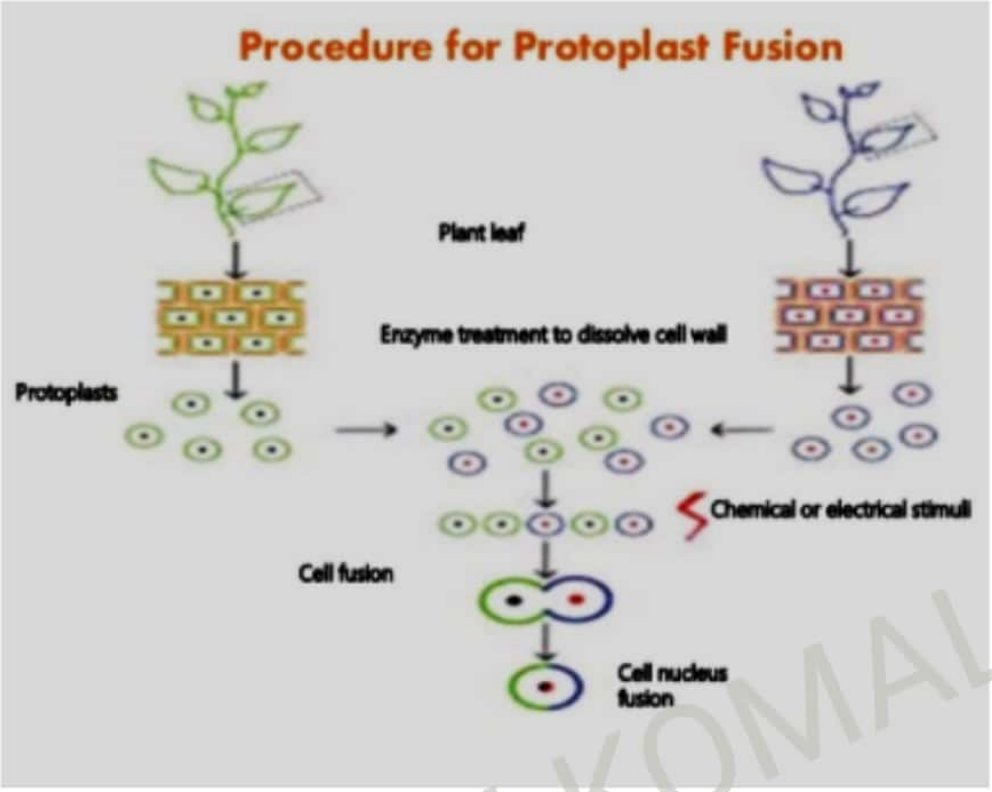
mixing of protoplasts of two different genomes This can be achieved by spontaneous, mechanical, or induced fusion methods.



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Spontaneous fusion:

Cell fusion is a natural process as is observed in case of egg fertilization. During the course of enzymatic degradation of cell walls, some of the adjoining protoplasts may fuse to form homokaryons.These fused cells may sometimes contain high number of nuclei (2- 40). Spontaneously fused protoplasts, however, cannot regenerate into whole plants, except undergoing a few cell divisions.

Mechanical fusion:

The protoplasts can be fused by pushing them together mechanically. Protoplasts of *Lilium* and *Trillium* can be fused in enzyme solution by gentle trapping in a depression slide. Mechanical fusion may damage protoplasts by causing injuries.

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Induced fusion:

Freshly isolated protoplasts can be fused by induction. There are several fusion-inducing agents which are collectively referred to as fusogens e.g. NaN03, high pH, Ca2+, polyethylene glycol, polyvinyl alcohol, lysozyme, dextran, dextran sulfate, fatty acids and esters as well as electro fusion. Some of the fusogens and their use in induced fusion are described.

Mechanism of fusion:

The fusion of protoplasts involves three phases agglutination, plasma membrane fusion and formation of heterokaryons.

1. Agglutination (adhesion):

When two protoplasts are in close contact with each other, adhesion occurs. Agglutination can be induced by fusogens e.g. PEG, high pH and high Ca2+.

2. Plasma membrane fusion:

Protoplast membranes get fused at localized sites at the points of adhesion. This leads to the formation of cytoplasmic bridges between protoplasts. The plasma membrane fusion can be increased by high pH and high Ca2+, high temperature and PEC, as explained below.

(a) High pH and high Ca2+ ions neutralize the surface charges on the protoplasts. This allows closer contact and membrane fusion between agglutinated protoplasts.

(b) High temperature helps in the intermingling of lipid molecules of agglutinated protoplast membranes so that membrane fusion occurs.

(c) PEG causes rapid agglutination and formation of clumps of protoplasts. This results in the formation of tight adhesions of membranes and consequently their fusion.

3. Formation of heterokaryons:

The fused protoplasts get rounded as a result of cytoplasmic bridges leading to the formation of spherical homokaryon or heterokaryon.

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B. Selection of Hybrid Cells:

About 20-25% of the protoplasts are actually involved in the fusion. After the fusion process, the protoplast population consists of a heterogenous mixture of un-fused chloroplasts, homokaryons and heterokaryons. It is, therefore, necessary to select the hybrid cells (heterokaryons). The commonly used methods employed for the selection of hybrid cells are biochemical, visual and cytometric methods.

Biochemical methods:

The biochemical methods for selection of hybrid cells are based on the use of biochemical compounds in the medium (selection medium). These compounds help to sort out the hybrid and parental cells based on their differences in the expression of characters.

Drug sensitivity and auxotrophic mutant selection methods are described below:

1. Drug sensitivity:

Drug sensitivity technique was originally developed by Power et. al. (1976) for the selection of hybrids of *Petunia sp*. A similar procedure is in use for the selection of other somatic hybrids e.g., hybrids between *Nicotiana silvestris* and *Nicotiana knightiana*. This method is useful for the selection hybrids of two plant species, if one of them is sensitive to a drug. Protoplasts of *Petunia hybride* (species A) can form macroscopic callus on MS medium, but are sensitive to (inhibited by) actinomycin D. *Petunia parodii* protoplasts (species B) form small colonies, but are resistant to actinomycin D. When these two species are fused, the fused protoplasts derive both the characters -- formation of macroscopic colonies and resistance to actinomycin D on MS medium. This helps in the selection of hybrids. The parental protoplasts of both the species fail to grow. Protoplasts of *P. parodii* form very small colonies while those of *P. hybrida* are inhibited by actinomycin D.

2. Auxotrophic mutants:

Auxotrophs are mutants that cannot grow on a minimal medium and therefore require specific compounds to be added to the medium. Nitrate reductase deficient mutants of tobacco (*N. tabacum*) are known. The parental protoplasts of such species cannot grow with nitrate as the sole source of nitrogen while the hybrids can grow. Two species of nitrate reductase deficiency — one due to lack of apoenzyme (nia-type mutant) and the other due to lack of molybdenum cofactor (cnx- type mutant) are known. The parental protoplasts cannot grow on nitrate medium while the hybrid protoplasts can grow.

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Visual methods:

Visual selection of hybrid cells, although tedious is very efficient. In some of the somatic hybridization experiments, chloroplast deficient (albino or non-green) protoplasts of one parent are fused with green protoplasts of another parent. This facilitates the visual identification of haterokaryons under a light microscope. The heterokaryons are bigger and green in colour while the parental protoplasts are either small or colourless. Further identification of these heterokaryons has to be carried out to develop the specific hybrid plant. There are two approaches in this direction — growth on selection medium, and mechanical isolation.

1. Visual selection coupled with differential media growth:
There exist certain natural differences in the sensitivity of protoplasts to the nutrients of a given medium. Thus, some media can selectively support the development of hybrids but not the parental protoplasts.

2. Mechanical isolation:
The visually identified heterokaryons under the microscope can be isolated by mechanical means. This involves the use of a special pipette namely Drummond pipette. The isolated heterokaryons can be cloned to finally produce somatic hybrid plants. The major limitation of this method is that each type of hybrid cell requires a special culture medium for its growth. This can be overcome by employing micro drop culture of single cells using feeder layers .

C. Identification of Hybrid (Cells) Plants:
The development of hybrid cells followed by the generation of hybrid plants requires a clear proof of genetic contribution from both the parental protoplasts. The hybridity must be established only from euploid and not from aneuploid hybrids. Some of the commonly used approaches for the identification of hybrid plants are briefly described.

Morphology of hybrid plants:

Morphological features of hybrid plants which usually are intermediate between two parents can be identified. For this purpose, the vegetative and floral characters are considered. These include leaf shape, leaf area, root morphology, flower shape, its structure, size and colour, and seed capsule morphology.
The somatic hybrids such as pomatoes and topatoes which are the fused products of potato and tomato show abnormal morphology, and thus can be identified. Although the genetic basis of the morphological characters has not been clearly known, intermediate

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morphological features suggest that the traits are under the control of multiple genes. It is preferable to support hybrid morphological characters with evidence of genetic data.

Isoenzyme analysis of hybrid plants:

The multiple forms of an enzyme catalysing the same reaction are referred to as isoenzymes. Electrophoretic patterns of isoenzymes have been widely used to verify hybridity. Somatic hybrids possess specific isoenzymes (of certain enzymes) of one or the other parent or both the parents simultaneously.
There are many enzymes possessing unique isoenzymes that can be used for the identification of somatic hybrids e.g. amylase, esterase, aspartate aminotransferase, phosphodiesterase, isoperoxidase, and hydrogenases (of alcohol, lactate, malate). If the enzyme is dimeric (having two subunits), somatic hybrids usually contain an isoenzyme with intermediate mobility properties. The isoenzymes are often variable within the same plant. Therefore, it is necessary to use the same enzyme from each plant (parents and somatic hybrids), from a specific tissue with the same age.

Symmetric and asymmetric hybrids:

If the chromosome number in the hybrid is the sum of the chromosomes of the two parental protoplasts, the hybrid is said to be symmetric. Symmetric hybrids between incompatible species are usually sterile. This may be due to production of 3n hybrids by fusing 2n of one species with n of another species. Asymmetric hybrids have abnormal or wide variations in the chromosome number than the exact total of two species. These hybrids are usually formatted with a full somatic complement of one parental species while all or nearly all of the chromosomes of other parental species are lost during mitotic divisions. Asymmetric hybrids may be regarded as cybrids but for the introgressed genes. Protoplast fusion between *N. tabacum* (2n = 48) and *N. nesophila* (2n = 24) results in a symmetric hybrids, while asymmetric hybrids are formed when *B. napus and B. junea* are fused.

Appications of Somatic Hybridization:

Somatic hybridization has opened new possibilities for the *in vitro* genetic manipulation of plants to improve the crops.

1. Disease resistance:
Several interspecific and intergeneric hybrids with disease resistance have been created. Many disease resistance genes (e.g., tobacco mosaic virus, potato virus X,

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club rot disease) could be successfully transferred from one species to another. For example, resistance has been introduced in tomatoes against diseases such as TMV, spotted wilt virus and insect pests.

2. Environmental tolerance:
The genes responsible for the tolerance of cold, frost and salt could be successfully introduced through somatic hybridization, e.g., introduction of the cold tolerance gene in tomatoes.

3. Quality characters:
Somatic hybrids for the production of high nicotine content, and low erucic acid have been developed.

4. Cytoplasmic male sterility:
Some of the genetic traits in certain plants are cytoplasmically controlled. This includes some types of male sterility, resistance to certain antibiotics and herbicides.

Limitations of Somatic Hybridization:

1. Somatic, hybridization does not always produce plants that give fertile and visible seeds.

2. Regenerated plants obtained from somatic hybridization are often variable due to somaclonal variations, chromosomal elimination, organelle segregation etc.

3. Protoplast culture is frequently associated with genetic instability.

4. Some of the somatic hybrids, particularly when produced by the fusion of taxonomically different partners, are unbalanced and not viable.

5. There are limitations in the selection methods of hybrids, as many of them.

Cybridization

The cytoplasmic hybrids where the nucleus is derived from only one parent and the cytoplasm is derived from both the parents are referred to as cybrids. The phenomenon of formation of cybrids is regarded as cybridization. Normally, cybrids are produced

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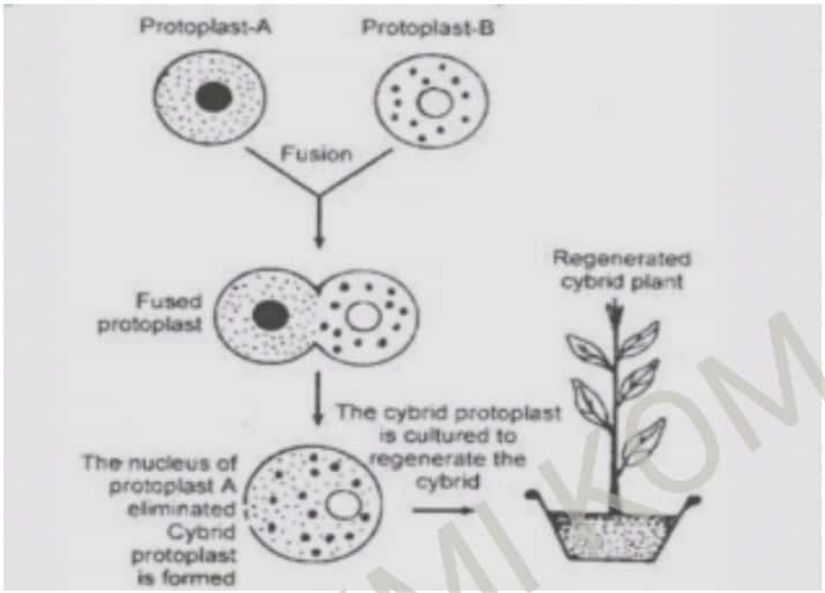
when protoplasts from two phylogenetically distinct species are fused. Genetically, cybrids are hybrids only for cytoplasmic traits.



A diagrammatic representation of the formation of hybrids and cybrids

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Applications of Cybrids:

Cybridization is a wonderful technique wherein the desired cytoplasm can be transferred in a single step. Cybrids are important for the transfer of cytoplasmic male sterility (CMS), antibiotic and herbicide resistance in agriculturally useful plants. Some of the genetic traits in certain plants are cytoplasmically controlled. This includes some types of male sterility, resistance to certain antibiotics and herbicides. Cybridization has been successfully used to transfer CMS in rice. Cybrids of *Brassica raphanus* that contain nucleus of *B. napus*, chloroplasts of atrazine resistant *B. campestris* and male sterility from *Raphanus sativus* have been developed.

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