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Studies on tissue and cell cultures for plant regeneration of Purple coneflower (*Echinacea purpurea* L.)

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Abstract: Explants of leaf, root and petiole were taken from *in vitro* grown Purple coneflower (*Echinacea purpurea* L.) plants and their regeneration ability was compared by culturing these explants on media with various supplements. The regeneration ability in root and petiole explants was higher than that in leaf ones, and a combination of 0.3 mg/l benzyladenine (BA) with 0.01 mg/l naphthaleneacetic acid (NAA) in Murashige and Skoog (MS) basal medium was the most effective, yielding 100% shoot regeneration frequency and associated with the highest number of 1.84 shoots per explant. Explants with higher chromosome level need higher cytokinine concentration to achieve higher regeneration frequency. In the experiments, haploid and diploid plants showed higher regeneration potential at 0.3 mg/l BA whereastetraploid (2n=44) required a higher BA concentration of 0.5 mg/l to accelerate the ability. All explants from two and a half months old plantlets produced buds in high frequency; 1143.9 buds were regenerated from 1g explants. With the established regeneration culture system, it was possible to obtain a large number (1124864) of buds within one year period from one mother plant. For initiation of roots from the regenerated shoots and stimulation of growth of the plantlets, all shoots from different explants responded equally well on medium with 0.01 mg/l NAA.

Keywords: *Echinacea purpurea, in vitro,* regeneration, haploid, diploid plants, tetraploid plants

Introduction

Purple coneflower (*Echinacea purpurea* L; Compositae or sunflower family) is one of the most popular medicinal herbs. Recently, because epidemic diseases caused by viruses have become much more threatening, global demand for products of purple coneflower has been increasing.

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 might be not stable. 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 ex-plants (Koroch *et al*. 2001; Roger *et al*. 2004; Choffe *et al*. 2000a; Kristen *et al*. 2000).

Plant regeneration in coneflower has been reported by culturing leaf and petiole explants.In the present paper, we report an efficient *in vitro* propagation culture protocol for this important medicinal plant.

Objectives

1). Establish simple and efficient *in vitro* culture system to regenerate buds and induction of roots which can be applied for agricultural practice such as propagation of elite clone.

2). To produce large number of uniform cells or cell clusters that is the most suitable material for re-differentiation studies.

Materials and methods

Investigation of the 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 and 0.01 mg/l NAA to investigate the regeneration ability with the age of downer plantlets. 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.

Estimation of the 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 on 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 1.5 cm regenerated from all explants types were isolated and inoculated on MS basal medium containing 0.01 mg/l NAA.

Data collection and analysis

All experiments were repeated at least once with a minimum of four replicates. Analysis of variance was carried out with the use of Statistical Analysis Systems (SAS version 9.2) software and DMRT tests were applied to compare the treatment means.

Results

Explants of leaf, petiole and root were inoculated on MS basal medium with BA at various combinations (0.1, 0.3, 0.9, 2.7 mg/l) and NAA at 0.01 mg/l. Most explants formed callus at the cut surface in two weeks, and the callus began to produce bud primodia in another one week. The primodia developed into adventitious buds afterwards. It was found that medium supplemented with 0.3 mg/l BA yielded the best results, allowing all the root and petiole explants and a higher percentage of leaf explants to regenerate adventitious buds (Table 1). It displayed 100% shoot regeneration form root and petiole explants associated with a high number of shoots per explants (1.84) without showing vitrification. A lower or higher concentration of BA was less effective; especially when higher concentration of BA used, not only the frequency of regeneration decreased, the quality of the regenerated buds also dropped as the symptoms of vitrification on the buds became evident. The callus observed with higher BA and NAA concentrations were brown and excessive necrosis, indicating toxic effects. Although difference in regeneration ability was observed among the three kinds of explants, quality of the regenerated buds from all the explants were alike. In most of the cases, regeneration ability in root and petiole explants was higher than that in leaf ones. Visual observations of the cultures revealed distinct routes of morphogenesis resulting in the formation of shoots in response to BA. According to these, the balance of auxin and cytokinine is a determining morphogenic factor of organogenesis.

On the bases of the above experiments, BA was used at 0.3 mg/l and NAA was tested at various concentrations (0.0, 0.01, 0.05, 0.15, 0.75 mg/l). Results of the experiments are summarized in Table.2. It is clear that concentration of NAA also played a very important role in regulating shoot regeneration. Explants of root and petiole were found to possess higher bud regeneration potential in all the NAA concentrations tested, and under the most suitable NAA concentration of 0.01 mg/l, explants of root and petiole had at least 30% higher regeneration potential than those of leaf.

Differences in maturity of plantlets greatly influenced the initiation of shoot buds (Table 3).

Percentage of shoot initiation from all root, petiole and leaf explants increased with increasing plantlet maturity upto two and a half months and after that negative effect was observed. Minimum regeneration potential (548.8 buds per 1 g of explants) was observed from one and a half months old plantlets whereas reached to maximum number (1143.9 buds per 1 g of explants) in two and a half months plantlets with showing less vitrification. The moderate regeneration ability was displayed from 3 months and 2 months old plantlets showing 745.7 and 782 buds per 1 g of explants respectively.

Discussion

Plant regeneration from petiole explants of *E. purpurea* was achieved by using only a small amount of BA (Choffe*et al*., 2000a), whereas, in the present study, BA (0.3 mg/l) in combination with NAA (0.01 mg/l) was the most effective in inducing adventitious shoot regeneration from all explants. Different explants are known to produce different types and frequencies of regenerative responses (Annadana*et al*., 2000); with hypocotyls being more responsive than other explants in many species (Gubis*et al*., 2003; Chae*et al*., 2004). It is apparent that the source of explants significantly affects the regenerative response of *E. purpurea*.

Conclusion

Regeneration ability in root and petiole explants of *E. purpurea* was higher than that in leaf ones.

All explants from two and a half months old plantlets produced buds in high frequency. Throughthe established regeneration system, it was possible to obtain a large number of bud productions within one year period.

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Table 1. Comparison of the ability of shoot regeneration of different explants on media containing 0.01mg/l and various concentration of 0.3 mg/l BA

Explant	BA concentration (mg/l)							
	0.1		0.3		0.9		2.7	
	Regeneration (%)	No. buds per explant	Regeneration (%)	No. buds per explant	Regeneration (%)	No. buds per explant	Regeneration (%)	No. buds per explant
Leaf	37.5 c*	0.28 c	75.0 b	1.05 b	12.5 b	0.13 b	22.5 b	0.10 c
Petiole	87.5 b	0.80 b	100.0 a	1.84 a	87.5 a	0.83 a	40.0 a	0.25 b
Root	100.0 a	1.13 a	100.0 a	1.75 a	87.5 a	1.00 a	40.0 a	0.40 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.

Table 2. Comparison of the ability of shoot regeneration of different explants on media with various concentration of NAA with 0.3 mg/l BA

Explant	NAA concentration (mg/l)									
	0.0		0.01		0.05		0.15		0.75	
	Regeneration (%)	No. buds per explant	Regeneration (%)	No. buds per explant	Regeneration (%)	No. buds per explant	Regeneration (%)	No. buds per explant	Regeneration (%)	No. buds per explant
Leaf	50.00a*	0.71 b	59.4 b	1.01 b	43.75a	0.63 b	20.83 b	0.21 b	8.33 c	0.08 b
Petiole	56.25 a	1.07 a	91.66 a	1.54 a	45.83a	1.13 a	40.00 a	0.83 a	29.16 b	0.46 a
Root	59.40 a	1.04 a	93.75 a	1.73 a	50.00 a	1.27 a	45.00 a	0.83 a	40.00 a	0.44 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.

Table 3. Effects of plantlet age on regeneration of buds

Age of plant (months)	No of buds from 1 g of leaf explants	No of buds from 1g of petiole explants	No of buds from 1g 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
2.0	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
3.0	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.