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Plant Regeneration from Protoplast Fusions of *Ipomoea batatas* (L.) Lam. and *I. triloba* L.

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Introduction

Plants of *Ipomoea* section *batatas* are classified into two groups based on the cross-compatibility with sweet potato, *I. batatas* (L.) Lam.: Group I cross-compatible with sweet potato and Group II cross-incompatible with sweet potato[11]. The transfer of valuable genes from Group II plants to sweet potato has failed due to cross-incompatibilities. Somatic hybridization by means of protoplast fusion offers a possibility to overcome such cross-incompatibility. Once somatic hybridization in *Ipomoea* section *batatas* is led to a success, it will open a wide road for the utilization of the related species in sweet potato breeding.

Plant regeneration from protoplasts of sweet potato and its related species has been reported[3,5,7–10]. Kokubu and Sato[1] and Liu *et al.*[4] studied conditions for protoplast fusion and culture of sweet potato and its related species. However, no regeneration of somatic hybrids has yet been achieved.

Sweet potato cv. Kokei No. 14 is the most important cultivar for table use in Japan. *I. triloba* L. is one of the related species cross-incompatible with sweet potato. This paper describes plant regeneration from protoplast fusions of them.

Materials and Methods

1. Plant materials

In vitro grown plants of Kokei No. 14 were prepared as described by Liu *et al.*[5]. *In vitro* grown plants of *I. triloba* L. were the regenerants obtained in our previous study[2].

2. Protoplast isolation

The petioles of three-week-old *in vitro* plants were rinsed with sterile distilled water and served for protoplast isolation. Protoplasts were isolated as described previously[3]. Prior to fusion, protoplasts were washed once in W_5 solution (125.0 mM $CaCl_2 \cdot 2H_2O$, 154.0 mM NaCl, 5.0 mM KCl, 5.0 mM glucose, and 5.0 mM 2-(N-Morpholino) ethane-sulfonic monohydrate (MES), pH 5.8) by centrifuging at 200×g for 4 min and suspended in W_5 solution at a final density of 10^6 protoplasts/ml.

3. Protoplast fusion

Protoplasts of both fusion partners were mixed in a 2 Kokei No. 14: 1 *I. triloba* L. ratio. Droplets of the fusion mixture were placed in a ϕ60 mm petri dish with a pasteur pipette. One or two droplets of the fusion medium were added onto each droplet of the fusion mixture, incubating

for 10 min. The fusion medium was composed of 30.0% polyethylene glycol (PEG), 0.1 M $Ca(NO_3)_2 \cdot 4H_2O$, and 0.5 M D-mannitol, pH 9.0. At fusion, fusion frequency (%) was estimated as follows:

$$\frac{\text{Number of double fusion products}}{\text{Total number of protoplasts counted}} \times 100$$

The fused protoplasts were washed once with W_5 solution and twice with protoplast culture medium.

4. Culture of fusion products

The fused protoplasts were cultured in a modified Murashige and Skoog[6] medium (mMS), which was composed of 1/2MS inorganic salts (minus NH_4NO_3), MS vitamins, 50.0 mg/l casein hydrolysate, 0.6 M D-mannitol, 0.05 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), 0.5 mg/l kinetin, and 1.0% (w/v) sucrose, pH 5.8, in the dark at 27°C. And then every 4 weeks the cultures were incubated in mMS medium in which D-mannitol concentration was reduced to 0.3 M and sucrose concentration was increased to 2.0% (w/v) and MS medium containing 0.05 mg/l 2,4-D, 0.5 mg/l kinetin, and 3.0% (w/v) sucrose, pH 5.8, in turn, in the dark at 27°C.

5. Callus proliferation and plant regeneration

Twelve weeks after plating, the obtained microcalli were transferred onto MS medium supplemented with 0.2 mg/l 2,4-D and cultured in the dark at 27°C. Three weeks after transfer, the resulting calli were cultured on MS medium supplemented with 1.0 mg/l 6-benzylaminopurine (BAP) and further on MS medium without plant growth regulators to induce plant regeneration under 13h day-light at 3,000 lux and 27 ± 1°C.

Results and Discussion

1. Protoplast fusion

Fusion of protoplasts from Kokei No. 14 and *I. triloba* L. was rapidly induced when the fusion medium was added (Fig. 1). The described fusion protocol gave a mean fusion frequency up to 28%. Multiple fusion (multifusion) was also observed. Heterokaryons can not be identified because the protoplasts of both fusion partners were of the same type. Thus, fused and unfused protoplasts were cultured together.

2. Cell division and callus formation

The cells underwent first division within 4–5 days (Fig. 2) and formed colonies (Fig. 3), indicating that the survival after fusion treatment was still high. Two weeks after plating, the plating efficiency was up to about 50%. After 8 weeks of plating, colonies developed into microcalli of 1.0–1.2 mm in diameter (Fig. 4) and after 12 weeks of plating, most of microcalli were up to 1.5–2.0 mm in diameter (Fig. 5).

Fig. 1. Fusion of protoplasts from Kokei No. 14 and *I. triloba* L..
Fig. 2. First cell division within 4–5 days.
Fig. 3. Colonies derived from fused protoplasts at 10 days after plating.

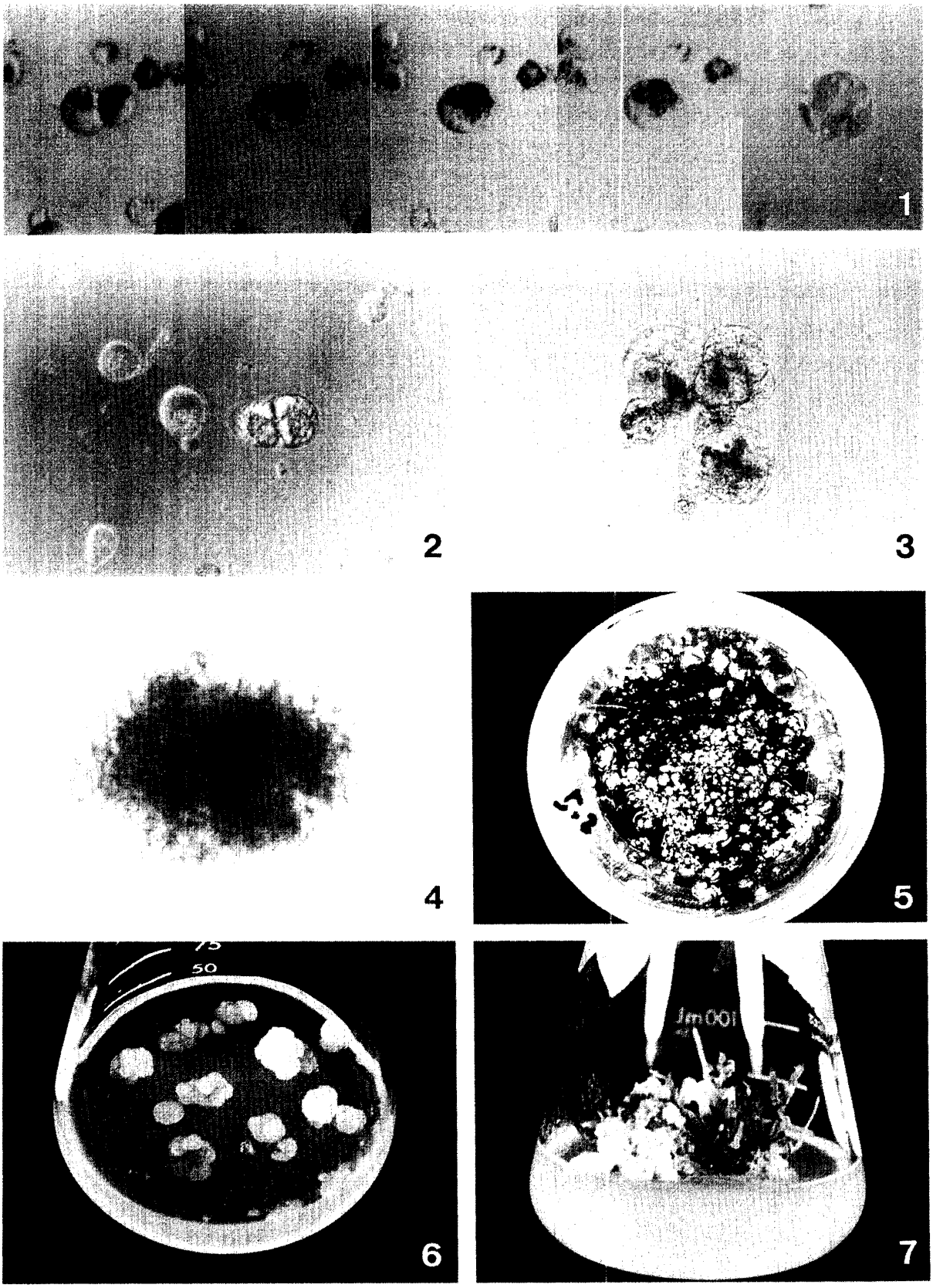

Fig. 4. Microcallus derived from a single fused protoplast at 8 weeks after plating (1.2 mm in diameter).
Fig. 5. Microcalli formed at 12 weeks after plating (1.5–2.0 mm in diameter).
Fig. 6. Rapidly proliferating calli on MS medium supplemented with 0.2 mg/l 2,4-D.
Fig. 7. Shoots regenerated from a callus on MS medium without plant growth regulators.

3. Callus proliferation and plant regeneration

The obtained microcalli were transferred onto MS medium supplemented with 0.2 mg/l 2,4-D, resulting in rapid proliferation of them (Fig. 6). Three weeks after transfer, these calli were cultured on MS medium supplemented with 1.0 mg/l BAP, and all of them produced adventitious roots. The calli with adventitious roots were further transferred onto MS medium without plant growth regulators. Of 44 calli transferred, 5 calli regenerated 16 shoots (Fig. 7). Transfer of regenerated shoots onto fresh MS medium resulted in development of whole plantlets (Fig. 8). These plants have not been verified as true somatic hybrids or not.

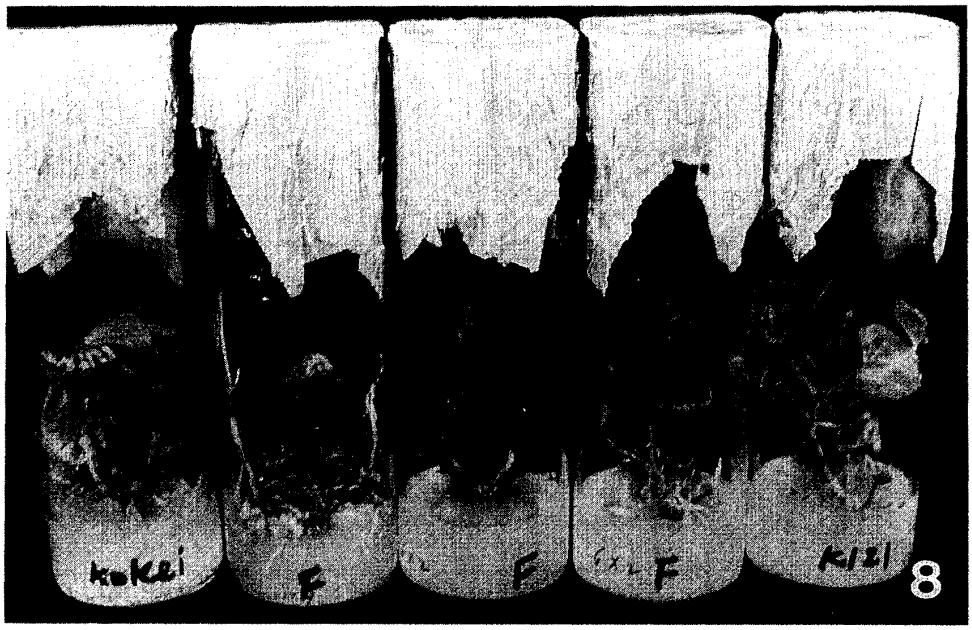

Fig. 8. Regenerated plantlets (middle three plantlets) and both parents (left: Kokei No. 14; right: *I. triloba* L.).

The purpose of this study is to establish a system for fusion and culture of protoplasts between sweet potato and its related species. The fusion protocol described here is effective. Once isolated, protoplasts can be fused at a high frequency, moreover, the survival after fusion treatment is high.

The present results have reached regeneration of putative somatic hybrid plants. It is a major step towards the application of somatic hybridization technique in sweet potato breeding. It is undoubted that this technique will provide an effective method for overcoming cross-incompatibilities and low seed sets in sweet potato breeding and advance it towards a new period.

Summary

Protoplasts of sweet potato cv. Kokei No. 14 were fused with protoplasts of *I. triloba* L. by PEG method. A mean fusion frequency of 28% was obtained. Fused protoplasts were cultured in a modified MS medium containing 0.05 mg/l 2,4-D and 0.5 mg/l kinetin. First cell division occurred within 4–5 days. Two weeks after plating, the plating efficiency reached 50%. Twelve weeks after plating, transfer of the calli up to 1.5–2.0 mm in diameter onto MS medium supplemented with 0.2 mg/l 2,4-D resulted in rapid proliferation of them. When these calli were

cultured on MS medium supplemented with 1.0 mg/l BAP and then on MS medium without plant growth regulators, 16 plants were regenerated from 5 calli.

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References

1) Kokubu, T. and Sato, M.: Protoplast fusion of *Ipomoea batatas* L. and its related species. *Japan. J. Breed.*, **37**(Suppl. 2), 56–57 (1987)
2) Liu, Q. C., Kokubu, T. and Sato, M.: Plant regeneration in stem, petiole and leaf explant cultures of *Ipomoea triloba* L.. *Japan. J. Breed.*, **40**, 321–327 (1990)
3) Liu, Q. C., Kokubu, T. and Sato, M.: Plant regeneration from *Ipomoea triloba* L. protoplasts. *Japan. J. Breed.*, **41**, 103–108 (1991a)
4) Liu, Q. C., Kokubu, T. and Sato, M.: Protoplast fusion and culture between *Ipomoea littoralis* Blune and *I. triloba* L.. *Proc. Intl. Colloquium Overcoming Breed. Barriers*, Miyazaki, Japan, 11–16 (1991b)
5) Liu, Q. C., Kokubu, T. and Sato, M.: Plant regeneration from callus and protoplasts of sweet potato, *Ipomoea batatas* (L.) Lam.. *Mem. Fac. Agr. Kagoshima Univ.*, **28**, 47–53 (1992)
6) Murashige, T. and Skoog, F.: A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant.*, **15**, 473–497 (1962)
7) Murata, T., Fukuoka, H. and Kishimoto, M.: Medium condition on plant regeneration from protoplast of sweet potato. *Japan. J. Breed.*, **41** (Suppl. 1), 54–55 (1991)
8) Murata, T., Hoshino, K. and Miyaji, Y.: Callus formation and plant regeneration from petiole protoplast of sweet potato, *Ipomoea batatas* (L.) Lam.. *Japan. J. Breed.*, **37**, 291–298 (1987)
9) Sihachakr, D. and Ducreux, G.: Plant regeneration from protoplast culture of sweet potato (*Ipomoea batatas* Lam.). *Plant Cell Rep.*, **6**, 326–328 (1987)
10) Suga, R., Takagi, Y. and Okutsu, Y.: Protoplast culture of sweet potato and its related wild species—plant regeneration from leaf protoplasts-derived callus of *Ipomoea trifida*. *Japan. J. Breed.*, **40** (Suppl. 1), 78–79 (1990)
11) Teramura, T.: Phylogenetic study of *Ipomoea* species in the section *Batatas*. *Mem. Coll. Agr. Kyoto Univ.*, **114**, 29–48 (1979)