Evaluation for Plant Regeneration Potential of Root Explants in *Echinacea purpurea*

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Abstract: For evaluation of the plant regeneration potential of root explants, explants of root, leaf and petiole were taken from *in vitro* grown purple coneflower, *Echinacea purpurea* L. plantlets and cultured on adventitious bud inducing media with different cytokinins and auxins at various concentrations. In most of the cases, the regeneration potential of root explants was much higher than that of leaf ones and similar to that of petiole explants, and a combination of 0.3 mg/L benzyladeine with 0.01 mg/L naphthaleneacetic acid was the most effective combination and concentrations for inducing adventitious bud regeneration. Although the best result of bud regeneration was obtained from culture of petiole explants, a good result in regeneration rate of 100% and a high number of 1.75 buds per explant were obtained from culture of root explants. Buds regenerated from root explants initiated roots and became intact plants readily upon transfer to a medium containing 0.01 mg/L naphthaleneacetic acid. Results of the experiments indicated that root was an ideal explant source for rapid propagation by means of tissue culture in this plant species.

Key words: *Echinacea purpurea*; *in vitro* culture; plant regeneration; root; explant
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松果菊根外植体植株再生能力的评价

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摘要:为了评价松果菊 *Echinacea purpurea* L. 根外植体的再生能力,将从松果菊无菌小苗得到的根外植体和叶片以及叶柄外植体接种到含有不同种类和浓度的细胞分裂素和生长素的培养基上,诱导不定芽的再生. 结果表明,在多数情况下,根外植体的再生能力显著高于叶片,和叶柄类似. 0.3 mg/L 的苄基腺嘌呤 和0.01 mg/L 的萘乙酸是诱导根外植体不定芽再生最合适的激素种类和质量浓度组合. 根外植体培养的不定芽再生频率为100%,每个根外植体得到再生芽1.75个. 当把这些由根再生的不定芽从母体组织切开并转移培养到含有0.01 mg/L 萘乙酸的培养基后,很容易生根并成为完整的植株. 可见根是组培快繁松果菊理想的外植体材料.

关键词:松果菊; 离体培养; 植株再生; 根;外植体

Since purple coneflower, *Echinacea purpurea* L., is one of the top three most important herbaceous medicinal plants in the world [1] ranking beside gingko and ginseng, application of biotechnical means for promoting the production of this plant has been emphasized recently [2]. Although purple coneflower seedlings are routinely

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propagated by seeds, quite a portion of the seeds are poor in germination [3] and most populations are strictly self-incompatible [4] and cultivation using these seeds results in many problems such as difficulty in managements and unreliability of the products due to the variation in the growing behavior of the seed-grown plants. To overcome these shortages, one of the strategies is to produce genetically identical plantlets in large scale by mass propagation of plantlets using *in vitro* culture means.

Plant regeneration in purple coneflower has been reported by culturing leaf explants [5-6] and petiole explants [7], and the use of root tissue as source of explant for plant regeneration purposes has not been reported in this species so far. In our preliminary experiments, we observed that shoots of purple coneflower rooted easily under *in vitro* culture conditions and quite a quantity of root tissue could be produced along with the growth of the shoots. With these observations and the facts that root tissue has been used successfully for regeneration in a range of other plant species [8-11], recently we investigated the regeneration potential of purple coneflower root by comparing with leaf and petiole. Details of the experiments are presented in this paper.

1 Materials and methods

1.1 Plant source

Seeds of purple coneflower were purchased at a supermarket provided by the 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.

1.2 Establishment of aseptic seedlings

Seeds were surface-sterilized by immersing in $\varphi = 70\%$ ethanol for 1 min and soaking in a $\varphi = 0.1\%$ mercuric chloride solution for 10 min followed by $\varphi = 1\%$ sodium hypochlorite solution containing one drop of Tween 20 per 50 mL for 10 min. Sterilized seeds were then rinsed three times in sterile deionized water and inoculated on a Phytagel-solidified medium comprised of half-strength MS (Murashige and Skoog)[12] salts, 10 g/L sucrose and 500 mg/L lactalbumin hydrolysis. After 14 d culture under dim-light, germinated seeds were transfered to a Phytagel-solidified medium containing full-strength MS salts and 10 g/L sucrose, and the pH was adjusted to 6.0. Cultures were then incubated in a room of 25 – 27 ℃ and with 12 h photoperiod under cool-white light (about 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

1.3 Regeneration and rooting cultures

Leaf, petiole and root explants were excised from 2-months old seedlings and cultured onto the MS basal medium with various combinations of plant growth regulators. In culture of leaf explants, leaf sections of about 0.5 cm^2 were placed on media with the adaxial surface toward the media. Petioles and roots were cut into about 5 mm and cultured by laying on the media. All the treatments were kept in the same room as mentioned above. As for the rooting culture of the regenerated buds, the buds were excised from the mother tissue and cultured on Phytagel-solidified full-strength MS media with 15 g/L sucrose and 0.01 mg/L naphthaleneacetic acid (NAA).

1.4 Data collection and analysis

Regeneration cultures were evaluated 40 d after initiation. All experiments were repeated at least once with a minimum of four replicates, each with eight explants per bottle. Statistical analysis was carried out using Duncan's Multiple Range Test.

2 Results

2.1 Comparison on plant regeneration potential of different explants on the media with BA

Explants taken from leaf, petiole and root were inoculated on MS basal medium with benzyladenine (BA) at various mass concentrations (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 primordia 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 (Tab. 1). Lower or higher concentration of BA was less effective; especially when higher concentration of BA was 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. Although difference in regeneration ability was observed among the three kinds of explants, quality of the regenerated buds from all the explants were all alike.

Tab. 1　Adventitious bud regeneration from different explants on the media with various mass concentration of BA and 0.01 mg/L NAA[1)]

Explant	0.1 mg · L^{-1} BA		0.3 mg · L^{-1} BA		0.9 mg · L^{-1} BA		2.7 mg · L^{-1} BA	
	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

1) Means followed by the same letter in each column are not significantly different at 5% level in Duncan's Multiple Range Test

2.2　Comparison on plant regeneration potential of different explants on the media with KT and TDZ

Two other cytokinins, KT (kinetin) and TDZ (thidiazuron) were tested separately at various mass concentrations (0.1, 0.3, 0.9, 2.7 mg/L) in combination with 0.01 mg/L NAA for their effects of inducing bud regeneration from explants. Although root and petiole explants had higher regeneration rates than those of leaf explants, regeneration rates of all the treatments were much lower than those cultures applied BA (data not shown).

2.3　Comparison on plant regeneration potential of different explants on the media with NAA

On the bases of the above experiment, BA was used at 0.3 mg/L and NAA was tested at various mass concentrations (0, 0.01, 0.05, 0.15, 0.75 mg/L). Results of the experiments are summarized in Tab. 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.

Tab. 2　Adventitious bud regeneration from different explants on the media with various mass concentration of NAA and 0.3 mg/L BA[1)]

Explant	0 mg · L^{-1} NAA		0.01 mg · L^{-1} NAA		0.05 mg · L^{-1} NAA		0.15 mg · L^{-1} NAA		0.75 mg · L^{-1} NAA	
	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	50.00 a	0.71 b	59.4 b	1.01 b	43.75 a	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	45.83 a	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	40.0 a	0.44 a

1) Means followed by the same letter in each column are not significantly different at 5% level in Duncan's Multiple Range Test

2.4　Comparison on plant regeneration potential of different explants on the media with IBA

IBA (indo-butyric acid) is an analog to NAA and various mass concentrations of IBA (0, 0.01, 0.05, 0.15, 0.75 mg/L) were tested in combination with BA at 0.3 mg/L. From the results shown in Tab. 3 and compared with those in Tab. 2, it is clear that IBA had almost the same function as that of NAA in regulating bud regeneration: 0.01 mg/L was again the most suitable concentration and explants of root and petiole had significantly higher regeneration potential than those of leaf in most of the IBA concentrations tested.

Tab. 3　Adventitious bud regeneration from different explants on the media with various mass concentration of IBA and 0.3 mg/L BA[1)]

Explant	0 mg · L^{-1} IBA		0.01 mg · L^{-1} IBA		0.05 mg · L^{-1} IBA		0.15 mg · L^{-1} IBA		0.75 mg · L^{-1} IBA	
	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	50.0 b	0.68 b	55.0 b	0.94 b	44.2 a	0.69 b	25.0 b	0.13 c	12.5 b	0.06 c
Petiole	55.0 ab	0.94 a	85.2 a	1.31 ab	46.0 a	1.01 a	37.5 a	0.69 b	26.0 ab	0.36 b
Root	60.0 a	0.88 a	91.5 a	1.69 a	48.0 a	1.19 a	37.5 a	0.88 a	36.0 a	0.50 a

1) Means followed by the same letter in each column are not significantly different at 5% level in Duncan's Multiple Range Test

2.5 Rooting of the regenerated buds and growth of the regenerated plants

Buds regenerated from root explants were isolated from the mother tissues and cultured on full-strength MS media with 0.01 mg/L NAA. Upon culture, these buds developed roots from the bases easily and became intact plants. These plants, after being transplanted to the soil, grew and set flowers normally.

3 Discussion

There have been a few reports on plant regeneration in purple coneflower by culturing tissues of leaf and petiole as explants[5-7,13]; Results of the present experiments indicated that explants of root had significantly higher regeneration potential than those of leaf and are comparable with those of petiole. It was found that the respond of root explants to culture conditions for regeneration was very similar to those of leaf and petiole, having the highest regeneration efficiency on media with 0.3 mg/L BA and 0.01 mg/L NAA or IBA.

Recently we have successfully obtained haploid plant regeneration in purple coneflower by anther culture for the first time[14], and a subsequent program which aims at breeding varieties with hybrid vigor by use of the haploid materials is under taken. Propagation of elite genotypes by tissue culture has important application value in purple coneflower production, especially when the genotypes of the varieties are heterogenous. Since root is one important part in purple coneflower occupying 20 or even higher percentage of the total fresh mass of the plant, high regeneration potential of root and equally high quality of the regenerated buds form root explants indicating that root is an important source of explants. By using root as explant material together with petiole and leaf, more number of plantlets can be produced to meet the demand of increased cultivation area.

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