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Somatic Embryogenesis and Plant Regeneration in Shoot Tip Cultures of Sweet Potato, *Ipomoea batatas* (L.) Lam.

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Introduction

In sweet potato, *Ipomoea batatas* (L.) Lam., petioles have been used for protoplast isolation, resulting in low frequency plant regeneration[4,6,8]. Thus, to successfully use somatic cell hybridization in sweet potato breeding, it is necessary to study the donor with high regeneration ability for protoplast isolation.

Vasil *et al.*[10] and He *et al.*[1] thought that embryogenic suspension culture was useful for obtaining totipotent protoplasts of wheat. Perhaps, such callus is also an ideal donor for obtaining regenerable protoplasts in sweet potato.

Somatic embryogenesis in sweet potato has been reported, but, in most cases the frequency was low[2,3,7,9]. Thus, somatic embryogenesis in sweet potato requires further study. This paper describes somatic embryogenesis and plant regeneration in shoot tip cultures of sweet potato.

Materials and Methods

Sixteen cultivars of sweet potato, Aikoku, Aooki, Ariakeimo, Genki (Kumamoto), Gifu No. 1, Kakei 4-153, Kanto No. 69, Kanto No. 97, Kintoki, Koganesengan, Kokei No. 14, Konpira, Kotobuki, Kuroshirazu, Lizhixiang, and White Star, were used in this study. These cultivars were randomly selected from sweet potato germplasm collection in a nursery of this laboratory.

About 30 mm-long shoot tips excised from the plants were fully washed with tap water, and sterilized with 70% ethanol for 10 s and 2% sodium hypochlorite solution for 5 min. They were immediately rinsed three times with sterile distilled water.

Shoot tips (about 0.5 mm in length) were excised with the aid of a dissecting microscope and cultured on Murashige and Skoog[3] (MS) medium supplemented with 0.2 and 2.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), 3.0% (w/v) sucrose, and 0.8% (w/v) agar, pH 5.8, under 13 h day-light at 3,000 lux and $27 \pm 1^{\circ}C$.

The obtained embryogenic calli were transferred onto MS medium supplemented with either abscisic acid (ABA) or 6-benzylaminopurine (BAP) and then MS basal medium to induce plantlet formation.

Results and Discussion

One week after culture, shoot tips started to lose their light green and to form white to light yellow, friable callus. The growth of calli was slower on MS medium supplemented with 2.0 mg/l 2,4-D than on MS medium supplemented with 0.2 mg/l 2,4-D except for the calli from

Koganesengan, Genki (Kumamoto), and Kintoki which showed more rapid growth on MS medium supplemented with 2.0 mg/l 2,4-D.

Ten days after culture, somatic embryos were formed from light yellow callus of Kokei No. 14 on MS medium supplemented with 0.2 mg/l 2,4-D, but afterwards they were covered by rapidly proliferating callus.

Two to 5 weeks after culture, a callus pale golden in colour, fine-grained in texture, and glossy in appearance was formed on the initial callus (Fig. 1). This callus was embryogenic, similar to that described by Jarret *et al.*[1] The frequency of embryogenic callus was markedly

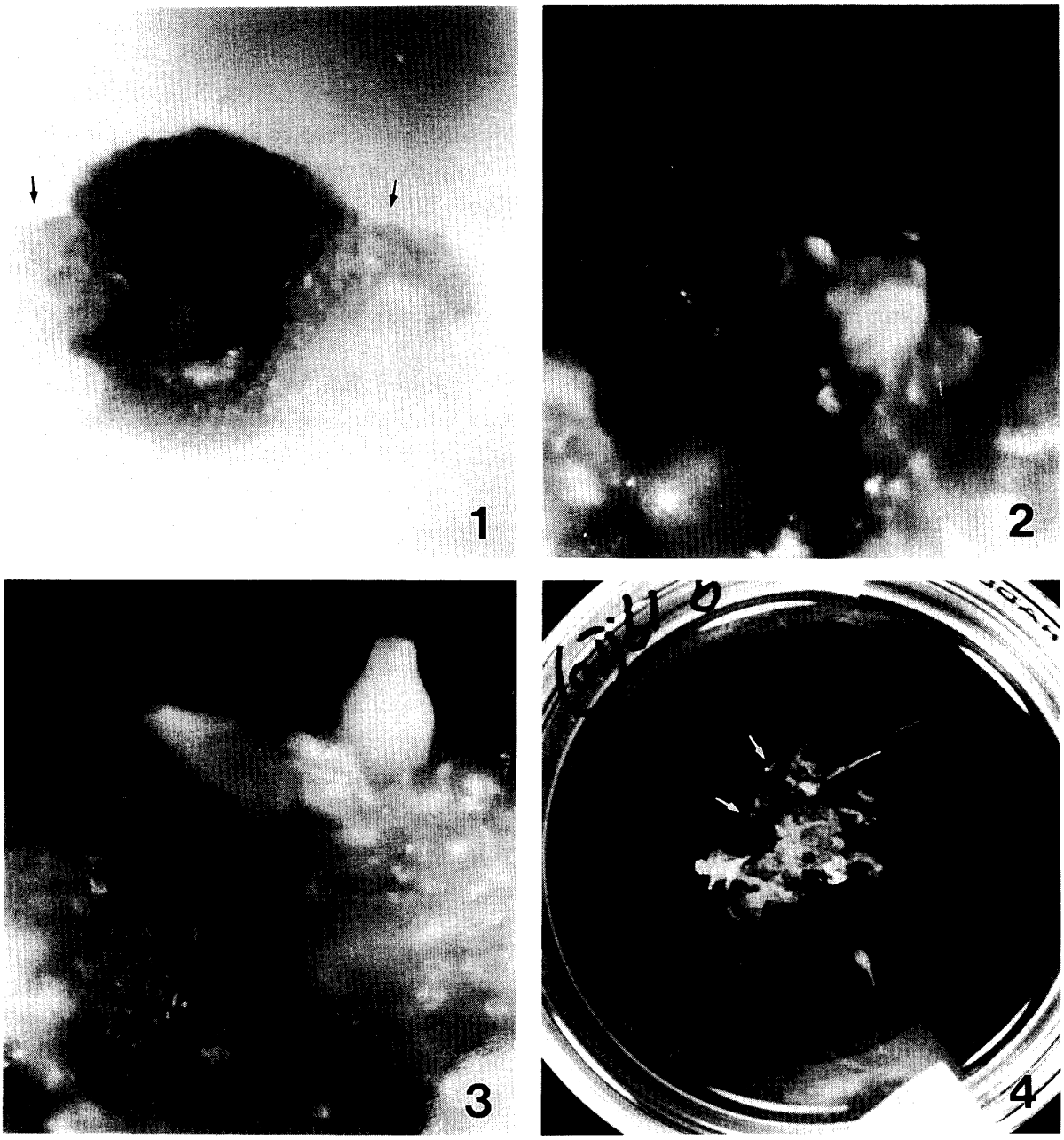

Fig. 1. Embryogenic callus derived from a shoot tip of White Star on MS medium supplemented with 2.0 mg/l 2,4-D (arrows).

Figs. 2–3. Somatic embryos formed from embryogenic callus of White Star on MS medium supplemented with 2.0 mg/l 2,4-D.

Fig. 4. Somatic embryos germinated on MS medium supplemented with 2.0 mg/l ABA.

Table 1. Formation of embryogenic callus from shoot tip explants of sweet potato on MS medium supplemented with 0.2 and 2.0 mg/l 2,4-D

Cultivar	2,4-D (mg/l)	No. of explants	Embryogenic callus No.	Embryogenic callus %
Aikoku	0.2	4	1	25.0
	2.0	4	0	0
Aooki	0.2	4	0	0
	2.0	5	0	0
Ariakeimo	0.2	4	0	0
	2.0	4	0	0
Genki (Kumamoto)	0.2	4	0	0
	2.0	3	1	33.3
Gifu No. 1	0.2	5	0	0
	2.0	5	0	0
Kakei 4-153	0.2	3	1	33.3
	2.0	0	0	0
Kanto No. 69	0.2	3	1	33.3
	2.0	4	0	0
Kanto No. 97	0.2	5	0	0
	2.0	4	0	0
Kintoki	0.2	4	1	25.0
	2.0	5	0	0
Koganesengan	0.2	4	0	0
	2.0	4	1	25.0
Kokei No. 14	0.2	10	1	10.0
	2.0	10	7	70.0
Konpira	0.2	4	1	25.0
	2.0	5	1	20.0
Kotobuki	0.2	5	1	20.0
	2.0	5	0	0
Kuroshirazu	0.2	5	1	20.0
	2.0	4	2	50.0
Lizhixiang	0.2	4	3	75.0
	2.0	4	0	0
White Star	0.2	10	2	20.0
	2.0	10	7	70.0

influenced by both cultivars and concentrations of 2,4-D. As shown in Table 1, 2,4-D of 0.2 mg/l gave a higher frequency of embryogenic callus than 2.0 mg/l in Aikoku, Kanto No. 69, Kintoki, Kakei 4-153, Kotobuki, and Lizhixiang, whereas in Genki, Koganesengan, Kokei No. 14, Kuroshirazu, and White Star 2.0 mg/l 2,4-D was more effective. In Konpira both concentrations of 2,4-D resulted in similar frequencies of embryogenic callus. No somatic embryogenesis was observed in Aooki, Ariakeimo, Gifu No. 1, and Kanto No. 97. High frequencies of embryogenic callus were obtained from Kokei No. 14, Lizhixiang, and White Star. Most of embryogenic calli formed somatic embryos on MS medium supplemented with 2,4-D (Figs. 2–3).

Five to 8 weeks after culture, when the obtained embryogenic calli were transferred onto MS medium supplemented either with 2.0 mg/l ABA (Kokei No. 14 and White Star) or 2.0 mg/l

BAP (the others tested), some somatic embryos developed further and germinated (Fig. 4). Eight weeks after transfer, somatic embryos which did not germinate were, with the callus, transferred onto MS basal medium and developed into shoots/plantlets. Regenerated shoots/plantlets grew vigorously on fresh basal medium (Fig. 5).

Fig. 5. Regenerated plantlets from White Star somatic embryos.

These results have demonstrated that somatic embryogenesis in sweet potato is markedly influenced by genotypes and the concentration of 2,4-D, in accordance with those reported by Jarret *et al.*[2]. High frequency somatic embryogenesis was achieved in Kokei No. 14, Lizhixiang, and White Star. However, most of the cultivars tested gave a low frequency of somatic embryogenesis. Thus, effects of auxin on somatic embryogenesis in sweet potato should be studied in detail.

Summary

Somatic embryogenesis and plant regeneration in shoot tip cultures of sweet potato was studied. Embryogenic callus was induced from shoot tip explants of 16 cultivars on MS medium supplemented with 0.2 and 2.0 mg/l 2,4-D. The frequency of embryogenic callus was markedly influenced by cultivars and concentrations of 2,4-D. When the obtained embryogenic calli were transferred onto MS medium supplemented either with 2.0 mg/l ABA or with 2.0 mg/l BAP, some somatic embryos germinated. When somatic embryos which did not germinate were, with the callus, transferred onto MS basal medium, they developed into shoots/plantlets.

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