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Efficient Embryogenic Callus Formation and Plant Regeneration in Shoot Tip Cultures of Sweet Potato

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Introduction

In order to use somatic hybridization successfully in the breeding program of sweet potato, it is necessary for us to study the donor having high regeneration capacity for protoplast isolation[3,5]. Vasil *et al.*[11] and He *et al.*[1] assumed that embryogenic suspension culture was useful for obtaining totipotent protoplasts of wheat. Such callus was also considered to be an ideal donor for obtaining regenerable protoplasts in sweet potato[3].

Somatic embryogenesis in sweet potato has been reported by Tsay *et al.*[10], Liu *et al.*[4], Jarret *et al.*[2], Takayanagi *et al.*[9], Shimonishi *et al.*[8], Kokubu *et al.*[3], Liu *et al.*[5-6], Otani *et al.*[7]. However, it was genotype-dependent. And the higher frequency of embryogenic callus formation, especially that of plant regeneration was obtained only in a few genotypes. This paper, therefore, describes the efficient somatic embryogenic callus formation and plant regeneration in shoot tip cultures of sweet potato.

Materials and Methods

1. Plant materials

Sweet potato cultivars, Bitambi, Beinong Xushu No.18, Kokei No.14, Kyushu No.66, Mishou No.1, Nanjing 51-93 and Kyushu No.85 were used as the sources of shoot tip explants in this study. About 20mm-long shoots excised from the plants were washed with running tap water, and were surface-sterilized with 70% ethanol for 10 seconds and with 2% sodium hypochlorite solution for 5 minutes. They were then washed twice in sterile distilled water.

2. Induction of embryogenic calli

Shoot tips (about 0.5mm in length) excised under a dissection microscope were cultured on the Murashige and Skoog (MS) medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) (0.2 or 2.0 mgl^{-1}), 3.0% (w/v) sucrose and 0.8% (w/v) agar at pH5.8, in the dark at 27℃. They were observed periodically under a dissection microscope for somatic embryogenesis.

3. Plant regeneration

After 4 to 10 weeks of culture, the embryogenic calli with somatic embryos were transferred onto hormone-free MS medium containing 3.0% (w/v) sucrose and 0.8% (w/v) agar at pH5.8, for the germination of somatic embryos. They were cultured under 13h day-light at 3,000lux and 27℃.

Results and Discussion

After 5 to 7 days of incubation, the shoot tips started to form calli. Two morphological types were observed. One was the slow growing type, being pale-brown in color, watery in appearance. Another callus type was pale-yellow to white in color, growing rapidly. It was a non-embryogenic friable callus. About 3 to 6 weeks after culture, the embryogenic calli, pale-yellow to purple in color, smooth and glossy in appearance, were formed from the first type calli (Fig.1 A). They were similar to those described by Jarret *et al.*[2]. Few embryogenic calli were formed from the 2nd type callus after 7 to 9 weeks of incubation (Fig.1 B). The result of the embryogenic callus formation, after 9 weeks of culture, was shown in Table 1 B. It was clearly demonstrated that the frequencies (10.0% to 84.8%) of embryogenic callus formation were different due to the cultivars and the concentrations of 2,4-D added to callus induction medium. This finding agrees with those reported by Jarret *et al.*[2], Kokubu *et al.*[3] and Liu *et al.*[5]. The high frequencies reaching up to 66.7%, 70.0% and 84.8% were obtained in Kokei No.14,

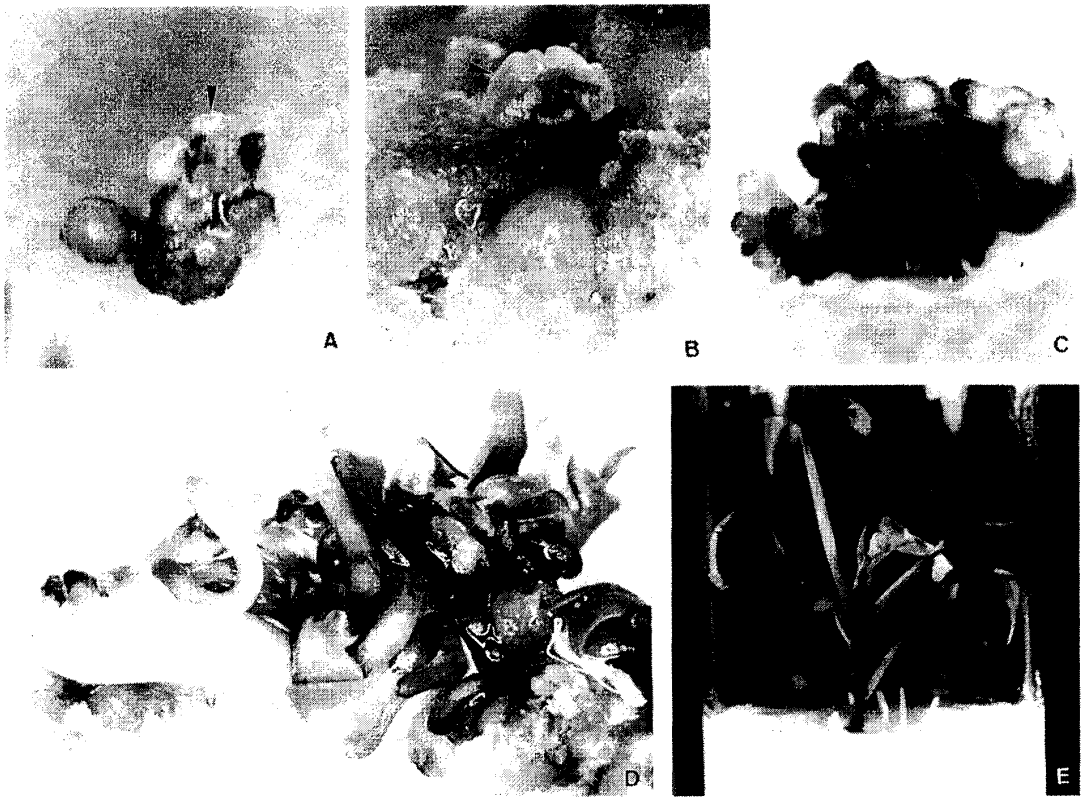

Fig. 1. Somatic embryogenesis and plant regeneration in shoot tip cultures of sweet potato.
A. Embryogenic callus derived from shoot tip of Bitambi on MS medium containing 0.2 mgl^{-1} 2,4-D.
B. Embryogenic callus formation from a nonembryogenic callus of Bitambi on MS medium containing 0.2 mgl^{-1} 2,4-D.
C. Somatic embryos formed from an embryogenic callus of Beinong Xushu No. 18 on MS medium containing 0.2 mgl^{-1} 2,4-D.
D. Germination of somatic embryos of Beinong Xushu No. 18 on hormone-free MS medium.
E. Regenerated plantlet from somatic embryos of Bitambi.

Table 1. Somatic embryogenesis on MS medium supplemented with 2,4-D in shoot tip cultures of sweet potato

Cultivar	Callus induction medium 2,4-D(mgl^{-1})	No. of shoot tips A	No. of embryogenic calli formed B(B/A×100)	No. of embryogenic calli forming somatic embryos C	No. of embryogenic calli regenerating plants D
Bitambi	0.2	33	28 (84.8)	27	27
	2.0	4	2 (50.0)	2	2
Beinong Xushu No.18	0.2	10	3 (30.0)	3	3
	2.0	10	4 (40.0)	4	4
Kokei No.14	0.2	10	1 (10.0)	1	1
	2.0	6	4 (66.7)	4	2
Kyushu No.66	0.2	9	4 (44.4)	1	1
	2.0	5	0	-	0
Mishou No.1	0.2	6	0	-	0
	2.0	6	1 (16.7)	1	1
Nanjing 51-93	0.2	10	7 (70.0)	2	0
Kyushu No.85	0.2	9	2 (22.2)	0	0
	2.0	4	0	-	0

Nanjing 51-93 and Bitambi. The callus induction medium supplemented with 0.2 mgl^{-1} 2,4-D was more effective to form embryogenic calli than 2.0 mgl^{-1} in Bitambi, Kyushu No.66 and Kyushu No.85. On the other hand, that supplemented with 2.0 mgl^{-1} 2,4-D was more efficient than 0.2 mgl^{-1} in Beinong Xushu No.18, Kokei No.14 and Mishou No.1.

After the embryogenic calli were incubated on callus induction medium for 4 to 9 weeks, some globular- and heart-shaped somatic embryos, pale-yellow to purple in color, were formed on most of them (Table 1 C). Some of the somatic embryos cultured further, developed into torpedo- and cotyledon-shaped stages (Fig.1 C), being similar to those described by Liu *et al.*[6].

When the embryogenic calli with somatic embryos were transferred onto MS basal medium, most of the somatic embryos germinated and developed into plants (Fig.1 D, Table 1 D). The most efficient plant regeneration was obtained in Bitambi that had been induced on the medium supplemented with 0.2 mgl^{-1} 2,4-D, with the frequency reaching over 80%. Whole plantlets were formed after transference onto the fresh basal medium (Fig.1 E).

Results of this study indicate that genotypes and the concentrations of 2,4-D significantly influence somatic embryogenesis and plant regeneration. The efficient embryogenic callus formation was achieved in Bitambi, Nanjing 51-93 and Kokei No.14. And the percentage of shoot tips regenerating plants reached over 80% in Bitambi. The culture system of this study may offer an efficient method for obtaining the ideal donor for producing somatic hybrids and gene transfer.

Summary

Shoot tips (about 0.5mm in length) of sweet potato were cultured on the MS medium supplemented with 0.2 or 2.0 mgl^{-1} 2,4-D. Up to 84.8% of shoot tips from cv. Bitambi formed

embryogenic callus on the medium supplemented with 0.2 mgl^{-1} 2,4-D. When the embryogenic calli with formed somatic embryos were transferred onto MS basal medium, most of the somatic embryos regenerated plants. Some differences of genotypes and 2,4-D concentrations on embryogenesis and plant regeneration were observed in the whole cultivars used.

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