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Full Length Research Paper

Improved *invitro* plant regeneration and micro propagation of *Rehmannia glutinosa* L.

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The establishment of an efficient protocol for plant regeneration and micropropagation from leaf cultures of *Rehmannia glutinosa* L. is reported. The regenerated shoots obtained from leaf cultures on solid MS medium containing different concentrations of TDZ. The highest number of shoots per explant (2.1) and shoot growth (1.2 cm) was obtained on MS medium containing 1 mg/L TDZ. The addition of 0.1 mg/L NAA and 3g/L Gelrite in MS medium containing 1 mg/L TDZ substantially improved the shoot regeneration of *R. glutinosa.* The rooted plants were hardened and transferred to soil with a 73% survival rate. The continuous production of *R. glutinosa* regenerated plants could be used as a possible micropropagation system.

Key words: Micropropagation, plant regeneration, shoot organogenesis, *Rehmannia glutinosa* L.

INTRODUCTION

Rehmannia glutinosa L. belongs to a member of the Scrophulariaceae family and is an important oriental medicinal plant. Rehmanniae radix (the dried root of *R.glutinosa* L.) containing iridoids, saccharides, amino acids, inorganic substances, other trace elements, etc., is commonly used as a traditional Chinese medicine and has a very high medicinal value. *R. glutinosa* and its main active components have been shown to have a variety of beneficial effects and pharmacological actions on the blood system, immune system, endocrine system, cardio-vascular system, and the nervous system (Hasegawa et al., 1982; Bi et al., 2008; Liu et al., 2008; Zhang et al., 2008).

The propagation of *R. glutinosa* is by division of the tuberous roots or planting seed. Propagation from seed is difficult method because of poor seed viability, low propagation rate, and delaying root harvest. Therefore, this plant is propagated conventionally through the division of roots. Therefore, some studies have reported in vitro plant regeneration and micropropagation of *R. glutinosa* from the culture of several explants for multiple propagation (Matsumoto et al., 1986; Nishioka, 1988; Hatano et al., 1997; Jeong et al., 2002) . However, the plant regeneration efficiency was low and not reliable. An efficient method for the regeneration of intact plants from tissue culture is essential to establish a multiple micropro-pagation system and a genetic transformation protocol.

In this paper, we describe the development of a method for shoot organogenesis and plant regeneration from leaf explant cultures of *R. glutinosa*.

MATERIALS AND METHODS

Shoot organogenesis

Young leaves of *R. glutinosa* were taken from greenhouse grown plants. Explants were surface-sterilized by a 10 se immersion in 70% (v/v) ethanol and for 10 min in aqueous solution of 1% (v/v) sodium hypochlorite containing a few drops of Tween 20. After three washes in sterilised water, leaves were cut aseptically at the ends, into sections of approximately 7 X 7 mm^2 in size. Explants were placed on the medium in the Petri dish (100 x 25 mm). Petri dish contained approximately 25 mL of culture medium. Seven explants were cultured in each Petri dish. The basal medium consisted of salts and vitamins of Murashige and Skoog (MS) medium (1962) and solidified with 0.7% (w/v) Phytagar. The pH of medium was adjusted to 5.8 before adding Phytagar. The media were sterilised by autoclaving at 1.1 kg cm^{-2} (121 °C) for 20 min.

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Table 1. Effect of different concentrations of TDZ on the regeneration and growth of shoots from excised leaf of *Rehmannia glutinosa* L. after six weeks in culture.

TDZ (mg/L)	Regeneration frequency** (%)	Number of shoots per explant**	Shoot length[a] (cm)
0.0	--	--	--
0.5	43	1.3 ± 0.1	0.8 ± 0.1
1.0	52	2.1 ± 0.2	1.2 ± 0.1
2.0	34	1.5 ± 0.2	1.1 ± 0.1
4.0	--	--	--

-- No response

*Basal medium consisted of MS salts and vitamins, and 30 g/L sucrose, solidified with 7 g/L Phytagar.

**From 100 leaf explants tested.

[a]Values represent the mean ± standard deviation of 50 shoots.

For shoot regeneration from leaf explants, the MS medium was supplemented with 0, 0.1, 0.5, 1, 2, 4 mg/L TDZ (1- phenyl-3-(1,2,3-thiadiazol-5-yl) urea; thidiazuron). For improvement of shoot regeneration, the medium was optimized by testing the effect of different concentrations of auxins (0.1 and 0.5 mg/L IAA (indole-3-acetic acid), IBA (indole-3-butyric acid), and NAA (1-naphthalene-acetic acid)) and gelling agents (6, 7, 8, and 9 g/L Phytagar or 2, 3, 4, and 5 g/L Gelrite) on shoot formation and growth. Cultures were maintained at 25 ± 1°C in a growth chamber with a 16-h photoperiod under standard cool white fluorescent tubes (35 ∝mol s^{-1} m^{-2}) for 6 weeks.

Rooting of regenerated shoots

Regenerated shoots (around 1 cm long) were placed in MS medium. The medium was solidified with 3 g/L Gelrite and dispensed at 30 ml per Magenta box and four shoots were cultured in each box. Regenerated shoots were incubated at 25±1°C in a growth chamber with a 16-h photoperiod under standard cool white fluorescent tubes (35 ∝mol s^{-1} m^{-2}) for 8 weeks. After eight weeks, the rooted plants were washed with sterile water to remove Gelrite, transferred to pots containing autoclaved vermiculite, and covered with polyethylene bags for one week to maintain high humidity. The plants were then transferred to soil and maintained in a growth chamber with a 16-h photoperiod, and a night/day temperature of 18/20°C for 2 weeks. These hardened plants then transferred to the greenhouse.

RESULTS AND DISCUSSION

Establishment of shoot regeneration

A simple and effective protocol has been developed for the *in vitro* plant regeneration and micropropagation of *R. glutinosa*. We investigated the effect of cytokinins, auxins, and gelling agents on the efficiency of shoot organogenesis. Shoot development from wounded callus tissue that formed on excised leaf did not occur in the absence of exogenous TDZ. Regenerated shoots were obtained from leaf cultures on MS medium containing different concentrations of TDZ except the treatment of 0.1 and 4 mg/L TDZ (Table 1). The addition of 1 mg/L TDZ was optimal for the development and growth of shoots of *R. glutinosa*.

To study the effects of different auxins on the regeneration and growth of shoots from excised leaf culture of *R. glutinosa*, leaf explants were grown for 6 weeks in basal medium (MS salts and vitamins, 30 g/L sucrose, and 1 mg/L TDZ, solidified with 7 g/L Phytagar) supplemented with various concentrations of different auxins. Our results revealed that all tested auxin treatments in basal medium marginally increased the shoot regeneration and growth rates of *R. glutinosa* leaf culture (Table 2). Among the different auxins treatment, NAA performed the best both on shoot regeneration and growth rates. With the concentration of 0.1 mg/L NAA produced the highest number of shoot (3.8) and the shoot length (1.5 cm).

For investigating the effects of different concentration of gelling agents (Phyagar and Gelrite) on the shoot regeneration of *R. glutinosa*, leaf explants were grown for 6 weeks in basal medium (MS salts and vitamins, 30 g/L sucrose, and 1 mg/L TDZ with 0.1 mg/L NAA) supplemented with various concentrations of Phyagar and Gelrite (Table 3). Although these initial experiments were performed on medium solidified with Phytagar, shoot organogenesis was found to be more efficient when Gelrite was used as the gelling agent. The number of shoots produced per leaf explant was 15% higher, and the growth of shoots was 12% greater, on 3 g/L Gelrite compared to 6 g/L Phytagar. The superiority of Gelrite over agar for the purposes of shoot regeneration has also been reported for apple (Saito and Suzuki, 1999) and Bacopa monnieri (L.) Pennell (Shrivastava and Rajani, 1999). Our optimized shoot regeneration medium consisted of MS salts and vitamins, 30 g/L sucrose, 1 mg/L TDZ, 0.1 mg/L NAA and 3 g/L Gelrite.

In 1957, Skoog and Miller performed classic experiments demonstrating that shoot and root initiation in callus cultures of tobacco could be regulated by manipulation of the ratio of auxin and cytokinin present in the growth medium. Generally in organogenesis protocols, high cytokinin to auxin ratios induce shoots, high auxin to cytokinin ratios produce roots, and more equal concentrations of theses phytohormones are found to cause callus proliferation. Currently, organogenesis is the most widely used method of in vitro plant regeneration in trans-

Table 2. Effect of different concentrations of auxins on the regeneration and growth of shoots from excised leaf of *Rehmannia glutinosa* L. after six weeks in culture.

Auxin* (mg/L)		Regeneration frequency** (%)	Number of shoots per explant**	Shoot length[a] (cm)
Control	0.0	52	2.1 ± 0.2	1.2 ± 0.1
IAA	0.1	54	2.3 ± 0.2	1.4 ± 0.2
	0.5	57	2.3 ± 0.3	1.3 ± 0.1
IBA	0.1	59	2.7 ± 0.4	1.3 ± 0.2
	0.5	63	3.4 ± 0.3	1.4 ± 0.1
NAA	0.1	67	3.8 ± 0.4	1.5 ± 0.2
	0.5	65	3.5 ± 0.5	1.3 ± 0.1

-- No response

*Basal medium consisted of MS salts and vitamins, 30 g/L sucrose, and 1 mg/L TDZ, solidified with 7 g/L Phytagar.

**From 100 leaf explants tested.

[a]Values represent the mean ± standard deviation of 50 shoots.

Table 3. Effect of different concentrations of gelling agents on the regeneration and growth of shoots from excised leaf of *Rehmannia glutinosa* L. after six weeks in culture.

Gelling agent (g/L)		Regeneration frequency** (%)	Number of shoots per explant**	Shoot length[a] (cm)
Phytagar	6.0	70	4.0 ± 0.4	1.6 ± 0.2
	7.0	67	3.8 ± 0.4	1.5 ± 0.2
	8.0	81	3.6 ± 0.3	1.1 ± 0.1
	9.0	68	2.9 ± 0.3	0.9 ± 0.1
Gelrite	2.0	73	3.9 ± 0.4	1.4 ± 0.1
	3.0	82	4.7 ± 0.5	1.8 ± 0.2
	4.0	78	3.6 ± 0.4	1.7 ± 0.2
	5.0	71	3.2 ± 0.3	1.3 ± 0.1

-- No response

*Basal medium consisted of MS salts and vitamins, 30 g/L sucrose, and 1 mg/L TDZ with 0.1 mg/L NAA.

**From 100 leaf explants tested.

[a]Values represent the mean ± standard deviation of 50 shoots.

formation systems. Most Agrobacterium-mediated transformation systems use the leaf disk procedure, or plant regeneration based on direct or indirect organogenesis.

Plant micropropagation

The rooted plants were hardened and transferred to soil with a 73% survival rate where they grew normally and flowered within 3 months. The continuous production of *R. glutinosa* regenerated plants could be used as a possible micropropagation system. Every year the growers have to save some roots from total harvested roots of their plant for conventional method for mass propagation, but we can produce about 5 plantlets from in vitro one leaf explant cultures of *R. glutinosa* all the year round.

REFERENCES

Bi J, Wang XB, Chen L, Hao S, An LJ, Jiang B, Guo L (2008). Catalpol protects mesencephalic neurons against MPTP induced neurotoxicity via attenuation of mitochondrial dysfunction and MAO-B activity. Toxicol. *in vitro*. 22: 1883-1889.

Hasegawa T, Koike K, Takahashi S, Ariyoshi U (1982). Constituents of leaves and roots of Kailei Jio (*Rehmammia glutinosa* Libosch. Forma hueichingensis Hsiao). Shoyakugaku Zasshi. 36: 1-5.

Hatano M, Nakai R, Kawanishi F, Kedo K, Shoyama Y (1997). Genetic diagnosis of Rehmannia species micropropagated by tip tissue culture and an F_1 hybrid by RAPD analysis. Plant Breeding. 116: 589-591.

Jeong JH, Yu KW, Chakrabarty D, Kim SJ, Paek KY (2002). In vitro regeneration and plantlet formation from adventitious roots of *Rehmannia glutinosa* liboschits. Propag.ation of Ornam.ental Plants. 2: 19-23,

Liu HR, Tang XY, Dai DZ, Dai Y (2008). Ethanol extracts of Rehmannia complex (Di Huang) containing no Corni fructus improve early diabetic nephropathy by combining suppression on the ET-ROS axis with modulate hypoglycemic effect in rats. J. Ethnopharmacol. 118: 466-472.

Matsumoto M, Nagano M, Shoyama Y, Nishioka I (1986). New vegetative propagation method of *Rehmannia glutinosa*. Shoyakugaku Zasshi. 40: 193-197.

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

Nishioka I(1988). Clonal multiplication of medical plant by tissue culture. Shoyakugaku Zasshi. 42: 1-11.
Saito A, Suzuki M (1999). Plant regeneration from meristem-derived callus protoplasts of apple (Malus x domestica cv. 'Fuji'). Plant Cell Rep. 18: 549-553.
Shrivastava N, Rajani M (1999). Multiple shoot regeneration and tissue culture studies on Bacopa monnieri (L.) Pennell. Plant Cell Rep. 18: 919-923.
Skoog F, Miller CO (1957). Chemical regulation of growth and organ formation in plant tissues cultivated in vitro. Symp. Soc. Exp. Bio. 11: 118-130.
Zhang RX, Li MX, Jia ZP (2008). *Rehmannia glutinosa*: review of botany, chemistry and pharmacology. J. Ethnopharmacol. 117: 199-214.