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Characteristics of Regenerated Plant from Protoplast Fusions between Sweet Potato and Its Related Species

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Introduction

Species of *Ipomoea* section *batatas* are classified into two groups, based on the cross-compatibility with sweet potato (*Ipomoea batatas* (L.) Lam.). The plants in group II have not been successfully utilized in sweet potato breeding due to their cross-incompatibility with sweet potato. Recent studies on several crops have shown that somatic hybridization through protoplast fusion is a possible means for overcoming such cross-incompatibility[2]. Up to now, however, the regeneration of somatic hybrid between sweet potato and its related species was only reported by Liu *et al.*[5] and Belarmino *et al.*[1]. For transferring various useful genes from the related species into sweet potato, it is necessary to improve their hybridization methods.

Therefore, in this report, we described some characteristics of the regenerated plant from protoplast fusions between sweet potato and its related species, *I. triloba* L. in group II.

Materials and Methods

1. Plant material

Embryogenic calli of sweet potato cv. Bitambi and petioles of *I. triloba* L. (K121) were used as the sources in this study. The embryogenic calli were induced from shoot tips on MS medium[6] supplemented with 0.2 mgl^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D), 3.0% sucrose and 0.8% agar (pH5.8) in the dark at 27℃.

2. Protoplast isolation and fusion

A large number of protoplasts were isolated from embryogenic calli and petioles by the means of Wang *et al.*[9].

Protoplasts of Bitambi and *I. triloba* were mixed in the ratio of 1 : 1 and then fused with Polyethylene Glycol (PEG) solution according to the method of Liu *et al.*[4]. The fusion-treated protoplasts were washed twice with W_5 solution[7] and washed once with protoplast culture medium.

3. Protoplast culture and plant regeneration

The fused protoplasts were cultured in the liquid medium in the dark at 27℃. The liquid medium was composed of 1/2 MS inorganic salts (except NH_4NO_3), MS vitamins, 50.0 mgl^{-1} casein hydrolysate, 2,4-D (0.05, 0.2 mgl^{-1}), kinetin (0.5, 1.0 mgl^{-1}), 0.6M mannitol and 1.0% (w/v) sucrose at pH5.8. After three to four weeks of culture, the mannitol content was reduced from 0.6M to 0.3M and the sucrose content was increased from 1.0% (w/v) to 2.0%

(w/v). Then, the formed colonies were cultured in liquid MS medium containing 3.0% (w/v) sucrose and the same concentrations of 2,4-D and kinetin not containing mannitol and casein hydrolysate.

After two months of culture in the liquid medium, the formed calli were transferred onto the solid MS media supplemented either with 2,4-D (0.05～0.2 mgl^{-1}) and kinetin (0～0.5 mgl^{-1}) or with 2.0m mgl^{-1} naphthalene acetic acid (NAA) and 1.0 mgl^{-1} 6-benzylaminopurine (BAP), together with 3.0% sucrose and 0.8% agar at pH5.8, for callus proliferation. Three weeks after transference, the calli were further transferred onto either the hormone-free MS basal medium or the medium supplemented with 3.0 mgl^{-1} BAP, 3.0% (w/v) sucrose and 0.8% (w/v) agar at pH5.8. They were further transferred onto MS basal medium, for plant regeneration. They were subcultured for three weeks intervals on the basal medium under 13h daylight at 3,000lux and 27℃.

4. Validation of regenerated plants

Morphology: The regenerated plantlet and the fusion parents were transplanted in pots with vermiculite at high humidity. Morphological characters were researched after they were grown in a green house.

Chromosome number: Root tips were used for counting chromosome number. They were treated by the means of Wang *et al.*[9].

RAPD: Total DNAs were isolated from young leaves, accrding to the method of Sakai *et al.*[8]. Polymerase chain reaction (PCR) and electrophoresis of the amplified DNA were carried out by the means of Wang *et al.*[9]. Eleven primers (Kit A, C, D, E, Operon Technologies) were used to develop the random amplified polymorphic DNA (RAPD) markers.

Results and Discussion

1. Protoplast fusion, culture and plant regeneration

The protoplasts of Bitambi and *I. triloba* were fused by PEG method (Fig. 1 A). The heterokaryons were to be observed because the embryogenic callus protoplasts of Bitambi were yellow-purple, while the petiole protoplasts of *I. triloba* were green. The first cell division occurred within 3 days of culture in the liquid medium (Fig. 1 B). Then some of the cells divided and developed into colonies (Fig. 1 C). Two months after plating, the small calli grew to 1～2mm in diameter.

The small calli were then transferred onto callus porliferation medium. Three weeks after, they grew to 8～15mm in diameter (Fig. 1 D). When they were transferred onto MS basal medium or the medium supplemented with BAP and transferred again onto MS basal medium, 23 plants were regenerated (Fig. 1 E).

2. Validation of regenerated plants

Out of the 23 regenerated plants, 10 plants showed the morphological characters of Bitambi and 11 plants were the same with *I. triloba*. These 21 plants were considered to be regenerated from non-fused protoplasts. One plant showed the morphologies somewhat similar to *I. triloba*, having 60 chromosomes as the double of that of *I. triloba*. This plant may be regenerated from the fused protoplasts of *I. triloba*. The other one plant (putative somatic hybrid, PH), the remainder, showed a pole-climbing habit as the same of that of *I. triloba*,

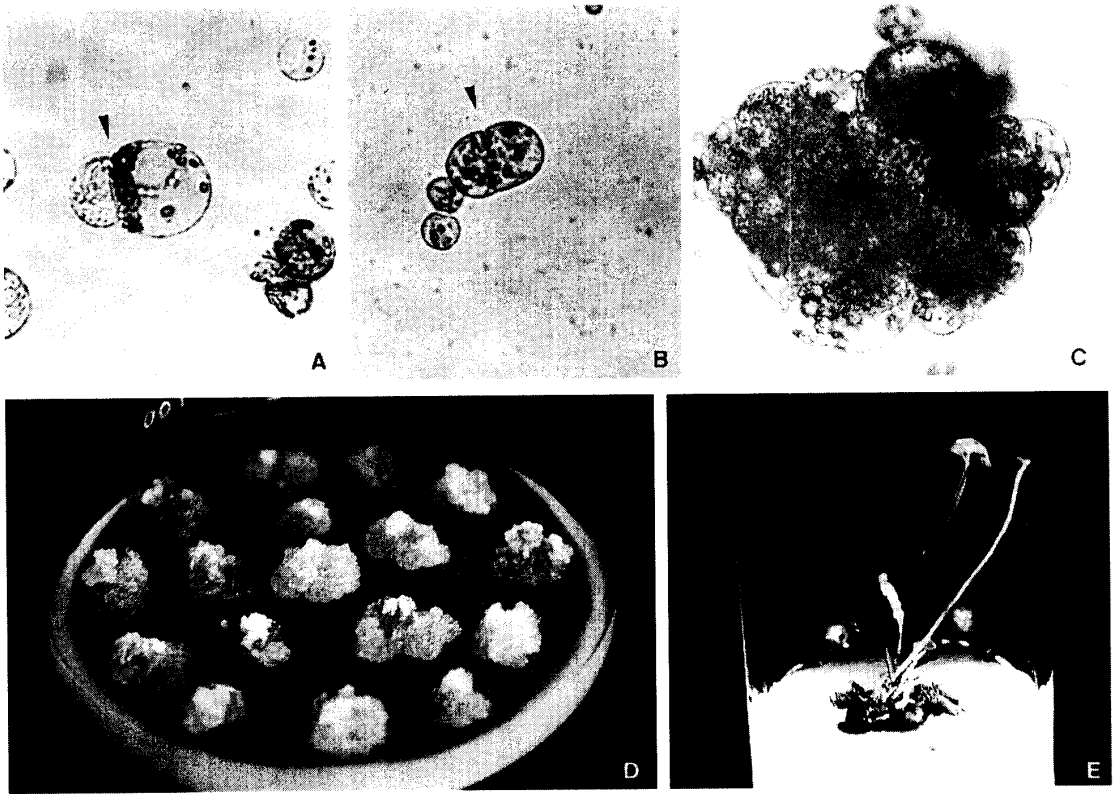

Fig. 1. Plant regeneration from protoplast fusions between sweet potato and *I.triloba*.
A. Fusion of protoplasts by PEG treatment.
B. First cell division, after 3 days of fusion.
C. Cell colony, after 3 weeks of fusion (0.2mm in diameter).
D. Calli derived from fusion-treated protoplasts on proliferation medium.
E. Shoot regeneration on hormone-free MS medium.

with internode length being intermediate between both the parents (Fig. 2). Chromosome number of the plant (PH) was counted to be from 45~65 in the 10 cells of root tip observed. Incidentally, the chromosome number of Bitambi is 90 and that of *I. triloba* is 30. With RAPD assay, the plant (PH) expressed no bands of Bitambi, while it expressed the unique bands besides some bands of *I. triloba* by using primer OPA-07, OPA-16, OPA-20 and OPC-06 (Fig. 3). From these observations of morphological characters, chromosome number and RAPD markers, this plant was reasoned to be somatic hybrid.

Chromosome number (2n) of the putative somatic hybrid was not the sum of the parents, 90+30=120, but was ranged from 45 to 65, being different with cells. The cause for these variations might be related to the loss of chromosomes in culture period[5].

In the present experiment, the bands of Bitambi and some bands of *I. triloba* disappeared and some unique bands appeared in the putative hybrid. The similar phenomenon was also observed by Hossain *et al.*[3] in somatic hybrids between tomato and night shade and by Xu *et al.*[10] both in somatic hybrids between *Solanum tuberosum* and S. *brevidens* and in their parental DNA mixture. The cause for this phenomenon has been left unknown. It may be either due to the fact that the putative somatic hybrid lost some chromosomes of its parents or the possible primer binding competition[10].

Fig. 2. Plants of Bitambi (Bi), putative somatic hybrid (PH) and *I. triloba* (T) in vitro grown (**A**) and grown in green house (**B**).

The putative somatic hybrid was obtained in the present experiment. By refining the method, it might become easy to introduce valuable genes from the related species to sweet potato.

Summary

The protoplasts of sweet potato cv. Bitambi and its related species, *I. triloba* L. were fused by PEG method, and cultured in the modified liquid MS medium containing 2,4-D and kinetin. After 2 months of culture, the formed calli were transferred onto the solid MS medium supplemented either with 2,4-D and kinetin or with NAA and BAP, for callus proliferation. When the calli were further transferred onto the MS basal medium or the medium supplemented with BAP, followed by being transferred onto the MS basal medium, 23 plants were regenerated. One of them showed the intermediate character in the internode length between the fusion parents, having 45 to 65 chromosomes, differing with cells. No RAPD bands of Bitambi were observed, however, some unique bands besides the bands of *I. triloba* were expressed. Therefore, this plant was estimated to be somatic hybrid.

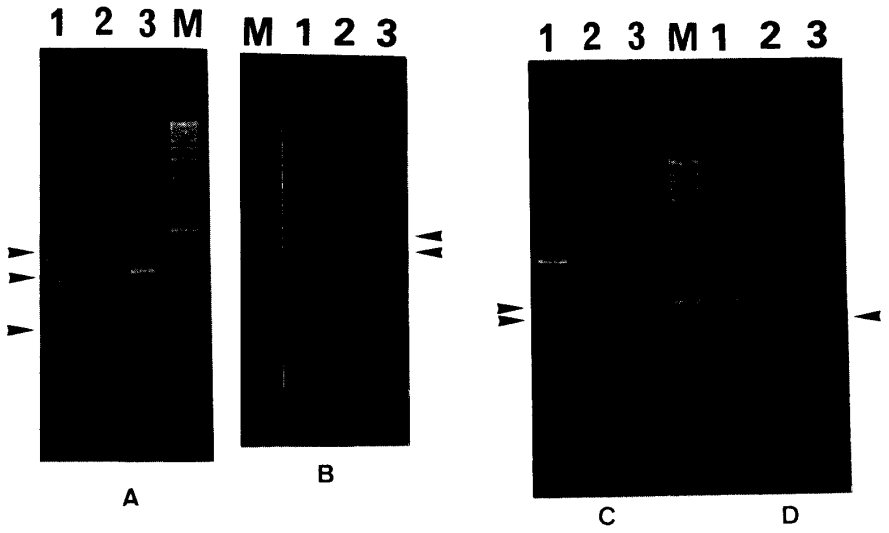

Fig. 3. RAPD profiles generated by primer OPA-07 (**A**), OPA-16 (**B**), OPA-20 (**C**) and OPC-06 (**D**).
M: Marker (1 kb ladder)
1: Bitambi
2: Putative somatic hybrid
3: *I. triloba*
Arrows indicate the unique bands expressed in hybrid

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