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Adventitious shoot regeneration from stem internode explants of *Adenophora triphylla* (Thunb.) A. DC. (Campanulaceae) - an important medicinal herb

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Abstract. Efficient plant regeneration system has been established from stem internode explants of *Adenophora triphylla* (Thunb.) A. DC. (Campanulaceae), an important medicinal herb. Adventitious shoots were induced by culturing the stem internode explants on Murashige and Skoog (MS) basal medium supplemented with 2.22-35.51 μM N^6-benzylaminopurine (BA) in combination with 0.54 μM α-naphthaleneacetic acid (NAA). The regeneration potential varied with the developmental stage of the stem explant and growth regulator combination. Adventitious shoots formed a new crop of multiple shoots when subcultured on MS medium supplemented with 17.75 μM BA. Shoots were rooted on 1/4-strength MS basal medium supplemented with 5.37 μM NAA. Plantlets produced from shoots were transferred to soil and acclimatized in a growth chamber. Plants appearing morphologically normal were obtained six to seven months after culture of stem internode explants.

Keywords: *Adenophora triphylla* (Thunb.) A. DC.; Campanulaceae; De novo regeneration; Germplasm conservation; Medicinal herb; Micropropagation; Sha shen.

Introduction

Adenophora triphylla (Thunb.) A. DC. [syn. *Adenophora verticillata* Fisch. or *Adenophora tetraphylla* (Thunb.) Fisch.], commonly known as "sha shen" in Chinese, is an important medicinal herb of the family Campanulaceae (Kao and DeVol, 1974). It is distributed in Japan (Kyushu, Ryukyus), mainland China and Taiwan (Kao and DeVol, 1974). The fleshy roots of *A. triphylla*, which contain saponins (Read, 1982), have been used in traditional Chinese medicinal preparations (Huang, 1993). It is used as an expectorant in the treatment of chronic bronchitis and whooping cough (Huang, 1993; Reid, 1996). It stimulates myocardial contraction and also has an antibacterial effect (Huang, 1993). It is also used as a general tonic and restorative of bodily vigour (Stuart, 1979).

In Taiwan, the commercial crude drug (dried roots of *A. triphylla*) is imported from mainland China as the roots collected from plants growing naturally in the mountains of Taiwan are insufficient to meet the local demand. Due to over-exploitation of the natural population for medicinal use and lack of systematic efforts at cultivation, *A. triphylla* is threatened with extinction in Taiwan. Hence, the present study was undertaken in an effort to conserve the wild populations of *A. triphylla* using in vitro propagation techniques. In vitro techniques are used in the conservation of rare and endangered species (Fay, 1992). Many important medicinal herbs have been propagated successfully either by shoot morphogenesis or somatic embryogenesis (Erdei et al., 1981; Shoyama et al., 1983; Hiraoka et al., 1986; Hiraoka and Oyanagi, 1988; Nishioka, 1988; Tsay et al., 1989; Yamada et al., 1991; Tsay and Huang, 1998; Huang et al., 2000). To our knowledge, reports on the in vitro regeneration of *A. triphylla* are not available to date. Recently, an in vitro propagation method was reported for *Platycodon grandiflorum*, another medicinally important herb of Campanulaceae (Yonemitsu et al., 1998). This paper describes an efficient method for in vitro propagation of *A. triphylla* using stem internode explants.

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Materials and Methods

Plant Material

Three mature plants of *A. triphylla* were collected from the mountainous area (altitude about 1,800-2,000 m) of Nantou County in central Taiwan and replanted in 21-cm diameter pots in potting mixture sand:soil (1:1 v/v). The plants were pruned and kept in a growth chamber (Hotech Instruments Corp., Model 624 HD, Taipei, Taiwan) under cool white fluorescent light at 100 μE m^{-2} s^{-1} (Philips, Holland), with a 16-h photoperiod per day, 80% relative humidity, and 22/16°C day/night temperature. The plants were irrigated every other day with tap water.

Induction and Development of Adventitious Shoots

New shoots 20-22 cm in length, sprouting after three to four weeks of replanting, were used as a source of leaf, stem node, and stem internode explants. All the leaves were cut and separated carefully from the stem. Stem sections (approximately 1.5 cm in length) were then surface sterilized in 70% ethanol for 30 sec, followed by 1% sodium hypochlorite (Clorox®, The Clorox Co., Oakland, USA) (with two drops of Tween 20® per 100 ml, Hayashi Pure Chemical Industries Ltd., Osaka, Japan) under ultrasonic vibration (Branson Ultrasonic Cleaner, Branson Cleaning Equipment Co., Shelton, CT, USA) for 10 min and rinsed three times with sterile distilled water. The leaves were surface sterilized in 70% ethanol for 10 sec, followed by 0.5% sodium hypochlorite for 5 min. After sterilization, stem node and internode sections were sliced into 3 mm thick segments and leaves cut into 1 cm^2 pieces. In a preliminary experiment, the leaf, stem node, and stem internode explants were cultured in glass test tubes, 22 mm diameter x 120 mm high, each containing 15 ml of medium. The stem node and internode sections were placed in a horizontal position in the medium. The abaxial side of each leaf piece was placed in contact with the medium. The medium consisted of Murashige and Skoog (MS) (Murashige and Skoog, 1962) basal medium (MS inorganic salts and vitamins + 100 mg/l inositol), 3% sucrose, 0.8% Difco Bacto-Agar (Difco Laboratories, Detroit Michigan, USA) and 8.88 µM 6-benzylaminopurine (BA) in combination with 0.54 µM α-naphthaleneacetic acid (NAA) (Table 1). Thirty explants were evaluated for induction of shoots. The cultures were incubated for 50 days at 25 ± 1°C under cool white fluorescent light at 38 µE m^{-2} s^{-1} (Philips, Holland) with a 16-h photoperiod per day.

As the stem internode explants produced more shoots than either the leaf or stem node explants (Table 1), further experiments were carried out using only stem internode explants. After removal of the shoot tip (about 0.5 cm from top), the rest of the stem was divided into three sections: (I) 0.5-5.0 cm from shoot tip; (II) 5.1-10.0 cm from shoot tip; and (III) 10.1-20.0 cm from shoot tip. The stem sections were surface sterilized as described earlier, sliced into 3 mm segments, and placed in a horizontal position in the medium. The medium consisted of MS basal medium, 3% sucrose, 0.8% Difco Bacto-Agar and 2.22, 4.44, 8.88, 17.75 or 35.51 µM BA in combination with 0.54 µM NAA (Table 2). Thirty explants were evaluated per treatment for induction of shoots. Frequency of shoot development and length of shoots were recorded after 60 days of incubation under the conditions described earlier. The pH of all media was adjusted to 5.7 ± 0.1 with 1 N NaOH before autoclaving at 121°C, 15 lb/in^2 for 15 min. The culture vessels were capped with two layers of aluminum foil before autoclaving and were sealed with three layers of parafilm M® (American National Can$_{TM}$, USA) after culture.

Multiplication of Adventitious Shoots

Adventitious shoots were separated and transferred to solid [0.15% Gelrite (Sigma Chemical Co., St. Louis)+0.4%

Table 1. Effect of explant type on regeneration of shoots in *Adenophora triphylla* (Thunb.) A. DC. after 50 days of culture on MS medium supplemented with 8.88 µM BA and 0.54 µM NAA.

Explant	No. of explants showing regeneration of shoots/No. of explants cultured	% Response	Average no. shoot buds produced per explant
Leaf	3/30	10.0 (2-27)*	1.7
Stem internode	20/30	66.6 (47-83)	10.0
Stem node	26/30	86.6 (69-96)	4.1

*Values in parentheses are 95% confidence limits of binomial distribution.

Table 2. Effect of growth regulator combination and position of stem internode explants on adventitious shoot regeneration in *Adenophora triphylla* (Thunb.) A. DC. after 60 days of culture.

BA µM	NAA µM	Position of stem explants*					
		I		II		III	
		(%)**	(No.)***	(%)	(No.)	(%)	(No.)
2.22	0.54	80	6.2[d]	60	1.6[b]	0	0[c]
4.44	0.54	80	7.2[cd]	70	2.5[b]	10	1.0[b]
8.88	0.54	100	11.8[a]	100	5.8[a]	30	2.5[a]
17.75	0.54	100	10.0[ab]	90	5.4[a]	20	1.3[b]
35.51	0.54	91	8.3[bc]	80	2.3[b]	0	0[c]

*I: 0.5-5.0 cm from shoot tip, II: 5.1-10.0 cm from shoot tip, III: 10.1-20.0 cm from shoot tip.
**Percent explants forming adventitious shoots.
***Average number of adventitious shoots per explant.
Means within a column followed by the same letter are not significantly different from each other at 5% level by Duncan's multiple range test.

Table 3. Effect of BA concentration on multiplication of adventitious shoots of *Adenophora triphylla* (Thunb.) A. DC. after 50 days of culture.

BA µM	% Explants producing new shoot buds	Average no. shoot buds per explant	Average length of new shoot buds (cm)
4.44	85	12.6[c]	1.0[c]
8.88	96	25.0[b]	2.0[b]
17.75	100	30.7[a]	2.5[a]
35.51	100	28.0[ab]	2.2[b]

Means within a column followed by the same letter are not significantly different from each other at 5% level by Duncan's multiple range test.

Table 4. Effect of NAA concentration on rooting of shoots of *Adenophora triphylla* (Thunb.) A. DC. after 50 days of culture.

NAA µM	% Shoots showing induction of roots	No. roots produced per shoot	Average length of roots (cm)
0	0	0[c]	0[a]
2.69	44.0	6.0[b]	0.4[c]
5.37	80.0	11.4[a]	1.1[a]
10.74	90.0 (40)*	13.0[a]	0.8[b]
21.48	52.4 (100)	6.4[b]	0.3[d]
42.96	41.0 (100)	11.0[a]	0.2[d]

*Values in parentheses shows percent shoot buds forming callus at the cut end along with rooting.
Means within a column followed by the same letter are not significantly different from each other at 5% level by Duncan's multiple range test.

Table 5. Effect of different potting mixtures on survival of micropropagated plants of *Adenophora triphylla* (Thunb.) A. DC.

Potting mixture (v/v/v)			No. of plants transplanted/
Soil	Vermiculite	Peat moss	No. of plants survived
2	0	0	25/24
2	1	0	25/24
2	0	1	25/25
2	1	1	25/25

agar-agar (No. A-7002, Sigma)] MS basal medium supplemented with 3% sucrose and 4.44, 8.88, 17.75 or 35.51 µM BA (Table 3). Thirty shoots were cultured on each of the four treatments. The shoots were also cultured in 20 ml liquid MS medium supplemented with 3% sucrose and 17.75 µM BA in 125-ml Erlenmeyer flasks. This medium was found to be optimal for inducing new shoot buds on solid medium. The flasks were placed on an orbital shaker (Model SK-302A, Sun Kuan Instruments Co., Taichung, Taiwan) with a rotary motion of 100 rev/min and incubated under the conditions described for induction of adventitious shoots. Frequency of shoot development and length of shoots were recorded after 50 days of incubation.

Rooting of Shoots

Elongated shoots, 2-3 cm in length, were rooted on 1/4-strength MS basal medium supplemented with 3% sucrose, 0.8% Difco Bacto-Agar and NAA at 0, 2.69, 5.37, 10.74, 21.48 or 42.96 µM (Table 4), in glass test tubes 22 mm diameter x 120 mm high, each containing 15 ml of medium. The cultures were incubated under the conditions described for induction of adventitious shoots. Twenty-five shoots were evaluated per treatment. The percentage of shoots producing roots, number of roots produced per shoot, and average length of roots were recorded after 50 days of incubation.

Transfer of Plantlets to Soil

Fully developed plants of 4-5 cm height were carefully removed from test tubes and the roots were washed thoroughly under tap water for 2-3 min to remove traces of agar-solidified medium sticking to them. Plants were then dipped in 0.1% (w/v) Benlate® (Du Pont de Nemours & Co. Inc., Taoyuan, Taiwan), a broad spectrum systemic fungicide for 1 min and transferred to 9-cm diameter pots containing different mixtures (v/v/v) of autoclaved soil, vermiculite and peat moss (Table 5), and the pots were kept in a growth chamber under a high light intensity of 100 µE m^{-2} s^{-1}, 16-h photoperiod per day, 80% relative humidity and 22/16°C day/night temperature, for one month. The plants were irrigated every other day with tap water. They were maintained for the first two weeks under plastic cover in order to avoid desiccation in the growth chamber. After one month, plants were irrigated once with a solution of full-strength MS salts and transferred outside the growth chamber. Twenty five plants were transplanted in each of the four potting mixtures. The percentage of surviving plants was calculated after two months.

Statistical Analysis

All treatments were performed three times. Duncan's multiple range test (Duncan, 1955) or a 95% confidence limit of binomial variation was used for statistical analysis.

Results and Discussion

In initial experiments, we failed to establish aseptic in vitro cultures of *A. triphylla* from the explants (leaf, stem node, and stem internode) collected from plants growing in the natural habitat due to endogenous bacterial and fungal contamination. To overcome this problem, plants collected from the natural habitat were transplanted to pots, pruned, and grown under the controlled, hygienic conditions described earlier (Debergh and Maene, 1981). New shoots, sprouting after three to four weeks of transplanting, were used in the present investigation. In a preliminary experiment, leaf, stem node, and stem internode segments were cultured on a medium containing 8.88 µM BA and 0.54 µM NAA. Regeneration of adventitious buds was observed in all three explants. However, shoot bud formation in leaf and stem node explants was less frequent than in the stem internode explants (Table 1). Hence further studies were carried out using only the stem internode explants. In earlier studies with chrysanthemum, Lu et al. (1990) reported that the morphogenetic potential varies with the developmental stage of the stem explants. Hence, to study the effect of maturity on morphogenetic potential in stem internode explants of *A. triphylla*, we divided stem cuttings into three sections: 0.5 to 5.0 cm (I), 5.1 to 10.0 cm (II), and 10.1 to 20.0 cm (III) from the shoot tip (Table 2).

Induction and Development of Adventitious Shoots from Stem Internode Explants

Various combinations of BA and NAA were used for the induction of adventitious shoots as they are among the growth regulators used most often for the shoot organogenesis (Tripepi, 1997). Stem internode explants when cultured on various combinations of BA and NAA (Table 2) showed swelling, two to three times to their original size, and induction of adventitious shoot bud primordia (arrows) in addition to callus after 21 days of culture (Figure 1A). Numerous shoot buds (arrows) were produced after thirty days of culture in addition to callus (c) at the cut end and surface of the stem internode explant (Figure 1B). The best response was obtained on MS medium supplemented with 8.88 µM BA and 0.54 µM NAA (Table 2). Adventitious shoot buds developed further and formed shoot(s) on the same bud induction medium after fifty days of culture (Figure 1C). The percentage of shoots forming explants and the average number of shoots per explant were highest in type I explants compared to type II and type III explants (Table 2). On a medium supplemented with 8.88 µM BA and 0.54 µM NAA, 100% of type I stem explants produced adventitious shoot buds compared to 100% from type II and 30% from type III explants (Table 2). Though 100% of type II explants produced adventitious shoots, the average number was lower (5.8) compared to (11.8) of type I explants (Table 2). Thus, type I stem explants, obtained from the uppermost region of the shoot, near to the shoot tip, were most competent for regeneration and the competence decreased as the stem matured, similar to earlier observations in chrysanthemum (Lu et al., 1990).

Multiplication of Adventitious Shoots

Adventitious shoots formed a new crop of multiple shoots when separated and transferred to solid MS medium containing 4.44, 8.88, 17.75 or 35.51 µM BA. The highest number of axillary shoots (30.7) per adventitious bud cultured was obtained on medium supplemented with 17.75 µM BA after 50 days of culture (Table 3). In liquid MS medium supplemented with 17.75 µM BA, more axillary shoots (44.5) per adventitious bud cultured were obtained than on solid medium after 50 days of culture. However, vitrification of shoots was observed when shoots multiplied in liquid medium, as reported for many crops cultured in liquid media (Ziv, 1991). To prevent vitrification, we adopted a solid-liquid-solid culture system as described by Pâques et al. (1987). The problem of vitrification can also be overcome by growing cultures on combinations of Gelrite and Sigma agar-gelled medium (Pasqualetto et al., 1986). In our studies we used 0.15% Gelrite in combination with 0.4% Sigma agar-agar as a gelling agent in solid shoot multiplication medium. Figures 1D and 1E depict shoot multiplication in liquid and solid medium, respectively.

Rooting of Shoots

Shoots 2-3 cm in length were separated and transferred to 1/4-strength MS basal medium supplemented with various concentrations of NAA for rooting (Table 4). Reduced strength of the basal medium is often used for rooting of adventitious shoots (Hu and Wang, 1983). A 1/4-strength of MS basal medium was used earlier for rooting of adventitious shoots in *Rosa* (Lloyd et al., 1988). NAA induced rooting of varying frequency (41-90%) at all the concentrations tested (Table 4). The highest number of roots formed without formation of callus at the cut end was achieved on a medium supplemented with 5.37 µM NAA within fifty days (Figure 1F). Eighty percent of shoots produced 11.4 roots per shoot with an average length of 1.1 cm on a medium with 5.37 µM NAA (Table 4). On media containing higher concentrations (10.74, 21.48 or 42.96 µM of NAA), rooting was accompanied with callusing at the base of shoots.

Transfer of Plantlets to Soil

A high survival rate of plants (100%) was obtained by using a potting mixture of soil:peat moss (2:1 v/v) or soil: vermiculite:peat moss (2:1:1 v/v/v) (Table 5) combined with a relative humidity of 80-90%. The hardening period of 4 weeks was essential for establishment of plants in soil. Regenerated plants showed no morphological differences from those grown naturally (Figure 1G).

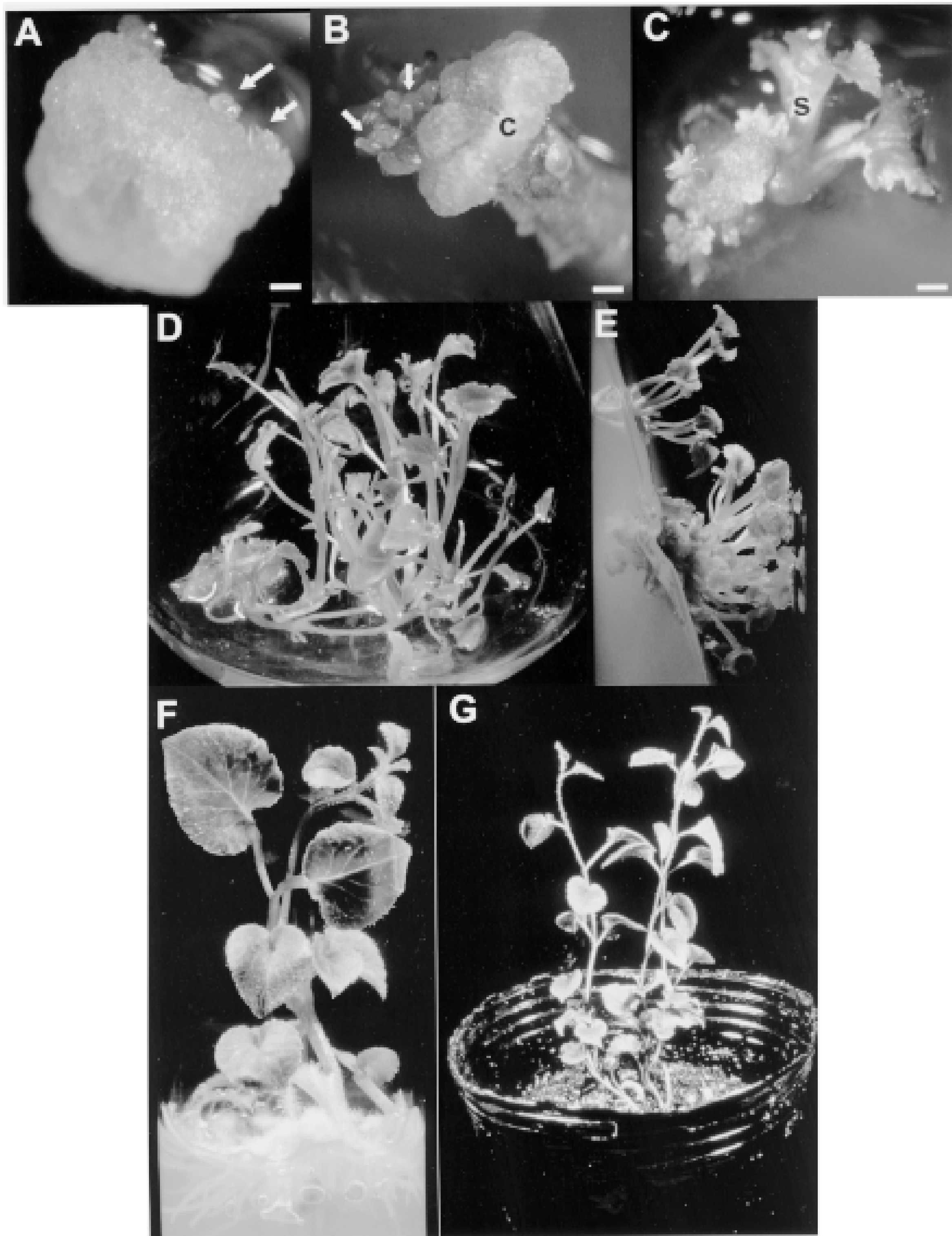

Figure 1. In vitro propagation of *Adenophora triphylla* (Thunb.) A. DC. A-C, induction and development of adventitious shoots from the stem internode explants cultured on MS medium supplemented with 8.88 µM BA and 0.54 µM NAA. A, induction of adventitious shoot bud primordia (arrows) at cut end of the stem internode explant after 21 days of culture. Bar, 2.58 mm; B, development of adventitious shoot buds (arrows) along with callus (c) on the surface after 30 days of culture of the explants. Bar, 2. 16 mm; C, elongation of adventitious shoot (s) after 60 days of culture. Bar, 1.71 mm. D-G, multiplication, rooting and ex vitro establishment of shoots derived from the stem internode explants. D, shoot multiplication in liquid MS basal medium supplemented with 17.75 µM BA in 125-ml Erlenmeyer flask; E, shoot multiplication in solid MS medium supplemented with 17.75 µM BA in 22 mm diameter x 120 mm high glass test tube; F, rooting of shoot in 1/4-strength MS basal medium supplemented with 5.37 µM NAA in 22 mm diameter x 120 mm high glass test tube after 50 days of incubation; G, potted plant 2 months after transfer to a soil: vermiculite (2:1 v/v) mixture.

Conclusions

This protocol could be used in the establishment of a large number of uniform plants of *A. triphylla* through tissue culture for: (i) detailed study of the saponins contained in the roots, (ii) pharmacological studies, (iii) germplasm conservation, (iv) commercial cultivation, and (v) replanting in the natural habitat. Tissue culture technology could help prevent further depletion of the natural population of this important medicinal herb in Taiwan.

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Literature Cited

Debergh, P.C. and L.J. Maene. 1981. A scheme for commercial propagation of ornamental plants by tissue culture. Scientia Hortic. **14:** 335-345.

Duncan, D.B. 1955. Multiple range and multiple *F* tests. Biometrics **11:** 1-42.

Erdei, I., Z. Kiss, and P. Maliga. 1981. Rapid clonal multiplication of *Digitalis lanata* in tissue culture. Plant Cell Rep. **1:** 34-35.

Fay, M.F. 1992. Conservation of rare and endangered plants using in vitro methods. In Vitro Cell. Dev. Biol. **28P:** 1-4.

Hiraoka, N. and M. Oyanagi. 1988. In vitro propagation of *Glehnia littoralis* from shoot-tips. Plant Cell Rep. **7:** 39-42.

Hiraoka, N., T. Kodama, M. Oyanagi, S. Nakano, Y. Tomita, N. Yamada, O. Iida, and M. Satake. 1986. Characteristics of *Bupleurum falcatum* plants propagated through somatic embryogenesis of callus cultures. Plant Cell Rep. **5:** 319-321.

Hu, C.Y. and P.J. Wang. 1983. Meristem, shoot tip, and bud cultures. *In* D.A. Evans, W.R. Sharp, P.V. Ammirato, and Y. Yamada (eds.), Handbook of Plant Cell Culture, Techniques for Propagation and Breeding, vol. 1. Macmillan Publishing Co., New York, pp. 177-227.

Huang, C.L., M.T. Hsieh, W.C. Hsieh, A.P. Sagare, and H.S. Tsay. 2000. *In vitro* propagation of *Limonium wrightii* (Hance) Ktze. (Plumbaginaceae), an ethnomedicinal plant, from shoot-tip, leaf- and inflorescence-node explants. In Vitro Cell. Dev. Biol.-Plant **36:** 220-224.

Huang, K.C. 1993. Sha Seng or Southern Sha Seng – the root of *Adenophora tetraphylla* (Thunb.) Fisch. or *A. stricta* Miq. *In* The Pharmacology of Chinese Herbs, CRC Press, Inc., Boca Raton, Florida, pp. 224-225.

Kao, M.T. and C.E. DeVol. 1974. The Campanulaceae of Taiwan. Taiwania **19:** 123-146.

Lloyd, D., A.V. Roberts, and K.C. Short. 1988. The induction *in vitro* of adventitious shoots in *Rosa*. Euphytica **37:** 31-36.

Lu, C.Y., G. Nugent, and T. Wardley. 1990. Efficient, direct plant regeneration from stem segments of chrysanthemum (*Chrysanthemum morifolium* Ramat. cv. Royal Purple). Plant Cell Rep. **8:** 733-736.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Plant. **15:** 473-497.

Nishioka, I. 1988. Clonal multiplication of medicinal plant by tissue culture. Shoyakugaku Zasshi **42:** 1-11.

Pâques, M., Ph. Boxus, and M. Dulos. 1987. "Vitrification" : an inducible and reversible phenomenon. Acta Hort. **212:** 253-258.

Pasqualetto, P.L., R.H. Zimmerman, and I. Fordham. 1986. Gelling agent and growth regulator effects on shoot vitrification of 'Gala' apple in vitro. J. Amer. Soc. Hort. Sci. **111:** 976-980.

Read, B.E. 1982. Chinese Medicine Series 5. Chinese Medicinal Plants from the Pen Ts'ao Kang Mu A.D. 1596 of a Botanical, Chemical and Pharmacological Reference List. Southern Materials Center, Inc., Taipei, The Republic of China, pp. 13.

Reid, D.P. 1996. *Adenophora tetraphylla* (Campanulaceae). *In* A. Amsel, M. McClellan, and D. Maitland (eds.), Chinese Herbal Medicine. Shambhala Publications, Inc., Boston, Massachusetts, pp. 152.

Shoyama, Y., K. Hatano, and I. Nishioka. 1983. Clonal multiplication of *Pinellia ternata* by tissue culture. Planta Med. **49:** 14-16.

Stuart, G.A. 1979. Chinese Materia Medica; Vegetable Kingdom, Southern Materials Center, Inc., Taipei, Republic of China, pp. 15-18.

Tripepi, R.R. 1997. Adventitious shoot regeneration. *In* R.L. Geneve, J.E. Preece, and S.A. Merkle (eds.), Biotechnology of Ornamental Plants, Biotechnology in Agriculture Series, No. 16. CAB International, Wallingford, UK, pp. 45-71.

Tsay, H.S. and H.L. Huang. 1998. Somatic embryo formation and germination from immature embryo-derived suspension-cultured cells of *Angelica sinensis* (Oliv.) Diels. Plant Cell Rep. **17:** 670-674.

Tsay, H.S., T.G. Gau, and C.C. Chen. 1989. Rapid clonal propagation of *Pinellia ternata* by tissue culture. Plant Cell Rep. **8:** 450-454.

Yamada, Y., Y. Shoyama, I. Nishioka, H. Kohda, A. Namera, and T. Okamoto. 1991. Clonal micropropagation of *Gentiana scabra* Bunge var. *buergeri* Maxim. and examination of the homogeneity concerning the gentiopicroside content. Chem. Pharm. Bull. **39:** 204-206.

Yonemitsu, H., M. Kusube, and M. Noguchi. 1998. Studies on propagation by tissue culture of *Platycodon grandiflorum* A. DC. Nat. Medicines **52:** 368-371.

Ziv, M. 1991. Vitrification: morphological and physiological disorders of *in vitro* plants. *In* P.C. Debergh and R.H. Zimmerman (eds.), Micropropagation *Technology and Application*, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 45-69.

輪葉沙參莖段培植體誘導不定芽再生

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輪葉沙參(桔梗科)始載於神農本草經列為上品，自古即用於鎮咳、去痰及排膿消腫，近代藥理分析更發現具有降血壓的效用，是一種相當重要且值得研究開發的中藥材。試驗結果顯示，取自上、中、下不同節位的培植體和生長調節劑濃度會影響再生作用的進行；將莖段培植體培養於 MS 基本鹽類添加 2.22～35.51 μM BA 及 0.54 μM NAA 的培養基可成功誘導不定芽再生；此種培植體如繼代培養於 MS 基本鹽類添加 17.75 μM BA 培養基時，能獲得大量增生的不定芽。當萌生的新芽培養於含 1/4 MS 基本鹽類添加 5.37 μM NAA 培養基則有促進根系生長的效果；已著生根系的瓶苗移出種植於土壤介質並置於生長箱中馴化栽培，總計自節間培植體開始培養至馴化後獲得完整的苗株，需時約 6～7 個月，據此可建立一套有效率的繁殖模式。

關鍵詞：輪葉沙參；桔梗科；Denovo再生；種源保存；中藥材；微體繁殖；沙參。