III.4 Electrofusion and Analysis of Potato Somatic Hybrids

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1 Introduction

1.1 Electrofusion

For experiments in somatic cell genetics of potato, the process of electrofusion has been used to generate fusion products of induced variants. Using this procedure rather than chemically-induced fusion, it is possible to exert a large degree of control over aggregation and fusion. Initially conceived by Zimmermann by combining the process of dielectrophoresis of charged particles and that of reversible membrane breakdown, chains of protoplasts are formed to obtain membrane contact in an alternating voltage field and fusion is induced by a short, high-voltage pulse which causes temporary local membrane breakdown at the point of contact (Zimmermann and Scheurich 1981; Zimmermann 1982).

It has taken a few years since the publication of this paper for others to take up this methodology and to make it suitable for sterile work and large-scale application on protoplasts. Recent articles from various sources (see below) have described systems permitting quick handling of millions of protoplasts. The viability of electrically treated protoplasts is no longer a contentious issue, and the system may also be used for electrical field-mediated transformation by DNA.

In a series of experiments on potato protoplasts, the electric field manipulations of aggregation and fusion have been used to analyze the fusion characteristics of protoplasts and to generate large numbers of heterokaryons of Solanum tuberosum variants which are difficult to fuse by chemical means, for genetic analysis.

1.2 Somatic Hybridization in Potato

So far, potato protoplasts from lines without specifically induced mutations or variations have been used as fusion partner of tomato (Melchers et al. 1978), Solanum chacoense (Butenko and Kuchko 1980), S. nigrum (Binding et al. 1982), tobacco (Skarzhynskaya et al. 1982), S. brevidens (Barsby et al. 1984), S. pinnatisectum, S. bulbocastanum and S. cardiophyllum (Sidorov et al. 1984) by means of conventional chemical fusion techniques. As for the intrageneric fusions with other Solanum species, the experiments involved were used to study the incorporation of commercially interesting traits in S. tuberosum.
Work on somatic cell genetics of potato has been hampered by a scarcity in available mutants. Some lines, resistant to 5-methyltryptophan, an amino acid analogue of tryptophan have been isolated by Carlson and Widholm (1978). These lines were used for studies on amino acid synthetic pathways which did not involve fusion experiments. Recently, workers in Groningen and Wageningen have characterized a number of variants in potato, i.e. lines resistant to various amino acid analogues (Jacobsen et al. 1985; Jacobsen 1986) and lines, using alternative carbon sources (Pennings, pers. commun.). Now that a suitable system for the culture and regeneration of potato cell suspension protoplasts has been established (de Vries and Bokelmann 1986), these variants can be used in somatic cell genetic experiments. Some of the amino acid resistant lines have been found suitable as fusion partner in homologous and heterologous situations. The heterologous fusions, which involve fusion with *Nicotiana plumbaginifolia* are carried out in order to map the potato genome. The homologous fusions serve to characterize the properties of putative mutations and are described in this chapter to illustrate the successful application of electrofusion on potato.

2 Protoplast Preparations

For optimal results, protoplast suspensions should be free of debris and the protoplasts themselves should be well swollen by means of suitable osmolarity of the medium. This is not a specific prerequisite for electrofusion, but it holds for chemical fusion as well. In the electrofusion situation, however, attention is very much drawn to these matters, as it is a microscopic technique in which the degree of aggregation and fusion can be monitored continuously. Normally, 3-h incubation in 1% cellulase/0.1% pectolyase/9% mannitol solution (de Vries and Bokelmann 1986) will release the suspension culture protoplasts, which may then be purified and concentrated by centrifugation. For electrofusion, the medium should have a low conductivity: a solution of mannitol is normally used to this end. However, addition of Ca$^{2+}$ or other substances may be necessary (see below).

Cytological investigation of the fresh protoplasts reveals a moderate amount of spontaneous fusion. Depending on the osmotic conditions, up to 20% of the protoplasts may be multinucleate, the majority being binucleate.

The presence of these multikaryons should not be neglected. In some cases, as in fusion between mutant cell lines, the untreated samples may serve as controls for homologous fusions. When analyzing genetic interaction in fusion products, it must be borne in mind that there is a chance that a product stems from multinucleated partners. Secondary doubling and instabilities during callus formation and regeneration (see Chap. IV.9, this Vol.) may further complicate the issue.