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Thidiazuron-induced Shoot Formation of *Hibiscus syriacus* L. 'Honghwarang' by Suspension Culture

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ABSTRACT This study was conducted to determine the optimum cultural condition and method for in vitro mass production of *Hibiscus syriacus* L. 'Honghwarang'. When callus induced in MS solid medium supplemented with 0.01 mg/L TDZ was cultured in liquid medium containing 0.01mg/L TDZ, callus growth and shoot primordia formation was most effective. Formed shoot primordia were regenerated into shoot in MS or 1/2 MS medium of growth regulator-free condition. Effects of mesh size, shaking speed on callus and shoot primordia formation were examined after 5 weeks. Callus and shoot primordia formation was formed most effectively at 10 mesh and 80 rpm shaking speed in liquid medium.

Additional key words: mass production, mesh size, shaking speed, shoot primordia, shoot regeneration,

Introduction

Suspension culture offers several distinct advantages over stationary cultures in solid media. When grown in liquid medium, callus or embryos are evenly exposed to nutrients and plant growth regulators. This allows more precise manipulation of media components, handling of callus and embryo, and control of plant development. In solid cultures, as gradients develop from medium to the top of tissue, uniform tissue response and control of plant develpment become more difficult. In suspension cultures, callus and proembyogenic clusters usually separate from each other and float freely in the medium. These large number of callus and cells can offer the prospect of large- scale cloning of plants. In many plants species, such as *Spinacia oleracea*(Xiao and Branchard, 1993), *Matricaria chamomilla*(Takano et al., 1991), *Lycopersicon chilense*(Greer and Tabaeizadeh, 1991), and *Populus alba* (Park and Son, 1988)

In vitro propagation of *Hibiscus syriacus* L. can be obtained via indirect organogenesis through callus culture, which was derived from shoot-tips at 2,4-D 2 mg/L, in the medium containing TDZ 0.5 mg/L (Yoo et al., 1996a, b). In order to induce shoot from callus, solid medium was used in this study. However, liquid suspension culture was more effective on induction and multiplication of shoot primordia induced from callus on MS liquid medium containing 0.1 mg/L TDZ. But, it is necessary that optimum shooting and rooting conditions should be studied for more efficient and rapid production.

The objectives of this study were to determine the most optimum cultural condition by the suspension culture in liquid medium, and to establish efficient in vitro mass production system for *Hibiscus syriacus* L. 'Honghwarang'.

Materials and methods

Hibiscus syriacus L. 'Honghwarang' used in this study had been grown at the campus of College of Agriculture & Life Sciences, Seoul National University. Shoots of about 10-20 mm length with 3-4 true leaves were taken. Leaves were removed, and then surface of shoots were sterilized by immersion in 70% ethanol for 15 sec, washed in sterile distilled water and 2.0% sodium hypochlorite solution for 20 min with 0.1% Tween 20, and then rinsed 3-5 times in sterilized water. Shoot apex tissue was obtained under dissecting microscope on air laminar flow bench and was placed upon MS medium.

Callus formed after 8 weeks of shoot-tip culture on MS medium containing TDZ 0.01 mg/L were dissociated with mesh 10, and transferred to a 50 ml Erlenmeyer flask containing 15 mL of MS liquid medium supplemented with TDZ 0, 0.001, 0.01, 0.1, or 1.0 mg/L. Callus suspension culture was conducted on a gyratory shaker maintained at 80 rpm, temperature of 25℃, a 16 hr photoperiod, and 2000 lx. Each treatment was repeated 4 times. Every 7 days, old medium was removed and replaced with the same volume of fresh medium, during the first 5 weeks, and then transferred to MS or 1/2 MS liquid medium with 0, 0.001 mg/L TDZ. After 5 and 10 weeks of suspension culture, callus growth, callus color, shoot primordia formation, and shoot regeneration were evaluated by visual ratings. Callus tissue of 0.5g induced at TDZ 0.01 mg/L were dissociated with mesh 10, 25, and 40. And then callus suspension cultures were kept on gyratory shaker at 80 rpm. Also, callus tissues sieved with mesh 10 were cultured at 20 and 80 rpm speed in MS liquid medium, and maintained under 25℃ room temperature, 16 hr photoperiod, and 2000 lux condition. After 5 weeks of suspension cultures callus growth, callus color, shoot primordia formation, and shoot regeneration were examined.

Results and Discussion

Effects of mesh size and shaking speed on callus and shoot primordia formation

Table 1 showed that sieving with mesh 10 was most effective on callus growth and shoot primordia formation after dissociation. Mesh 25 and 40 with the smaller pore size were not effective on callus growth and shoot primordia formation,

Table 1. Effect of mesh size on callus growth and shoot primordia formation in suspension culture of *Hibiscus syriacus* L. 'Honghwarang' at 5 weeks after treatment.

Mesh size[z]	Callus[y] growth	Callus[x] color	Shoot primordia[w] formation	Shoot[w] regeneration
10	++++	G	+++	-
25	+	LG	+	-
40	+	YG	+	-

[z]Mesh 10(1.5 × 1.5 mm), mesh 25(1.0 × 1.0 mm), mesh 40(0.5 × 0.5 mm).
[y]-: none; +: low; ++: medium; +++: high; ++++: very high.
[x]G: green; LG: light green; YG: yellow green.
[w]-: none; +: 1~5; ++: 6~10; +++: 11~20; ++++: more than 20.

Table 2. Effect of shaking speed on callus growth and shoot primordia formation in suspension culture of *Hibiscus syriacus* L. 'Honghwarang' at 5 weeks after treatment.

shaking speed	Concentrations of TDZ (mg/L)	Callus[z] growth	Callus[y] color	Shoot primordia[x] formation	Shoot[x] regeneration
20 rpm	0.0	+	LG	+	+
	0.001	+	LG	+	+
	0.01	+++	YG	+	+
	0.1	+	LG	+	+
	1.0	-	W	-	-
80 rpm	0.0	-	-	+	+
	0.001	++	LG	++	++
	0.01	++++	WG	++	+
	0.1	+++	YB	-	-
	1.0	-	W	-	-

[z]-: none; +: low; ++: medium; +++: high; ++++: very high.
[y]LG: light green; W: white; YG: yellow green; YB: yellow brown.
[x]-: none; +: 1~5; ++: 6~10; +++: 11~20; ++++: more than 20.

Table 3. Effect of TDZ on callus growth and shoot primordia formation in suspension culture of *Hibiscus syriacus* L. 'Honghwarang' at 5 weeks after treatment.

TDZ (mg/L)	Callus[z] growth	Callus[y] color	Shoot primordia[x] formation	Shoot[x] regeneration
0.0	-	-	+	+
0.001	++	LG	++	++
0.01	++++	WG	++++	+
0.1	+++	YB	-	-
1.0	-	W	-	-

[z]-: none; +: low; ++: medium; +++: high; ++++: very high.
[y]LG: light green; WG: white green; YB: yellow brown; W: white.
[x]-: none; +: 1~5; ++: 6~10; +++: 11~20; ++++: more than 20.

Table 4. Effect of TDZ on callus growth and shoot primordia formation in suspension culture of *Hibiscus syriacus* L. 'Honghwarang' at 5 weeks after transferring to growth regulator-free or TDZ-supplemented media.

TDZ 0.01[z] (mg/L)	Subculture 1 TDZ(mg/L)	Callus[y] growth	Callus[x] color	Shoot primordia[w] formation	Shoot[w] regeneration
0.0	MS 0.0	-	-	-	-
0.001	MS 0.0	+	YB	+	+
0.01	MS 0.0	++++	WG	+++	+++
	1/2MS 0.0	++++	WG	+++	+++
	MS 0.001	++++	W	+	+
	1/2MS 0.001	++++	WG	+++	+
0.1	MS 0.0	++++	YG	++++	+
1.0	MS 0.0	-	B	-	-

[z]Source of callus was cultured in MS solid medium supplemented with 0.01 mg/L TDZ for 8 weeks.
[y]-: none; +: low; ++: medium; +++: high; ++++: very high.
[x]WG: white green; YB: yellow brown; W: white; B: browning; YG: yellow green
[w]-: none; +: 1~5; ++: 6~10; +++: 11~20; ++++: more than 20.

and callus color was light green or yellow green.

Effect of shaking speed on callus and shoot primordia formation in suspension culture culture was shown in Table 2. Callus growth and shoot primordia formation was not effective in TDZ containing medium at 20 rpm. It was too slow to proliferate the callus and shoot primordia and callus color turned to be white gradually. The treatment of TDZ 0.01 mg/L at 80 rpm speed was the best in callus growth and shoot primordia formation. But, TDZ 0.001 mg/L was the most effective in shoot regeneration after 5 weeks of suspension culture.

Effect of TDZ on callus and shoot primordia formation

Table 3 showed the effect of TDZ concentration on callus growth and shoot primordia formation. Formation of shoot primordia and callus growth was best at the MS meidum supplemented with 0.01 mg/L TDZ. At concentrations over 0.1mg/L TDZ, shoot primordia and shoots were not formed. The suspension was transferred to growth regulator-free medium and shoot primordia formation and shoot regeneration were examined for 5 weeks. Entering the 5th week, the green spots (shoot primordia) protruded from the callus surface (Table 4). After 5 weeks of culture, the suspensions were transferred to the MS or 1/2 MS media containing.

Shoot primordia induced at 0.01 mg/L TDZ were regenerated into shoot in MS or 1/2 MS medium of growth regulator-free condition(Table 4). However, few shoot primordia were regenerated into shoot in medium containing 0.001mg/L TDZ. Also, induced shoot or shoot primordia could not elongate continuously in the presence of TDZ. It was assumed that TDZ may have relationship to ethylene biosynthesis, since elevated endogenous ethylene level in explant may inhibit the development of induced primordia(Elstner et al., 1983). Development stage of shoot primordia and shoot from callus induced at 0.01 mg/L TDZ in liquid suspension culture were observed through SEM and stereoscope (Fig. 1, 2). Shoot primordia were initiated from callus surface, and callus turned to green color(Fig. 1a, Fig. 2a), and then were continuously protruded(Fig. 1 b, c, Fig. 2 b, c). Protruded shoot primordia were continuously developed into shoots in growth regulator-free mdium(Fig. 1d, Fig. 2d).

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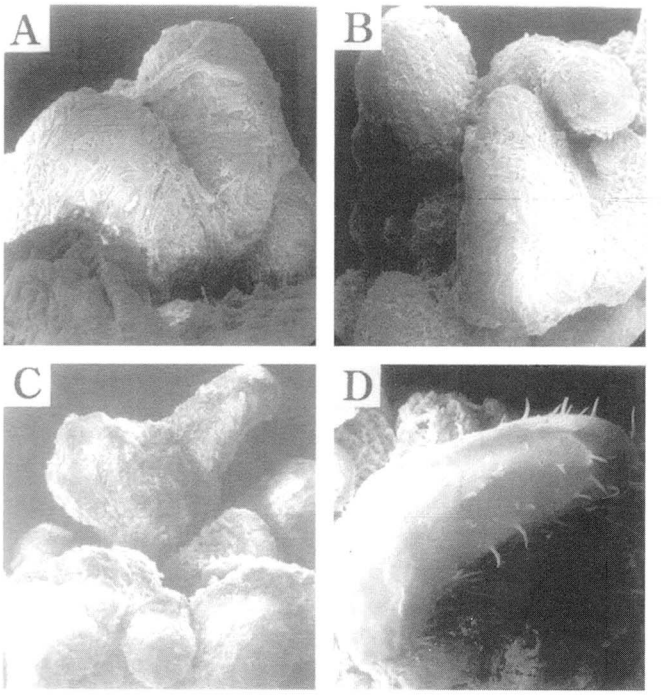

Fig. 1. Scanning electron micrographs of different development stages of shoot formed callus after TDZ 0.01 mg/L treatment in suspension culture from callus induced in TDZ 0.01 mg/L of *Hibiscus syriacus* L. 'Honghwarang'. (A) Initiation of shoot primordia (×100) (B) Protruding of shoot primordia from callus surface (×60) (C) Second protruding shoot primordia from original shoot primordium (×60), (D) Completely leafy shoot formation (×78).

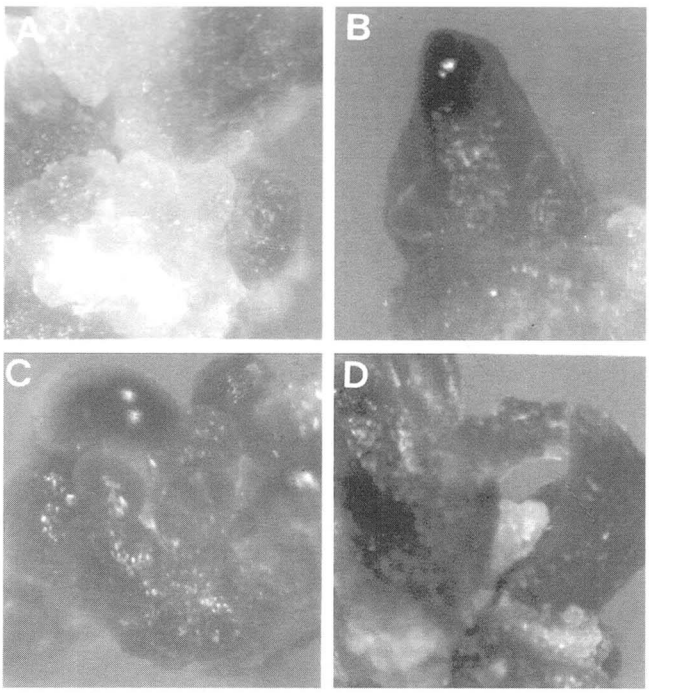

Fig. 2. Shoot development of *Hibiscus syriacus* L. 'Honghwarang' in suspension culture from callus previously induced at MS solid medium containing TDZ 0.01 mg/L. (A) Vigorous callus growth and occurrence of green spots. (B, C) Protruding of green spots. (D) Shoot development from green spots.

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Thidiazuron이 무궁화 '홍화랑' 품종 액체 현탁 배양시 신초형성에 미치는 영향

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초 록

본 실험은 무궁화 '홍화랑' 품종의 기내 대량생산을 위한 액체 현탁배양에서의 배양조건과 방법을 구체적으로 규명하고자 수행되었다. Mesh 종류별로 비교해 보았을 때 가장 굵은 10 mesh를 사용한 것이 callus형성에 효과적이었으며, 회전속도에 따른 callus의 형성은 80 rpm에서 가장 효과적이었다. TDZ 0.01 mg/L 첨가된 MS 고체배지에서 유기된 callus를 TDZ 0.001 mg/L가 첨가된 액체배지에 현탁배양했을 경우 callus 형성과 신초원기 형성이 가장 왕성하였다. 형성된 신초원기를 MS와 1/2 MS 배지에 생장조절제가 첨가되지 않은 조건에서 현탁배양시 신초 재분화율이 가장 높았다. 따라서, 이러한 액체 현탁배양 조건과 방법의 구체적인 규명은 무궁화 급속 대량 번식체계 확립을 위한 가능성을 보여 주었다.

추가 주요어 : 대량생산, mesh 크기, shaking 속도, 신초원기, 신초재분화