Shoot organogenesis of *Echinacea angustifolia* DC as influenced by polyamines

Soo Cheon Chae

Department of Horticultural Science, College of Industrial Sciences, Kongju National University, Daehak-ro, Yesan-gun, Chungnam, 340-702, Korea
scchae@kongju.ac.kr

Abstract: Polyamines, low-molecular-weight polycationic molecules are present in all plant cells that play a vital role in cell division and differentiation. Therefore, an attempt was undertaken to evaluate the effects of polyamines i.e., putrescine, spermidine and spermine on shoot organogenesis of *Echinacea angustifolia* DC. Leaf explants were cultured in initial shoot-regeneration media with four different concentrations (10, 30, 70, and 100 mg/L) of putrescine, spermidine and spermine. All polyamines showed better regeneration as well shoot growth except a few than that of control. With increasing the concentration of putrescine, spermidine and spermine, increased regeneration capacity and shoot growth upto the concentration 70 mg/L and then started to decrease in all the cases. Among the polyamines, putrescine at 70 mg/L performed the best achieving for both regeneration and shoot growth. Putrescine at 70 mg/L showed 20% more regeneration and produced the longest shoot length achieving 1.5 times longer shoot than that of control. Our study suggests that polyamines especially putrescine may be utilized efficiently in micropropagation for regeneration of *E. angustifolia*.
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1. Introduction

The species *Echinacea*, belongs to the family Asteraceae, is considered the most popular medicinal plants in both North America and Europe (Pepping, 1999; Percival, 2000). *E. angustifolia* DC has been used for the treatment of different ailments such as venomous bites and stings, to infectious or inflammatory conditions like cold and flu, toothaches, cough, sore eyes, and rheumatism (Kindscher, 1989; Barnes et al., 2005).

Polyamines (particularly putrescine, spermidine and spermine) are present in both eukaryotic and prokaryotic cells (Galston, 1983), play a vital role in cell division and differentiation (Yamada et al., 1986; Basu et al., 1989) and also help in the regulation of plant growth and development, and help to protect from abiotic and biotic stress (Tisi et al., 2011). Besides, polyamines have been engaged in several morphogenic processes i.e., somatic embryogenesis in *Dactylis glomerata* L. (Li and Burritt, 2003) and in *Daucus carota* cells (Bastola and Minocha, 1995), t regeneration of shoot from the leaves of Passiflora (Desai and Mehta, 1985), and cotyledonary explants of *Brassica campestris* (Chi et al., 1994) and *Cucumis melo* (Tian et al., 1994).

Here, we report the establishment of an improved method for plant regeneration applying polyamines on shoot organogenesis of *E. angustifolia* from stem explants.

2. Material and Methods

2.1. Plant materials and culture media

Seeds of *E. angustifolia* were purchased from Otto Richter and Sons Limited (Goodwood, Canada) and stored at 4°C. The seeds were surface-sterilized with 70% (v/v) ethanol for 30 s and 2% (v/v) sodium hypochlorite solution for 10 min and then rinsed 3 times in sterilized water. Ten seeds were placed in each petri dish (100 × 15 mm) containing 25 mL of agar-solidified culture medium. The basal medium consisted of salts and Murashige and Skoog (MS) vitamins (Murashige and Skoog, 1962) and 0.7% (w/v) agar. The medium was adjusted to pH 5.8 before adding agar and then sterilized by autoclaving at 121°C for 20 min. The seeds were germinated in a growth chamber at 25°C under standard, cool-white fluorescent tubes with a flux rate of 35 $\mu mol \cdot s^{-1} \cdot m^{-2}$ and a 16-h photoperiod.

2.2. In vitro shoot organogenesis

Stems of *E. angustifolia* were obtained from plants grown in vitro, and they were cut aseptically at the ends into sections of approximately 0.7 cm. Explants were placed in petri dishes (100 × 25 mm) containing approximately 25 mL of basal medium supplemented with 30 g/L of sucrose, 7 g/L of Phytagar, and 2 mg/L of 6-benzylaminopurine (BAP). Seven explants were cultured in each petri dish. The pH of the medium was adjusted according to that required for the germination medium, and the medium was sterilized using the same procedure mentioned

above. Leaf explants were cultured in initial shoot-regeneration media with four different concentrations (10, 30, 70, and 100 mg/L) of putrescine, spermidine and spermine Cultures were maintained at 25 ± 1°C in a growth chamber with a 16-h photoperiod under standard, cool-white fluorescent tubes (35 µmol·s^{-1}·m^{-2}) for 6 weeks.

2.3. Rooting of regenerated shoots

Regenerated shoots (~1.5 cm in length) were transferred to 1/2 MS medium in a Magenta box. The medium was solidified using 8 g/L of plant agar, and 50 mL of the medium was placed in each culture vessel. Four shoots were cultured in each vessel. Regenerated shoots were incubated at 25 ± 1°C in a growth chamber with a 16-h photoperiod under standard, cool-white fluorescent tubes (35 µmol·s^{-1}·m^{-2}) for 5 weeks. After 5 weeks, the rooted plants were washed with water to remove the agar, transferred to pots containing autoclaved vermiculite, and covered with polyethylene bags for 1 week to maintain high humidity. The plants were then transferred to the soil and maintained in a growth chamber with a 16-h photoperiod and a day/night temperature of 18/20°C for 2 weeks. These hardened plants were then transferred to the greenhouse.

2.4. Statistical analysis

Data for the 50 tested leaf explants are expressed as mean ± standard deviation.

3. Results and Discussion

For in vitro shoot organogenesis of *E. angustifolia*, Kim et al., 2010, reported a protocol, which was not efficient for shoot regeneration. In order to get better shoot regeneration, we used polyamines i.e., putrescine, spermidine and spermine at different concentrations to find out the efficiency of shoot organogenesis in *E. angustifolia.*

From the results of our study, it was found that polyamines acted well for regeneration of *E. angustifolia* (Table 1). Shoot regeneration as well as shoot growth increased with increasing the concentration of putrescine, spermidine and spermine upto 70 mg/L and then started to decline. Among the treatments, putrescine performed the best for higher regeneration and better shoot growth of *E. angustifolia*. It was observed that putrescine at 70 mg/L acted well and helps to produce 20% more shoot regeneration and 1.5 times longer shoot than that of control. This concentration of putrescine (70 mg/L) showed 26% and 21% higher regeneration capacity than that of spermidine and spermine 70 mg/L, respectively and this (putrescine at 70 mg/L) also produced 17% and 21% longer shoot than that of spermidine and spermine 70 mg/L, respectively.

Table 1. Effect of polyamines on shoot regeneration and growth from *Echinacea angustifolia* explants after 6 weeks of culture on regeneration medium (Murashige and Skoog medium with 2.0 mg/L BAP).

Polyamines (mg/l)	Regeneration %*	No. of shoots per explant*	Shoot length* (mm)
Control	68	3.3 ± 0.3	13.5 ± 1.5
Putrescine 10	70	3.6 ± 0.2	15.8 ± 1.3
Putrescine 30	78	4.2 ± 0.3	17.1 ± 1.3
Putrescine 70	85	5.2 ± 0.4	20.3 ± 2.3
Putrescine 100	83	4.9 ± 0.5	19.6 ± 2.0
Spermidine 10	69	3.4 ± 0.3	13.9 ± 1.3
Spermidine 30	73	3.6 ± 0.3	14.3 ± 1.5
Spermidine 70	63	4.3 ± 0.4	17.4 ± 3.0
Spermidine 100	52	3.8 ± 0.3	17.5 ± 1.8
Spermine 10	71	3.1 ± 0.3	13.9 ± 1.4
Spermine 30	70	3.5 ± 0.4	13.8 ± 1.4
Spermine 70	67	4.1 ± 0.4	15.6 ± 1.6
Spermine 100	62	3.8 ± 0.3	15.7 ± 1.8

Regeneration frequency (%) = (No. of explants with shoot differentiation/all explants) × 100
* Values represent the mean ± standard deviation of 50 shoots

Results revealed that the addition of polyamines were effective for regeneration shoot growth of *E. angustifolia* (Table 1). The best concentration of polyamine was putrescine at 70 mg/L for regeneration and shoot growth. Similar results were reported in *M. charantia*, where putrescine performed better for regeneration and increase in fresh weights of organogenic calli of *Momordica charantia* (Paul et al., 2009; Thiruvengadam et al., 2012). Some other reports also suggested that PAs are important for cell growth, somatic embryogenesis, and shoot morphogenesis (Kaur-Sawhney et al. 1986; Galston and Kaur-Sawhney, 1990; Chi et al. 1994; Walden et al., 1997). Earlier, it was reported that spermidine is essential for shoot multiplication in cucumber (Vasudevan et al., 2008) and sugarcane (Uma shankar et al., 2011). However, here in this study putrescine performed better than spermidine for shoot regeneration and shoot growth of *E. angustifolia*.

In contrast, spermine performed better for adventitious shoots formation *in vitro* from cotyledons of cucumber (Zhu and Chen, 2005) and *Brassica rapa* (Chi et al., 1994). In another study, it was reported that putrescine, spermidine, and spermine were involved in adventitious shoot formation through *in vitro* in cotyledons of melon (Tian et al., 1994). This evidence suggests that putrescine, spermidine, and spermine may play dissimilar roles in different explants which was supported by Zhu and Chen, (2005). Martin-Tanguy (2001) reported that spermidine and spermine play significant roles in adventitious shoot formation, which can be promoted by exogenous polyamines treatment. Polyamines interacted with phytohormones have been shown positive response in *in vitro* culture of plant species (Altman, 1982; Scholten, 1998; Tonon et al., 2001).

An efficient protocol is essential to the practice of genetic engineering for plant improvement. In this study, we developed an improved method for regeneration of *E. angustifolia* by using polyamines that might be facilitated future research on the genetic enhancement of *E. angustifolia*.

Corresponding Authors:
Dr. Soo Cheon Chae
Department of Horticultural Science, College of Industrial Sciences, Kongju National University, Daehak-ro, Yesan-gun, Chungnam, 340-702, Korea
E-mail: scchae@kongju.ac.kr

References

1. Pepping J. Echinacea. Am J Health Syst Pharm 1999;56:121-122.
2. Percival SS. Use of echinacea in medicine. Biochem Pharmacol 2000;60:155-158.
3. Kindscher K. Ethnobotany of purple coneflower (*Echinacea angustifolia*, Asteraceae) and other *Echinacea* species. Econ Bot 1989;43:4498–507.
4. Barnes J, Anderson LA, Gibbons S, Phillipson JD. Echinacea species (*Echinacea angustifolia* (DC.) Hell., *Echinacea pallida* (Nutt.) Nutt., *Echinacea purpurea* (L.) Moench): a review of their chemistry, pharmacology and clinical properties. J Pharm Pharmacol 2005;57: 929-954.
5. Galston AW. Polyamines as modulators of plant development. Bioscience 1983; 33: 382-388.
6. Yamada Y, Kumpaisal R, Hashimoto T, Sugimoto Y (1986). Growth and aspartate kinase activity in wheat cell suspension culture: Effects of lysine analogs and aspartate-derived amino acids. Plant Cell Physiol 1986; 27: 607-617.
7. Basu A, Sethi U, Guha-Mukherjee S. Regulation of cell proliferation and morphogenesis by aminoacids in *Brassica* tissue cultures and its correlation with threonine deaminase. Plant Cell Rep 1989; 8: 333-335.
8. Tisi A, Angelini R, Cona A. Does polyamine catabolism influence root development and xylem differentiation under stress conditions? Plant Signal Behav 2011; 6:1844-1847.
9. Li ZL, Burritt DJ. Changes in endogenous polyamines during the formation of somatic embryos from isogenic lines of *Dactylis glomerata* L. with different regenerative capacities. Plant Growth Regul 2003; 40: 65-74.
10. Bastola DR, Minocha SC (1995). Increased putrescine biosynthesis through transfer of mouse ornithine cDNA in carrot promotes somatic embryogenesis. Plant Physiol 1995; 109: 63-71.
11. Desai HV, Mehta AR. Changes in polyamine levels during shoot formation, root formation and callus induction in cultured *Passiflora* leaf discs. J. Plant Physiol 1985;119: 45-53.
12. Chi GL, Lin WS, Lee JEE, Pua EC. Role of polyamines on de novo shoot morphogenesis from cotyledons of *Brassica campestris* spp. Pekinensis (Lour) Olsson *in vitro*. Plant Cell Rep 1994; 13: 323-329.
13. Tian CE, Li RG, Guan H. Relationship between polyamines andmorphogenesis in cotyledons of *Cucumis melo* L. cultured *in vitro*. Acta Bot. Sin 1994. 36: 219-222.
14. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 1962;15:473-497.
15. Kim JS, Lee SY, Eom SH, Park SU. Improved shoot organogenesis and plant regeneration of

16. *Echinacea angustifolia* DC. J Med Plants Res 2010;4:587-591.
17. Paul A, Mitter K, Raychaudhuri SS. Effect of polyamines on *in vitro* somatic embryogenesis in *Momordica charantia* L. Plant Cell Tiss Organ Cult 2009; 97: 303-311.
18. Thiruvengadam M, Chung IM, Chun SC. Influence of polyamines on *in vitro* organogenesis in bitter melon (*Momordica charantia* L.). J Med Plants Res 2012; 6(19): 3579-3585.
19. Kaur-Sawhney R, Tiburcio AF, Galston AW. Polyamine-mediated control of organogenesis in thin layer explants of tobacco. Plant Physiol 1986; 80: 37.
20. Galston AW, Kaur-Sawhney R. Polyamines in plant physiology. Plant Physiol 1990; 94: 406-410.
21. Walden R, Cordeiro A, Tiburcio AF. Polyamines: Small molecules triggering pathways in plant growth and development. Plant Physiol 1997; 113: 1009-1013.
22. Vasudevan A, Selvaraj N, Ganapathi A, Kasthurirengan S, Ramesh Anbazhagan V, Manickavasagam M, Choi CW. Leucine and spermidine enhance shoot differentiation in cucumber (*Cucumis sativus* L.). *In Vitro* Cell Dev Biol Plant 2008; 4: 300-306.
23. Uma shankar C, Ganapathy A, Manickavasagam M. Influence of polyamines on shoot regeneration of sugarcane (*Saccharum officinalis* L*)*. Egypt J Biol 2011; 13: 44-50.
24. Zhu C, Chen Z. Role of polyamines in adventitious shoot morphogenesis from cotyledons of cucumber *in vitro*. Plant Cell Tiss Organ Cult 2005; 81: 45-53.
25. Tian CE, Li RG, Guan H. Relationship between polyamines andmorphogenesis in cotyledons of *Cucumis melo* L. cultured *in vitro*. Acta Bot Sin 1994; 36: 219-222.
26. Martin-Tanguy J. Metabolism and function of polyamines in plants: Recent development (new approaches). Plant Growth Regul 2001; 34: 135-148.
27. Altman A (1982). Retardation of radish leaf senescence by polyamines. Physiol Plant 1982; 54: 189-193.
28. Scholten HJ. Effect of polyamines on the growth and development of some horticultural crops in micropropagation. Sci Hortic 1998; 77: 83-88.
29. Tonon G, Kevers C, Gaspar T. Changes in polyamines, auxins and peroxidase activity during *in vitro* rooting of *Fraxinus angustifolia* shoots: An auxin independent rooting model. Tree Physiol 2001; 2110: 655-663.

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