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Plant Regeneration in Leaf and Petiole Explant Cultures of *Ipomoea littoralis* Blune

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Introduction

The wild related species of sweet potato, *Ipomoea batatas* (L.) Lam., are classified into two groups based on the cross-compatibility with sweet potato: Group I cross-compatible with sweet potato and Group II cross-incompatible with sweet potato[6]. *I. littoralis* is one of the related species cross-compatible with sweet potato, and has genomes $B_2B_2B_2B_2$ homologous to sweet potato ($B_1B_1B_2B_2B_2B_2$)[4]. Therefore, *I. littoralis* is especially an interesting related species as a partner of somatic hybridization with Group II diploid species which have genomes AA. To produce somatic hybrids through protoplast fusion, it is necessary to establish a plant regeneration system from the callus of these related species.

Plant regeneration in tissue cultures of sweet potato and its related species is difficult partly because of its genotype-dependency[2]. Belarmino *et al.*[1] reported plant regeneration from leaf callus of *I. trifida* (4x) through the use of the callus induction medium containing NAA (2.0 mg/l) and BAP (0.1 mg/l). In this study, using the callus induction medium containing various concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and kinetin we succeeded in plant regeneration from leaf- and petiole-derived calli of *I. littoralis*.

Materials and Methods

The basal medium included the inorganic salts and vitamins of Murashige and Skoog[3], 3.0% (w/v) sucrose, and 0.8% (w/v) agar (MS medium). The pH was adjusted to 5.8 with 1 N sodium hydroxide before autoclaving at 120°C for 20 min.

1. Plant materials

Plants *in vitro* cultured from shoot tips of *I. littoralis* strain K233-1 were used as the sources of explants in this study. The culture was carried out on basal medium under 13 h day-light at 3000 lux and 27±1°C. The shoots were subcultured every 3 weeks.

2. Culture of explants

Explants were prepared through cutting leaves into 5 mm-long, 2 mm-wide strips and petioles into 5 mm-long segments.

Experiment 1: Explants were placed on MS medium containing 0.02, 0.2, 1.0, 2.0 mg/l 2,4-D and 0, 0.5 mg/l kinetin. The cultures were incubated under 13 h day-light at 3000 lux and 27± 1°C. After 8 weeks, the induced calli were transferred onto MS medium supplemented with 3-indoleacetic acid (IAA; 0, 0.2 mg/l) and 6-benzylaminopurine (BAP; 1.0, 2.0 mg/l) and

cultured under 13 h day-light at 3000 lux and 27±1°C.

Experiment 2: Explants were incubated on MS medium containing 0.02 mg/l 2,4-D and 0.5, 1.0, 1.5, 2.0 mg/l kinetin under 13 h day-light at 3000 lux and 27±1°C. Five weeks after incubation, some calli were transferred onto basal medium and cultured under 13 h day-light at 3000 lux and 27±1°C, and the others were continuously cultured on the callus induction medium.

Results and Discussion

Experiment 1

Callus formation began within 1 week. The response of two kinds of explants to various media was very similar. The calli induced on the media containing 0.02 mg/l 2,4-D and 0, 0.5 mg/l kinetin were compact and flat, and most of them formed adventitious roots on the callus induction media (Fig. 1, Table 1).

Table 1. Callus and adventitious root formation from leaf and petiole explants of *I. littoralis* strain K233-1

Explant	Callus formation medium		No. of explants cultured	No. of calli induced	Callus forming roots	
	2,4-D (mg/l)	Kinetin (mg/l)			No.	%
Leaf	0.02	0	26	22	22	85.0
	0.02	0.5	26	26	14	54.0
	0.2	0	26	6	0	0
	0.2	0.5	26	20	0	0
	1.0	0	26	0	0	0
	1.0	0.5	26	20	0	0
	2.0	0	26	0	0	0
	2.0	0.5	26	10	0	0
Petiole	0.02	0	24	20	20	83.0
	0.02	0.5	24	22	12	50.0
	0.2	0	24	7	0	0
	0.2	0.5	24	22	0	0
	1.0	0	24	0	0	0
	1.0	0.5	24	14	0	0
	2.0	0	24	0	0	0
	2.0	0.5	24	12	0	0

The calli induced on the media containing 0.2, 1.0, 2.0 mg/l 2,4-D and 0.5 mg/l kinetin were soft and grew rapidly at earlier stage of culture, but at later stage stopped growth and became light brown. Moreover, the number of calli induced on the media containing 0.2, 1.0, 2.0 mg/l 2,4-D but no kinetin was remarkedly less than on the media containing 0.2, 1.0, 2.0 mg/l 2,4-D and 0.5 mg/l kinetin (Table 1).

Eight weeks after incubation, the calli were transferred onto regeneration media supplemented with 0, 0.2 mg/l IAA and 1.0, 2.0 mg/l BAP. The calli induced on callus induction media containing 0.02 mg/l 2,4-D and 0, 0.5 mg/l kinetin produced many rapidly growing adventitious roots, but no shoot formation was observed. The calli induced on callus induction

media containing 0.2, 1.0, 2.0 mg/l 2,4-D and 0, 0.5 mg/l kinetin (except media containing only 1.0, 2.0 mg/l 2,4-D) produced no adventitious roots or shoots.

Eight weeks after transfer onto the regeneration medium, transferred onto MS medium without plant growth regulators, the calli stopped growth and then withered.

As mentioned above, when transferred onto the regeneration medium, only calli induced on the callus induction medium containing 0.02 mg/l 2,4-D produced adventitious roots, and when 2,4-D added to the callus induction medium was over 0.02 mg/l, neither adventitious roots nor shoots were formed. These results have demonstrated that the concentration of 2,4-D added to the callus induction medium markedly influences the formation of adventitious roots and shoots.

Experiment 2

The growth of calli derived from leaf and petiole explants was very similar on all callus induction media used. Two weeks after incubation, a few calli began to form adventitious roots.

Five weeks after incubation, some calli were transferred onto basal medium. Two weeks after transfer, shoots were formed either directly from the callus or from the adventitious roots, and at almost the same time the calli remaining on the callus induction medium also formed shoots (Fig. 2, Table 2).

In this experiment the callus induced on the medium containing 0.02 mg/l 2,4-D and 1.0 mg/l kinetin gave the highest frequency of shoot formation. No shoot formation was observed from the callus induced on the medium containing 0.02 mg/l 2,4-D and 2.0 mg/l kinetin.

In the study of Belarmino *et al.*[1], plants were regenerated from leaf callus of *I. trifida* (4x) through the use of the callus induction medium containing 2.0 mg/l NAA and 0.1 mg/l BAP, but leaf callus obtained on the callus induction medium containing 0.5 mg/l 2,4-D and 0.1 mg/l BAP failed to produce plants. Suga and Irikura[5] reported that the concentrations of 2,4-D and BAP added to the callus induction medium influenced plant regeneration in *I. trifida* (2x, strain K221) and sweet potato cultivars, Kokei No. 14 and DJ-13-2. The present results show that extremely low concentration of 2,4-D (0.02 mg/l) and 1.0 mg/l kinetin included in the callus induction medium is effective in plant regeneration from the callus of *I. littoralis*. Thus, it appears that the compositions of plant growth regulators included in callus induction medium have marked effects

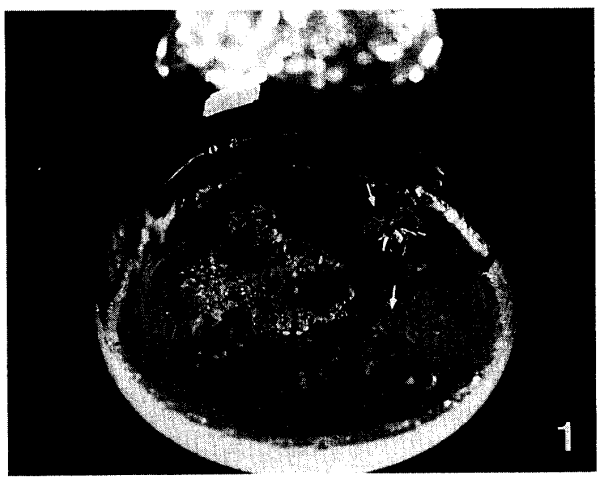

Fig. 1. Adventitious roots formed from calli of *I. littoralis* on MS medium containing 2,4-D and kinetin (arrows).

Fig. 2. Shoot regenerated from a callus of *I. littoralis* on MS basal medium.

Table 2. Adventitious root and shoot formation from the calli induced on media containing 0.02 mg/l 2,4-D and various concentrations of kinetin

Explant	Callus induction medium		No. of explants cultured	Callus forming roots		Callus forming shoots	
	2,4-D (mg/l)	Kinetin (mg/l)		No.	%	No.	%
Leaf	0.02	0.5	25	25	100.0	0	0
	0.02	1.0	25	19	76.0	5	20
	0.02	1.5	25	12	48.0	2	8
	0.02	2.0	25	8	32.0	0	0
Petiole	0.02	0.5	25	16	64.0	3	12
	0.02	1.0	25	12	48.0	4	16
	0.02	1.5	25	4	16.0	2	8
	0.02	2.0	25	1	4.0	0	0

on plant regeneration in sweet potato and its related species, and the optimal composition is different with cultivars or strains.

Summary

Plant regeneration from callus of *I. littoralis* strain K233-1 was studied. Only calli induced on the medium containing 0.02 mg/l 2,4-D and various concentrations of BAP produced adventitious roots. When 2,4-D was over 0.02 mg/l, no adventitious root formation was observed. Of the callus induction media tested, the medium containing 0.02 mg/l 2,4-D and 1.0 mg/l BAP was optimal to plant regeneration from the callus of *I. littoralis* strain K233-1.

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