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Somatic Embryogenesis and Shoot Organogenesis from Leaf Callus of *Ipomoea triloba* L.

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Introduction

Utilization of most of the related species in sweet potato (*Ipomoea batatas* (L.) Lam.) breeding has been limited by their cross-incompatibility with sweet potato. Recently, it has been attached importance to overcome such cross-incompatibility through somatic cell hybridization. The successful application of this technique requires a reliable and efficient system of plant regeneration from a callus.

Somatic embryogenesis in sweet potato has been obtained[1,3,5,7,14]. There has been no report of somatic embryogenesis in the related species of sweet potato. A few studies on shoot organogenesis in cell and tissue cultures of the related species have been carried out[6,8–10,12,13,15]. *I. triloba* L. is one of the related species that are cross-incompatible with sweet potato. This paper describes somatic embryogenesis and efficient shoot organogenesis from leaf callus of *I. triloba* L..

Materials and Methods

The basal medium included the inorganic salts and vitamins of Murashige and Skoog[11], 3.0% (w/v) sucrose, and 0.8% (w/v) agar (MS medium). The pH was adjusted to 5.8 with 1N sodium hydroxide before autoclaving at 120°C for 20 min.

In vitro grown plants of *I. triloba* L. were obtained as previously described[8]. Young well-expanded leaves were cut into 3–5 mm-long, 2 mm-wide strips, and incubated on MS medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D; 0.05, 0.2, 1.0, 3.0, 5.0 mg/l; callus formation medium) at 27±1°C in the dark. Callus formation was observed with a dissecting microscope. Embryogenic and non-embryogenic callus was formed.

Seven weeks after incubation, non-embryogenic callus was transferred onto MS medium supplemented with 6-benzylaminopurine (BAP; 0, 1.0, 2.0 mg/l; regeneration medium) and cultured for 7 weeks under 13 h day-light at 3000 lux and 27±1°C. And 9 weeks after incubation, embryogenic callus was cultured on the regeneration medium under the same conditions as above. Plantlets or shoots were regenerated from them. The remaining non-shoot forming calli were further cultured for 4 weeks on the basal medium under 13 h day-light at 3000 lux and 27±1°C.

Regenerated shoots were transferred onto fresh basal medium to induce roots. Resulting plantlets were transplanted in pots with vermiculite and grew to maturity.

Results and Discussion

1. Formation of embryogenic and non-embryogenic callus

One week after incubation, leaf explants started to form callus. Five to 7 weeks after incubation, two kinds of callus distinct in color and texture were formed. One callus, pale-yellow, fine-grained and watery, was embryogenic (Fig. 1A) and the other, white and friable, was

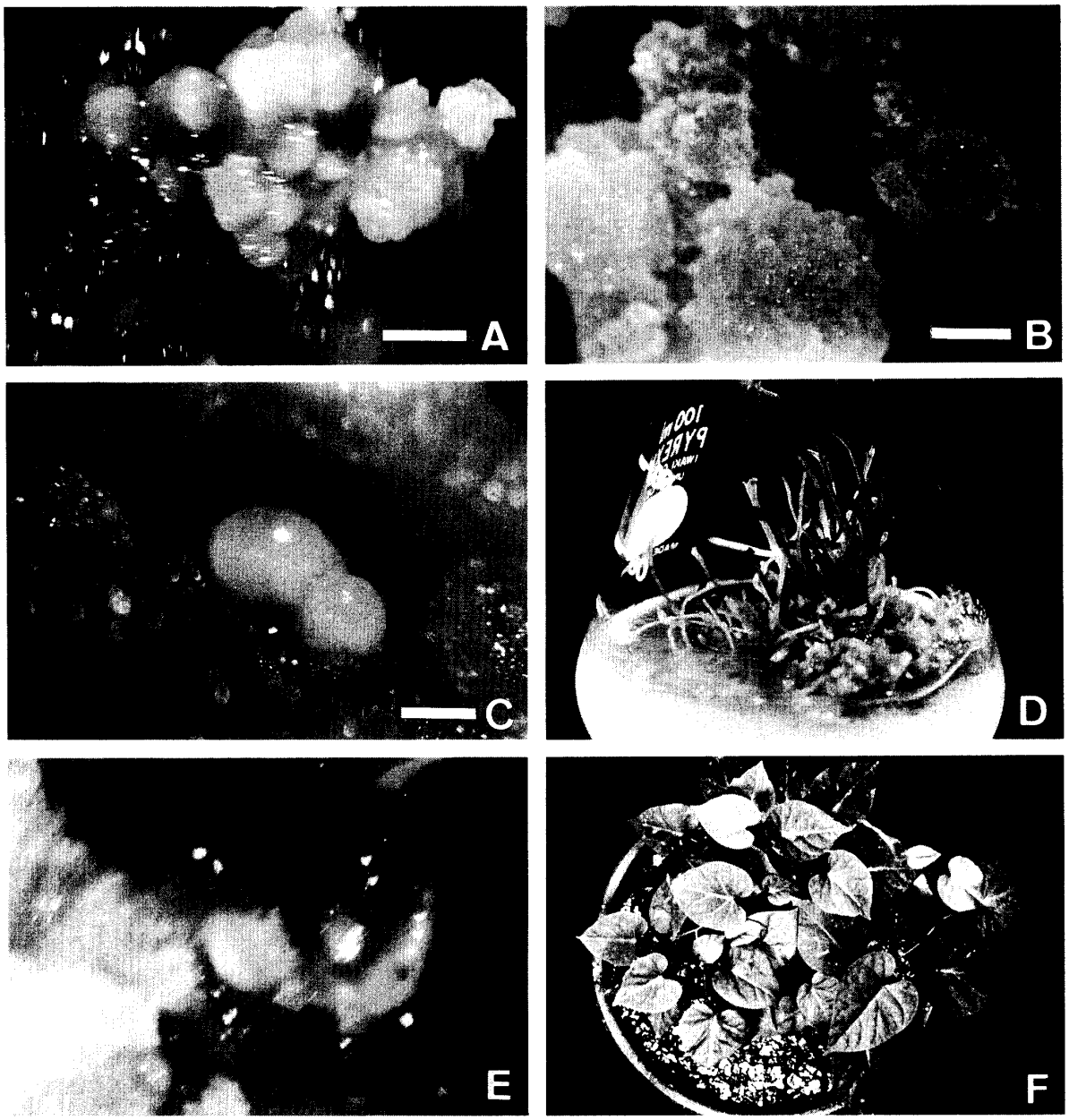

Fig. 1A-F. Somatic embryogenesis, shoot organogenesis and plant regeneration from leaf callus of *I. triloba* L. Scale bars=0.5 mm. A: Embryogenic callus formed on MS medium supplemented with 0.05 mg/l 2,4-D at 7 weeks after incubation. B: Non-embryogenic callus formed on MS medium supplemented with 0.2 mg/l 2,4-D at 7 weeks after incubation. C: Somatic embryos emerging from an embryogenic callus at 9 weeks after incubation. D: Multiple shoot regeneration via organogenesis from a non-embryogenic callus cultured on MS medium supplemented with 1.0 mg/l BAP. E: Regenerated plantlet from a somatic embryo. F: Regenerated mature plants in a pot with vermiculite.

non-embryogenic (Fig. 1B).

Of 2,4-D concentrations used, only 0.05 mg/l was effective in the induction of embryogenic callus (Table 1). On callus formation medium with 0.2 mg/l 2,4-D, even if some calli were cultured for 12 to 15 weeks, no somatic embryogenesis was observed. Nine weeks after incubation, embryogenic callus gave rise to globular to heart-shaped embryos (Fig. 1C).

Callus formation frequencies differed with 2,4-D concentrations. All explants formed callus at 0.05 to 1.0 mg/l 2,4-D. 2,4-D over 1.0 mg/l was not beneficial to callus formation (Table 1).

Table 1. Formation of embryogenic and non-embryogenic callus from leaf explants of *I. triloba* L. on MS medium containing various 2,4-D concentrations

2,4-D (mg/l)	No. of explants cultured	Embryogenic callus		Non-embryogenic callus	
		No.	%	No.	%
0.05	60	9	15	51	85.0
0.2	157	0	0	157	100.0
1.0	49	0	0	49	100.0
3.0	45	0	0	33	73.3
5.0	53	0	0	26	49.1

2. Plant regeneration from embryogenic callus

Embryogenic calli with globular to heart-shaped embryos were transferred onto either the basal medium or regeneration medium to promote further development of the somatic embryos. Two to 3 weeks after transfer, some of the somatic embryos developed into plantlets on regeneration medium with 2.0 mg/l BAP, and on the basal medium plantlet development from some of the somatic embryos was observed at 5 weeks after transfer (Fig. 1E, Table 2). The somatic embryos formed only roots but not shoots on regeneration medium with 1.0 mg/l BAP. Regenerated plantlets were transplanted in pots with vermiculite and vigorously grew to maturity.

Chée and Cantliffe[2] thought that shoot development was the limiting factor for plant formation from somatic embryos in sweet potato because all embryo developmental stages were able to produce roots but not shoots. The study of Chée *et al.*[4] on plant recovery from sweet potato somatic embryos demonstrated that BAP increased shoot formation but reduced root and whole plantlet formation from somatic embryos. In the present study the effects of BAP on shoot, root or whole plantlet formation from the somatic embryos of *I. triloba* L. were not significant.

Table 2. Plantlet regeneration from embryogenic callus of *I. triloba* L. on MS medium with various BAP concentrations

BAP (mg/l)	No. of embryogenic callus transferred	Embryogenic callus forming plantlets	
		No.	%
0	4	1	25.0
1.0	2	0	0
2.0	3	1	33.3

3. Plant regeneration from non-embryogenic callus

Non-embryogenic callus formed on callus formation medium was organogenic. Seven weeks after incubation, when transferred onto regeneration medium, non-embryogenic callus began to produce multiple shoots via organogenesis at 2 weeks after transfer (Fig. 1D). Shoot regeneration at 7 weeks after transfer was shown in Table 3.

Table 3. Shoot regeneration from non-embryogenic callus of *I. triloba* L. on MS medium with various BAP concentrations (regeneration medium) and the basal medium

Callus formation medium 2,4-D (mg/l)	Regeneration medium BAP (mg/l)	No. of callus cultured on regeneration medium	Callus producing shoots on regeneration medium		Callus producing shoots on the basal medium		Total % of callus producing shoots
			No.	%	No.	%*	
0.05	0	12	0	0	—	—	0
0.05	1.0	20	4	20.0	1	5.0	25.0
0.05	2.0	19	0	0	2	10.5	10.5
0.2	0	55	0	0	—	—	0
0.2	1.0	26	15	57.7	6	23.1	80.8
0.2	2.0	24	12	50.0	2	8.3	58.3
1.0	1.0	32	0	0	6	18.8	18.8
1.0	2.0	16	0	0	5	31.3	31.3
3.0	1.0	21	0	0	0	0	0
3.0	2.0	12	0	0	0	0	0

* Percentage to number of callus cultured on regeneration medium.

Shoot regeneration occurred only on the non-embryogenic calli obtained on callus formation medium with 0.05 and 0.2 mg/l 2,4-D. Regeneration frequency differed with BAP concentrations added to regeneration medium. The non-embryogenic callus transferred onto the basal medium formed neither adventitious shoots nor somatic embryos.

Non-embryogenic calli that did not form shoots on regeneration medium were further transferred onto the basal medium and produced adventitious shoots within 4 weeks (Table 3).

Regenerated shoots formed white roots and developed into whole plantlets after transfer to fresh basal medium. Plantlets were transplanted in pots with vermiculite and vigorously grew to maturity (Fig. 1F).

In this study 2,4-D concentrations included in callus formation medium markedly affected not only callus formation from leaf explants of *I. triloba* L. but also shoot regeneration from the non-embryogenic callus. BAP concentrations included in regeneration medium also influenced the shoot regeneration.

When the callus obtained on callus formation medium with 0.2 mg/l 2,4-D was cultured on regeneration medium with 1.0 mg/l BAP and further on the basal medium, a regeneration frequency up to 80.8% was achieved. This percentage was much bigger than that of our earlier study[8]. In the present study the callus regenerated adventitious shoots at a high frequency on MS medium with BAP, but no shoot regeneration was observed on MS medium with indole-3-acetic acid (IAA) and BAP in the earlier study. These are mainly due to the improvement of the callus formation medium. In the earlier study the callus formation medium contained high concentrations of kinetin and 2,4-D, whereas in the present study only 0.2mg/l 2,4-D gave the high regeneration frequency, and as 2,4-D concentration increased from 0.2 mg/l to 1.0mg/l, the

regeneration frequency reduced from 80.8% to 31.3%. Other factors such as dark incubation during callus induction probably affected the regeneration efficiency.

Summary

Leaf explants of *I. triloba* L. produced embryogenic callus and non-embryogenic callus on MS medium supplemented with 0.05 to 5.0 mg/l 2,4-D. When embryogenic calli were transferred onto MS medium supplemented with 0 to 2.0 mg/l BAP, some of the somatic embryos germinated. Non-embryogenic calli regenerated multiple shoots via organogenesis at a high frequency on MS medium with BAP. Further transfer of the non-embryogenic callus that did not form shoots to the basal medium resulted in additional shoot regeneration.

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