Mem. Fac. Agr. Kagoshima Univ., **30**, 31-34, 1994

Studies on Basic Conditions of Protoplast Electrofusion in Sweet Potato (*Ipomoea batatas* (L.) Lam.) and Its Related Species

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Received for Publication September 10, 1993

Introduction

By applying powerful electric fields to electrically induced cell chains (pearl chains), many attempts have been done to stimulate cell fusion[8]. It has become apparent that such techniques would offer more controllable and more efficient methods for protoplast fusion than chemical treatments.

Through electrofusion, somatic hybrid plants have been regenerated in *Nicotiana*[1,3,5,6], *Solanum*[7], *Lactuca*[2], etc. There is, however, no report on the electrofusion of protoplasts of *Ipomoea batatas* (L.) Lam. and its related species. The aim of this work is to find the optimum conditions for the electrofusion of protoplasts of those species.

Materials and Methods

Plant materials

I. batatas (L.) Lam. cv. Nakamurasaki (6x) and its related species, *I. littoralis* (K233, 4x) and *I. triloba* (K121,2x), were used as the sources of protoplasts.The plants of cv. Nakamurasaki and K233 were grown in a greenhouse (15～32℃). Their protoplasts were isolated and fused. *In vitro* grown plants of K233 and K121 were also served as the parents of cell fusion.

Protoplast preparation

By the methods in our previous report[4], protoplasts were isolated from the young petioles of cv. Nakamurasaki, K233, and K121. The protoplasts purified with 20% sucrose solution were suspended in 0.2M, 0.4M or 0.6M D-mannitol solution and centrifuged at 200xg for 4min. After harvest, protoplasts were resuspended in the equivalent D-mannitol solution at a final density of 10^6 protoplasts/ml. Prior to fusion, the protoplasts of either Nakamurasaki and K233 or of K233 and K121 were mixed together in a ratio of 1:1.

Protoplast electrofusion

Electrical fields were generated by SHIMADZU SOMATIC HYBRIDIZER SSH-1. The fusion chamber (SSH-C02; 1.0mm electrode separation, a volume of 0.4ml) was sterilized by autoclaving for 20min. at 120℃. Generally, chain formation in an AC (alternative currency) field requires a medium of low conductivity, so the protoplasts were fused in a medium containing 0.2M, 0.4M or 0.6M D-mannitol without inorganic salts. Protoplasts placed in the fusion chamber were exposed to an 80~200V/cm, 1~2MHz AC field for 5~70s, and then one pulse of 2000~2200V/cm and 50~200 μs duration was applied. After giving the pulse, the AC field was smoothly damped to 0V for a period of 60s.

Results and Discussion

Preparative fusion experiments demonstrated that, among fusion media compared, the one with 0.6M D-mannitol best suited for the protoplast fusion. The following results were obtained when 0.6M D-mannitol was used as the fusion medium.

The application of AC electrical fields resulted in the aligning of protoplasts into pearl chains of various lengths (Fig.1). Some repulsion of aligned protoplasts was observd when the fusion pulse was applied (Fig.2). Parameters were selected to favor the formation of protoplast pairs. Table 1 and Table 2 show the electrical parameters for protoplast fusions of cv. Nakamurasaki+K233 and of K233+K121, respectively.

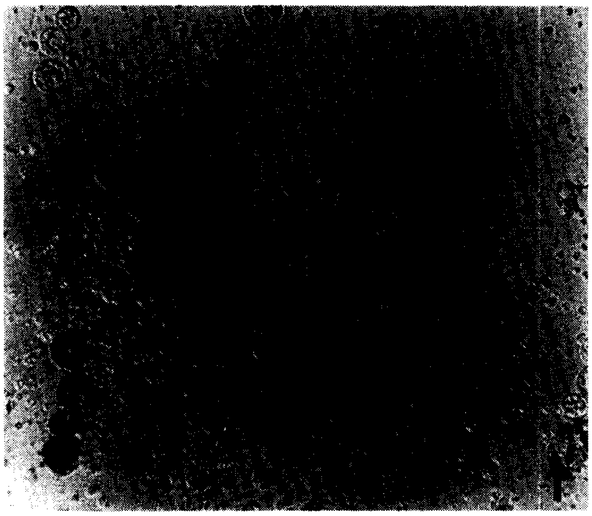
The values selected for the AC and DC (direct currency) electric fields markedly influenced the extent of fusion. Higher fusion frequencies were obtained at 1.0MHz than at 2.0MHz. In a field strength of 150~200 V/cm for 70s, most of the protoplasts formed chains and at the same

Table 1. Electrical parameters for protoplast fusion between Nakamurasaki and *I.littoralis*

Parameter	Value					
Alignment frequency (MHz)	1	1	1	1	1	2
Alignment voltage (V/cm)	200	200	80	150	200	200
Initial time (s)	5	60	70	70	70	60
Fusion voltge (V/cm)	2000	2000	2000	2000	2000	2000
Number of DC pulse	1	1	1	1	1	1
Pulse duration (μs)	50	150	200	200	150	150
Fusion frequency (%)	2.5	10.0	8.5	15.0	15.0	2.5

Table 2. Electrical parameters for protoplast fusion between *I.littoralis* and *I.triloba*

Parameter	Value	
Alignment frequency (MHz)	1	1
Alignment voltage (V/cm)	200	200
Initial time (s)	70	80
Fusion voltage (V/cm)	2000	2200
Number of DC pulse	1	1
Pulse duration (μs)	150	180
Fusion frequency (%)	12.5	11.0



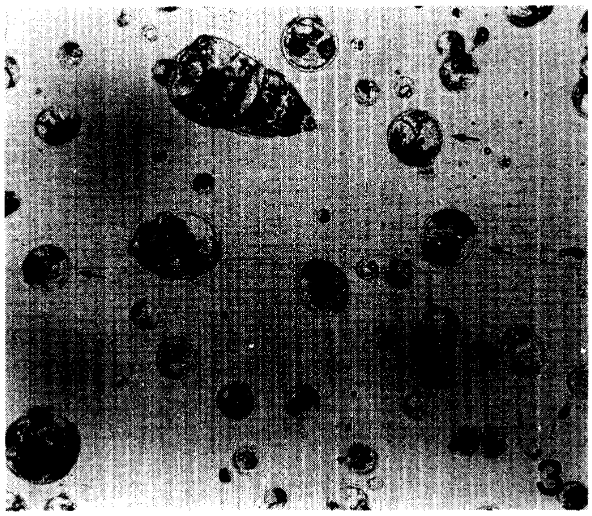


Fig. 1. Pearl chains formed between Nakamurasaki and *I. littoralis* when AC electrical field was applied.
Fig. 2. Aligned protoplasts of *I. littoralis* and *I. triloba* fused when DC pulse was applied.
Fig. 3. Fused protoplasts of *I. littoralis* and *I. littoralis* and *I. triloba*.

field strength protoplast pairing occurred most frequently.

Fusion frequency was also dependent on the strength of DC pulse. Fusion occurred most at an electric pulse of 2000V/cm and 150~200 μs (Fig.3). Further increases in DC pulse strength induced protoplasts migration during pulse application.

In this study, when the electric field of 150~200V/cm at 1MHz for 70s and the pulse of 2000 V/cm and 150~200 μs were applied, the maximum fusion frequencies were obtained in the combinations of cv. Nakamurasaki+K233 and of K233+K121. They were 15.0% and 12.5%, respectively (Tables 1 and 2). The frequencies were, however, lower than those reported by Zimmermann and Scheurich[8], Kohn *et al.*[3] and Vries *et al.*[7]. But considering the fact that this study was the first one made on electrofusion of *I. batatas* and its related species, the results might be regarded as good enough to suggest the usefulness of electrofusion. Electrofusion may provide a useful system for the improvement of sweet potato.

Summary

A series of pilot experiments were done on electrofusion of protoplasts in sweet potato and its related species. Optimal fusion conditions were determined by changing the fusion media and the electrical parameters. The maximum fusion frequencies of *I. batatas* (L.) cv. Nakamurasaki+*I. littoralis* and of *I. littoralis*+*I. triloba* were 15.0% and 12.5%, respectively.

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