Mem. Fac. Agr. Kagoshima Univ., **35**, 1-5, 1999

High Frequency Plant Regeneration in Leaf and Petiole Explant Cultures of Sweet Potato

Jing-Shan WANG*[1], Muneharu SATO and Satoru TAURA*[2]
(*Laboratory of Crop Science*)
Received for Publication September 10, 1998

Introduction

Efficient plant regeneration in tissue cultures of sweet potato (*Ipomoea batatas* (L.) Lam.), is an important step for genetic manipulation in sweet potato. In general, plant regeneration in tissue cultures of sweet potato is difficult because of its genotype-dependency[2]. Some studies showed that plants can be induced from the calli derived from stem, petiole and leaf explants of sweet potato. However, the frequency of plant regeneration was reached at the adequate level in few cultivars such as Chugoku No.25[5], Jewel[6], and Beniazuma[1]. Therefore, in this study, the development of an efficient plant regeneration method from leaf and petiole calli of sweet potato was attempted.

Materials and Methods

1. Plant materials

Three weeks old *in vitro*-grown plants of sweet potato cvs. Genki, Bitambi, White Star, and Kokei No.14 were used as the source of leaf and petiole explants in this study.

2. Callus induction and plant regeneration

The leaves were cut into 5mm-long, 3mm-wide strips, and the petioles were cut into 5mm -long segments. These explants were cultured on the MS medium supplemented with 2, 4-D (0.02, 0.05mg/l), 0.5mg/l kinetin, 3.0% (w/v) sucrose and 0.8% (w/v) agar at pH5.8, under 13h day-light at 3,000lux and 27°C.

After two to four weeks of culture, the formed calli were transferred onto either the MS basal medium or the MS medium supplemented with 3.0mg/l BAP, 3.0% (w/v) sucrose and 0.8% (w/v) agar at pH5.8 followed by being transferred onto MS basal medium, for plant regeneration. The calli were subcultured for two to three weeks interval on the basal medium under 13h day-light at 3,000 lux and 27°C.

Results and Discussion

1. Effect of 2, 4-D concentration on plant regeneration from leaf and petiole calli of sweet potato

Leaf and petiole calli of Genki and Bitambi formed on callus induction media supple-

*[1] Lyan Agricultural University, Lyan, China
*[2] Gene Research Center, Kagoshima University

mented with different concentrations of 2, 4-D (0.02, 0.05mg/l) and 0.5mg/l kinetin were transferred onto hormone-free medium. Two to three weeks after transference, the shoot formation was observed (Fig.1; A and B). As shown in Table 1, the frequency of shoot regeneration was influenced by the concentration of 2, 4-D included in callus induction medium. The one supplemented with 0.05mg/l 2, 4-D combined with 0.5mg/l kinetin was the most suitable medium for shoot regeneration both in Genki and in Bitambi. Their regeneration frequencies reached up to 91.3% and 78.3%, respectively.

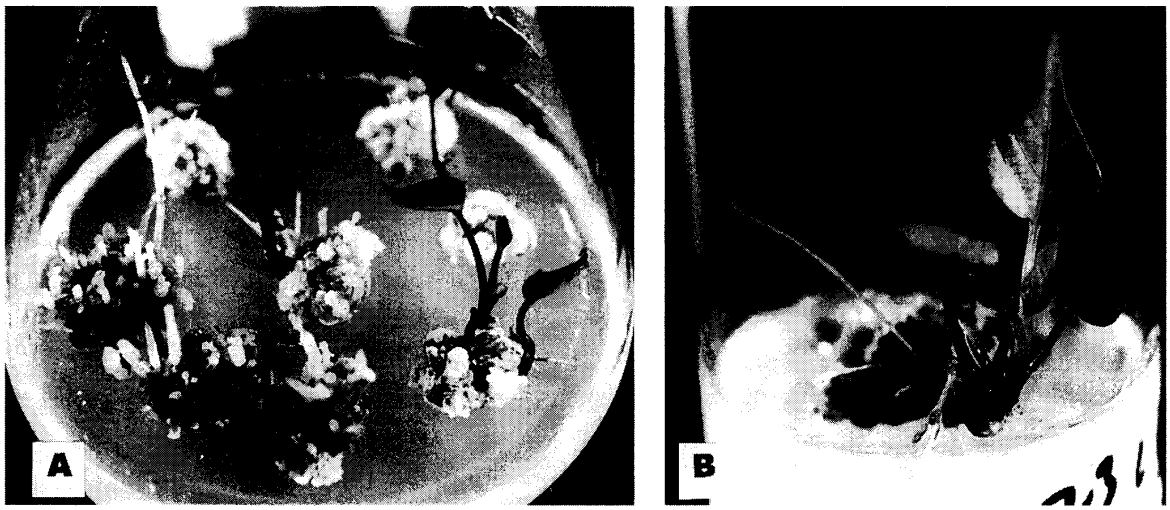

Fig. 1. Plant regeneration from leaf and petiole calli of sweet potato.
A: Shoot regeneration from leaf calli of Genki on hormone-free MS medium.
B: Shoot regeneration from leaf calli of Bitambi on hormone-free MS medium.

Table 1. Effect of 2, 4-D concentration on shoot regeneration from leaf and petiole calli of sweet potato

Cultivar	Explant	Callus induction medium		No. of calli transferred	No. of calli regenerating shoots (%)
		2,4-D (mg/l)	kinetin (mg/l)		
Genki	Leaf	0.02	0.5	25	1(4.0)
		0.05	0.5	23	21(91.3)
	Petiole	0.02	0.5	23	6(26.1)
		0.05	0.5	18	14(77.8)
Bitambi	Leaf	0.02	0.5	20	0
		0.05	0.5	23	15(65.2)
	Petiole	0.02	0.5	20	6(30.0)
		0.05	0.5	23	18(78.3)

*Regeneration medium: hormone-free MS medium.

2. Effect of BAP on plant regeneration from leaf and petiole calli of sweet potato

To determine the effect of BAP on shoot regeneration, the calli induced from the leaf and petiole explants of Genki and Bitambi on callus induction medium supplemented with 0.05mg /l 2, 4-D and 0.5mg/l kinetin were transferred onto the medium supplemented with or without BAP, followed by being transferred onto basal medium.

As shown in Table 2, the petiole calli of Genki that had been cultured on the regeneration medium supplemented with 3.0mg/l BAP gave a higher regeneration frequency than those on the hormone-free medium. However the higher frequencies were obtained by using hormone-free medium from leaf calli of Genki and from leaf and petiole calli of Bitambi. BAP played an important role in plant regeneration in tissue cultures and protoplast cultures of *I. trilobaa*[2),3)]. Otani and Shimada[4)] showed that shoot regeneration from leaf calli of *I. trichocarpa* was induced only on the medium supplemented with more than 2mg/l BAP, and the one with 10mg/l was the most effective. In contrast, Otani *et al.*[5)] mentioned that BAP inhibited the shoot regeneration in tissue cultures of sweet potato. They observed that the higher frequency of shoot regeneration was obtained on the hormone-free medium and the percentage of shoot regeneration was reduced with an increase in BAP concentration.

Table 2. Effect of BAP on shoot regeneration from leaf and petiole calli of sweet potato

Cultivar	Explant	Regeneration medium BAP(mg/l)	No. of calli transferred	No. of calli regenerating shoots (%)
Genki	Leaf	0	23	17(73.9)
		3.0	23	8(34.8)
	Petiole	0	31	21(67.7)
		3.0	22	18(81.8)
Bitambi	Leaf	0	22	15(68.2)
		3.0	24	3(12.5)
	Petiole	0	21	16(76.2)
		3.0	22	4(18.2)

*Callus induction medium: MS medium supplemented with 0.05mg/l 2,4-D and 0.5mg/l kinetin.

In the present experiment, the addition of BAP to regeneration medium was effective for shoot regeneration from petiole calli of Genki, but inhibited the shoot regeneration both from leaf calli of the same cultivar and from leaf and petiole calli of Bitambi. This suggests that the role of BAP in plant regeneration is different with genotypes. This difference may be related to the endogenous levels of hormone in various genotypes[1)].

3. Effect of different genotypes on plant regeneration from leaf and petiole calli of sweet potato

Calli formed from leaf and petiole explants of various sweet potato cultivars on the medium supplemented with 0.05mg/l 2, 4-D and 0.5mg/l kinetin were transferred onto hormone-free medium for plant regeneration. It was obvious that there existed some differences in regenerability among genotypes. Genki and Bitambi gave higher regeneration frequencies than those of White Star and Kokei No.14 as shown in Table 3. This experiment also showed that the frequencies of calli regenerating shoots were different among explants. The leaf calli gave a higher frequency than petiole calli in Genki, however, the petiole calli performed better than leaf calli in Bitambi, White Star, and Kokei No.14. Differences in regenerability were also observed among different genotypes in sweet potato and its related species[1,6] and among different explants of *I. batatas*[6] and *I. triloba*[2].

Table 3. Effect of different genotypes on shoot regeneration from leaf and petiole calli of sweet potato

Cultivar	Explant	No. of calli cultured	No. of calli regenerating shoots(%)
Genki	Leaf	16	14(87.5)
	Petlole	38	24(63.2)
Bitambi	Leaf	26	16(61.5)
	Petiole	43	34(79.1)
White Star	Leaf	24	3(12.5)
	Petiole	21	10(47.6)
Kokei No.14	Leaf	23	6(26.1)
	Petiole	19	6(31.6)

*Callus induction medium: MS medium supplemented with 0.05mg/l 2,4-D and 0.5mg/l kinetin.

The regeneration rates obtained in the present study were higher than those previously reported in tissue cultures of sweet potato. The vital point to achieve efficient shoot regeneration is considered to be the use of the lower concentration of 2, 4-D (0.05mg/l) combined with kinetin into the medium. This result further confirms the important role of lower 2, 4-D concentration and kinetin on plant regeneration in sweet potato[7]. Therefore, the plant regeneration system established here could be useful for the studies of genetic transformation in sweet potato.

Summary

The culture conditions for efficient plant regeneration in tissue cultures of sweet potato (*Ipomoea batatas* (L.) Lam.) were studied. Leaf and petiole explants of sweet potato cultivars, Genki, Bitambi, White Star, and Kokei No.14 were cultured on MS medium supplemented with 2, 4-dichlorophenoxyacetic acid (2, 4-D) (0.02 and 0.05mg/l) and 0.5mg/l kinetin, for callus induction. The formed calli were transferred onto either hormone-free MS medium or the medium supplemented with 3.0mg/l 6-benzylaminopurine (BAP) followed by being transferred onto hormone-free medium, for plant regeneration. The result showed that the callus induction medium supplemented with 0.05mg/l 2, 4-D and 0.5mg/l kinetin was useful for shoot regeneration and the effect of BAP was different with genotypes and explants. BAP promoted the shoot regeneration in petiole calli of Genki, but inhibited the shoot regeneration in leaf calli of Genki as well as in leaf and petiole calli of Bitambi.

References

1) Belarmino, M. M., Abe, T. and Sasahara T. : Efficient plant regeneration from leaf calli of *Ipomoea batatas* (L.) Lam and its related species. *Japan J. Breed.*, **42**, 109-114 (1992).
2) Liu, Q. C., Kokubu, T. and Sato, M. : Plant regeneration in stem, petiole and leaf explant cultures of *Ipomoea triloba* L. *Japan J. Breed.*, **40**, 321-327 (1990).
3) Liu, Q. C., Kokubu, T. and Sato, M. : Plant regeneration from *Ipomoea triloba* L. Protoplasts. *Japan J. Breed.*, **41**: 103-108 (1991).
4) Otani, M. and Shimada, T.: Plant regeneration from leaf calli of *Ipomoea trichocarpa* Ell. *Japan J. Breed.*, **38**, 205-211 (1988).
5) Otani, M., Mii, M. and Shimada, T.: High frequency plant regeneration from leaf calli in sweet potato cv. Chugoku 25. *Plant Tiss. Cult. Lett.*, **13**, 23-27 (1996).
6) Pido, N., Kowyama, Y., Kakeda, K. and Shiotani, I.: Plant regeneration from leaf disks and stem segments of sweet potato using only NAA as supplementary growth regulator. *Plant Tiss. Cult. Lett.*, **12**, 289-296 (1995).
7) Wang, J. S., Sato, M., Taura S. and Kokubu, T.: Efficient plant regeneration from protoplasts of sweet potato, *Ipomoea batatas* (L.) Lam. *Mem. Fac. Agr. Kagoshima Univ.*, **34**, 65-70 (1998).