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# Recent advances in the electrofusion of plant cells

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Electrofusion (EF) and electroporation (EP) offer a way to modify the cellular functions. Plant cells exposed to a field pulse of high intensity and short duration (ns to $\mu$s) lead to reversible electrical breakdown of the membrane. Such a field reversibly increases the membrane electrical conductivity and permeability, which facilitates the introduction of large molecules like proteins, DNA etc. through the plasma membrane, which are otherwise impermeable. This technique has many advantages over conventional methods of fusion. Electrofused cells remain viable and offer a convenient means to breed new plants of desired characteristics.

**Key words: Electrofusion, electroporation, reversible membrane breakdown**

## INTRODUCTION

Somatic hybridization and genetic engineering offer a way of modifying plant cells and, in turn, of improving crops. The disadvantages in the conventional fusion method based on the use of chemical agents or virus particles have been overcome by the electrofusion (EF) technique [1]. This technique is a combination of dielectrophoresis and controlled reversible electrical breakdown of the membrane. The original membrane properties are restored after a certain time interval. The mechanism of EF and electroporation are similar. The former requires the destabilization of two membranes at close approach and the latter, one single membrane. This review paper highlights the technical aspects EF and EP and its potential applications in plant breeding, agriculture.

## ELECTROFUSION

The experimental set up for EF is shown in Fig. 1 [1]. The fusion is carried out in an isotonic nonelectrolyte medium. The technique consists of two stages: (i) subjecting cells in a nonuniform alternating field of low intensity to induce "pearl chain" formation [2] and (ii) a d.c. pulse of short duration (ns to $\mu$s) and higher field strength (KV range) to elicit breakdown of the cell membrane [1] to induce fusion. Optimal experimental conditions for EF of plant cells are given in Table I [1]. It has been reported that the electrofused hybrid plants of cells are given in Table I [1]. It has been reported that the electrofused hybrid plants of Nicotiana remain stable and the seeds keep the hybrid character as predicted by Mendelian laws [3]. Fusion between any two cells appears to be possible. EF has resulted in the production of viable and high yield of fused cells (upto 100%).

## ELECTROPORATION

Electric impulses (1-20 kV.cm$^{-1}$ 1-5 $\mu$s) lead to reversible

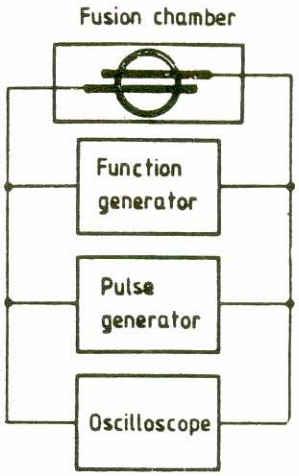


*Fig. 1: Experimental set up for EF*

pore formation and at subcritical field strengths, the pore radius increases [4]. Electropermeabilization facilitates direct transfer of DNA into cytoplasm and for the production of improved varieties and disease resistant plants [3]. Transient expression of genes in carrot protoplasts and stable expression in tobacco mesophyll protoplasts [5] and in Brassica [6] using shorter pulsing periods have been reported. Detection of somatic embryos in carrot cultures 45 days after electroporation with Ti plasmid DNA has been reported [7].

## CONCLUSION

Electrofusion technique may be applied to any kind of plant cells. This has great potentialities in agriculture, genetic engineering and in the production of improved varieties, disease resistant plants etc. They have advantages over the conventional method of fusion. High yield of viable and fused cells up to 100% may be obtained.

TABLE–I: EF of plant cells

| Fusion between | | Medium | Minimum electrode-distance | Dielectrophoretic collection | | Electrical breakdown pulse | | Fusion time |
|---|---|---|---|---|---|---|---|---|
| Cell 1 | Cell 2 | | | Maximum field strength | Frequency | Field strength | Duration | |
| | | | ($\mu$m) | (V/cm) (a) | (MHz) | (V/cm) (a) | ($\mu$s) | (min) |
| Mesophyll-protoplast, oats | as 1 | 0.5M mannitol | 200 | 200 | 0.5 | 750 | 20 | 3-10 |
| Mesophyll-protoplast, bean | as 1 | 0.5M mannitol | 200 | 200 | 0.5 | 750 | 50 | 30-60 |
| Mesophyll-protoplast, petunia | as 1 | 0.5M | 200 | 200 | 0.5 | 750 | 50 | 30-60 |
| Guard cell-protoplast, bean | Mesophyll-protoplast, bean | 0.6M mannitol | 200 | 200 | 0.5 | 2000 | 50 | 20-40 |
| Mesophyll-protoplast, Kalanchoe | as 1 | 0.3M sorbitol | 300 | 70 | 1 | 570 | 20 | 0.5 |
| Mesophyll-protoplast, Kalanchoe | Vacuole, Kalanchoe | 0.3M sorbitol | 300 | 70 | 1 | 500 | 20 | 0.5 |
| Vacuole, Kalachoe | as 1 | 0.4M sorbitol | 300 | 50 | 1 | 500 | 20 | 0.1-0.2 |
| Mesophyll-protoplast, oats | as 1(b) | 0.5 mannitol | 200 | 200 | 0.5 | 750 | 20 | 3-10 |
| Mesophyll-protoplast, Kalanchoe | Mesophyll-protoplast, oats | 0.4 M sorbitol | 300 | 70 | 1 | 570 | 20 | 1 |

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