J. Plant Biotechnology(2003) Vol. 5(4). pp. 221~224

Journal of Plant Biotechnology

High Frequency Adventitious Shoot Formation and Plant Regeneration in Leaf Explant Cultures of *Ixeris sonchifolia* Hance, a Newly Proposed Model Plant for Organogenesis

Sung Ran Min[1], Young Hoe Kim[1], Won Joong Jeong[1], Su Kyung Han[1], Dong Su In[2], Jang R. Liu[1]*

[1]Laboratory of Plant Cell Biotechnology, Korea Research Institute of Bioscience and Biotechnology (KRIBB), P.O. Box 115, Daejeon 305-600, Korea; [2]Eugentech Inc., P.O. Box 115, Daejeon 305-600, Korea

Abstract

Leaf explants of *Ixeris sonchifolia* produced adventitious shoots at a frequency of 100% when cultured on MS medium supplemented with combinations of various concentrations of 6-benzyladenine (BA) (0.44, 4.44, or 8.87 μM) and 0.54 μM NAA, or MS medium supplemented with 22.19 μM BA and 2.69 μM α-naphthaleneacetic acid (NAA) after four weeks of culture. Each explants (approximately 3 × 6 mm) produced greater than 70 shoots at a combination of 0.44 μM BA and 0.54 μM NAA. Leaf explants produced shoots at a frequency of greater than 80% even at as low as 0.13 μM BA as the sole growth regulator. Upon transfer to one-third strength MS with 0.54 μM NAA, excised adventitious shoots were rooted at a frequency of 100%. Regenerated plantlets were transplanted to potting soil and grown to maturity in a greenhouse. The competence of *I. sonchifolia* for plant regeneration via organogenesis appears to be greater than the competence of tobacco, currently the best model plant for organogenesis.

Key words: Compositae, model plant, plant regeneration

Abbreviations: BA - 6-benzyladenine; *MS* - Murashige and Skoog; NAA - α-naphthaleneacetic acid

Introduction

Ixeris sonchifolia Hance is an herbaceous medicinal plant belonging to the Compositae. The whole herb is used as an anti-inflamatory and hemostatic remedy in herbal medicine. The two new sesquiterpene lactones 8-desoxyartelin and ixerin Z, and the known 9-hydroxyzaluzalin C were isolated from the whole plant (Ma et al. 1998). However, as far as we know, this species has not been the subject of a prior plant tissue culture study. In this study, we have demonstrated that *I. sonchifolia* has a great competence for plant regeneration via organogenesis, probably even greater than the competence of tobacco that is the best-known model plant for organogenesis. The plant regeneration system of *I. sonchifolia* can be useful for investigating the determination event of cells to undergo development into shoots at the molecular level.

* Corresponding author, E-mail: jrliu@kribb.re.kr
Received Jul. 3, 2003; accepted Oct. 28, 2003

Materials and Methods

Plant materials

Open-pollinated seeds of *Ixeris sonchifolia* Hance purchased from Jeil Seed Co. (Korea) were surface-sterilized in a 1% (v/v) sodium hypochlorite solution for 15 min, and then rinsed three times with sterile distilled water. After surface-sterilization, seeds were placed onto Murashige and Skoog's (MS) (1962) basal medium contained in 250 mL wide-mouth jars for germination. Leaf explants (approximately 3 × 6 mm) were excised from four-week-old plants maintained in 250 mL wide-mouth jars.

Culture medium and culture conditions

The basal medium used for the induction of adventitious shoots from explants consisted of Murashige and Skoog (MS) (1962) inorganic salts, 100 mg l^{-1} myo-inositol, 0.4 mg l^{-1} thi-

amine · HCl, 3% (w/v) sucrose, and 4 g l^{-1} Phytagel (MS medium; Shoot inducing medium). MS medium in which the inorganic salts were reduced to one-third strength (rooting medium) was used for rooting of adventitious shoots excised from explants. The pH of all media was adjusted to 5.8 before autoclaving at 121°C for 15 min. Twenty-five ml of shoot inducing medium was dispensed into each plastic Petri dish (87 × 15 mm) and 80 mL of rooting medium was dispensed into each 108 mm high Phytacon vessel (Sigma). Unless mentioned otherwise, all cultures were maintained under light (approximately 3 Wm^{-2} from cool-white fluorescent lamps with 16-h photoperiods) at 25°C .

Induction of adventitious shoots

Leaf explants were placed onto MS medium supplemented with combinations of various concentrations of BA (0, 0.44, 2.22, 4.44, or 8.87 μM) and various concentrations of NAA (0, 0.54, 2.69, or 5.37 μM) to induce adventitious shoots. Leaf explants were also placed onto MS medium supplemented with combinations of lower concentrations of BA (0, 0.04, 0.13, 0.44, or 1.33) and NAA (0, 0.05, 0.16, or 0.54 μM). Each treatment consisted of 10 explants per dish with three replicates. After four weeks of culture, the numbers of explants producing adventitious shoots and adventitious shoots produced per explant were determined.

Plant regeneration

Adventitious shoots formed on leaf explants were excised and transferred to rooting medium supplemented with 0, 0.005, 0.054, or 0.54 μM NAA. Each treatment consisted of three explants per vessel with three replicates. The number of shoots producing roots was determined after four weeks of culture. Regenerated plantlets were subjected to acclimation, transplanted to potting soil (vermiculite:peatmoss = 1:1), then maintained in a growth chamber (27°C day/22°C night, 70 μmol $m^{-2}s^{-1}$ from cool-white fluorescent lamps with 16-h photoperiods) before transfer to a greenhouse.

Chromosome analysis

Root tips from two seedlings and five randomly selected plantlets regenerated from leaf explants were treated with a saturated 1-bromonaphthalene solution for 5 to 6 h at 25°C for chromosome counts. After fixation in an ethanol:acetic acid (3:1, v:v) solution at 4°C overnight, root tips were kept in 95% ethanol at 4°C overnight. They were subsequently hydrolyzed with 1 N HCl for 30 s at 60°C, and then stained with Feulgen solution.

Results and Discussion

The cut edges of leaf explants cultured on medium with BA and NAA began to produce numerous adventitious buds without an intervening callus phase after 10 days of culture (Figure 1A). As culture proceeded, adventitious buds were elongated to shoots (Figure 1B). The frequency of adventitious shoot formation was enhanced at a wide range of the concentration of BA in combination with low concentrations of NAA.

The greatest frequency (100%) of adventitious shoot formation on leaf explants was obtained with combinations of BA

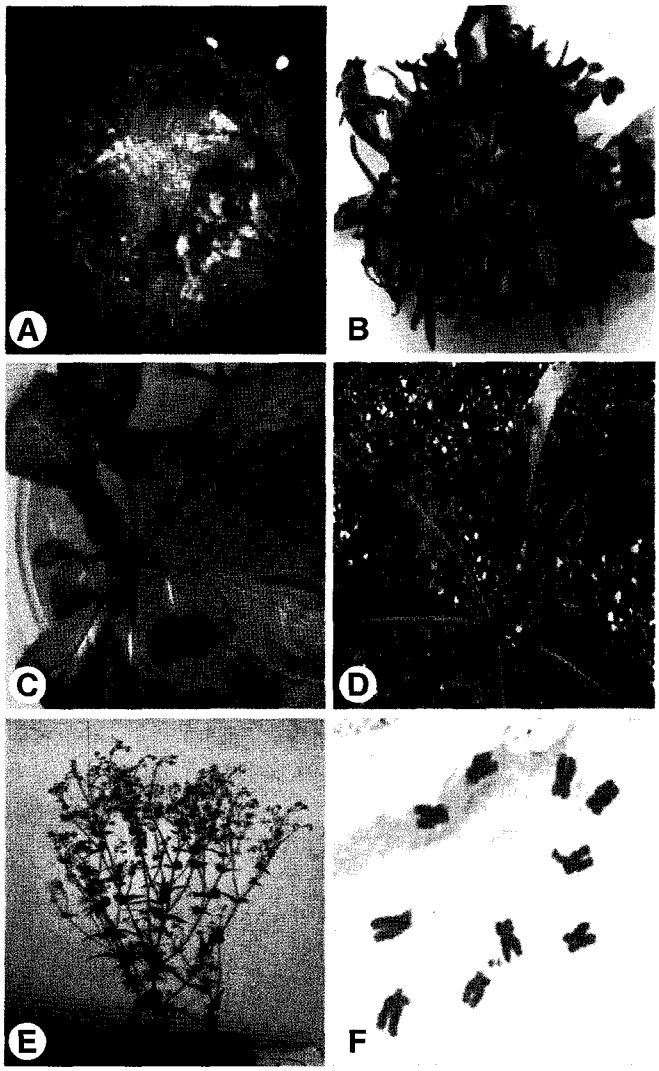

Figure 1. Adventitious shoot formation and plant regeneration in leaf explant cultures of *I. sonchifolia* Hance. A: Adventitious shoots formed on leaf explant (bar = 200 μM); B: Elongated adventitious shoots on leaf explant (bar = 100 μM); C: Rooted adventitious shoot (bar = 1 cm); D: Regenerated plantlet transplanted to potting soil (bar = 1 cm); E: Metaphase chromosomes of a plantlet from "C" (2n = 2x = 10) (bar = 10 μM).

(0.44, 4.44, or 8.87 μM) and 0.54 μM NAA, or with a combination of 22.19 μM BA and 2.69 μM NAA after four weeks of culture (Figure 2A). At combinations of BA and NAA for a great frequency of adventitious shoot formation, leaf explants produced a great number of adventitious shoots. For instance, at 0.44 μM BA and 0.54 μM NAA, each leaf explant produced greater than 70 shoots on average (Figure 1B). The frequency of callus formation on leaf explants increased with an increasing concentration of NAA (data not shown). Calluses formed on leaf explants were not competent to produce any organized structures. The elongation of adventitious buds was suppressed as the concentration of BA increased (data not shown). Leaf explants produced shoots at a frequency of greater than 80% even at as low as 0.13 μM BA as the sole growth regulator (Figure 2B). An increased concentration of NAA in combination with greater than 0.13 μM BA (Figure 2A)

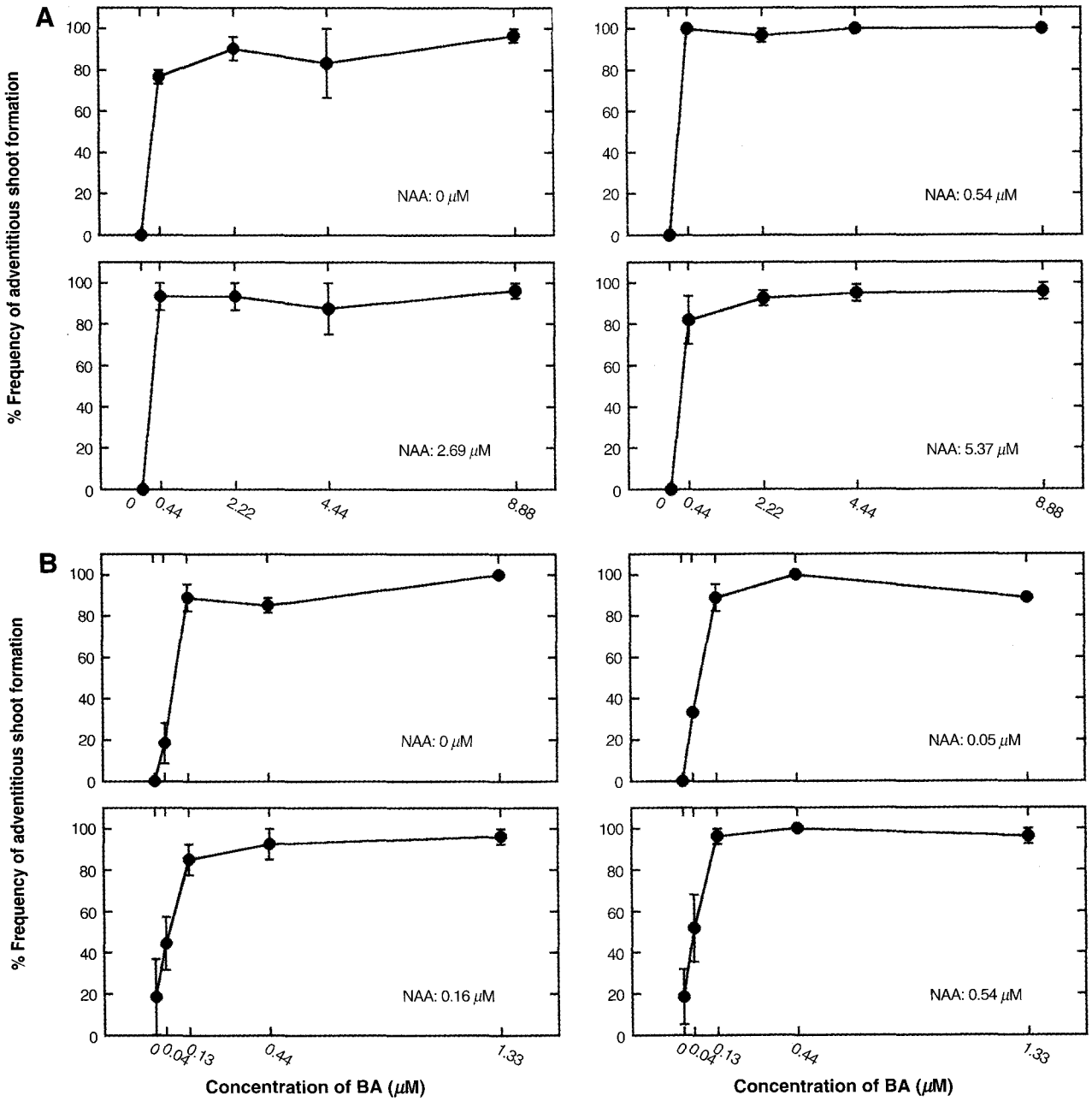

Figure 2. Effects of combinations of various concentrations of BA and NAA supplemented to MS medium on the frequency of adventitious shoot formation in leaf explant cultures of *I. sonchifolia*. A: Combinations of BA (0 to 8.88 μM) and NAA (0 to 5.37 μM); B: Combinations of BA (0 to 1.33 μM) and NAA (0 to 0.54 μM). Data were collected after four weeks of culture. Each point represents the mean of three replicates. Vertical bars indicate S.D.

or 0.44 μM BA (Figure 2B) did not significantly enhance the frequency of adventitious shoot formation. In a preliminary experiment, the frequency of adventitious shoot formation and the number of shoots formed per explant were compared by placing leaf explants both adaxial side up and down onto shoot-inducing medium. No significant differences were observed (data not shown).

Leaf explants produced roots without an intervening callus phase when cultured on medium with NAA alone. Excised adventitious shoots began to root after six days of transfer to rooting medium. The frequency of rooting tended to increase with an increasing concentration of NAA (Figure 3). Adventitious shoots were rooted at the greatest frequency (100%) at 0.54 μM NAA. Greater than 90% of regenerated plantlets were successfully transplanted to potting soil (Figure 1D) after acclimation and were grown to maturity (Figure 1E). All seedlings and plantlets derived from leaf explants exhibited a chromosome number of 2n = 2x = 10 (Figure 1F), indicating that *in vitro* organogenesis of this species does not cause noteworthy chromosomal abnormalities.

Species that belong to the Compositae are known to have a high competence for plant regeneration via organogenesis. Lettuce (Jung et al. 1999) and *Artemisia annua* (Vergauwe et al. 1996) are the cases. We demonstrated that *I. sonchifolia* also has a great competence in plant regeneration via organogenesis. As far as we know, no species of higher plants has been reported to have a competence comparable to *I. sonchifolia*. Evidently, *I. sonchifolia* has a competence for plant regeneration via organogenesis than tobacco, the best-known model plant for organogenesis. In addition, a few cells are usually committed to produce adventitious shoots in tissue culture, resulting in the formation of a limited number of shoots per explant. However, in this study, a 3×6 mm-leaf explant of *I. sonchifolia* produced greater than 70 shoots, indicating that a great number of cells of the explant were determined to produce shoots. Therefore, the system for high frequency adventitious shoot formation in leaf explant cultures of *I. sonchifolia* established in this study will be useful for investigating the determination event of cells to undergo development into shoots at the molecular level.

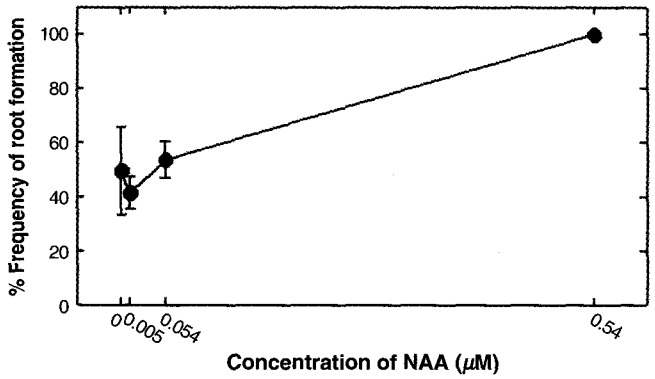

Figure 3. Effect of NAA supplemented to one-third strength MS medium on the frequency of root formation in excised adventitious shoot cultures of *I. sonchifolia*. Data were collected after four weeks of culture. Each point represents the mean of three replicates. Vertical bars indicate S.D.

Acknowledgments

This work was supported by a grant (No. CGM0400111) to JRL from the Crop Functional Genomics Center of the 21st Century Frontier Research Program, a grant (BDM0100211) to JRL from the Genetic Resources and Information Network Center of the Strategic National R&D Program funded by the Korean Ministry of Science and Technology, a grant (No. ABM0030211) to JRL from the BioGreen 21 Program funded by the Korean Rural Development Administration, and a grant to JRL from the Korea Science and Engineering Foundation through the Plant Metabolism Research Center (Kyung Hee University).

References

Jung M, Woo JW, Jeong WJ, Liu JR (1999) High frequency organogenesis and plant regeneration in tissue cultures of lettuce seedling explants. Korean J. Plant Tiss. Cult. 26: 219-222

Ma JY, Wang ZT, Xu LS, Xu GJ, Kadota S, Namba T (1998) Sesquiterpene lactones from *Ixeris sonchifolia*. Phytochemistry 48: 201-203

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

Vergauwe A, Cammaert R, Vandenberghe D, Genetello C, Inze D, Van Montagu M, Van Den Eeckhout E (1996) *Agrobacterium tumefaciens*-mediated transformation of *Artemisia annua* L. plants and the regeneration of transgenic plants. Plant Cell Rep. 15: 929-933