

CELL FUSION

Abstract

Naked plant cells called as protoplast. protoplast can be called as a functional individual. Cell with plasma membrane as outermost layers and the only barrier between environment and the cell contents. Cell fusion is a cellular process in which several uninucleate cells combine to form a multinucleate cell fusion called as cell - cell fusion is a normal biological process playing essential roles in organ formation, tissue differentiation, repair and regenerations.

Keywords: Cell, Gametic fusion, Plant protoplast, Breeding, Molecules

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I. INTRODUCTION

In nature, gametic fusion forms zygote with a new combination of genes. Diploid nature is restored by haploid gametic fusion. Sexual crossing is one of the major source of genetic variability, incompatibility at various levels, before fertilization and after fertilization restricts recombination between distantly related species and species specific characters are maintained. A limited success has been achieved us obtaining interspecific and intergeneric hybrids by conventional breeding programmes and in the understanding of cytological and genetical behavior of chromosomes in these hybrids. However, it is not possible to obtain hybrids by conventional method in all the desired plant materials. Protoplasts fusion provides a novel approach overcoming sexual incompatibility and obtaining somatic hybrids. Fusion of somatic cells (2n) and production of hybrids is known as somalic cell fusion or somatic hybridization.

Plant protoplast isolated from species which are related but sexually incompatible can be fused either by using chemicals such as polyethylene glycol, dextran or poly-L-ornithine to act as fusogen or by electrofusion. After fusion, the nuclear and cytoplasmic genomes reassert and recombine, resulting in wide array of gene combination not obtainable through conventional breeding. The ability to transfer plastids is specially promising since traits such as cytoplasmic male sterility, disease resistance and herbicide resistance are enclosed by the organelles and not by the nuclear genome when fusion are mad between protoplasts of totally unrelated species e.g., tobacco and soyabean, the nuclei either fail to fuse or else one set of chromosomes is lost following fusion. In contrast, fusions between sexually incompatible species of same family have produced hybrid plants that can retain some chromosomes from both parents.

It was Kuster (1909) who proposed the ptotoplast fusion as tool for production of hybrids. Micheli (1937) demonstrated protoplasts fusion by treatment of sodium nitrate. It was only after 1970's that somatic hybridization has been used bot in basic plant science and for production of new variants for plant breeding. The man-mouse hybrid produced during 1960's thrilled the world. But animal cells are not totipotent and unfortunately this hybrid cell did not survive long.

II. PROCEDURE OF PARASEXUAL HYBRIDIZATION

The procedure of parasexual hybridization involves following steps.

1. Isolation of ptotoplasts
2. The induction of membrane fusion.
3. The mixing of cytoplasms and organelles.
4. The formation of synkaryones during the culture of hybrid cells.
5. The selection of fusion products
6. The regeneration of hybrid cells.
7. Molecular and bio-chemical characterization of primary regenerates and plants from the subsequent generation.

Membrane Properties: Plant membrane are flexible, asymmetric structure of membrane protiens integrated intophospholipid molecules. The fluid mosaic model with lipid bilayer is the established structure of intact membranes. The process of protoplasts fusion requires the

direct contact between membranes of two different cells. The adhesion of protoplasts depends on surface charge. According to electrophoretic studies outer membrane surface is negatively charged. This charge varies from -10 mV to -50 mV and can significantly be reduced by Ca^{2+} . Positively charged protoplasts can be obtained in the presence of poly-L-lysine, poly-L-ornithine or synthetic phospholipids. After establishment of protoplasts - protoplast contact, membrane fusion results by structural modifications and can be enhanced by fusogenic agents. Protoplast fusion is a non-specific event and hence fusion can be induced between any two protoplasts types. Due to these fusion properties of the membranes, protoplast fusion has been achieved between dicot and dicot, dicot and monocots, plant and insect cells (*Drosophila*), amphibian cells and mammalian cells. Several methods were proposed to induced protoplast fusion between haploid (gametic) and diploid (Somatic) cells has been achieved which clearly demonstrated that fusion is independent of cell type.

III. FUSION PROCEDURE

Protoplast of both types are mixed in an equal proportion attaining density of 5×10^4 to 2×10^5 protoplasts per ml. Protoplasts density is usually determined by haemocytometer. There are several chemical and physical agents which enhance fusion between protoplasts known as fusogen or fusion agent; e.g. sodium nitrate, polyethylene glycol of different molecular weight (PEG1500, PEG4000, PEG6000), dextran sulphate and gelatin etc. high calcium ion concentration and pH of the incubation mixture have profound effect on fusion percentage. Fusion is stimulated under electric charge as membranes are negatively charged.

Isolated protoplasts are suspended in an aggregation mixture (5.5% sodium nitrate 20 – 40%, PEG in 10% sucrose solution), kept at 35°C for 5 minutes and then centrifuged at 200g for 5 min to obtain dense pellet. The tube with pellet of protoplasts is once again transferred to a waterbath maintained at 30°C for 30 minutes during which most of the protoplasts undergo fusion. The protoplasts are gently washed and transferred to liquid medium or plated. The fusion and fusion products are observed in inverted microscope

IV. FUSION METHODS

Several methods were proposed to induce protoplasts fusion, earliest success was achieved using sodium nitrate, (Carlson et al., 1972). Plant protoplasts have uniformly negatively charged membranes. Divalent cations, especially Ca^{2+} , modify the electrophoretic mobility of protoplasts aggregation mediated by neutralization of plasma membrane negative charges by 50 mM Ca^{2+} mixed with 0.4 M mannitol in a solution of high pH (10.5) which has led to the genuine cytoplasmic fusions of tobacco protoplasts and *Petunia* protoplasts. The other fusogens are as follows.

1. **PEG:** Various fusogenic agents have been used for getting protoplasts fusion. The discovery of the powerful aggregation effect of polyethylene glycol (Kao and Michayluk, 1972) provided the awaited tool. A few minutes after being immersed in 20 – 40% (W/v) solution of PEG (1500 to 6000 MW) virtually all protoplasts exhibit adhesion. However, actual fusion occurs upon dilution of PEG. A combination of PEG induced adhesion followed by dilution with PEG, high Ca^{2+} , high pH was even more reliable actual role of PEG in the process of membrane fusion remains yet to be clearly determined. Addition of dimethyl sulphoxide (DMSO) with PEG has stimulatory effect

on protoplast fusion. It has been suggested that bridge formation mediated by Ca^{2+} and PEG through its slightly negative polarity might be the cause of PEG induced fusion of protoplasmic.

- 2. Dextran:** Furogenic properties of dextran and of polyvinyl alcohol suggest that weak non-ionic surfactant properties be involved. It seems perturbation of negative charges of the protoplast plasma lemma seems essential. This is illustrated by fusion induced by a positively charged phospholipids and all electrified induced fusion.
- 3. Electrofusion:** In electrofusion method of Zimmerman (1982) protoplasts are brought in close contact by a non-uniform alternating field between two electrodes, the fusion is initiated by field pulse of high voltage ($500\text{V}-3\text{KV}$, cm^{-1}) for a very short time (10-100 microseconds). This is most widely used apparatus and method in the studies on membrane fusion and for the production of somatic hybrid colonies or plants.

Examples of Protoplast Fusion

- The best example of protoplast fusion is 'Pomato' which is somatic hybrid of potato and tomato.
- The most useful hybrids produced are cytoplasmic male-sterile rice, oilseed rape resistant to the fungus *Phoma lingam*, and potato varieties resistant to certain viral disease.

V. APPLICATIONS OF PROTOPLASTS IN PLANT RESEARCH

Plant cells are normally connected to each other via many plasmodesmata in multicellular tissues. Plant protoplasts cultures provide an excellent system to obtain single cells of higher organism and study its functioning in a controlled environment. Protoplast isolation provides millions of cells which is comparable to microbial system and can be used many ways.

1. Single cell cloning can easily be performed with protoplast.
2. Somatic cell fusion can only be performed with protoplasts.
3. Genetic transformations through DNA or organelles uptake can be achieved using protoplasts.
4. Complete plants of single cell origin can be obtained, as plant cells are totipotent.
5. It is a unique experimental system to study de novo cell wall formation.
6. Protoplasts are very useful objects of membrane studies.
7. Protoplasts are also excellent experimental material for ultra-structural studies.
8. Protoplasts are very suitable for isolation cell organelles and because of absence of thick cell wall barrier.

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