Plant Cell Reports (2000) 19:727–732

W. Tang

High-frequency plant regeneration via somatic embryogenesis and organogenesis and in vitro flowering of regenerated plantlets in *Panax ginseng*

Received: 1 December 1998 / Revision received: 13 September 1999 / Accepted: 20 September 1999

Abstract The morphogenesis ability of light yellowish globular callus derived from cotyledons of mature zygotic embryos of *Panax ginseng* was investigated. The optimal media for somatic embryogenesis and shoot organogenesis were MS medium containing 0.5 mg l^{-1} 2,4-dichlorophenoxyacetic acid, 0.1 mg l^{-1} 6-benzyladenine (BA), and 500 mg l^{-1} lactoalbumin hydrolysate, and SH medium supplemented with 0.5 mg l^{-1} α-naphthaleneacetic acid, 0.1 mg l^{-1} BA, and 500 mg l^{-1}casein hydrolysate. The influences of glucose, mannose, fructose, and sorbose in the media on somatic embryogenesis and shoot organogenesis were revealed as differences in the numbers of somatic embryos and adventitious shoots per gram of morphogenic callus. The best regeneration of somatic embryos was obtained on medium containing glucose, with a mean of 8.7 somatic embryos per gram of callus. The best regeneration of shoots was observed on medium containing fructose, with an average of 12.2 adventitious shoots per gram of callus. Of the somatic embryos 95% were converted into regenerated plantlets, and 100% of adventitious shoots rooted to form regenerated plantlets. Regenerated plants were successfully established in soil. Flowering was observed in 5.7% of the regenerated plants derived from shoot organogenesis and in 1.4% of the regenerated plants derived from somatic embryogenesis.

Key words *Panax ginseng* · C.A. Meyer · Somatic embryogenesis · Organogenesis · In vitro flowering of regenerated plantlets

Communicated by: G.C. Philips

W. Tang
Institute of Chemical Metallurgy, Chinese Academy of Sciences, Beijing 100080, P.R. China

Present address:
Forest Biotechnology Group, Department of Forestry, North Carolina State University, Raleigh, NC 27695-8008, USA
e-mail: wtang@unity.ncsu.edu
Fax: 919-515-7801

Abbreviations *BA*: 6-Benzyladenine · *2,4-D*: 2,4-Dichlorophenoxyacetic acid · *GA_3*: Gibberellic acid · *MS medium*: Murashige and Skoog medium · *NAA*: α-Naphthaleneacetic acid · *SH medium*: Schenk and Hildebrandt medium

Introduction

Medical plant cell and tissue culture has advanced rapidly in recently years, allowing researchers to develop propagation techniques that have the potential to significantly shorten the breeding cycle of outstanding clones and to establish efficient in vitro plant regeneration protocols that can be used in genetic modification studies using different gene transfer systems (Choi et al. 1998; Fetlt-Neto et al. 1992; Yoshikava and Furuya 1987). Despite the various methods of clonal propagation applicable to medical plants, somatic embryogenesis and organogenesis are rapidly becoming acceptable techniques for the clonal propagation of superior medical plant species (Gary and Brent 1986; Choi et al. 1998). Moreover, the establishment of in vitro flowering systems, based on somatic embryogenesis and organogenesis in higher plants, is expendient to the genetic analysis of the expression of particular gene products during floral organ formation.

Panax ginseng C.A. Meyer is one of the most economically important medical plants in the world. Although there are a few reports on callus induction (Jhang et al. 1974), low-temperature preservation (Butenko et al. 1984; Mannonen et al. 1990), saponin production (Furuya et al. 1983), and plant regeneration (Shoyama et al. 1988) in *Panax ginseng*, the regeneration of plantlets via somatic embryogenesis and organogenesis from somatic tissues remains difficult with a low frequency of occurrence (5–15%) (Chang et al. 1980b; Shoyama et al. 1988). Callus from leaf, stem segments, and root of *Panax ginseng* has been successfully induced, but this callus failed to regenerate (Jhang et al. 1974). Chang and Hsing (1980b) obtained regener-

ated plants via somatic embryogenesis from callus derived from the root of ginseng. Tae et al (1982) reported plant regeneration through organogenesis from callus and leaflet cultures of ginseng. Choi et al. (1997, 1998) reported direct somatic embryogenesis from cotyledons of ginseng at a frequency of 12–66%. However, the regeneration frequency still needs to be improved. In vitro flowering of regenerated ginseng plantlets from somatic embryos has been reported (Chang et al. 1980a; Lee et al. 1990). However, methods for inducing in vitro flowering of the regenerants also need to be improved. This investigation reported here examined the development of high-frequency plant regeneration system via somatic embryogenesis and organogenesis in ginseng and in vitro flowering of regenerated plantlets from somatic embryos and adventitious buds.

Materials and methods

Plant material

Mature seeds of *Panax ginseng* C.A. Meyer were collected from Hunan Farm, Heilongjiang Province, Northeast China. All seeds were stored at 4 °C for 3 months so as to finish the after-ripening. Seeds were immersed for 30 s in 70% (v/v) ethanol, surface-sterlized for 15 min with 0.1% mercuric chloride, and rinsed five times with sterile distilled water. Cotyledons, hypocotyls, and radicles of mature zygotic embryos were aseptically removed from seeds and placed on solid MS medium (Murashige and Skoog 1962) supplemented with 0.5 mg l^{-1} 2,4-D, 500 mg l^{-1} lactoalbumin hydrolysate, 30 g l^{-1} sucrose, and 6.5 g l^{-1} agar for inducing callus formation. The cotyledons used were 0.3–0.5 cm in length.

Effects of medium compositions on somatic embryogenesis and organogenesis

Four weeks after the explants were cultured on MS medium with 0.5 mg l^{-1} 2,4-D in darkness, light yellowish globular calli derived from cotyledons, hypocotyls, and radicles were transferred to four different media used for somatic embryos and adventitious bud production. These media were selected for this experiment after promising results had been obtained from several preliminary experiments. They were: (1) MS medium with 0.5 mg l^{-1} 2,4-D, 0.1 mg l^{-1} BA, and 500 mg l^{-1} lactoalbumin hydrolysate; (2) SH medium (Schenk and Hildebrandt 1972) with 0.5 mg l^{-1} 2,4-D, 0.1 mg l^{-1} BA, and 500 mg l^{-1} lactoalbumin hydrolysate; (3) MS medium with 0.5 mg l^{-1} NAA, 0.1 mg l^{-1} BA, and 500 mg l^{-1} casein hydrolysate; (4) SH medium with 0.5 mg l^{-1} NAA, 0.1 mg l^{-1} BA, and 500 mg l^{-1} casein hydrolysate. All four media were supplemented with 30 g l^{-1} sucrose and 6.5 g l^{-1} agar. Adventitious buds over 0.5 cm in height were excised and cultured on SH medium with 0.1 mg l^{-1} NAA, 20 g l^{-1} sucrose, and 6.5 g l^{-1} agar for rooting. Each treatment was replicated three times, and each replicate consisted of 50 explants. A completely randomized design was used. Data on the frequency of somatic embryo and shoot regeneration were recorded after 6 weeks of culture.

Effects of carbon sources on somatic embryogenesis and organogenesis

Calluses of *Panax ginseng* induced on MS callus induction medium were transferred to MS medium with 0.5 mg l^{-1} 2,4-D, 0.1 mg l^{-1} BA, 6.5 g l^{-1} agar, and 500 mg l^{-1} lactoalbumin hydrolysate as well as to SH medium with 0.5 mg l^{-1} NAA, 0.1 mg l^{-1} BA, 6.5 g l^{-1} agar, and 500 mg l^{-1} casein hydrolysate. The effects of glucose, mannose, fructose, and sorbose on both regeneration frequency and mean number of somatic embryos or adventitious buds per gram of callus were evaluated. The concentration of each of the four kinds of carbon sources was 30 g l^{-1}. Adventitious buds over 0.5 cm in height were excised and cultured on SH medium with 0.1 mg l^{-1} NAA, 30 g l^{-1} fructose, and 6.5 g l^{-1} agar for rooting. Each treatment was replicated four times, and each replicate consisted of 30 pieces of callus, an average of 1.1 gram per callus cultures: The numbers of somatic embryos and adventitious buds per gram of callus cultures were recorded after 6 weeks of culture.

In vitro flowering of regenerated plantlets

Regenerated plantlets derived from somatic embryos and adventitious buds were transferred to MS medium supplemented with 0.5, 1, 2, 4, or 8 mg l^{-1} GA_3, 20 g l^{-1} glucose, and 6.5 g l^{-1} agar. After 4 weeks of culture, plantlets were transferred to fresh medium containing the same composition of ingredients. Following two consecutive passages, data on number of flowering plantlets from somatic embryogenesis and organogenesis were collected. Subsequently, the flowering plantlets were transferred to MS medium without any growth regulators. Each treatment comprised 30 plantlets and the experiment was repeated three times. The pH of all media was adjusted to 5.8 with 1 *N* KOH or 1 *N* HCl prior to autoclaving at 121 °C for 20 min. All cultures were incubated at 25° ± 1 °C under cool-white fluorescent light with a 16-h photoperiod and 60% relative humidity. All data were analyzed using analysis of variance.

Acclimatization and transfer of plantlets to soil

Regenerated plantlets over 1.5 cm in height were removed from agar medium and transferred to liquid medium containing SH basal salts for 1 week to harden the root system. Plantlets were subsequently transferred to an autoclaved soil mixture containing coarse sand and soil (1:2). Potted plants were gradually acclimatized by increasing the temperature from 22 °C to 28 °C over a 10-day period in controlled growth chambers.

Results and discussion

Callus induction and proliferation

The mature zygotic embryo explants expanded rapidly and began to form callus at the end of the cotyledon and radicle within 7–14 days when cultured on MS callus induction medium containing 0.5 mg l^{-1} 2,4-D and 500 mg l^{-1} lactoalbumin hydrolysate. As the calli started to proliferate, three types could be distinguished: light-yellow globular (LYG), light-green globular (LGG), and light-brown globular (LBG). Three types of callus were initiated at frequencies of 57.1%, 21.3%, and 16.8%, respectively, on MS callus induction medium (Fig. 1). Light-yellow globular callus formed from the cotyledons of the mature zygotic embryos, while both light-green globular and light-brown globular callus formed from the hypocotyls and radicles. Light-yellow globular callus was morphogenic and was selectively proliferated on MS medium supple-

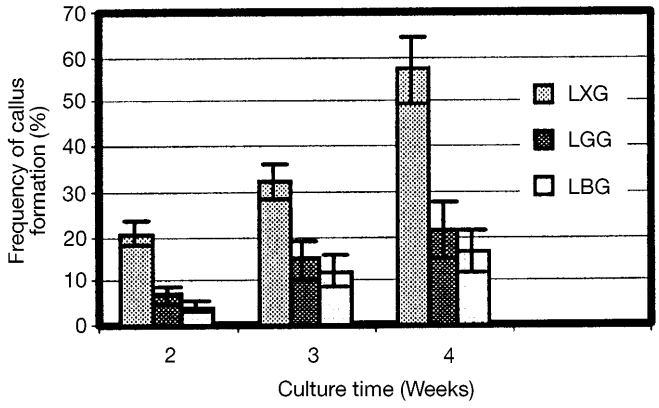

Fig. 1 Frequency of callus formation in ginseng embryo explants after 4 weeks of culture on MS medium supplemented with 2,4-D and lactoalbumin hydrolysate. Each treatment consisted of 90 explants. *LYG* Light-yellow globular callus, *LGG* light-green globular callus, *LBG* light-brown globular callus. *Bars*: Standard error of mean

mented with 0.5 mg l^{-1} 2,4-D and 500 mg l^{-1} lactoalbumin hydrolysate.

Effects of different medium compositions on somatic embryogenesis and organogenesis

Upon transfer of the light yellowish globular callus to each of the four media used for somatic embryos and adventitious bud production, somatic embryos were induced from callus cultured on MS and SH medium with 0.5 mg l^{-1} 2,4-D, 0.1 mg l^{-1} BA, and 500 mg l^{-1} lactoalbumin hydrolysate (Fig. 2A). The highest induction frequency of somatic embryos was 87% on MS medium with 2,4-D and BA (Table 1). Adventitious buds were induced from callus cultured on SH and MS medium with 0.5 mg l^{-1} NAA, 0.1 mg l^{-1} BA, and 500 mg l^{-1} casein hydrolysate (Fig. 2B). The highest induction frequency of adventitious buds was 85% on SH medium with NAA and BA (Table 1). When calli were transferred to a fresh medium with the same compositions, secondary somatic embryogenesis and organogenesis were observed, and increased numbers of somatic embryos and adventitious buds were obtained. In contrast, the regeneration frequencies of somatic embryos or adventitious buds were only about 1% after morphogenic calli were transferred to MS and SH medium containing NAA and BA, and SH and MS medium supplemented with 2,4-D and BA, respectively (Table 1). Somatic embryos from the best treatment were transferred to a growth hormone-free MS medium, and over 90% converted into regenerated plantlets within 1 month (Fig. 2C). Adventitious buds over 0.5 cm long were transferred to SH medium supplemented with 0.1 mg l^{-1} NAA, and over 95% rooted and converted into regenerated plantlets within 1 month. Morphogenic callus was observed to maintain its embryogenic or organogenic potential for over 12 months.

Effects of different carbon sources on somatic embryogenesis and organogenesis

Light yellowish globular calli were transferred to MS medium (used for somatic embryo production) with 0.5 mg l^{-1} 2,4-D, 0.1 mg l^{-1} BA, 500 mg l^{-1} lactoalbumin hydrolysate, and different carbon sources and to SH medium (used for adventitious bud production) with 0.5 mg l^{-1} NAA, 0.1 mg l^{-1} BA, 500 mg l^{-1} casein hydrolysate, and different carbon sources. The induction frequencies of both somatic embryos and adventitious buds were over 80%. However, both average somatic embryo number and average adventitious bud

Fig. 2A–D Morphogenesis and regeneration of flowering plantlets in *Panax ginseng*. **A** Cotyledonary somatic embryos on callus (*bar*: 0.2 cm), **B** adventitious buds on callus (*bar*: 0.3 cm). **C** regenerated plantlet derived from somatic embryo on callus (*bar*: 0.4 cm). **D** flowering regenerated plantlet derived from shoot organogenesis (*bar*: 0.5 cm)

Table 1 Effect of medium composition on somatic embryo and adventitious bud regeneration of *Panax ginseng*. Each treatment consisted of about 50 pieces of calli, an average of 1.2 gram per callus tissue. Tests were replicated three times. Values represent the mean ± SD

Medium composition	Number of calli tested	Number of calli forming somatic embryos	Percentage of calli producing somatic embryos[a]	Number of calli forming adventitious buds	Percentage of calli producing shoots[a]
MS + 0.5 mg l^{-1} 2,4D + 0.1 mg l^{-1} BA + 500 mg l^{-1} LH	150	131	87.0 ± 6.1a	1	1.0 ± 0.1c
SH + 0.5 mg l^{-1} 2,4D + 0.1 mg l^{-1} BA + 500 mg l^{-1} LH	150	59	39.2 ± 4.8bc	2	1.2 ± 0.1c
MS + 0.5 mg l^{-1} NAA + 0.1 mg l^{-1} BA + 500 mg l^{-1} CH	150	2	1.1 ± 0.1c	54	36.4 ± 3.5bc
SH + 0.5 mg l^{-1} NAA + 0.1 mg l^{-1} BA + 500 mg l^{-1} CH	150	1	0.9 ± 0.1c	128	85.0 ± 4.7a

[a] Data followed by different letters differ siginicantly within columns

number per gram of callus were observed to be different with the various carbon sources. An average of 8.7 somatic embryos per gram of callus and a regeneration frequency of 92.1% were observed in MS medium with 0.5 mg l^{-1} 2,4-D, 0.1 mg l^{-1} BA, 500 mg l^{-1} lactoalbumin hydrolysate, and 30 g l^{-1} glucose. An average of 12.2 adventitious buds per gram of callus and an induction frequency of 96.5% were observed in SH medium with 0.5 mg l^{-1} NAA, 0.1 mg l^{-1} BA, 500 mg l^{-1} casein hydrolysate, and 30 g l^{-1} fructose (Table 2). Of the somatic embryos 95% were converted into regenerated plantlets on a growth hormone-free MS medium with 30 g l^{-1} glucose, and 100% of the adventitious shoots rooted to form regenerated plantlets on SH medium containing 0.1 mg l^{-1} NAA and 30 g l^{-1} fructose. The induction frequency of somatic embryos was below 84%, and the mean number of somatic embryos per gram of callus was below 5 on MS medium (used for somatic embryo production) with either mannose, fructose, or sorbose as the carbon source. The induction frequency of shoots was below 85% and the mean number of shoots per gram of callus was below 7 on SH medium (used for adventitious bud production) with either glucose, mannose, or sorbose as the carbon source.

Regeneration of flowering plantlets

Regenerated plantlets derived from somatic embryogenesis and organogenesis were transferred to MS medium containing various concentrations of GA_3 (0.5–8 mg l^{-1}). The highest frequency (5.7%) of flowering plantlets from adventitious bud organogenesis (PAB) was obtained on the MS medium with 2 mg l^{-1} GA_3 (Fig. 2D), and the highest frequency (1.4%) of flowering plantlets from somatic embryogenesis (PSE) was observed on MS medium containing 4 mg l^{-1} GA_3 (Fig. 3). The frequency of flowering plantlets was below 0.5% when regenerated plantlets from adventitious bud organogenesis were cultured on MS medium with 0.5, 1, 4, or 8 mg l^{-1} GA_3 and when regenerated plantlets from somatic embryogenesis were cultured on MS medium with 0.5, 1, 2, or 8 mg l^{-1} GA_3 (Fig. 3). After

Table 2 Effect of carbohydrate sources on adventitious bud and somatic embryo induction in *Panax ginseng*. Each treatment consisted of about 30 pieces of calli, an average of 1.1 gram per callus tissue. Tests were replicated four times. Values represent the mean ± SD

Carbohydrates		Somatic embryo regeneration			Adventitious bud regeneration			
	Number of calli tested	Number of calli forming somatic embryos	Percentage of calli producing somatic embryos[a]	Mean no. of somatic embryos per gram of callus[a]	Number of calli tested	Number of calli forming adventitious buds	Percentage of calli producing adventitious buds[a]	Mean Number of adventitious buds per gram of callus[a]
Glucose	120	110	92.1 ± 5.0a	8.7 ± 1.5a	120	96	80.6 ± 3.7b	5.3 ± 1.6b
Mannose	120	100	83.2 ± 4.7b	4.8 ± 1.3b	120	97	81.3 ± 4.2b	5.7 ± 1.2b
Fructose	120	97	81.7 ± 5.2b	4.2 ± 1.1b	120	115	96.5 ± 3.1a	12.2 ± 2.1a
Sorbose	120	96	80.1 ± 4.2b	3.8 ± 0.9b	120	101	84.3 ± 3.5b	6.3 ± 1.6b

[a] Data followed by different letters differ siginicantly within columns

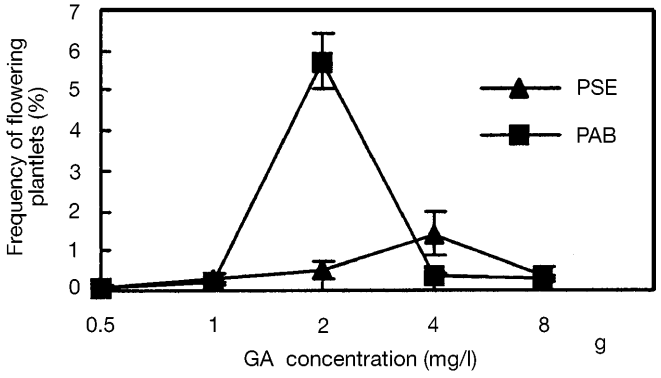

Fig 3 Effects of GA_3 concentrations on the in vitro flowering frequency of regenerated plantlets. *PSE* Plantlet from somatic embryogenesis, *PAB* plantlet from adventitious bud. *Bars*: Standard deviation of the mean

the flowering plantlets were transferred to hormone-free MS medium, the growth of plantlets improved.

Soil establishment

After acclimatization was finished, 101 plantlets derived from somatic embryos and 87 plantlets from adventitious buds were transferred to a greenhouse maintained at 25 °C. Ninety-five percent of the regenerated plantlets from somatic embryogenesis and all of the rooted plantlets from shoot organogenesis were successfully established in soil, and all regenerated plants derived from somatic embryogenesis and organogenesis produced morphologically and physiologically normal seeds. Twelve flowering plantlets from somatic embryogenesis and 17 flowering plantlets from shoot organogenesis were tested for seed production under in vitro conditions. All flowering, regenerated plantlets derived from somatic embryogenesis and organogenesis produced physiologically normal seeds under the conditions of in vitro culture, however, most of the plants (21 of 29) failed to survive in the field.

An efficient protocol for plantlet regeneration from callus of *Panax ginseng* is described in this report. A prerequisite in establishing this procedure is the use of mature zygotic embryos suitable for the induction of morphogenic callus. Another important factor in this high-frequency somatic embryogenesis and shoot organogenesis system is the use of different carbohydrates as sugar sources for the differentiation culture. The use of glucose for differentiation culture of callus resulted in a comparably high induction frequency of somatic embryo regeneration and mean number of somatic embryos per gram of callus. High-frequency shoot organogenesis was induced from morphogenic callus on differentiation medium with fructose as the sugar source. These results suggest that the choice of carbon source for the differentiation medium is one of the most important aspects in the regeneration pathway of callus derived from mature zygotic embryos of *Panax ginseng*, equal in importance to the choice of auxin for the induction medium. Glucose and fructose probably play an important role in the morphogenesis process of callus, not only as carbon source, but also as a factor regulating the differentiation pathway of totipotent cells. A similar finding was observed by Mukherjee et al. (1991) in *Solanum melongena* L. and Marino et al. (1993) in apricot.

In vitro flowering of regenerated plantlets from somatic embryos of ginseng has been reported (Chang et al. 1980a; Lee et al. 1990). Here, we demonstrated the potential of regenerated plantlets from mature zygotic embryo callus of ginseng to undergo in vitro flowering. The flowering plantlets described in the present study may have a practical value in wide hybridization and in vitro fertilization studies, especially in studying the mechanism of fertilization of ginseng under experimental conditions because in vitro flowering of regenerated plantlets from somatic embryogenesis and organogenesis can be induced at any time of the year. In vitro flowering was induced from regenerated plantlets cultured on MS medium supplemented with GA_3. The use of 2 mg l^{-1} GA_3 for regenerated plantlets from organogenesis resulted in a comparably high frequency of in vitro flowering, and the highest frequency of flowering plantlets from somatic embryogenesis was obtained on MS medium containing 4 mg l^{-1} GA_3. These results suggest that GA_3 concentration is one of the important factors regulating in vitro flowering of regenerated plantlets from somatic embryogenesis and organogenesis in ginseng. GA_3 probably plays an important role in the flowering process not only as a plant growth regulator but also as a factor regulating the floral organ formation of regenerated plantlets derived from somatic embryogenesis and organogenesis. However, the mechanism of in vitro flowering of regeneration plantlets of ginseng deserves a thorough biological analysis going far beyond the present study.

Acknowledgement This work was supported by the China Postdoctoral Science Foundation (97–11).

References

Butenko RF, Popov AS, Volkova LA, Chernyak ND, Nossov AM (1984) Recovery of cell cultures and their biosynthetic capacity after storage of *Dioscorea deltoidea* and *Panax ginseng* cells in liquid nitrogen. Plant Sci Lett 33:285–292

Chang WC, Hsing YI (1980a) In vitro flowering of embryoids derived from mature root callus of ginseng (*Panax ginseng*). Nature 284:341–342

Chang WC, Hsing YI (1980b) Plant regeneration through somatic embryogenesis in root derived callus of ginseng. Theor Appl Genet 57:133–135

Choi YE, Yang DC, Kim HS, Choi KT (1997) Distribution and changes of reserve materials in cotyledon cells of ginseng related to direct somatic embryogenesis and germination. Plant Cell Rep 16:841–846

Choi YE, Yang DC, Park JC, Soh WY, Choi KT (1998) Regenerative ability of somatic single and multiple embryos from cotyledons of Korean ginseng on hormone-free medium. Plant Cell Rep 17:544–551

Fetlt-Neto AG, Dicosmo F, Reynolds WF, Sakata K (1992) Cell culture of *Taxu*s as a source of the antineoplastic drug taxol and related taxanes. Bio/Technology 10:1572–1575

Furuya T, Yoshikawa T, Orihara Y, Oda H (1983) Saponin production in cell suspension cultures of *Panax ginseng*. Plant Med 48:83–87

Gary AT, Brent HM (1986) Establishing a micropropagation system for American ginseng (*Panax quinquefolium*). HortScience 21:232–236

Jhang JJ, Staba EJ, Kin JY (1974) American and Korean ginseng tissue cultures: growth, chemical analysis, and plantlet production. In Vitro 9:253–259

Lee SH, Liu EJ, Yang SG, Lee YH, Lee KW (1990) In vitro flowering of plantlets regenerated from zygotic embryo-derived somatic embryos of ginseng. HortScience 25:1652–1654

Mannonen L, Toivonen L, Kauppinen V (1990) Effects of long term preservation on growth and productivity of *Panax ginseng* and catharanthus roseus cell cultures. Plant Cell Rep 9:173–177

Marino G, Bertazza G, Magnanini E, Alta AD (1993) Comparative effects of sorbitol and sucrose as main carbon energy sources in micropropagation of apricot. Plant Cell Tissue Organ Cult 34:235–244

Mukherjee SK, Rathinasabapathi B, Gupta N (1991) Low sugar and osmotic requirements for shoot regeneration from leaf pieces of *Solanum melongena* L. Plant Cell Tissue Organ Cult 25:13–16

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

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

Shoyama Y, Kamura K, Nishoka I (1988) Somatic embryogenesis and clonal multiplication of *Panax ginseng*. Plant Med 54:155–156

Tae CK, Kim MW, Shin HS (1982) Root and shoot formation from callus and leaflet cultures of ginseng (*Panax ginseng* C. A. Meyer). In: Fugiwara A (ed) Proc 5th Int Cong Plant Tissue Cell Cult. Marizen Co Ltd, Tokyo, pp 171–172

Yoshikava T, Furuya T (1987) Saponin production by cultures of *Panax ginseng* transformed with *Agrobacterium rhizogenes*. Plant Cell Rep 6:449–453