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AN EFFICIENT *IN VITRO* PROPAGATION SYSTEM FOR PURPLE CONE-FLOWER (*ECHINACEA PURPUREA* L)

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ABSTRACT

An efficient *in vitro* propagation culture system was developed for purple coneflower. In the system, adventitious buds were first regenerated from leaf, petiole and root explants of aseptic seedlings on MS medium with 0.3 mg/l BA and 0.01 mg/l NAA, and then these adventitious buds were rooted and grown into more mature plantlets on MS media with 0.01 mg/l NAA for providing more explant materials for a subsequent adventitious bud regeneration culture. It was found that explants taken from plantlets of different maturity had different adventitious bud regeneration ability, with those from two and a half months old plantlets having the highest regeneration ability, and with root explants having the highest number of adventitious buds regenerated. Through this culture system, it was possible to obtain at least one million plantlets ready for transplantation within one year.

Key words: *In vitro* propagation, Purple coneflower

INTRODUCTION

Echinacea purpurea L (Asteracae) is a perennial herb that has been used as a medicinal plant for hundreds of year. The main prerequisite for the development of high-quality medicinal products is a consistent source of high-quality plant material (Murch *et al*. 2004). However, purple coneflower is heterozygous, the content of medicinal compounds might differ significantly among individual plants and the quality of the medicine manufactured from these plants may not be the same. Because of this, techniques for *in vitro* propagation of seedlings of elite genotype in purple coneflower have high application value. Plant regeneration in coneflower has been reported by culturing leaf and petiole explants (Koroch *et al*. 2002; Roger *et al*. 2004; Choffe *et al*. 2000a; Kristen *et al*. 2000). In the present paper, we report an efficient *in vitro* propagation system for this important medicinal plant.

MATERIALS AND METHODS

Plant source

Seeds of purple coneflower were purchased at a supermarket (Supplier Company of Plantation Products, Norton, MA 02766, USA) and cultivated at the Chinese Medicinal Plant Garden in the campus of South China Agricultural University. Seeds were collected from these seed-grown plants and used for the present studies.

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Establishment of aseptic cultures

Seeds were surface-sterilized by immersing in 70% ethanol for 1 minute and soaking in a 0.1% mercuric chloride for 10 minutes followed by 1% sodium hypochlorite solution containing one drop of Tween 20 per 50ml for 10 minutes. Sterilized seeds were then rinsed three times in sterile deionized water and inoculated on a medium comprised of half-strength MS (Murashige and Skoog 1962) salts, 1% sucrose and 500mg/l lactalbumin hydrolysis and the medium was solidified with 0.2% Phytagel prior to autoclaving. The seeds were cultured first under dim-light for 14d and then the seedlings were transferred to a medium containing full-strength MS salts, 1% sucrose and 0.2% Phytagel and kept at 25^0-27^0C with 12h photoperiod under cool-white light (50μmol $m^{-2}s^{-1}$).

Preparation of medium

All the media used were adjusted to a pH value of 6.0 with 1N NaOH or 1N HCl solution, gelled with 0.6% agar (except those for seed germination and growth) prior to autoclaving at 1.4kgcm^{-2} for 20 minutes.

Regeneration ability of explants from different maturity plantlets

Leaf, petiole and root explants of aseptic plantlets were cultured on MS basal medium supplemented with 0.3mg/l BA (Benzyladenine) and 0.01mg/l

NAA (Naphthaleneacetic acid) to investigate the regeneration ability with the age of mother plantlets. In cultures, leaves were cut into sections ($0.5cm^2$) and placed on media with the adaxial surface toward the media, while petioles and roots were cut into about 5 mm and cultured by laying randomly on the media. The optimum plantlet age for shoot initiation was determined by comparing the regeneration ability of roots, petioles and leaves taken from plantlets of one and a half months, 2 months, 2 and a half months, and 3 months old.

Capacity of plantlet production with the established methods

Five healthy plantlets were randomly selected as explant source and 50% of each type (root, petiole and leaf) of explants taken from these plantlets were cultured onto the above mentioned regeneration medium. Regenerated buds were rooted and the resulted plantlets were again divided into explants of different kinds and cultured for regeneration of buds. This cycle was repeated again and again and all the healthy buds and plantlets produced from all explants were counted.

Rooting of adventitious buds

Healthy shoots longer than 1.5cm regenerated from all explants types were isolated and cultured on MS basal medium containing 0.01 mg/l NAA. All cultures were kept in 12-h under cool-white light (50 µ mol $m^{-2}s^{-1}$) at 25^0C for 40 days.

Data collection and analysis

Experiment was arranged according to the Randomized Complete Block Design. Regeneration cultures were evaluated 40 days after initiation and regeneration percentage of adventitious buds was calculated on the ratio of number of explants regenerated buds to the number of explants cultured. All experiments had four replicates, each with eight explants per bottle. Statistical analysis was carried out using the Student Newman-Kuells Means Separation Test of SAS (SAS Institute, Cary, NC, 1995).

RESULTS AND ANALYSIS

Regeneration ability of explants

Differences in maturity of the material plantlets greatly influenced the regeneration of shoot buds from the explants (Table1). Number of shoot buds

Table 1 Effects of the age of the mother plantlet on regeneration of buds

Age of plantlets (months)	No of buds from 1 g of leaf explants	No of buds from 1 g of petiole explants	No of buds from 1 g of root explants	No of vitrified buds	Time taken to regenerate (Days)
1.5	74.6 c*	235.4 d	236.8 c	38.8 a	24.4 c
2	102.0 b	352.0 b	328.8 b	24.2 b	24.6 c
2.5	194.5 a	443.6 a	505.8 a	11.6 c	28.2 b
3	94.6 b	319.9 c	331.2 b	13.0 c	30.6 a

*Means fallowed by the same lower case letters in each column are not significantly different at 5% level in Duncan's Multiple Range Test.

Figure 1 Regeneration of buds from explants of two and a half months old plantlets. Photos were taken after 40 days of culture. a. leaf explants; b. petiole explants; c. root explants.

regenerated from all root, petiole and leaf explants increased with increasing plantlet maturity up to two and a half months. Petiole and root explants of different maturity exhibited much higher regeneration ability than leaf explants with no exception, while vitrification of the regenerated buds was generally slight (Figure 1).

Capacity of plantlet production

It took 40 days to regenerate buds of 1.5cm height, and two and a half months was necessary for a bud to grow to a plantlet of suitable maturity for providing explant material with high regeneration potential. It means, to complete a propagation culture cycles required about 4 months. In that case, it is possible to get 3 propagation culture cycles within one year duration.

According to the data shown in Table 2, explants of leaf, petiole and root taken from a 75 days old plantlet could in average regenerate 104 buds in 40 days, and after subsequent 75 days of rooting culture of these buds, these buds would become plantlets explants taken from which had high adventitious bud regeneration potential and would regenerate 10816 (104 times 104) buds in another 40 days. As culture continuing, more number of plantlets would be propagated. Details in the estimated production capacity of buds and plantlets are summarized in Table 3. From Table 3 it is clear that within one year more than one million plantlets ready for transplanting could be propagated by the established culture methods.

DISCUSSION

Previous work in our laboratory has demonstrated that purple coneflower root explants easily regenerate shoots under *in vitro* culture conditions (Dahanayake 2009). Root tissue has been used successfully for regeneration in a range of plant species (Bhat *et al*. 1992; Knoll *et al*. 1997; Sankhla *et al*. 1995; Vinocur *et al*. 2000; Chaudhuri *et al*. 2004). It is obvious that making full use of all the organs including the root as explant source can effectively increase the number of buds from a certain plantlet.

Efficient plant regeneration systems are required in this species to propagate unique lines and to improve the quality based on somatic cell genetics and recombinant DNA technology. Age of plantlets greatly influenced the regeneration ability of explants excised from garlic root tips, which reached the maximum value (95%) in 15 days old plantlets (Muhammad *et al*. 1997). In addition the highest number of shoots per explant was obtained from 30 days old seedlings in *Aeschynomene sensitiva* (Claudine *et al*. 1996). Up to the date the effect of planting material with plantlets age on shoot regeneration has not been reported in *E. purpurea.* In the present experiment, it was clear that explant age had a positive effect on shoot induction and

Table 2 Regeneration of buds from different explants

Plant code	Number of buds observed after 40 days			
	Leaf	Petiole	Root	Total number of buds
1	28	46	49	123
2	25	37	33	95
3	20	52	45	117
4	21	39	32	92
5	22	42	29	93
Average	23.2	43.2	37.6	104

Table 3 Details of the propagation culture system

Purpose of culture	Days required for the purpose	Number of buds regenerated	Number of plantlet suitable for providing explants	Number of plantlets suitable for transplanting	Total number of days required
--*	--	--	1	--	--
Regeneration of buds	40	104	0	0	40
Growth of buds	75	--	104	0	115
Regeneration of buds	40	10,816	--	0	155
Growth of buds	75	--	10,816	0	230
Regeneration of buds	40	1,124,868	--	0	270
Growth of buds	45	--	--	1,124,868	315

*No sence.

also it seemed to be a factor interfering with the level of regeneration. These results agreed with the previous findings in *Brassica* (Sharma and Thorpe 1989), garlic (Muhammad *et al*. 1997), legume (Claudine *et al*. 1996) and *Tylophora indica* (Chaudhuri *et al*. 2004).

The present study clearly demonstrates that all parts of a plant can be used as explants for regeneration of adventitious shoots and two and a half months old plantlets were most suitable for providing explant materials. By culturing the explants on MS basal medium with 0.3mg/l BA and 0.01mg/l NAA, large number of buds could be regenerated in 40 days. Such a prolific rate of multiplication cannot be expected by any of the *in vivo* methods of clonal propagation. Because it was difficul to maintain a so large number buds, then we had to randomly select 5 plantlets to continue the study. All of the hardened plants transferred to the field survived, and no phenotypic variations were observed.

A novel shoot regeneration methodology from root, petiole and leaf explants has been developed for *E. purpurea*. This method is promising for rapid multiplication of purple coneflower.

REFERENCES

Bhat SR, Chitralekha P and Chandel KPS 1992 Regeneration of plants from long-term root culture of lime, *Citrus aurantifolia* (Christm) Swing. Plant Cell Tissue and Organ Cult. 29: 19-25

Chaudhuri KN, Ghosh B and Jha S 2004 The root: a potential source of competent cells for high-frequency regeneration in *Tylophora indica*. Plant Cell Reports, 22: 731-740

Choffe KL, Victor JMR, Murch SJ and Saxena P K 2000 In vitro regeneration of *Echinacea purpurea* L.: Direct somatic embryogenesis and indirect shoot organogenesis in petiole culture. In Vitro Cell Dev-Pl 36(1):30-36

Claudine Nelf-Campa, Clemence Chaintreuil-Dongmo and Bernard Louis Dreyfus. 1996. Regeneration of the tropical legume Aeschynomene sensitive Sw. from root explants. Plant Cell, Tissue and Organ Culture 44: 149-154, 1996

Dahanayake N, Zha Fu-cheng, Yang Yue-sheng and Wu Hong 2009 Evaluation for PlantRegeneration Potential of Root Explants in Purple Coneflower. Journal of South China Agricultural University 30: 51-54

Knoll KA, Short KC, Curtis IS, Power JB and Davey MR 1997 Shoot regeneration from cultured root explants of spinach (*Spinacia oleracea* L.): a system for Agrobacterium transformation. Plant Cell Reports, 17: 96-101

Koroch A, Kapteyn J, Juliani JE and Simon HR 2002 In vitro regeneration of *Echinacea pallida* from leaf explants. In Vitro Cell. Dev. Biol.-Plant 39:415-418

Kristen L, Choffe, Jerrin M R, Victor, Susan J, Murch, Praveen K and Saxena 2000 In Vitro regeneration of *Echinacea purpurea* L.: Direct somatic embryogenesis and indirect shoot organogenesis in petiole culture. In Vitro Cell. Dev. Biol.-Plant 36:30-36

Muhammad Shahidul Haque, Tomikichi Wada and Kazumi Hattori 1997 High frequency shoot regeneration and plantlet formation from root tip of garlic. Plant Cell, Tissue and Organ Culture 50: 83-89, 1997

Murch SJ, Liu CZ, Romero RM and Saxena PK 2004 In vitro culture and temporary immersion bioreactor production of *Crescentia cujete*. Plant Cell Tissuse Organ Cult. 78(1): 63-68

Roger J Sauve, Margaret T, Mmbaga and Suping Zhou 2004 *In vitro* regeneration of the Tennessee coneflower (*Echinacea tennesseensis*). In Vitro Cellular and Developmental Biology; 40, 3; AGRICOLA pg. 325

Sankhla D, Sankhla N and Davis TD 1995 Promotion of *in vitro* shoot formation from excised roots of silk tree (*Albizzia julibrissin*) by oxime ether derivate and other ethylene inhibitors. Plant Cell Reports, 15, 143-146o regeneration of shoot buds and plantlets from seedling root segments of Brassica napus L. Plant Cell Tiss. Org. Cult. 18: 129-141

Vinocur B, Carmi T, Altman A and Ziv M 2000 Enhanced bud regeneration in aspen (*Populus tremula* L.) roots cultured in liquid media. Plant Cell Reports. 19,:1146-1154