Journal of Plant Biology, December 2007, 50(6) : 681-686

Rapid Shoot Propagation from Micro-Cross Sections of Kiwifruit (*Actinidia deliciosa* cv. 'Hayward')

Misun Kim[1,2], Seong-Cheol Kim[1]*, Doo-Young Moon[1], and Kwan Jeong Song[2]
[1]National Institute of Subtropical Agriculture, Rural Development Administration, Jeju 690-150, Korea
[2]Faculty of Bioscience and Industry, Cheju National University, Jeju 690-756, Korea

Kiwifruit shoots can be rapidly propagated through a micro-cross section (MCS) system we established here. Optimal culture conditions were determined for different explant types, section sizes, and concentrations of inorganic salts and plant growth regulators. Rates of survival and callus formation were higher in half-strength MS salts than in full-strength MS media. Similar performance (i.e., survival and callus formation) was achieved with section sizes of either 800 μm or 1200 μm. Proliferation efficiency was greatest when explants from stem tissue were cultured on 1/2 MS supplemented with 4.5×10^{-3} μM 2,4-dichlorophenoxyacetic acid and 4.6×10^{-1} μM zeatin. The number of shoots averaged 2.61 per explant, representing an efficiency of 94%. RAPD analysis revealed that the regenerated plants from our MCS system were genetically stable. These results show that the culturing of micro-cross sections from stem tissue is a powerful method for kiwifruit propagation.

Keywords: kiwifruit, normal shoots, section sizes, tissue types, vibratory microtome

The genus *Actinidia*, or kiwifruit, includes 66 species and 118 taxa that originated in China and neighboring countries. These primarily deciduous plants are climbing or scrambling perennial vines (Ferguson, 1999; Huang et al., 2004). This fruit is popular world wide because of its nutritional and medicinal benefits. The main cultivar in the market is 'Hayward', which was selected from seeds of *Actinidia deliciosa* var. *deliciosa* imported into New Zealand from China. However, newer varieties have now been bred that have a range of flesh colors and high contents of vitamin C and other functional substances. Several cross-breeding and biotechnology techniques have been investigated for developing more varieties (Janssen and Gardner, 1993; Ferguson, 1999; Kobayashi et al., 2000; Jung et al., 2003; Kim et al., 2003, 2004; Huang et al., 2004 Kim et al., 2007a).

Tissue culture has been the best method for rapid propagation and genetic improvement of kiwifruit, relying mainly on inductions from shoot tips (Marino and Bertazza, 1990) and axillary buds (Shen et al., 1990). Although culturing of the leaf, stem, and petiole, has recently been examined (González et al., 1995; Zhang et al., 1998; Takahashi et al., 2004), results have varied according to species, cultivar, sex, explant type, and culturing conditions.

A thin cell layer (TCL) technique, using small, thin explants derived from plant organs, was first introduced with tobacco (*Nicotiana tabacum*; Tran Thanh Van, 1973). This method has been successful for the rapid proliferation of certain plant species, including the common bean (*Phaseolus vulgaris*), large crabgrass (*Digitaria sanguinalis*), lily (*Lilium longiflorum*), oilseed rape (*Brassica napus*), and trifoliate orange (*Poncirus trifoliate*) (Klimaszewska and Keller, 1985; Van et al., 1998, 1999; Cruz de Carvalho et al., 2000; Nhut et al., 2002). Although two sectioning methods – hand or machine – have been tested for TCL culture, explants obtained from by former have been irregular and difficult to propagate stably. Therefore, Lee-Stadelmann et al. (1989) have developed micro-cross section (MCS) techniques using a vibratory microtome, and have achieved stable propagation of uniform products from poplar (*Populus* spp.).

Although the ultimate goal of tissue culture is rapid multiplication, *in vitro* techniques can lead to genetic mutation, especially somaclonal variation (Larkin and Scowcroft, 1981). This becomes a critical issue when proliferating valuable germplasms that are genetically fixed. Therefore, micro-propagation should be accompanied by a molecular marker approach, e.g., AFLP, RFLP, RAPD, or SSR, that can detect somaclonal variants, as has been done with garlic (Al-Zahim et al., 1999), cauliflower (Leroy et al., 2000), oil palm (Jaligot et al., 2002), kiwifruit (Palombi and Damiano, 2002), and pine (Goto et al., 1998; Burg et al., 2007).

Here, we describe the development of a new MCS method. Our objective was to show more rapid and stable propagation of kiwifruit shoots compared with conventional methods, as verified via RAPD marker analysis.

MATERIALS AND METHODS

Preparation of Explants and Cultures

Branches from a 10-year-old vine of *Actinidia deliciosa* var. 'Hayward' were placed in water for 3 weeks. Tips from newly emerged shoots without leaves were immersed in 70% (v/v) ethanol for 1 min, then sterilized for 7 min in 1% sodium hypochlorite solution (v/v) containing one drop of Tween 20. After they were rinsed four times with sterile distilled water, the explants were placed into test tubes containing an MS medium (Murashige and Skoog, 1962) supplemented with 3% sucrose (w/v) and solidified with 0.8% agar (w/v) at pH 5.8 (adjusted with 1N NaOH before autoclaving at 121°C for 12 min). The cultures were incubated at 25±2°C under a 16-h photoperiod supplied by fluorescent lamps (45 μmol m^{-2} s^{-1}). The shoots were later transferred to a 500 mL jar containing 100 mL of a prolifer-

*Corresponding and co-first author; fax +82-64-749-2066
e-mail kimsec@rda.go.kr

ation medium with 4.44 μM benzyladenine (BA) and 0.49 μM indole-3-butyric acid (IBA). All cultures were incubated at 25±2°C, with a 16-h photoperiod under fluorescent lamps (45 μmol m^{-2} s^{-1}).

The section segments were prepared with a Vibratory Microtome (Vibratome® Series 1000 Sectioning System, USA). The effect of inorganic salt concentrations was studied by using either half- or full-strength MS media. Three segment thicknesses -- 400 μm, 800 μm, or 1200 μm – were tested for their suitability in regeneration. We also evaluated the optimal concentrations of auxins [naphthaleneacetic acid (NAA), IBA, and 2,4-dichlorophenoxyacetic acid (2,4-D)], cytokinins [benzyladenine (BA), kinetin, zeatin], and a cytokinin-like substance [thidiazuron (TDZ)]. Micro-cross sections of tissues were prepared by the modified procedure of Lee-Stadelmann et al. (1989). Briefly, the interior of the Vibratory Microtome was sterilized with 70% (v/v) ethanol for 1 h, then rinsed with sterile distilled water. A Styrofoam block (2×2 cm) and rubber string for fixing the tissues were immersed in absolute alcohol for 1 d, then rinsed with sterilized water. Segments obtained from this transversal micro-cross section system were stored in a 1/2 MS liquid medium prior to beginning the experiments. The entire micro-cross sectioning process is illustrated in Figure 1.

Tissue segments from the petiole, stem, or leaf were placed on 1/2 MS supplemented with sucrose (3%, w/v) plus a combination of 2,4-D (4.5×10^{-3}, 4.5×10^{-2}, 4.5×10^{-1}, or 4.5 μM) and zeatin (4.6×10^{-3}, 4.6×10^{-2}, 4.6×10^{-1}, or 4.6 μM). The pH was adjusted to 5.8, and plant agar (0.8%, w/v, Duchefa, The Netherlands) was included as a gelling agent. Zeatin also was added to the medium after filter-sterilization. All treated cultures were replicated 3 times in 87×15 mm Petri dishes. After 7 weeks, we examined their regeneration characteristics, including survival rate, fresh plant weight, and the number of shoots produced per explant.

RAPD Analysis

RAPD analysis was conducted on 29 plants obtained with our MCS method. DNA was extracted from 1 g of tissue according to a method modified from that of Dellaporta et al. (1983). Random decamer primers included OPO 01 to OPO 13, and OPP 01 to OPP 20 (Operon Technologies, USA). DNA (10 ng) was used as template in a reaction mixture (total volume 25 μL) that contained 2.5 μL of 10× reaction buffer, 500 μM of each dNTP, 0.25 μM of primer, and 1.5 units iTaq DNA polymerase (iNt RON Bio, Korea). PCR amplifications were performed in a GeneAmp PCR System 9600 Thermal Cycler (Perkin Elmer Cetus, USA), with initial denaturation for 2 min at 94°C; then 35 cycles of 1 min at 94°C, 1 min at 38°C, and 2 min at 72°C; followed by 5 min of final extension at 72°C. The RAPD products were separated on 1.0% Agarose LE gels (Promega, USA) at 100 V for 40 min with 1× Tris-borate-EDTA (TBE) buffer, then finally stained with ethidium bromide.

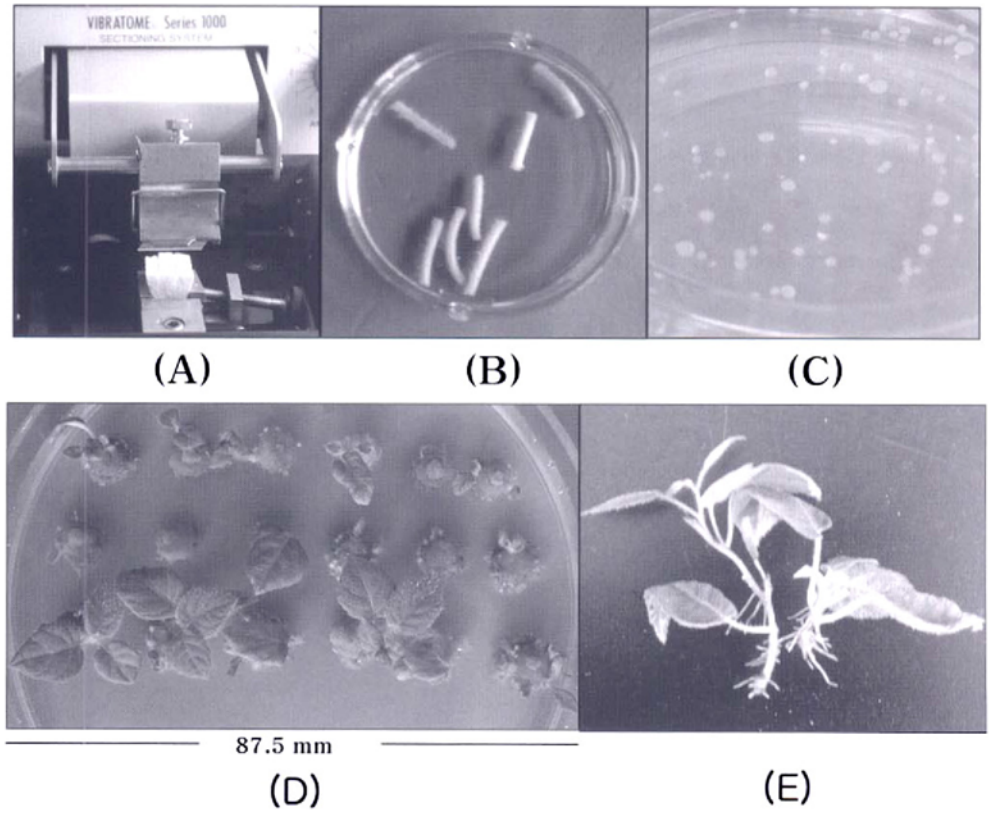

Figure 1. Process for rapid propagation of shoots from stem segments of *Actinidia deliciosa* cv. 'Hayward' using micro-cross section method. (**A**) Vibrating microtome, (**B**) stem segments, (**C**) sliced stem segments, (D) organogenesis from micro-cross sections on 1/2 MS + 4.5×10^{-3} μM 2,4-D + 4.6×10^{-1} μM zeatin + 3% sucrose after 7 weeks, (**E**) rooted plants after 3 weeks.

Statistical Analysis

This study was designed to distinguish between normal shoots and those with abnormal morphologies that possibly resulted from somaclonal variation. Data were analyzed using SAS (2002) Version 6.1.2, and significant differences between means were assessed by Duncan's multiple range tests at P 0.05.

RESULTS AND DISCUSSION

To determine how the growth response of kiwifruit cultures was associated with the concentration of inorganic salts, we tested petiole segments (800 μm) on 1/2 MS and full-strength MS media, and obtained survival rates of 100% and 88.9%, respectively. The size of the callus did not differ between them, although some segments gradually died without forming any calli on the MS medium (Table 1), perhaps because of higher osmotic stress than from the 1/2 MS salts.

The optimum size for segments was 800 μm (Table 2), which differs from the 400 to 500 μm recommended for poplar leaf sections (Lee-Stadelmann et al., 1989). However, our results were similar to a previous report that the number of developing shoots increases in proportion to explant size

Table 1. Effect of various concentrations of inorganic salts in MS media[z] used for micro-cross section cultures of kiwifruit petiole segments.

Medium	Survival (%)	Callus formation (%)	Callus size[x] (cm)
MS[y]	88.9	88.9	1.12 ± 0.09
1/2 MS	100.0	100.0	0.99 ± 0.02

[z]All materials were cultured on inorganic salts medium + 3% sucrose + 0.8% agar + 0.45 μM 2,4-D (pH 5.8).
[y]MS= Murashige and Skoog (1962) medium.
[x]Callus size is given as mean ± SE.

Table 2. Effect of fragment size from micro-cross sections for culturing of kiwifruit[z].

Tissue type	Size (μm)	Survival (%)	Callus formation (%)	Callus size[y] (cm)
Leaf	400	6	6	0.02 ± 0.02
	800	100	100	0.53 ± 0.05
	1200	100	100	0.69 ± 0.04
Stem	400	100	100	0.62 ± 0.02
	800	100	100	0.67 ± 0.02
	1200	100	100	0.72 ± 0.02
Petiole	400	100	100	0.61 ± 0.03
	800	100	100	0.62 ± 0.01
	1200	100	100	0.66 ± 0.01

[z]All materials were cultured on 1/2 MS medium + 3% sucrose + 0.8% agar + 0.45 μM 2,4-D (pH 5.8).
[y]Callus size is given as mean ± SE.

Table 3. Effect of plant growth regulators on shoot induction and development from kiwifruit leaf, petiole, and stem micro-cross section segments after 7 weeks of culture[zy].

Growth regulator (μM)		Leaf				Petiole				Stem			
2,4-D	Zeatin	Shoot formation (%)	No. of shoots per explant	No. of leaves	Shoot length (cm)	Shoot formation (%)	No. of shoots per explant	No. of leaves	Shoot length (cm)	Shoot formation (%)	No. of shoots per explant	No. of leaves	Shoot length (cm)
0	0	0	0b	0b	0b	0	0c	0c	0c	5.6	0.06c	0.06d	0.03e
0	4.6×10^{-3}	0	0b	0b	0b	0	0c	0c	0c	11.1	0.17c	0.14cd	0.05de
0	4.6×10^{-2}	0	0b	0b	0b	0	0c	0c	0c	38.9	0.39c	0.61c	0.22cd
0	4.6×10^{-1}	5.6	0.12ab	0.06b	0b	5.6	0.06c	0.17c	0.03c	38.9	0.44c	0.50cd	0.25c
0	4.56	0	0b	0b	0b	33.3	0.56b	0.77b	0.16b	61.1	1.44b	1.46b	0.26c
4.5×10^{-3}	4.6×10^{-3}	0	0b	0b	0b	0	0c	0c	0[c]	72.2	1.22b	1.96ab	0.80a
4.5×10^{-3}	4.6×10^{-2}	0	0b	0b	0b	5.6	0.06c	0.06c	0[c]	88.9	1.28b	1.93ab	0.76a
4.5×10^{-3}	4.6×10^{-1}	0	0b	0b	0b	5.6	0.06c	0.06c	0[c]	94.4	2.61a	2.16a	0.70a
4.5×10^{-3}	4.56	11.1	0.17a	0.42a	0.05a	50.0	1.06a	1.13a	0.46a	83.3	2.44a	1.54b	0.51b
4.5×10^{-2}	4.6×10^{-3}	0	0b	0b	0b	0	0c	0c	0c	0	0c	0d	0e
4.5×10^{-2}	4.6×10^{-2}	0	0b	0b	0b	0	0c	0c	0c	0	0c	0d	0e
4.5×10^{-2}	4.6×10^{-1}	0	0b	0b	0b	0	0c	0c	0c	0	0c	0d	0e
4.5×10^{-2}	4.56	0	0b	0b	0b	0	0c	0c	0c	0	0c	0d	0e
4.5×10^{-1}	4.6×10^{-3}	0	0b	0b	0b	0	0c	0c	0c	0	0c	0d	0e
4.5×10^{-1}	4.6×10^{-2}	0	0b	0b	0b	0	0c	0c	0c	0	0c	0d	0e
4.5×10^{-1}	4.6×10^{-1}	0	0b	0b	0b	0	0c	0c	0c	0	0c	0d	0e
4.5×10^{-1}	4.56	0	0b	0b	0b	0	0c	0c	0c	0	0c	0d	0e
4.52	4.6×10^{-3}	0	0b	0b	0b	0	0c	0c	0c	0	0c	0d	0e
4.52	4.6×10^{-2}	0	0b	0b	0b	0	0c	0c	0c	0	0c	0d	0e
4.52	4.6×10^{-1}	0	0b	0b	0b	0	0c	0c	0c	0	0c	0d	0e
4.52	4.56	0	0b	0b	0b	0	0c	0c	0c	0	0c	0d	0e

[z]Means separation within each column by Duncan's multiple range test at $P \leq 0.05$.
[y]All materials were cultured on 1/2 MS medium + 3 % sucrose + 0.8% agar (pH 5.8).

for poplar leaves (Lee-Stadelmann et al., 1989), while the number of shoots diminishes by increment in internode size for native spearmint tissues (Poovaiah et al., 2006).

In our hormone trials, all explants formed calli after 10 d of exposure to various plant growth regulators. Among the auxins, 2,4-D was the most effective in promoting callus formation. Shoot production only occurred with zeatin or TDZ treatments; BA and kinetin were ineffective. The positive effect of TDZ on multiple-shoot induction has also been shown in fig (Kim et al., 2007b). Nevertheless, an excessive number of abnormal shoots also appeared from our TDZ-treated tissues (data not shown). Similar observations have been made with *Arabidopsis* (Gleddie, 1989) and kiwifruit (Kim and Oh, 1998). Not only do morphological abnormalities increase with higher TDZ concentrations (Huetteman and Preece, 1993), but even lower levels are associated with numerous malformations and the production of hyperhydric shoots (Fraser et al., 1995).

Because Kim and Oh (1998) also have reported that zeatin is more effective than other cytokinins and cytokinin-like substances for tissue culturing of kiwifruit, we chose that hormone for our micro-cross section protocol. Table 3 presents a comparison among various parameters evaluated to determine the most suitable conditions for regenerating normal shoots. Leaf and petiole explants were more successfully utilized on culture media containing 4.5×10^{-3} μM 2,4-D and 4.6 μM zeatin than other combinations. The frequency of normal shoot formation also was higher from petiole segments (50.0%) than from leaves (11.1%). Although no shoots formed from leaf or petiole explants cultured on media lacking growth regulators, it was possible to produce a few shoots from stem tissues under the same constraint. When stem segments were cultured on media containing zeatin, the number of normal shoots and the rate of shoot formation increased in proportion to the concentration of zeatin applied. Moreover, the number and rate of normal shoots increased at higher zeatin concentrations in combination with 2,4-D at a level below 4.5×10^{-3} μM.

The number of regenerated shoots averaged 2.61 per explant, at a formation rate of 94% (Table 3). Takahashi et al. (2004) have reported that, on a zeatin-supplemented medium, leaf explants from *A. polygama* Miq. show obviously poorer shoot formation than do stem or petiole explants. However, they have also found that particular tissues respond differently to phytohormones. In contrast, Sugawara et al. (1994) have demonstrated that tissue type (leaf, petiole, or stem) is not a significant determining factor when inducing *A. polygama* shoots on media containing BA and NAA. In the current study, we noted that stems were the best tissue source for kiwifruit propagation.

In contrast to traditional nodal culture, which generally requires tissues of at least 1.0 cm, our micro-cross section method utilized 800-μm-thick segments, thereby enabling us to obtain more than 8 explants from a single 1.0-cm stem. Marino and Bertazza (1990) have reported achieving their best proliferation efficiency with shoot tip cultures from *A. deliciosa* cv. 'Hayward', producing 3.12 normal shoots per explant in a medium containing 4.44 μM BA. In comparing these culturing methods using 1.0-cm nodes, MCS resulted in >20 shoots versus 3.12 shoots from shoot tip cultures. This means that the micro-cross section system is approximately seven times more effective for the proliferation of kiwifruit shoots.

To determine their genetic stability when employing MCS, we randomly selected 29 regenerated shoots for RAPD analysis. In all, 19 of 24 primers produced 63 bands that ranged from 400 to 3500 bps. Palombi and Damiano (2002) have reported that SSR and RAPD analyses indicate few soma-

Table 4. Polymorphism analysis of regenerated kiwifruit plants using decamer primers.

Primer	Sequence (5′ → 3′)	No. of bands	Band size (base pairs)	No. of polymorphic bands
OPP 01	GTAGCACTCC	2	800, 1300	0
OPP03	CTGATACGCC	6	750, 1500, 1700, 2100, 2500, 3000	0
OPP04	GTGTCTCAGG	2	600, 1300	0
OPP05	CCCCGGTAAC	4	650, 800, 1250, 2500	0
OPP08	ACATCGCCCA	4	750, 1300, 1400, 1800	0
OPP10	TCCCGCCTAC	5	400, 750, 1400, 2,300, 3000	0
OPP11	AACGCGTCGG	5	600, 1000, 1200, 1800, 2300	0
OPP12	AAGGGCGAGT	3	600, 900, 1500	0
OPP13	GGAGTGCCTC	2	1000, 1500	0
OPP14	CCAGCCGAAC	4	750, 1000, 1400, 2000	0
OPP17	TGACCCGCCT	2	1200, 2000	0
OPP20	GACCCTAGTC	3	750, 2000, 2500	0
OPO01	GGCACGTAAG	2	750, 900	0
OPO02	ACGTAGCGTC	5	1000, 1100, 1700, 3000, 3500	0
OPO03	CTGTTGCTAC	3	850, 1000, 2000	0
OPP08	CCTCCAGTGT	1	1500	0
OPO09	TCCCACGCAA	5	1100, 1300, 1500, 1800, 2000	0
OPO10	TCAGAGCGCC	4	1000, 1400, 2500, 3500	0
OPO11	GACAGGAGGT	1	750	0

clonal variants occur during the tissue-culturing of kiwifruit. Here, we could not detect polymorphic bands among our primers (Table 4). Therefore, we suggest that MCS-regenerated plants should be analyzed by several methods, e.g., chromosome-counting and morphological assessments.

In summary, we have determined that, for normal shoot regeneration from kiwifruit, the optimal MCS method includes the following components: 1) a 1/2 MS medium with inorganic salts; 2) 800-μm segments from petioles, stems, or leaves; and 3) a combination of 2,4-D (4.5×10^{-3} to 10^{-1} μM) and zeatin ($4.\times10^{-3}$ to 10^{-1} μM) in the culture medium. Shoots can be formed in approximately 20 d, with survival rates of 100% achieved from petiole and stem explants. Our results demonstrate that micro-cross sectioning is not only a good method for rapidly propagating rare kiwifruit cultivars, but is also useful to biotechnology research efforts that require numerous samples for greater success.

ACKNOWLEDGEMENTS

We thank Dr. Ho Bang Kim for reading this manuscript.

Received May 1, 2007; accepted November 12, 2007.

LITERATURE CITED

Al-Zahim, MA, Ford-Lloyd BV, Newbury HJ (1999) Detection of somaclonal variation in garlic (*Allium sativum* L.) using RAPD and cytological analysis. Plant Cell Rep 18: 473-477

Burg K, Helmersson A, Bozhkov P, Arnold S (2007) Developmental and genetic variation in nuclear microsatellite stability during somatic embryogenesis in pine. J Exp Bot 58: 687-698

Cruz de Carvalho MH, Van LB, Zuily-Fodil Y, Pham Thi AT, Trân Thanh Vân K (2000) Efficient whole plant regeneration of common bean (*Phaseolus vulgaris* L.) using thin-cell-layer culture and silver nitrate. Plant Sci 159: 223-232

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: VersionII. Plant Mol Biol Rep 1: 19-21

Ferguson AR (1999) Kiwifruit cultivars: Breeding and selection. Acta Hort 498: 43-51

Fraser LG, Kent J, Harvey CF (1995) Transformation studies of *Actinidia chinensis* Planch. NZ J Crop Hort 23: 407-413

Gleddie S (1989) Plant regeneration from cell suspension culture of *Arabidopsis thaliana* Heynh. Plant Cell Rep 8: 1-5

González MV, Rey M, Rodriguez R (1995) Plant regeneration from petioles of kiwifruit microshoots. HortSci 30: 1302-1303

Goto S, Thakur RC, Ishii K (1998) Determination of genetic stability in long-term micropropagated shoots of *Pinus thunbergii* Parl. using RAPD markers. Plant Cell Rep 18: 193-197

Huang H, Wang Y, Zhan Z, Jiang Z, Wang S (2004) *Actinidia* germplasm resources and kiwifruit industry in China. HortSci 39: 1165-1172

Huetteman CA, Preece JE (1993) Thidiazuron: A potent cytokinin for woody plant tissue culture. Plant Cell Tiss Org Cult 33: 105-119

Jaligot E, Beul T, Rival A (2002) Methylation-sensitive RFLPs: Characterisation of two oil palm markers showing somaclonal variation-associated polymorphism. Theor Appl Genet 104: 1263-1269

Janssen BJ, Gardner RC (1993) The use of transient GUS expression to develop an *Agrobacterium*-mediated gene transfer system for kiwifruit. Plant Cell Rep 13: 28-31

Jung YH, Kim KH, Kang SH, Jun SJ, Oh MY, Kim SC (2003) Phylogenetic analysis of the Genus *Actinidia* in Korea inferred from two non-coding chloroplast DNA sequences. Kor J Genet 25: 95-101

Kim CH, Kim SC, Jang KC, Song EY, Kim M, Moon DY, Seong KC, Lee JS, Suh HD, Song KJ (2007a) A new kiwifruit cultivar, "Jecy Gold" with yellow flesh. Kor J Breed Sci 39: 258-259

Kim KM, Kim MY, Yun PY, Chandrasekhar T, Lee HY, Song PS (2007b) Production of multiple shoots and plant regeneration from leaf segments of fig tree (*Ficus carica* L.). J Plant Biol 50: 440-446

Kim SC, Jung YH, Kim M, Kim CH, Koh SC, Kang SH (2003) Genetic relationships of genus *Actinidia* based on random amplified polymorphic DNA analysis. J Kor Soc Hort Sci 44: 340-344

Kim SC, Jung YH, Kim M, Koh SC, Song KJ, Kim HB (2004) Characterization of a RAPD fragment unique to species with hairy fruit skin in the genus *Actinidia*. J Plant Biol 47: 210-215

Kim Y, Oh S (1998) Plant regeneration from leaf and petiole culture of kiwifruit (*Actinidia deliciosa*). Kor J Plant Tiss Cult 25: 305-308

Klimaszewska K, Keller WA (1985) High frequency plant regeneration from thin cell layer explants of *Brassica napus*. Plant Cell Tiss Org Cult 4: 193-197

Kobayashi S, Ding CK, Nakamura Y, Nakajima I (2000) Kiwifruits (*Actinidia deliciosa*) transformed with a *Vitis* stilbene synthase gene produce piceid (resveratol-glucoside). Plant Cell Rep 19: 904-910

Larkin PJ, Scowcroft WR (1981) Somaclonal variation a novel source of variability from cell culture for plant improvement. Theor Appl Genet 60: 197-214

Lee-Stadelmann OY, Lee SW, Hackett WP, Read PE (1989) The formation of adventitious buds in vitro on micro-cross sections of hybrid *Populus* leaf midveins. Plant Sci 61: 263-272

Leroy XJ, Leon K, Charles G, Branchard M (2000) Cauliflower somatic embryogenesis and analysis of regenerant stability by ISSRs. Plant Cell Rep 19: 1102-1107

Marino G, Bertazza G (1990) Micropropagation of *Actinidia deliciosa* cvs. 'Hayward' and 'Tomuri'. Sci Hort 45: 65-74

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

Nhut DT, Le BV, Minh NT, de Silva JT, Fukai S, Tanaka M, Tran Thanh Van K (2002) Somatic embryogenesis through pseudobulblet transverse thin cell layer of *Lilium longiflorum*. Plant Growth Regul 37: 193-198

Palombi MA, Damiano C (2002) Comparison between RAPD and SSR molecular markers in detecting genetic variation in kiwifruit (*Actinidia deliciosa* A. Chev). Plant Cell Rep 20: 1061-1066

Poovaiah CR, Weller SC, Jenks MA (2006) In vitro adventitious shoot regeneration of native spearmint using internodal explants. HortSci 41: 414-417

SAS (2002) SAS 9.1.2 for Windows. SAS Institute, Inc., Cary, NC, USA

Shen XS, Wan JZ, Luo WY (1990) Propagation in vitro of Chinese Gooseberry (*Actinidia chinensis*) through the development of axillary buds. Sci Hort 42: 45-54

Sugawara F, Yamamoto N, Tanaka O (1994) Plant regeneration in *in vitro* culture of leaf, stem and petiole segments of *Actinidia polygama* Miq. Plant Tissue Cult Lett 11: 14-18

Takahashi W, Sugawara F, Yamamoto N, Bando E, Matsushita J, Tanaka O (2004) Plant regeneration in *Actinidia polygama* Miq. by leaf, stem, and petiole culture with zeatin, and from stem-derived calli on low-sucrose medium. J For Res 9: 85-88

Tran Thanh Van M (1973) Direct flower bud neoformation from superficial tissue of small explants of *Nicotiana tabacum* L. Planta 115: 87-92

Van LE, Jeanneau M, Do My NT, Vidal J, Thanh Vân K (1998) Rapid regeneration of whole plants in large crabgrass (*Digitaria sanguinalis* L.) using thin-cell-layer culture. Plant Cell Rep 18: 166-172

Van LE, Thanh HN, Anh Hong LT, Tran Thanh Van K (1999) High frequency shoot regeneration from trifoliate orange (*Poncirus trifoliate* L. Raf.) using the thin cell layer method. CR Acad Sci Paris 322: 1105-1111

Zhang YJ, Qian YQ, Mu XJ, Cai QG, Zhou YL, Wei XP (1998) Plant regeneration from in vitro-cultured seedling leaf protoplasts of *Actinidia eriantha* Benth. Plant Cell Rep 17: 819-821