Plant Regeneration in Tissue Cultures of Ipomoea tiliacea(Willd.)Choisy

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Plant Regeneration in Tissue Cultures of *Ipomoea tiliacea* (Willd.) Choisy

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Introduction

Based on the cross-compatibility with sweet potato, *Ipomoea batatas* (L.) Lam., the wild related species of sweet potato are classified into two groups: Group I cross-compatible with sweet potato and Group II cross-incompatible with sweet potato. The related species belonging to Group II have not successfully been applied in sweet potato breeding. To overcome such cross-incompatibility through somatic cell hybridization, it is essential to establish a plant regeneration system from a callus.

Plant regeneration from leaf- and petiole-derived calli of a few diploid species cross-incompatible with sweet potato, *I. trichocarpa, I. triloba, I. lacunosa* have been reported[1–3,5]. However, no plant regeneration of tetraploid species cross-incompatible with sweet potato has been reported.

I. tiliacea, possessing one genome A_1 homologous to a genome A from *I. trichocarpa* and another genome labelled T, namely, the genome A_1A_1TT, is a tetraploid species cross-incompatible with sweet potato[6]. In this paper we report plant regeneration in tissue cultures of strain K270-3 of *I. tiliacea.*

Materials and Methods

1. Plant material

Plants *in vitro* grown from shoot tips of *I. tiliacea* strain K270-3 were used as the stock material of explants in this study.

The stems were grown in pots in a greenhouse (15–32°C). About 5 mm-long shoot tips excised from the plants were surface-sterilized with 70% ethanol for 10 s and then soaked in 2% sodium hypochlorite solution for 15 min. They were immediately rinsed three times with sterile distilled water. Sterilized shoot tips were inoculated on Murashige and Skoog[4] (MS) medium containing 3.0% (w/v) sucrose and 0.8% (w/v) agar but no plant growth regulators, i.e. basal medium. The pH was adjusted to 5.8 with 1 N sodium hydroxide before autoclaving for 120 min at 120°C. The culture was carried out under 13 h day-light at 3000 lux and 27 ± 1°C. The shoots were subcultured every 3 weeks.

2. Preparation of explants

Three-week-old shoots were rinsed with sterile distilled water. Leves and petioles were taken from the first four well-expanded leaves. Explants were prepared by cutting leaves into 5

mm-long, 2 mm-wide strips and petioles into 5 mm-long segments.

3. Culture of explants

Explants were incubated on MS medium supplemented with 0.02, 0.2, 1.0, 2.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), 0, 0.5 mg/l kinetin, 3.0% (w/v) sucrose, and 0.8% (w/v) agar, pH 5.8, at 27°C in the dark. Four explants were placed in each of at least 15 culture bottles per treatment. The cultures were weekly evaluated for callus growth, root formation and growth, and shoot formation.

4. Plantlet regeneration

Three or 4 weeks after incubation, the obtained calli were transferred onto MS medium supplemented with 3-indoleacetic acid (IAA; 0, 0.2 mg/l), 6-benzylaminopurine (BAP; 0, 1.0, 2.0 mg/l), 3.0% (w/v) sucrose, and 0.8% (w/v) agar, pH 5.8 (regeneration medium), and cultured for 6 weeks under 13 h day-light at 3000 lux and 27 ± 1°C. The calli formed many adventitious roots, and then were cultured on basal medium under 13 h day-light at 3000 lux and 27 ± 1°C.

Regenerated shoots were excised and transferred onto fresh basal medium to induce roots. Regenerated plantlets were rinsed carefully with tap water and transplanted in pots with vermiculite.

Results and Discussion

1. Callus induction

Callus formation began within 1 week after incubation. The response of two kinds of explants to various media was quite similar.

Table 1. Callus formation on media containing various concentrations of 2,4-D and BAP in tissue cultures of *I. tiliacea* strain K270-3[1)]

Explant	2,4-D (mg/l)	Kinetin (mg/l)	No. of large callus	No. of small callus	No. of explants withered
Leaf	0.02	0	5	54	1
	0.02	0.5	55	5	0
	0.2	0	0	9	51
	0.2	0.5	50	3	7
	1.0	0	0	19	41
	1.0	0.5	56	1	3
	2.0	0	0	39	21
	2.0	0.5	47	10	3
Petiole	0.02	0	20	36	4
	0.02	0.5	58	2	0
	0.2	0	0	12	48
	0.2	0.5	53	1	6
	1.0	0	0	54	6
	1.0	0.5	49	2	9
	2.0	0	11	29	20
	2.0	0.5	54	1	5

[1)] Sixty explants per treatment were used.

The callus grew slowly and the explants gradually withered on the medium containing only 2,4-D. However, growth of callus was markedly quick on media containing various concentrations of 2,4-D and 0.5 mg/l kinetin, especially 0.02 mg/l 2,4-D and 0.5 mg/l kinetin (Table 1).

Calli induced on various media showed different characteristics in colour and texture. Calli formed on media containing 0.02 mg/l 2,4-D and 0, 0.5 mg/l kinetin were compact, flat, and milk white. Calli formed on media containing 0.2, 1.0, 2.0 mg/l 2,4-D and 0.5 mg/l kinetin were friable, round, and light yellow.

Eight weeks after incubation, adventitious root formation was observed from the callus on the medium containing 0.02 mg/l 2,4-D and 0.5 mg/l kinetin.

2. Adventitious root and shoot formation from callus on regeneration medium and basal medium

Eight weeks after incubation, the calli induced on media containing either only 0.02 mg/l 2,4-D or 0.02, 0.2, 1.0, 2.0 mg/l 2,4-D and 0.5 mg/l kinetin were transferred onto the regeneration medium containing various concentrations of IAA and BAP. No calli induced on media containing 0.2, 1.0, 2.0 mg/l 2,4-D were, however, transferred onto the regeneration medium because they grew very slowly and then gradually withered.

After transfer onto the regeneration medium, the calli induced on the medium containing 0.02 mg/l 2,4-D and 0, 0.5 mg/l kinetin gradually became light green. Two weeks after transfer, adventitious root formation was observed from the calli on regeneration media containing 0 mg/l IAA+1.0 mg/l BAP and 0.2 mg/l IAA+1.0 mg/l BAP, but the calli transferred onto the regeneration medium without IAA and BAP gradually became light brown, then light green, and after 5 weeks a few calli produced adventitious roots.

The calli induced on media containing 0.2, 1.0, 2.0 mg/l 2,4-D and 0.5 mg/l kinetin kept light yellow in colour after transfer onto the regeneration medium, but later gradually became light brown to death.

In a word, only calli induced on media containing 0.02 mg/l 2,4-D and 0, 0.5 mg/l kinetin formed adventitious roots on the regeneration medium, and the calli induced on the medium containing over 0.02 mg/l 2,4-D did not form adventitious roots.

Six weeks after transfer onto the regeneration medium, the calli were up to 20 mm and formed many adventitious roots (Table 2). The calli with adventitious roots were transferred onto fresh regeneration medium or basal medium. Five weeks after transfer onto basal medium, shoots were formed from the petiole-derived callus which had been cultured on the callus induction medium containing 0.02 mg/l 2,4-D and 0.5 mg/l kinetin, and further on the regeneration medium containing 0.2 mg/l IAA and 1.0 mg/l BAP (Fig. 1). The half of this callus was light brown and the other half formed many adventitious roots. Three shoots were formed from green areas of the half forming adventitious roots. Four weeks after shoot formation, roots formed on the shoots. Regenerated plantlets were washed carefully with tap water and transplanted in pots with vermiculite.

Otani and Shimada[5] obtained regenerated plants from leaf callus of *I. trichocarpa*. They emphasized the role of BAP in plant regeneration. Suga and Irikura[7] investigated plant regeneration from leaf callus of *I. tiliacea* and *I. trifida* (2x) and observed plant regeneration only from leaf callus of *I. trifida*. They emphasized the role of low concentration of 2,4-D added to the callus induction medium in plant regeneration. We reported high frequency plant regeneration from stem, petiole, and leaf calli of *I. triloba*[2,3].

Table 2. Adventitious root and shoot formation from callus of *I. tiliacea* strain K270-3 on regeneration medium and basal medium

Explant	Callus induction medium		Regeneration medium		No. of callus cultured	No. of callus forming roots	No. of callus forming shoots
	2,4-D (mg/l)	KT[1] (mg/l)	IAA (mg/l)	BAP (mg/l)			
Leaf	0.02	0	0	0	2	0	0
	0.02	0	0	1.0	2	0	0
	0.02	0	0.2	1.0	1	0	0
	0.02	0	0.2	2.0	1	0	0
	0.02	0.5	0	0	14	1	0
	0.02	0.5	0	1.0	14	1	0
	0.02	0.5	0.2	1.0	14	2	0
	0.02	0.5	0.2	2.0	13	1	0
Petiole	0.02	0	0	0	5	1	0
	0.02	0	0	1.0	5	2	0
	0.02	0	0.2	1.0	5	1	0
	0.02	0	0.2	2.0	5	2	0
	0.02	0.5	0	0	15	8	0
	0.02	0.5	0	1.0	15	12	0
	0.02	0.5	0.2	1.0	14	13	1[2]
	0.02	0.5	0.2	2.0	14	10	0

[1] KT=kinetin.

[2] Shoot regeneration was observed on basal medium.

Fig. 1. Shoots regenerated from petiole-derived callus of *I. tiliacea* on MS basal medium.

In this study only a few plantlets were regenerated from petiole-derived callus of *I. tiliacea*. Only calli induced on media containing 0.02 mg/l 2,4-D and 0, 0.5 mg/l kinetin formed adventitious roots and shoots on the regeneration medium, and the calli induced on the medium

containing over 0.02 mg/l 2,4-D did not form adventitious roots at all. It is probable that efficient plant regeneration in *I. tiliacea* requirs lower concentration of 2,4-D included in callus induction medium than *I. triloba*.

Summary

Plant regeneration from callus of *I. tiliacea* strain K270-3 was studied. The growth of the callus induced on media containing 0.02, 0.2, 1.0, 2.0 mg/l 2,4-D but no kinetin was slow, but the callus induced on media containing various concentrations of 2,4-D and 0.5 mg/l kinetin, especially 0.02 mg/l 2,4-D and 0.5 mg/l kinetin showed rapid growth.

Eight weeks after incubation, when the calli well proliferating on media containing 0.02–2.0 mg/l 2.4-D and 0.5 mg/l kinetin were transferred onto regeneration media containing 0, 0.2 mg/l IAA and 0, 1.0, 2.0 mg/l BAP, only calli induced on media containing 0.02 mg/l 2,4-D and 0, 0.5 mg/l kinetin formed roots on the regeneration medium. Six weeks after transfer, the calli with adventitious roots were further transferred onto fresh regeneration media and then basal medium. Five weeks after transfer onto basal medium, shoots were formed from a petiole-derived callus which had been cultured on the callus induction medium containing 0.02 mg/l 2,4-D and 0.5 mg/l kinetin and further on the regeneration medium containing 0.2 mg/l IAA and 1.0 mg/l BAP. After 4 weeks, the shoots formed roots and developed into plantlets.

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