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Cybrids modern application of plant biotechnology

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Cybrids are cells with nucleus of one species but cytoplasm from both the parental species. It may be single cell or a complete growing plant called cybrid plant.

The process of protoplast fusion resulting in the development of cybrid is known as cybridization. Cybrids are basically cells produced from two parental cells, one complete cell cytoplasm with nucleus and other should be without nucleus or inactive nucleus.

Hence nucleus of one cell inactivated by using gamma rays and X-rays. Therefore, self-activeness of chromosome loses through this. Otherwise we can also remove the nucleus from one parental cells.

Cybrids contain nucleus of one species but cytoplasm of both the parents (has genetic material of only one parent), whereas somatic **hybrids** contain genetic material of both the parents. Somatic hybrids don't have species barrier, whereas cybrids are bound to species barrier.

History:

In 1892 Klercker was first to isolate protoplast using mechanical method. Cocking in 1960 was first to report isolation of protoplast from tomato root tips using concentrated solutions of cellulase from fungus. Therefore, enzymes for protoplast isolation was first employed by Takebe and his coworkers in 1968. Kao and Michayluk in 1974 first proposed PEG for fusion of protoplast. Gleba 1979 fused tobacco protoplast which produced a cybrid.

Need for cybrid?

The conventional method to improve characteristics of cultivated plants, for years, has been sexual hybridization. Hence species barriers for plant improvement come across in sexual hybridization can be overcome by cybrids that form a viable hybrid. Consequently, this method is a way to combine those species which does not perform sexual reproduction.

Hence make a desired species combination (Sexually incompatible). In this mitochondrial gene also combined with chloroplast gens of another species. Therefore protoplast fusion is a wonderful approach to overcome sexual incompatibility between different species of plants.

- Production of full hybrids through protoplast fusion of distantly related plants. Impractical wide spread instability of the two different genomes in common cytoplasm.
- Undesirable-exhibit structural and developmental abnormalities.
- Partial genome transfer. Fusion of normal protoplasts of the recipient with enucleated protoplasts of the donor-cybridization.

How to produce cybrids?

1. From two phylogenetically distant species cybrids are produced during fusion.
2. Regeneration from phylogenetically distant species will have plastomes from both parental species but the functional genome of only one species through chromosomal elimination.
3. The extranuclear genes(that occur outside the nucleus) which control agronomically important characters are of considerable interest.

Steps involved in protoplast fusion:

1. Isolation of protoplasts
2. Culture of protoplasts
3. Fusion of protoplasts
4. Identification and selection of hybrid cells. Then their subsequent regeneration of protoplast into whole plants.

Cybridization technique:

1. Before protoplast fusion nuclear genome of one parent “donor” is inactivated chemically or by using irradiations.
2. Irradiation with x-rays or gamma rays, in doses of 50 to 300 Gy. That is effective in partial or complete inactivation of donor cells.
3. Fusion of untreated “recipient” protoplast with “donor” and culturing result in cybrid plants. That contain the nucleus of the “recipient” and the cytoplasm of both parental species.
4. Hence genetically distant species produce cybrids during protoplast fusion.

Are cybrids useful in plant breeding?

At first look it seems of little importance to work with organelle DNA. Since it represents between two of the total higher plant DNA. In fact, this DNA carries very important traits, chloroplastic as well as mitochondrial, as illustrated by the following observations:

1. Depending on the nucleus, a chloroplast genome can lead to different levels of chlorophyll content
2. Chloroplast DNA also codes some herbicide resistance traits, such as atrazine resistance
3. Furthermore, it had revealed by the protoplast fusion experiments that the cytoplasmic male sterile (CMS) trait is a result of a nucleus-mitochondrial interaction.

Applications of Cybrids

- ### Disease Resistance:

Hybrids have been created that are inter-specific and inter generic. Hence from one species to another many disease resistance genes (e.g., tobacco mosaic virus, potato virus X) could be successfully transferred. Therefore, In tomato, resistance has been introduced against diseases such as TMV, spotted wilt virus and insect pests.

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Environmental Tolerance:

The genes responsible for tolerance of cold, frost and salt could be successfully introduced e.g., introduction of cold tolerance gene in tomato.

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Cytoplasmic male sterility:

A modification of hybridization in the form of cybridization has made it possible to transfer cytoplasmic male sterility (tomato, tobacco).

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Nuclear cytoplasmic combinations:

Protoplast fusion will help in the combination of mitochondria and chloroplasts to result in a unique nuclear-cytoplasmic genetic combination.

Advantages

- Two different parental genomes that cannot reproduce sexually (asexual or sterile) are recombined.
- Cybrids overcome sexual incompatibility barriers.
- Cybrids used in study of cytoplasmic genes & their activities- plant breeding experiments.
- Used to transfer antibiotic resistance character (tobacco)
- Cybrids are used to transfer herbicide resistance (brassica).
- Also used in mitochondrial research.

Limitations

- Biparental inheritance of cytoplasm during sexual reproduction occurs in only a few genera.
- Plant regeneration from proplast is often a difficult or even impossible task.
- Instability of transferred genes in somatic hybrids.
- Recovering controlled asymmetric hybrids due to factors like cell fusion, nuclear fusion and recombination.