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Efficient Plant Regeneration from Protoplasts of Sweet Potato, *Ipomoea batatas* (L.) Lam.

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Introduction

Sweet potato cultivars are classified into a few cross-incompatible groups. Since their crossings are limited among different cross-incompatible groups, no hybrid can be obtained from the crossings between cultivars belonging to the same cross-incompatible group. To overcome this problem, somatic hybridization has been considered to be an effective means for producing new gene recombination of sweet potato. And for the production of somatic hybrids, plant regeneration from protoplasts has been one of the prerequisites.

Plant regeneration from protoplast-derived calli of sweet potato has been reported by Murata *et al.*[7-9], Sihachakr *et al.*[14], Perera *et al.*[12], Liu *et al.*[5-6] and Belarmino *et al.*[2]. However, in terms of the regeneration frequency, most of these studies failed in reaching the practical level. The lack of a system of efficient plant regeneration from protoplasts has been a main barrier against the application of somatic hybridization in sweet potato breeding.

Therefore, this paper described an efficient method for the plant regeneration from the protoplasts derived from petioles and from embryogenic calli of sweet potato.

Materials and Methods

1. Plant material

The petioles of *in vitro* grown plants of sweet potato cv. Genki and White Star and embryogenic calli of cv. Bitambi were used as the sources of protoplasts. The embryogenic calli were induced from the shoot tips on Murashige and Skoog (MS) medium supplemented with 0.2 mgl^{-1} 2,4-D, 3.0% sucrose and 0.8% agar (pH5.8) in the dark at 27℃.

2. Protoplast isolation

The petioles were cut into slices and isolated by the means of Liu *et al.*[4]. The embryogenic callus-derived protoplasts were isolated according to the method of Wang *et al.*[15].

3. Protoplast culture and plant regeneration

The isolated protoplasts were cultured in the modified liquid MS medium composed of 1/2 MS inorganic salts (except NH_4NO_3), MS vitamins, 50.0 mgl^{-1} casein hydrolysate, 0.05 mgl^{-1} 2,4-D, 0.5 mgl^{-1} kinetin, 0.6M mannitol and 1.0% (w/v) sucrose at pH5.8, in the dark at 27℃. After 3 to 4 weeks of plating, the cultures were continued to incubate for 2 to 3 weeks in the medium in which mannitol concentration was reduced from 0.6M to 0.3M and sucrose concentration was increased from 1.0% (w/v) to 2.0% (w/v). Then this medium was again

replaced by the medium containing 3.0% (w/v) sucrose and the same concentrations of 2,4-D and kinetin not containing mannitol and casein hydrolysate.

The calli derived from protoplasts up to 1~2mm in diameter were then cultured for callus proliferation on the solid MS media supplemented with 2,4-D (0.05~0.2 mgl^{-1}), kinetin (0~0.5 mgl^{-1}), 3.0% (w/v) sucrose and 0.8% (w/v) agar at pH5.8, under 13h day-light at 3,000lux and 27℃. After 3 to 5 weeks, the calli were further transferred onto either the MS basal medium or the medium supplemented with BAP (2.0~3.0 mgl^{-1}), 3.0% (w/v) sucrose and 0.8% (w/v) agar at pH5.8, followed by being transferred onto the hormone-free MS basal medium, for plant regeneration. They were subcultured at three weeks intervals on the MS basal medium, under 13h day-light at 3,000lux and 27℃.

Results and Discussion

1. Protoplast culture and callus proliferation

The isolated protoplasts were cultured in the liquid MS medium. The first cell division occurred within 3 to 4 days after culture initiation (Fig.1 A and B). Then some of the cells

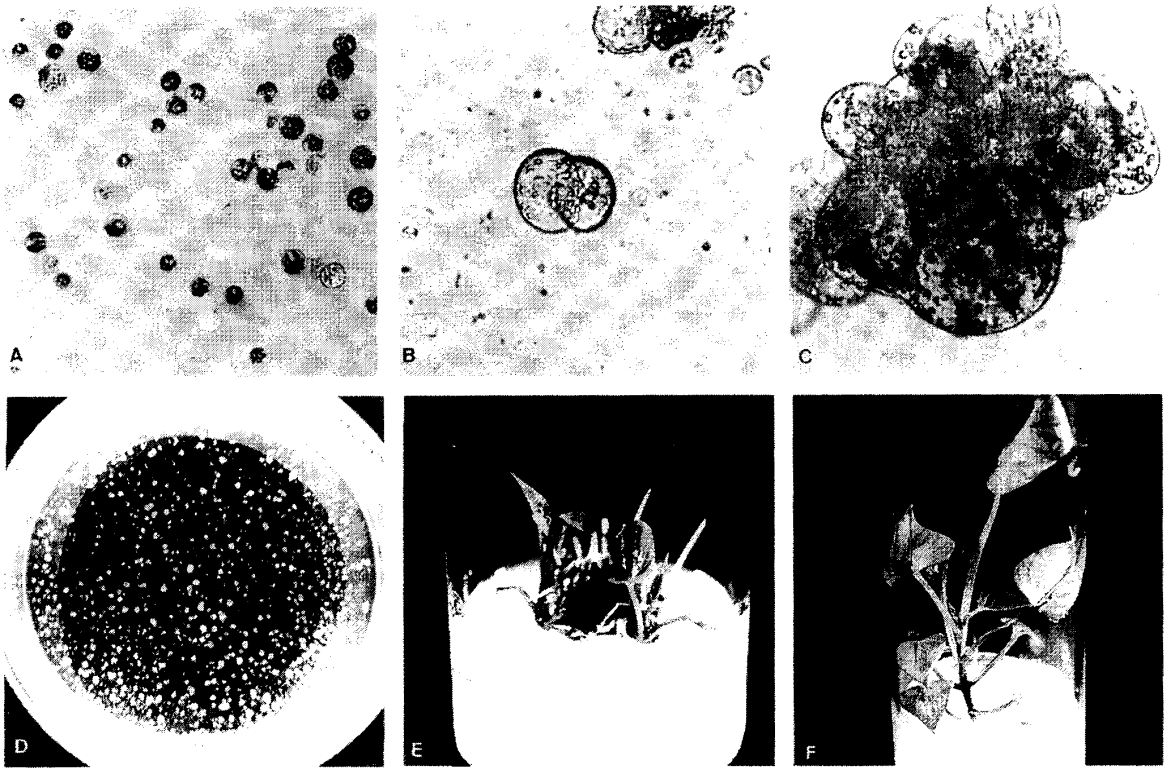

Fig. 1. Plant regeneration from protoplasts of sweet potato.
A. Freshly isolated protoplasts of Genki.
B. First cell division of White Star, after 4 days of culture.
C. Cell colony derived from protoplast of Bitambi, after 3 weeks of culture (0.2mm in diameter).
D. Calli derived from protoplasts of White Star, after 7~8 weeks of culture (1~2mm in diameter).
E. Shoot regeneration from protoplast-derived callus of Bitambi on hormone-free MS medium.
F. Plantlet derived from regenerated shoot of Bitambi.

divided and developed into colonies (Fig.1 C). After 7 to 9 weeks of plating, the small calli grew to 1～2mm in diameter (Fig.1 D).

The transfer of small calli onto proliferation medium resulted in a rapid callus proliferation. After 3 to 5 weeks, the calli grew to 7～12mm in diameter.

2. Plant regeneration

The protoplast-derived calli described above were then transferred onto the regeneration medium (Table 1). After 4 weeks of transfer, the calli regenerating plants were observed on the hormone-free medium in all the cultivars used (Fig.1 E).

The calli cultured on the regeneration medium supplemented with BAP were further transferred onto hormone-free medium. One week after the transferrence, the calli started to regenerate shoots in Genki. While no plant regeneration was obtained in White Star and Bitambi.

The results of plant regeneration from the protoplast-derived calli, obtained after 8 weeks of transfer onto hormone-free medium, are shown in Table 1. It was obvious that the percentages of plant regeneration depended both on the genotypes and on the concentrations of 2,4-D and kinetin added to the proliferation medium and on BAP added to the regeneration medium. The frequencies ranged from 0 to 60.0% in Genki, 0 to 25.7% in White Star and 0 to

Table 1. Plant regeneration from protoplasts of sweet potato on hormone-free MS medium

Cultivar	Proliferation medium 2,4-D (mgl^{-1})	kinetin (mgl^{-1})	Regeneration medium BAP (mgl^{-1})	No. of calli transferred	No. of calli regenerating plants (%)
Genki	0.05	0	0	29	1 (3.4)
	0.1	0	0	20	0
	0.05	0.5	0	18	1 (5.6)
	0.1	0.5	0	22	1 (4.5)
	0.2	0.5	0	20	0
	0.05	0	3	26	1 (3.8)
	0.1	0	3	20	0
	0.2	0	3	25	0
	0.05	0.5	3	20	12 (60.0)
	0.1	0.5	3	20	7 (35.0)
	0.2	0.5	3	24	5 (20.8)
White Star	0.05	0.5	0	35	9 (25.7)
	0.1	0.5	0	50	7 (14.0)
	0.05	0.5	2	24	0
	0.1	0.5	2	19	0
Bitambi	0.05	0	0	32	1 (3.1)
	0.1	0	0	18	1 (5.6)
	0.2	0	0	21	1 (4.8)
	0.05	0.5	0	24	12 (50.0)
	0.1	0.5	0	43	16 (37.2)
	0.2	0.5	0	23	3 (13.0)
	0.5	0	3	32	0
	0.05	0.5	3	20	0
	0.1	0.5	3	24	0

50.0% in Bitambi, in the respective cultivars.

As shown in Table 1, the proliferation medium supplemented with 0.05 mgl^{-1} 2,4-D and 0.5 mgl^{-1} kinetin was most effective for plant regeneration in all the cultivars used. When the calli of White Star and Bitambi were transferred onto MS basal medium, they gave regeneration frequencies of 25.7% and of 50.0%, respectively. High frequency of shoot regeneration reaching up to 60.0% was obtained from the calli of Genki on MS basal medium by using this proliferation medium and subsequently by the regeneration medium supplemented with 3.0 mgl^{-1} BAP. The percentage of calli-regenerating plants was clearly reduced with an increasing in 2,4-D concentration. Similarly, when the proliferation medium contained no kinetin, the calli gave a quite low frequency of plant regeneration both in Genki and in Bitambi. This result showed the important role of lower concentration of 2,4-D and kinetin in plant regeneration from protoplasts of sweet potato.

As shown in Table 1, BAP added to regeneration medium was useful for plant regeneration in Genki, when no BAP was used in the regeneration medium, the calli gave a very low frequency of shoot regeneration. In White Star and Bitambi, however, BAP inhibited the plant regeneration. If BAP was added to regeneration medium, the protoplast-derived calli regenerated no plant. BAP played an important role in plant regeneration in tissue cultures of *I. triloba*[3], *I. trichocarpa*[10], *I. trifida*, *I. lacunosa* and sweet potato[1] as well as in protoplast cultures of *I. triloba*[4-6]. However, both Pido *et al.*[13] and Otani *et al.*[11] reported that BAP inhibited plant regeneration in tissue cultures of sweet potato. The result of the present experiment showed that the effect of BAP on plant regeneration might be varied with genotypes.

Regenerated shoots developed into whole plantlets after being transferred onto fresh basal medium (Fig.1 F). No morphological variations were observed.

Murata *et al.*[7,9], Belarmino *et al.*[2] and Perera *et al.*[12] reported the plant regeneration from protoplasts of sweet potato by making use of the callus proliferation medium supplemented with 2,4-D, kinetin and ABA. But in these cases the regeneration frequencies were low. In this study, however, plants were easily regenerated. The vital point for obtaining efficient plant regeneration was considered to be the use of the lower concentration of 2, 4-D combined with kinetin added to the proliferation medium. The effect of BAP on plant regeneration should not be neglected too. That varies with genotypes, however. Therefore, the plant regeneration system established in this study should be useful for somatic hybridization through protoplast fusion.

Summary

Protoplasts isolated from petioles of sweet potato cv. Genki and White Star and from embryogenic calli of cv. Bitambi were cultured in the modified liquid MS medium containing 0.05 mgl^{-1} 2,4-D and 0.5 mgl^{-1} kinetin. The first cell division occurred within 3 to 4 days. After 8 to 9 weeks of incubation, protoplast-derived calli up to 1～2mm in diameter were plated on the solid MS medium supplemented with 2,4-D (0.05～0.2 mgl^{-1}) and kinetin (0～0.5 mgl^{-1}) for callus proliferation. Furthermore, the calli were transferred onto either the hormone-free medium or the medium supplemented with BAP (2～3 mgl^{-1}), followed by being transferred onto the hormone-free medium for plant regeneration. The result showed that the callus proliferation medium supplemented with 0.05 mgl^{-1} 2,4-D and 0.5 mgl^{-1} kinetin was most efficient for plant regeneration in all the cultivars used, and the effect of BAP was varied with

genotypes. BAP played an important role in promoting the plant regeneration in Genki, but inhibited the plant regeneration in White Star and Bitambi.

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