นิพนธ์ต้นฉบับ

Studies on Tissue Culture of *Angelica sinensis* (Oliv.) Diels

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Abstract

Rhizomal buds of *Angelica sinensis* (Oliv.) Diels were collected from a field in Kunming Institute of Botany, China. Rhizomal buds were sterilized with orthocide and Clorox. The buds were removed aseptically from the rhizome and cultured onto Murashige and Skoog (MS) basal medium. After one month, shoot tips of plantlets induced from the buds were subcultured onto MS containing 3 mg/l benzyl amino purine (BAP or B) and 0.1 or 1 mg/l naphthalene acetic acid (NAA), and MS58 (0.1 mg/l BAP, 5 mg/l giberellic acid, 150 mg/l citric acid and double the strength of iron) for multiple shoot induction. The best media for multiple shoot induction were MS containing 3 mg/l BAP and 1 mg/l NAA which had an average of 3.45 shoots per culture in one month. The rooting medium was MS containing 0.2 mg/l BAP and 2 mg/l 3-indoleacetic acid (IAA), 0.1 mg/l kinetin (KN) and 0.02 mg/l NAA, and filter paper moistened with ½ MS liquid medium. The result of the best rooting medium was 0.1 mg/l KN and 0.02 mg/l NAA which became plantlets. The percentage of survival for plantlets grown in a tissue culture room (controlled temperature = 25˚C, with 16 hours of light) was 100 per cent after one and two months, compared with those grown in a nursery, which were 83.5 and 72.6 percent, respectively.

Key words: **Angelica sinensis, tissue culture**

Introduction

The plant know as *dang gui* (*Angelica sinensis* (Oliv.) Diels), family Umbelliferae, is a very valuable Chinese herb. It has been used for thousands of years in traditional Chinese medical prescriptions (Zhang and Cheng, 1989). It is claimed to enrich the blood, relieve headache and fatigue, and act as a laxative.[1] It is used for balancing hormone levels in the body, including female estrogen hormone, antispasmodic, stimulant for blood circulation, and act as a regulator of menstruation;[2] it relieves an abnormal urinary system, stimulates blood circulation balance in females, and increases blood flow to the skin.[3] Its estrogen-like action relieves menopausal syndrome.[1,4] The active constituents of *dang gui*-enriched essential oil (0.4 - 0.7%) are ligustilide and *n*- butylphthalide, phytosteiols, ferulic acid and coumarins (angelol, angelicone),[1,5] buty-lidenephthalide *allo*-ocimene and angelicide,[5] homosenkyunolide H, homosukyunolide I, neoligustilide, 6-methoxycoumarin, hypoxanthine-9-beta D-ribofuranoside.[6] The purpose of this study was to develop a propagation system for *A. sinensis* from rhizomal buds.

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Materials and methods

Rhizomal buds of *A. sinensis* (Oliv.) Diels were collected from a field in Kunming Institute of Botany, China. Rhizomal buds were of sterilized with half a spoonful of orthocide in 100 ml of sterilized water for 30 minuetes, washed with 10% and 5% Clorox, containing 1-2 drops of Tween 20 for 10 and 5 min, respectively, and finally rinsed 2-3 times with sterilized water. The buds were removed aseptically from the rhizomes and cultured onto Murashige and Skoog (MS) basal medium. After one month, shoot tips induced from the buds were subcultured onto MS containing 3 mg/l benzyl amino purine (BAP or B) and 0.1 or 1 mg/l naphthalene acetic acid (NAA), and MS58 (0.1 mg/l BAP, 5 mg/l giberellic acid (GA), 150 mg/l citric acid and double the strength of iron for the induction of multiple shoots for one month. The shootlets were subcultured in rooting media, which were MS containing 0.2 mg/l BAP and 2 mg/l 3-indole acetic acid (IAA), 0.1 mg/l kinetin (KN) and 0.02 mg/l NAA and filter paper moistened with $\frac{1}{2}$ MS liquid medium. The plantlets were those grown in a tissue culture room (controlled temperature = 25°C, with 16 hours of light) compared with those grown in a nursery. The medium for growing in the tissue culture room using sterilized vermiculite in pots and the medium for growing in the nursery using sterilized soil : ash : fertilizer in the ratio of 2:2:1 in black plastic packets.

Results

The best media for multiple shoot induction was MS containing 3 mg/l BAP and 1 mg/l NAA, which produced an average of 3.45 shoots per culture and an average shoot length of 3.23 cm in one month (Table 1, Fig. 1). The best rooting medium contained 0.1 mg/l KN and 0.02 mg/l NAA (Table 2, Figs. 2-3), the average root induction of which was 100 percent for one month and two months, and the average number of roots 1.85 and 7.40 shoots for one month and two months, respectively. The average root length, average shoot length, average number of shoots were 1.78 cm, 4.15 cm and 1.05 for one month, respectively. The average root length, average shoot length and average number of shoots were 4.78 cm, 8.15 cm and 2.95, respectively, for two months. The percentage of survival for plantlets grown in the tissue cul-

Table 1 Average number of shoots, average shoot length of *A. sinensis* (Oliv.) Diels after culturing shoot tips on MS medium containing 3 mg/l BAP and 0.1 mg/l NAA, 3 mg/l BAP and 1 mg/l NAA and MS58 for one month.

Hormone	Multiple shoots	
	Average number of shoots	Average shoot length (cm)
BAP3NAA0.1	2.55	3.2
BAP3NAA1	3.45	3.23
MS58	1.25	5.40

Note: MS58 = MS medium containing 0.1 mg/l BAP,5 mg/l GA, 150 mg/l citric acid and double the strength of iron.

Table 2 Average percentage of root induction, average number of roots, average root length, average shoot length and average number of shoots of *A. sinensis* (Oliv.) Diels after culturing shootlets on MS medium containing 0.2 mg/l BAP and 2 mg/l IAA, 0.1 mg/l KN and 0.02 mg/l NAA and $\frac{1}{2}$ MS liquid for 1 month and 2 months

Hormone	Average% of root induction		Average number of roots		Average root length (cm)		Average shoot length (cm)		Average number of shoots	
	1M	2M	1M	2M	1M	2M	1M	2M	1M	2M
BAP0.2IAA2	20	95	0.35	3.6	0.11	3.64	4.6	6.18	2.6	3.05
KN0.1NAA0.02	100	100	1.85	7.40	1.78	4.78	4.15	8.15	1.05	2.95
$\frac{1}{2}$ MS liquid	0.05	100	0.05	3.20	0.03\	3.08	3.15	5.14	1.30	1.70

Note: M = month; $\frac{1}{2}$ MS liquid = filter paper moistened with $\frac{1}{2}$ MS liquid.

Table 3 Average percentage of survival for young *A. sinensis* (Oliv.) Diels plants after growing in a tissue culture room and a nursery for 1 month and 2 months

Tissue culture room		Nursery	
Average % of survival after growing for 1 month	Average % of survival after growing for 2 months	Average % of survival after growing for 1 month	Average % of survival after growing for 2 months
100%	100%	83.5%	72.6%

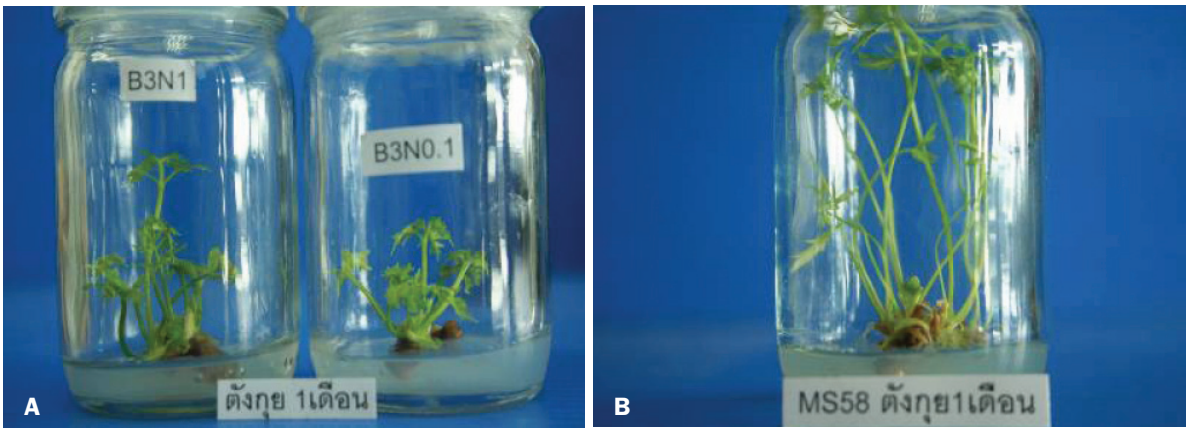

Fig. 1 Multiple shoot induction of *A. sinensis* (Oliv.) Diels after culturing for 1 month on MS medium containing A. 3 mg/l BAP and 1 mg/l NAA (left), 3 mg/l BAP and 0.1 mg/l NAA (right), B. MS 58.

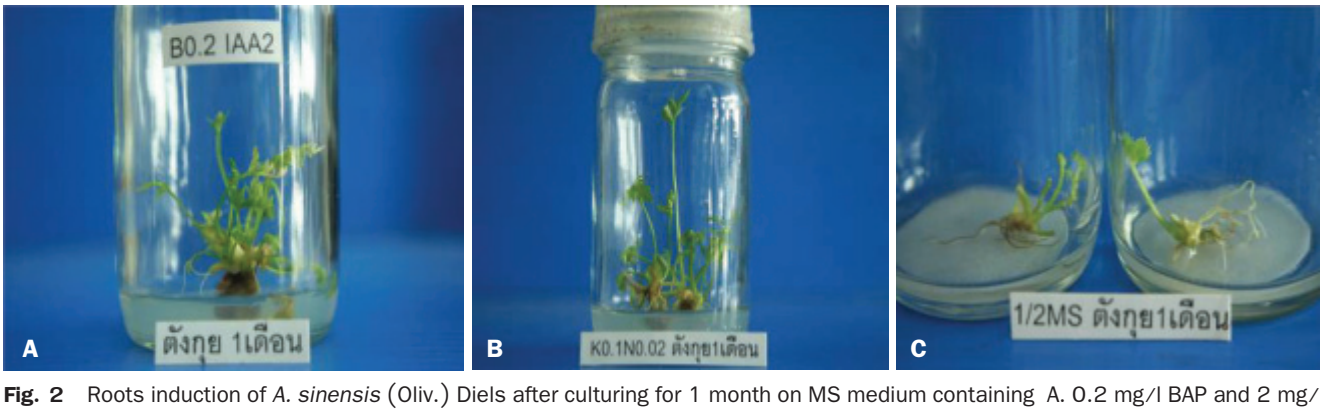

Fig. 2 Roots induction of *A. sinensis* (Oliv.) Diels after culturing for 1 month on MS medium containing A. 0.2 mg/l BAP and 2 mg/l IAA, B. 0.1 mg/l kinetin and 0.02 mg/l NAA, C. filter paper moistened with $\frac{1}{2}$ MS liquid.

Fig. 3 Plantlets of *A. sinensis* (Oliv.) Diels after culturing for 2 months on MS medium containing A. 0.2 mg/l BAP and 2 mg/l IAA, B. 0.1 mg/l kinetin and 0.02 mg/l NAA, C. filter paper moistened with $\frac{1}{2}$ MS liquid.

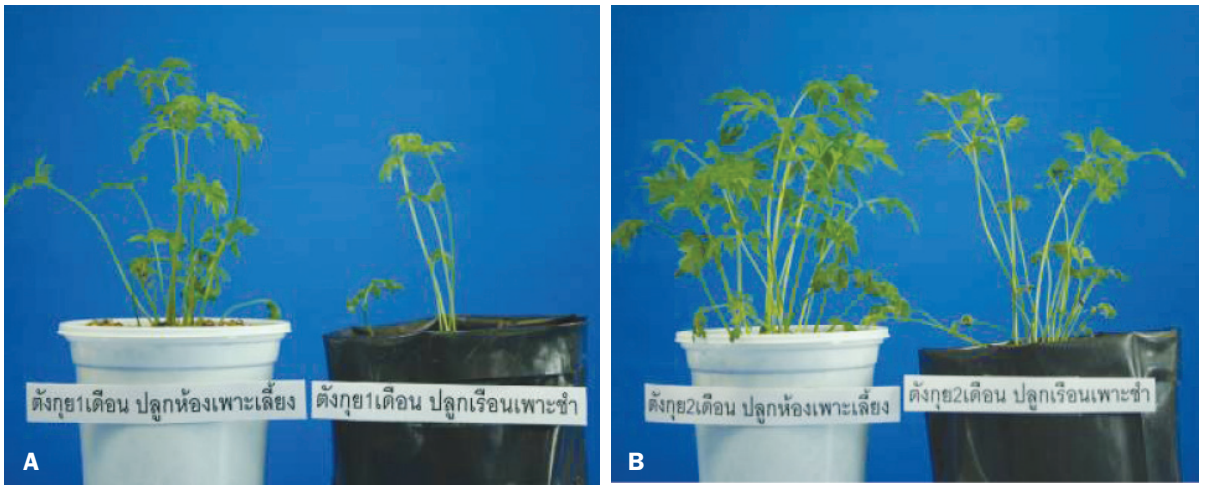

Fig. 4 Comparison of young *A. sinensis* (Oliv.) Diels young plants after growing in a tissue culture room (left) and a nursery (right) A. 1 month; B. 2 months.

Fig. 5 Comparison of stems and roots of *A. sinensis* (Oliv.) Diels after growing in a nursery (left) and a tissue culture room (right) A. 1 month; B. 2 months.

Fig. 6 *A. sinensis* (Oliv.) Diels growing in a nursery: A. young plants; B. young plants with flowers.

Fig. 7 Flowers of *A. sinensis* (Oliv.) Diels growing in a nursery. A. budding; B. young flowers; C. blooming flower.

ture room was 100 per cent after one month and two months, compared with those grown in the nursery, which were 83.5 and 72.6 percent, respectively (Table 3). The stems and roots of those grown in the tissue culture room were taller and bigger when compared with those grown in the nursery, (Figs. 4-5). The young plants were successfully transferred into soil and had flowers (Figs. 6-7).

Discussion

Our experiment used rhizomal buds which induced multiple shoots. The value of this mass propagation method was that it produced no abnormal plantlets, no immature embryo-derived cultured cells.[5] The plantlets grown in the tissue culture room fared better than those grown in the nursery: they had taller stems, bigger roots and a higher rate of survival because the nature of these plants is to grow in a cold climate (5.5-11.4°C) and on high mountains (1,500-3,000 m).[8] These young plants were successfully transferred into soil and flowered in the nursery. The study shows that Thailand could successfully plant *dang gui* if growing this plant on high mountains and in a cold climate such as Doi Angkhang in Chiang Mai Province.

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บทคัดย่อ

การเพาะเลี้ยงเนื้อเยื่อพืชของตังกุย

ธัญญ์วัณณ์ มงคลชัยภักดิ์, ปภาวดี สุฉันทบุตร
สถาบันวิจัยสมุนไพร กรมวิทยาศาสตร์การแพทย์

นำตาจากเหง้าของต้นตังกุย (*Angelica sinensis* (Oliv.) Diels) ที่ได้รับมาจากสถาบันพฤกษศาสตร์คุนหมิง (Kunming Institute of Botany) สาธารณรัฐประชาชนจีน มาฟอกฆ่าเชื้อด้วยออร์โทไซด์ (orthocide) และคลอร๊อกซ์ (Clorox) ตัดตาออกจากเหง้าอย่างปราศจากเชื้อ และเพาะเลี้ยงบนอาหาร MS ที่ไม่มีฮอร์โมน หลังจาก ๑ เดือนเกิดต้นอ่อน นำปลายยอดมาเพาะเลี้ยงต่อบนอาหาร MS ที่มีฮอร์โมน BAP ขนาดความเข้มข้น ๓ มิลลิกรัม/ลิตร ที่รวมกับ NAA ขนาดความเข้มข้น ๐.๑ หรือ ๑ มิลลิกรัม/ลิตร และ MS58 (๐.๑ มิลลิกรัม/ลิตร BAP, ๕ มิลลิกรัม/ลิตร gibelleric acid, ๑๕๐ มิลลิกรัม/ลิตร citric acid และเหล็กความเข้มข้นเท่าตัว) เพื่อให้เกิดยอดอ่อนหลายยอด สูตรอาหารที่ทำให้เกิดยอดอ่อนหลายยอดได้ดีที่สุด คือ สูตรอาหาร MS ที่มีฮอร์โมน BAP ขนาดความเข้มข้น ๓ มิลลิกรัม/ลิตร รวมกับ NAA ขนาดความเข้มข้น ๑ มิลลิกรัม/ลิตร ซึ่งจะให้ยอดอ่อนเฉลี่ย ๓.๔๕ ยอดต่อ ๑ ชิ้นเนื้อเยื่อ เมื่อเพาะเลี้ยงเป็นเวลา ๑ เดือน นำยอดอ่อนที่ได้มาเพาะเลี้ยงต่อบนอาหารเพื่อให้เกิดราก โดยเพาะเลี้ยงบนอาหาร MS ที่มีฮอร์โมน BAP ขนาดความเข้มข้น ๐.๒ มิลลิกรัม/ลิตร รวมกับ IAA ขนาดความเข้มข้น ๒ มิลลิกรัม/ลิตร, KN ขนาดความเข้มข้น ๐.๑ มิลลิกรัม/ลิตร รวมกับ NAA ขนาดความเข้มข้น ๐.๐๒ มิลลิกรัม/ลิตร และอาหารเหลว ½ MS ที่แช่บนกระดาษกรอง สูตรอาหารที่ทำให้เกิดรากได้ดีที่สุด คือ สูตรอาหาร MS ที่มีฮอร์โมน KN ขนาดความเข้มข้น ๐.๑ มิลลิกรัม/ลิตร รวมกับ NAA ขนาดความเข้มข้น ๐.๐๒ มิลลิกรัม/ลิตร ซึ่งจะกลายเป็นต้นอ่อน นำต้นอ่อนมาปลูกในห้องเพาะเลี้ยงเนื้อเยื่อพืช (ควบคุมอุณหภูมิ ๒๕๐ °ซ ที่มีแสงสว่าง ๑๖ ชั่วโมง) เปรียบเทียบกับปลูกในเรือนเพาะชำ พบว่าเมื่อปลูกต้นอ่อนในห้องเพาะเลี้ยงเนื้อเยื่อพืช มีเปอร์เซ็นต์รอดชีวิต ๑๐๐% ที่ ๑ และ ๒ เดือน เมื่อเปรียบเทียบกับปลูกในเรือนเพาะชำ มีเปอร์เซ็นต์รอดชีวิต ๘๓.๕% และ ๗๒.๖% ตามลำดับ

คำสำคัญ : **ตังกุย, เพาะเลี้ยงเนื้อเยื่อ**