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Varietal Differences of Somatic Embryogenesis in Shoot Tip Cultures of Sweet Potato, *Ipomoea batatas* (L.) Lam.

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Introduction

Plant regeneration in sweet potato, *Ipomoea batatas* (L.) Lam., is genotype-dependent[1–3,6]. Thus, to successfully use somatic hybridization technique in sweet potato breeding, it is extremely important to select genotypes with high regeneration ability.

Somatic embryogenesis in sweet potato has been reported[2,4,6,7]. However, in most cases the frequency of somatic embryogenesis was low, only a few cultivars such as White Star and Hi-starch gave a high frequency of somatic embryogenesis. In this paper we report varietal differences of somatic embryogenesis in shoot tip cultures of sweet potato.

Materials and Methods

Seven cultivars of sweet potato, Kokei No. 14, Kyushu No. 31, Lizhixiang, Nongdahong, Qunli No. 2, Taiwan, and Xushu No. 18, were used in this study. About 20 mm-long shoot tips excised from highly proliferating plants in a green house 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[5] (MS) medium supplemented with 0.2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), 3.0% sucrose (w/v), and 0.8% agar, pH 5.8, at $27\pm1^{\circ}C$ in the dark. The cultures were observed periodically under a dissecting microscope for somatic embryogenesis.

Eight to 9 weeks after culture, the obtained embryogenic calli with/without somatic embryos were transferred onto MS medium without plant growth regulators (basal medium) and cultured for the germination of somatic embryos under 13 h day-light at 3,000 lux and $27\pm1^{\circ}C$.

Results and Discussion

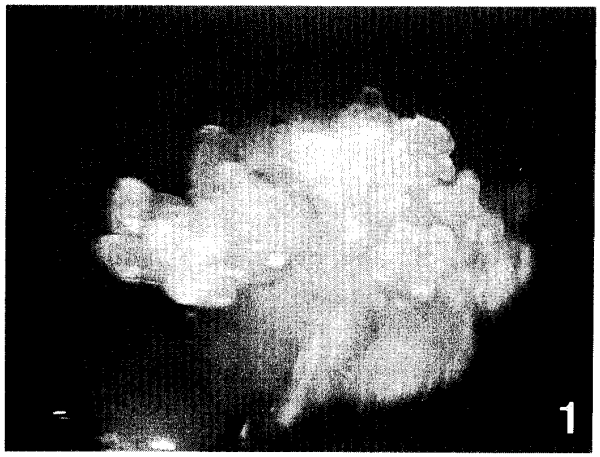
Five to 7 days after culture, shoot tips started to produce white, friable callus on their surface. This callus was non-embryogenic.

Four to 6 weeks after culture, bright-yellow and compact callus was formed on non-embryogenic callus (Fig. 1). This callus was embryogenic and gave rise to somatic embryos on MS medium supplemented with 0.2 mg/l 2,4-D.

The formation of embryogenic callus in the cultivars tested was influenced significantly by cultivars (Table 1). Nongdahong and Kyushu No. 31 gave a high frequency of embryogenic callus

Table 1. Formation of embryogenic callus on MS medium supplemented with 0.2 mg/l 2,4-D

Cultivar	No. of shoot tips	Embryogenic callus No.	Embryogenic callus %
Kokei No. 14	14	2	14.3
Kyushu No. 31	20	14	70.0
Lizhixiang	18	7	38.9
Nongdahong	14	10	71.4
Qunli No. 2	15	6	40.0
Taiwan	16	0	0
Xushu No. 18	14	2	14.3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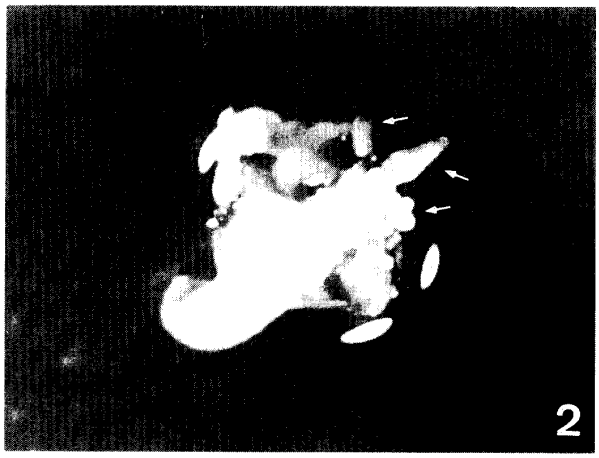
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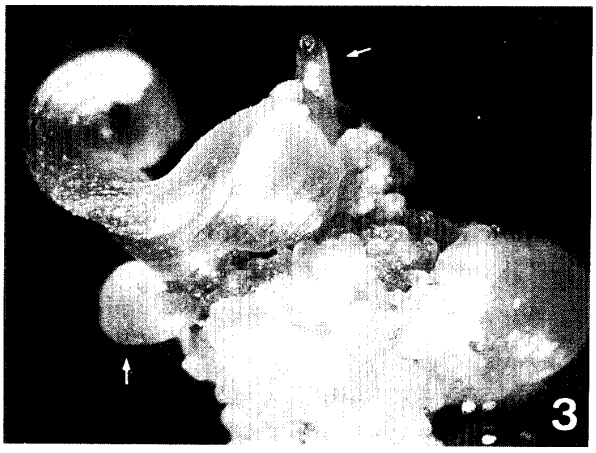
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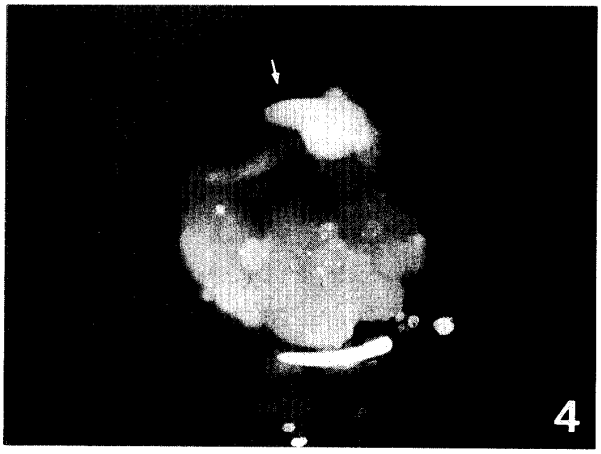
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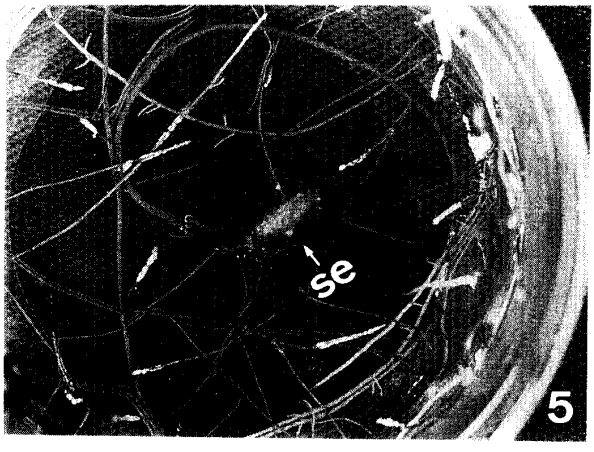

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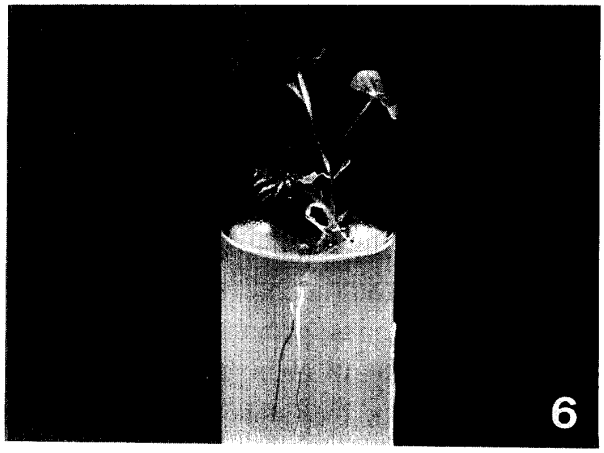
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over 70.0%. No embryogenic callus was formed from Taiwan. In other cultivars frequencies of embryogenic callus ranged from 14.3% to 40.0%.

Eight to 9 weeks after culture, heart- and torpedo-shaped somatic embryos appeared on the surface of embryogenic callus (Fig. 2). They were attached either directly to the callus or to the callus through suspensor-like structures, similar to those described by Jarret *et al.*[2]. There were also some embryogenic calli not forming somatic embryos.

When embryogenic calli with somatic embryos were transferred onto basal medium, somatic embryos developed to maturity and then germinated. Somatic embryo germination resulted in either shoot/plantlet formation (Figs. 3, 4) or only root initiation (Fig. 5).

The percentages of embryogenic calli with shoots/plantlets (35.7% to 100.0%) were different with cultivars (Table 2). As reported by Liu and Cantliffe[4], regenerated shoots/plantlets often had poorly or atypically developed cotyledons. These shoots/plantlets vigorously grew on fresh basal medium (Fig. 6). When embryogenic calli without somatic embryos were transferred onto basal medium, only adventitious root formation was observed on them.

The studies of Jarret *et al.*[2] and Shimonishi *et al.*[6] demonstrated that somatic embryogenesis in sweet potato was influenced significantly by genotypes. The present results have also shown it. In this study high frequency somatic embryogenesis was achieved in Nongdahong and Kyushu No. 31, and even in Qunli No. 2 and Lizhixiang. However, some somatic embryos failed to develop into shoots or plantlets on basal medium. This is a main difficulty in plant regeneration via somatic embryogenesis in sweet potato. Thus, somatic embryo germination in sweet potato should be improved.

Table 2. Formation of shoots / plantlets from somatic embryos on MS medium without plant growth regulators

Cultivar	No. of embryogenic callus transferred	Embryogenic callus with shoots/plantlets	
		No.	%
Kokei No. 14	2	1	50.0
Kyushu No. 31	14	5	35.7
Lizhixiang	7	3	42.9
Nongdahong	10	5	50.0
Qunli No. 2	6	3	50.0
Xushu No.18	2	2	100.0

Fig. 1. Embryogenic callus derived from a shoot tip of Qunli No. 2 on MS medium supplemented with 0.2 mg/l 2,4-D.

Fig. 2. Somatic embryos formed from a embryogenic callus of Kyushu No. 31 on MS medium supplemented with 0.2 mg/l 2,4-D (arrows).

Fig. 3. Germination of Qunli No. 2 somatic embryos on MS medium without plant growth regulators (arrows).

Fig. 4. Germination of Kyushu No. 31 somatic embryo on MS medium without plant growth regulators (arrow).

Fig. 5. Root initiation from Xushu No. 18 somatic embryo on MS medium without plant growth regulators (se: somatic embryo).

Fig. 6. Regenerated plantlet from Nongdahong somatic embryo.

Summary

Somatic embryogenesis in sweet potato was studied. Shoot tips (about 0.5 mm in length) of seven cultivars gave rise to non-embryogenic callus on MS medium supplemented with 0.2 mg/l 2,4-D. Four to 6 weeks after culture, embryogenic callus was formed on non-embryogenic callus, and then produced somatic embryos. Transfer of embryogenic callus with somatic embryos resulted in the germination of somatic embryos. Somatic embryogenesis in the cultivars tested was significantly different with cultivars.

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