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Rapid Propagation through Tissue Culture of *Cudrania tricuspidata*, Medicinal Plant

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ABSTRACT : An effective rapid propagation method was established through *in vitro* cultures of the medicinal plant, *Cudrania tricuspidata*. *In vitro* plantlets were obtained from *in vitro* germinated seeds. The various levels of cytokinins (BAP, Kinetin and TDZ) were tested on multiple shoot formation from plantlets. BAP (1.0 ㎎/ℓ) treatment induced highest number of multiple shoots. Single shoot cultures gave higher initial shoot numbers than 5 shoots per culture. Among the various culture media, the shoot elongation was optimal on 2 MS basal medium without growth regulators. The IAA (2.0 ㎎/ℓ) treatment induced highest number of roots. IBA (2.0 ㎎/ℓ) treatment more promoted *in vitro* root growth than other concentrations. Rooted shoots were transferred directly to small pots with an artificial soil and successfully acclimatized.

Key words : *Cudrania tricuspidata*, cytokinin, multiplication, *in vitro* rooting

INTRODUCTION

Cudrania tricuspidata is belongs to Moraceae family and deciduous tree distributed over Korea, China and Japan. The cortex and root bark of this species have been used as a traditional medicine for treatment abdominal region bleeding, women's disease, impaired hearing, myoma of the uterus, exhaustion and others. Previous workers reported that root bark of this species contains biologically active isoprenylated xanthones (Fujimoto *et al*., 1984) and flavonoids (Lee *et al*., 1996), and also reported that extracts of root bark have been to be shown effective on high blood pressure (Kang *et al*., 2002), inflammation, and cytotoxic to human tumor cell lines (Lee *et al*., 1996). Isoprenylated xanthones are known to have a variety of biological activities, such as hypertensive effect, anti-rhinoviral activity, inhibition of the formation of some prostanoids, and anti-tumor promoting activity (Fujimoto *et al*., 1984). Lee *et al*. (1996) isolated Gericudranin from *C. tricuspidata* that is a new flavonoids group and they found out its structure. These compounds have a powerful activity against liver cancer, colon cancer, uterine cancer, lung cancer, nervous system cancer and skin cancer of cell (Choi *et al*., 2001).

In recent years there has been renewed interest in natural medicines that are obtained from plant parts or plant extracts. These many valuable medicinal plants are under the treat of extinction and rapidly depleted. Plant tissue culture is an alternative method of commercial production and conservation of endangered plants (He *et al*., 2007).

Conventional vegetative propagation of medicinal plants involved woody plants is not successful and is considerably limited by seasonal growth. Especially, propagation of medicinal woody plants is time consuming and does not yield homogeneous progeny (Ostrolucka *et al*. 2004). As an alternative to these disadvantages *in vitro* culture methods have been intensively used for the clonal propagation of many medicinal woody plants of economic importance (Gomez & Segura, 1995).

Micropropagation of many medicinal plants has been described by many authors during last two decades (Skirvin *et al*., 1990). Micropropagation of medicinal plants has been achieved through rapid proliferation of shoot-tips and axillary bud in culture. Numerous factors are reported in influence the success of *in vitro* propagation of different medicinal plants. However, it is observed that many tree species are difficult to propagate through *in vitro* methods due to their slow growth rates and low survivability (Gurel & Gulsen, 1998). The reports on the rapid propagation of *C. tricuspidata* through *in vitro* methods are few. Thus, this study has been conducted to determine efficient *in vitro* propagation method of *C. tricuspidata*.

MATERIAL AND METHODS

Plant material and *in vitro* culture condition

C. tricuspidata seeds were collected from Korea National Arboretum in Korea. The seeds were processed by washing in

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running water for 30 minutes. There after the seed surface was sterilized with 70% (v/v) ethanol for 1 minute and 3% (v/v) NaClO solution for 10 minutes. Finally the sterilized seeds were rinsed five times with sterilized distilled water. The processed seeds were then transferred onto MS (Murashigae & Skoog, 1962) basal medium supplemented with 3% (w/v) sucrose and 0.4% (w/v) gelrite for germination. For further growth, germinated plantlets were transferred to MS basal medium without growth regulators. All cultures were carried out on 16/8 h (light/dark) photoperiod at 25 ± 1 ℃ The resulting plantlets were further used in experiments and subcultured 4 weeks interval.

Effects of cytokinins on shoot multiplication

The three cytokinins; BAP, Kinetin and TDZ at different concentrations (0.5, 1.0, 2.0, 5.0 and 10.0 ㎎/ℓ) were added on MS medium to compare their effect on multiple shoot induction and shoot elongation. Shoots (3 ㎝ long) were inoculated into test-tube containing 10 ㎖ MS solid medium supplemented with various concentrations of cytokinins. All the cultures were carried out at 25 ± 1 ℃ in a chamber maintaining 16/8 h (light/dark) photoperiod. All the experiments were conducted in triplicates.

Effect of various culture media on shoot growth

To determine the optimal culture conditions for shoot elongation from multiplied shoots were tested onto various culture media; 1/2-MS (1/2MS), MS, 2 x MS (2MS), 4 x MS (4MS), B5 (Gamborg *et al*., 1968), WPM (Lloyd & McCown, 1981), LS (Linsmaier & Skoog, 1965), LP (Quoirin & Lepoivre, 1977), SH (Schenk & Hildebrandt, 1972), White (White, 1963) and NN (Nitsch & Nitsch, 1969). All media contained 3% (w/v) sucrose as carbon source and 0.4% (w/v) gelrite. In all cases of medium was adjusted to pH of 5.7 before autoclaving at 121 ℃ for 15 minutes. The multiplied shoots were cut (3 ㎝ long), and inoculated into test-tube containing 10 ㎖ of various basal culture media. These test tubes were incubated for 4 weeks under 3000 lux light condition. The growth rate of shoots was represented by growth index (GI, %) which is calculated using the equation; GI = (final shoot length − inoculated shoot length)/inoculated shoot length × 100.

In vitro rooting

The *in vitro* rooting of the shoots carried out with MS medium supplemented various concentrations of two-auxins; IBA and IAA (0.5, 1.0, 2.0 and 5.0 ㎎/ℓ, respectively). The elongated shoots were cut (3 ㎝ long) and inoculated into test-tube containing 10 ㎖ solid culture media. The root numbers and growth were counted after 4 weeks of culture under 3000 lux light condition.

Acclimatization of *in vitro* plantlets

Rooted plantlets transferred from the culture vessels, washed gently under running tap water, and transplanted to pots containing autoclaved peatmoss, sand and vermiculite mixture (1 : 1 : 1, w/w/w). The pots were covered with polythene bags. The plants were irrigated daily with tap water. The pots were placed in a growth chamber maintained at 25 ± 1 ℃ with fluorescent light for 4 weeks before they were transferred to soil in the greenhouse.

Statistical analysis

All above experiments were conducted at least three replications. The data generated was subjected to statistical analysis by using SAS for Window Version 6.12 (SAS Institute Inc., 2001). One-way analysis of variance (ANOVA) followed by Duncan's multiple-range test (DMRT) to evaluate differences among the treatments.

RESULTS AND DISCUSSION

Effect of cytokinins on shoot multiplication

Seeds of *C. tricuspidata* were germinated within 3 weeks of culture (Fig. 4A). When germinated plantlets were transferred to MS basal medium without growth regulators, shoots number were increased (Fig. 4B). The effects of various cytokinins (BAP, Kinetin and TDZ) on shoot multiplication were investigated (Fig. 1). The plantlets were produced multiple shoots on MS medium supplemented with cytokinins (Fig. 4C). Differences among various treatments started to appear within 3 weeks, although the difference was more defined after 4 weeks. The most effective cytokinin was 1.0 ㎎/ℓ BAP for multiple shoot induction (5.1 per explant), followed by 2.0 ㎎/ℓ BAP (4.7 per explants). However, Kinetin has lower effect on shoot multiplication than BAP and TDZ. Shoot mul-

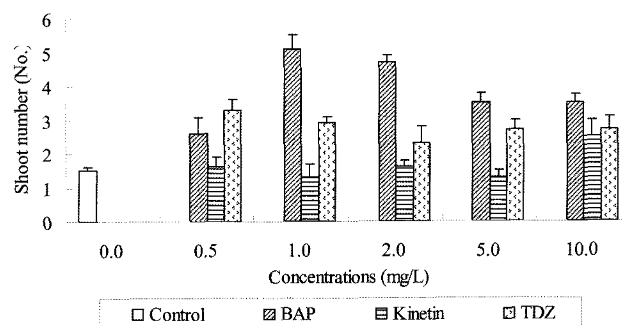

Fig. 1. Effect of various cytokinins on shoot multiplication of *C. tricuspidata*. The plantlets were cultured on MS medium supplemented with various cytokinins and 3% (w/v) sucrose for 4 weeks. Bars represent the standard deviation from the mean.

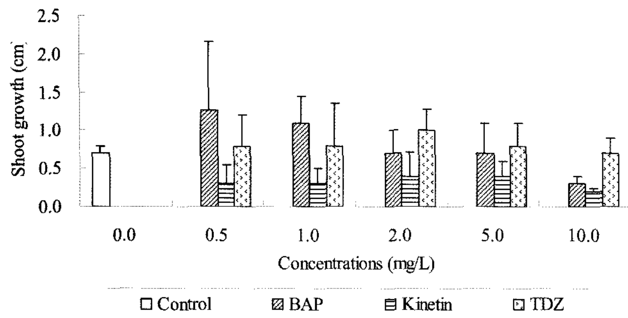

Fig. 2. Effect of various cytokinins on shoot growth of *C. tricuspidata*. The plantlets were cultured on MS medium supplemented with various cytokinins and 3% (w/v) sucrose for 4 weeks. Bars represent the standard deviation from the mean.

Table 1. Effects of IBA and IAA on *in vitro* rooting of *C. tricuspidata*

Concentration (mg/ℓ)		Root number (No.) (Mean ± S.E)	Root length (cm) (Mean ± S.E)
Control		1.33 ± 0.09 c	0.60 ± 0.06 d
IBA	0.5	2.00 ± 0.12 b	2.11 ± 0.71 b
	1.0	1.22 ± 0.03 c	2.78 ± 0.54 b
	2.0	2.22 ± 0.92 b	2.22 ± 0.39 b
	5.0	0.44 ± 0.07 de	3.89 ± 0.55 a
IAA	0.5	1.44 ± 0.06 c	1.42 ± 0.03 c
	1.0	0.98 ± 0.05 dc	0.78 ± 0.13 dc
	2.0	3.08 ± 0.15 a	0.92 ± 0.08 dc
	5.0	0.10 ± 0.06 e	0.96 ± 0.23 dc

tiplication rate gradually improved as the number of subcultures (Data not shown).

The multiplied shoots were cut, and transferred to various culture media containing cytokinins for shoot elongation (Fig. 2). Treatment of BAP at low concentration promoted shoot growth compared to cytokinins free medium, however, high concentration of cytokinins were inhibited shoot elongation. Maximum shoot growth was induced at 0.5 mg/ℓ BAP treatment, however, Kinetin treatment has lower effect of shoot growth than other cytokinins.

The concentrations of exogenous cytokinins appear to be the main factor affecting shoot multiplication. Our experimental results indicated that BAP concentration significantly influenced shoot multiplication and elongation. The BAP is the most commonly preferred cytokinin (Vuylsteke, 1989). Rao & Purohit (2006) have showed that BAP appeared to be more effective than other growth regulators for production of more shoot buds per explants.

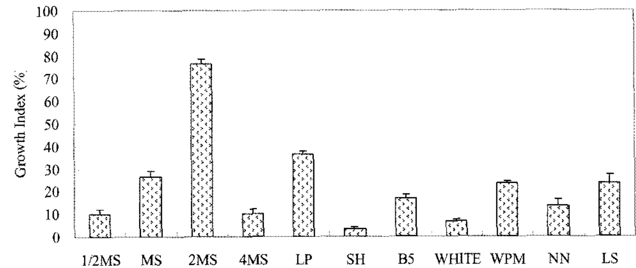

Fig. 3. Effect of culture media on shoot growth of *C. tricuspidata*. The multiplied shoots were cultured on various basal medium supplemented with 3% (w/v) sucrose for 4 weeks. Bars represent the standard deviation from the mean.

Effect of various culture media on shoot growth

For determination of optimal growth medium, shoots were cultured on various culture media such as 1/2MS, MS, 2MS, 4MS, LP, SH, B5, White, WPM, NN and LS were tested for 4 weeks (Fig. 3). The maximum shoot growth was found in two strength MS medium with over 20-fold increase as compared to SH medium. Culture media were different major and minor elements, vitamins, growth regulators and carbon sources, but the optimal concentration of each many vary with plant species. This result caused by component of culture medium.

In vitro rooting and acclimatization

Root was induced from cut shoot base after 1 week of culture. Shoots could be rooted in MS medium supplemented with or without auxins. Root number and growth was increased gradually during 4 weeks of culture (Fig. 4D and E). The root number per plantlet and average root length in culture medium supplemented with auxin was higher than those grown on growth regulator free medium. Between the two auxins, IAA treatment was found to be comparatively more effective than IBA. The maximum root number was obtained on 2.0 mg/ℓ IAA treatment with about 2-fold increase compared to control. The best treatment for root growth was found at 2.0 mg/ℓ IBA treatment.

The rooted plantlets were transferred to pots containing autoclaved peatmoss, sand and vermiculite mixture (1 : 1 : 1, w/w/w) (Fig. 4F). Plantlets after 3 weeks hardening process were transferred to soil with about 90% survival. Generally, it is well known that woody plants are difficult to undergo *in vitro* propagation.

In conclusion, the *in vitro* techniques reported here offer a powerful tool for mass propagation of this threatened plant species. These results provide a basis for germplasm conservation of medicinally important species. Also, this micropropagation method will be available to supply of useful biological compounds as Gericudranin. Further study will be determined characterization of *in vitro* propagated plantlet and biosynthetic ability of secondary metabolites.

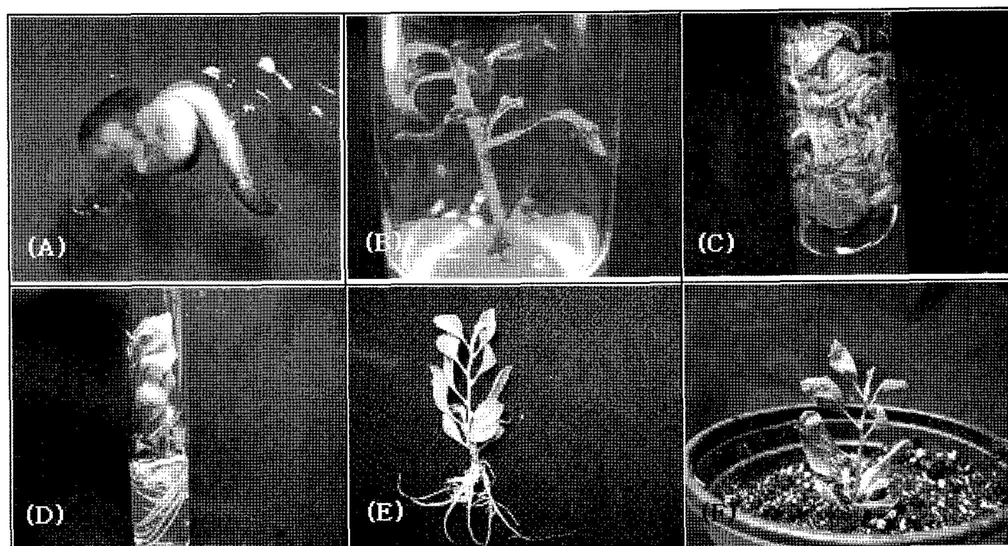

Fig. 4. Micropropagation of *C. tricuspidata* through *in vitro* cultures. A: Seed germination on MS basal medium, B: Germinated plantlet from seed, C: Multiple shoot induction on MS medium with 1.0 mg/ℓ BAP, D: Shoot elongation of multiplied plant on MS medium supplemented 2.0 mg/ℓ IAA, E: *In vitro* rooted plant, and F: Acclimatized plantlet in pot.

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LITERATURE CITED

Choi MS, Kwak SS, Liu JR, Lee IK, Yoo ID (2001) Production of novel flavonolids in cell cultures of *Cudrania tricuspidata*. Korean J. Plant Tissue Cult.28(3):159-164.

Fujimoto T, Hano Y, Nomura T (1984) Components of Root Bark of *Cudrania tricuspidata* 1.1,2 Structures of Four New Isoprenylated Xanthones, Cudraxanthones A, B, C and D. Planta Med 50:218-221.

Gamborg OL, Miller RA, Ojimaki K (1968) Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50:51-158.

Gomez MP, Segura J (1995) Axillary shoot proliferation in cultures of explants from mature *Juniperus oxycedrus* trees. Tree Physiol. 15:625-628.

Gurel S, Gulsen Y (1998) The effects of different sucrose, agar and pH levels on *in vitro* shoot production of almond (*Amygdalus communis* L.). Tr. J. Botany 22:363-373.

He SS, Liu CZ, Saxena PK (2007) Plant regeneration of an endangered medicinal plant *Hydrastis canadensis* L. Scientia Horticulturae 113(5):82-86.

Kang DG, Hur TY, Lee GM, Oh H, Kwon TO, Sohn EJ, Lee HS. (2002) Effects of *Cudrania tricuspidata* water extract on blood pressure and renal functions in NO-dependent hypertension. Life Sciences, 70(22):2599-2609.

Lee IK, Kim CJ, Song KS, Kim HM, Koshino H, Uramoto M, Yoo ID (1996) Cytotoxic benzyl dihydroflavonols from *Cudrania tricuspidata*. Phytochem 41:213-216.

Linsmaier EM, Skoog F (1965) Organic growth factor requirements of tobacco tissue cultures. Physiol. Plant 18:100-127.

Lloyed GB, McCown BH (1981) Commercially feasible micropropagation of mountain laurel (*Kalmia laticolia*) by used of shoot tip culture. Proc. Int. Plant Propagators Soc. 30:421-437.

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant 15:473-497.

Nitsch JP, Nitsch C (1969) Haploid plants from pollen grains. Science 163:85-87.

Ostrolucka MG, Libiakova G, Ondruskova E, Gajdosova A (2004) *In vitro* propagation of *Vaccinium* species. Acta Universitatis Latviensis, Biology 676:207-212.

Quoirin M, Lepoivre P (1977) Improved media for *in vitro* culture of *Prunus* sp. Acta Hort. 78:437-442.

Rao MS, Purohit SD (2006) *In vitro* shoot bud differentiation and plantlet regeneration in *Celastrus paniculatus* Willd. Biol. Plantarum 50:501-506.

Schenk RU, Hildebrandt AC (1972) Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. Can. J. Bot. 50:199-204.

Skirvin RM, Chu MC, Young HJ (1990) Rose. In: Ammirato, P.V., Evans, D.R., Sharp, W.R. and Bajaj, Y.P.S., Editors, Handbook of Plant Cell Cultures, Vol. 5, MacMillan, New York p. 716-43.

Vuylsteke D (1989) Shoot-tip culture for the propagation, conservation and exchange of *Musa* germplasm. IBPGR, Rome.

White PR (1963) The cultivation of animal and plant cells. Ronald Press, New York. p. 199-203.