Optimization conditions of adventitious buds regeneration from petiole explants of *Echinacea purpurea*

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Abstract. *Echinacea purpurea* as a perennial herb has high ornamental and medicinal value. While the tissue culture technologies of *E. purpurea* are contradictory. In order to improve the culture conditions of the adventitious buds regeneration of *E. purpurea*, petioles from the aseptic seedlings were used as the explants. Different concentrations of sodium nitrophenolate (SN), copper sulfate (CuSO₄), amine fresh ester (DA-6), sodium nitroprusside (SNP) and ascorbic acid (Vc) were added into MS medium containing cytokinin and auxin, respectively, to investigate their effects on inducing regeneration of adventitious buds. The results showed that the best induction effects of adventitious buds were obtained under 1 mg/L CuSO₄, 1 mg/L DA-6, 0.2 mg/L SN, 2 mg/L SNP and 20 mg/L Vc conditions. Our results can provide references for the induction of adventitious buds regeneration and rapid propagation of *E. purpurea*.

1. Introduction

*Echinacea purpurea* is a perennial herb of Echinacea in Compositae [1], which has high ornamental and medicinal value [2]. Although there are many reports on the tissue culture of *E. purpurea*, many of them are contradictory, and the regeneration efficiency of adventitious bud of numerous culture technology is still very low [3], so it is necessary to optimize the regeneration system of culture and improve induction efficiency of adventitious bud regeneration in *E. purpurea*. It has been reported that during the *Cotinus coggyria* tissue culture, adding SN and GA3 will improve the growth, and shorten the culture cycle [4]. The Cu²⁺ has been known as an key cofactor of some enzymes which play important roles in plant tissue culture [5]. On the other hand, SNP, nitric oxide donor, has been shown helpful in improving plant regeneration in chrysanthemums [6]. To date, most studies on the culture of *E. purpurea in vitro* are compared and optimized for the concentrations of the hormone components, but few studies concerning with the factors such as SN, DA-6, CuSO₄, Vc and SNP on the culture effect of *E. purpurea* have been reported. This study can provide references for the induction of adventitious buds regeneration and rapid propagation of *E. purpurea*.

2. Methods

2.1. Plant sources

In this study, the purple coneflower seeds were provided by professor Yue-sheng Yang from College
of Life Sciences, South China Agricultural University.

2.2. Preparation of explants
The purple coneflower seeds were sterilized by 75% ethanol solution for 1 min, and 2% sodium hypochlorite (NaClO) for 20 min, respectively, finally, using sterile distilled water rinsed 5 times. Aseptic seedlings could be gained by inoculating sterile seeds on MS (Murashige and Skoog) medium [7] after 45 days of culture. Petioles were isolated from the 45-day-old sterile seedlings, and cut them into 5 cm pieces as petiole explants.

2.3. Preparation of mediums and condition of culture
The same conditions of culture were suitable for all experiments. The medium pH was controlled from 5.8 to 6.0 by 1 mol/L NaOH before the mediums were autoclaved (LX-C75, Hefei, China) for 20 min at 1.4 kg·cm⁻². The mediums were placed at 25 ± 2°C with 12 h photoperiod of cool white fluorescent tubes with a 60-80 μmol·m⁻²·s⁻¹ intensity.

2.4. Adventitious buds regeneration
In order to induce new adventitious buds, the explants of petiole were inoculated on different concentrations of SN (0, 0.02, 0.2 and 2 mg/L), DA-6 (0, 0.01, 0.1 and 1 mg/L), CuSO₄ (0, 0.1, 1 and 10 mg/L), Vc (0, 20, 40 and 80 mg/L) and SNP (0, 2, 4 and 8 mg/L) were added into MS medium supplemented with 0.5 mg/L 6-BA and 0.01 mg/L NAA for inducing adventitious buds regeneration respectively for 30 days of culture.

2.5. Data analysis
Adventitious buds regeneration rate (%) = (number of adventitious buds differentiated/number of petiole explants inoculated) ×100%.
Average number of buds (number of buds) = total number of adventitious buds/number of petiole explants inoculated.

The test data was analyzed by statistical analysis method. All the tables are made by Microsoft Excel.

3. Results

3.1. Results of CuSO₄ on regeneration of adventitious buds from petiole explants
As shown in table 1, the regeneration efficiency of adventitious buds was affected by concentration of CuSO₄ distinctly. The biggest regeneration percentage of adventitious buds (50%) was acquired, when 1 mg/L of CuSO₄ was added into medium on the induction of adventitious buds (table 1). Moreover, although the maximum number of buds was obtained at 0.1 mg/L, the buds were relatively small and poor quality.

| Concentration (mg/L) | Adventitious buds regeneration rate (%) | Average number of buds |
|----------------------|----------------------------------------|------------------------|
| 0                    | 15                                     | 0.30                   |
| 0.1                  | 30                                     | 0.85                   |
| 1                    | 50                                     | 0.45                   |
| 10                   | 25                                     | 0.50                   |

3.2. Influences of DA-6 on regeneration of adventitious buds from petiole explants
From the results summed up in table 2, regeneration of adventitious buds might be promoted by adding DA-6 in mediums. While the regeneration efficiency would be raised with adding high concentrations (0-1 mg/L) of DA-6 in the induction mediums (table 2). When 1 mg/L of DA-6 was
applied into mediums on the induction of adventitious buds, the best regeneration percentage of adventitious buds (35%) and the maximum number of buds (0.9) were gained (table 2).

Table 2. Results of different concentrations of DA-6 on adventitious buds regeneration of petiole explants in *E. purpurea*.

| DA-6 concentration (mg/L) | Adventitious buds regeneration rate (%) | Average number of buds |
|--------------------------|----------------------------------------|------------------------|
| 0                        | 15                                     | 0.30                   |
| 0.01                     | 15                                     | 0.30                   |
| 0.1                      | 20                                     | 0.55                   |
| 1                        | 35                                     | 0.90                   |

3.3. Effects of sodium nitrophenolate (SN) on regeneration of adventitious buds

As shown in table 3, the concentrations of SN influenced adventitious buds regeneration visibly. From the results summarized in table 3 and figure 1(A-D), it’s explicit clear that when the concentration of SN was 0.2 mg/L, the best results of regeneration of adventitious buds (35%) and the biggest buds number (0.8) were obtained respectively (table 3 and figure 1C).

Table 3. Effects of different concentrations of SN on adventitious buds regeneration of petiole explants in *E. purpurea*.

| Concentration of SN (mg/L) | Adventitious buds regeneration rate (%) | Average number of buds |
|----------------------------|----------------------------------------|------------------------|
| 0                          | 15                                     | 0.30                   |
| 0.02                      | 25                                     | 0.45                   |
| 0.2                       | 35                                     | 0.80                   |
| 2                         | 33.3                                   | 0.73                   |

Figure 1. Effect of SN on adventitious buds regeneration. A: 0 mg/L; B: 0.02 mg/L; C: 0.2 mg/L; D: 2.0 mg/L. (Bars=1 cm).

3.4. Effects of SNP on adventitious buds induction from petiole explants of *E. purpurea*

The responses of adventitious buds induction were significantly influenced by the concentrations of SNP in mediums (table 4). The addition of 2 mg/L SNP made the highest adventitious buds regeneration rate and the most buds (1.35) (table 4). While, if SNP concentration was more than 2 mg/L, the adventitious buds regeneration rate was dramatically decreased (table 4).

Table 4. Effects of different concentrations of SNP on adventitious buds regeneration of petiole explants in *E. purpurea*.

| Concentration of SNP (mg/L) | Adventitious buds regeneration rate (%) | Average number of buds |
|----------------------------|----------------------------------------|------------------------|
| 0                          | 15                                     | 0.30                   |
| 2                          | 65                                     | 1.35                   |
| 4                          | 30                                     | 0.40                   |
| 8                          | 0                                      | 0.00                   |
3.5. Effects of vitamin C (Vc) on adventitious buds induction by petiole explants of E. purpurea

The results show that Vc affected the induction of adventitious buds from petiole explants of E. purpurea, significantly (table 5). When 20 mg/L Vc was added in the mediums, the highest adventitious buds induction percentage (35%) and the maximum number of buds (1.45) were achieved (table 5).

| Concentration of Vc (mg/L) | Adventitious buds regeneration rate (%) | Average number of buds |
|-----------------------------|----------------------------------------|------------------------|
| 0                           | 15                                     | 0.30                   |
| 20                          | 35                                     | 1.45                   |
| 40                          | 30                                     | 0.70                   |
| 80                          | 15                                     | 0.25                   |

4. Discussion

Cu²⁺ is one of the trace elements essential for plant growth, but it can be toxic to plants when excess. Previous studies show that the regeneration efficiency of adventitious buds can be promoted by the addition of a small amount of copper sulfate into mediums [3]. In the present study, when the concentration of copper ions exceeded 1 mg/L, the number of regenerated adventitious buds decreased observably. Furthermore, the regeneration of adventitious buds was facilitated at low concentrations of copper ions added into mediums.

DA-6 is a new type of plant growth regulator in plants. It is suitable for many plants and their entire growth period, and widely used in vegetable production [8,9]. However, DA-6 is rarely used in plant tissue culture. Sodium nitrophenolate (SN), a potent cell activator, could significantly increase cell viability [10]. In plant tissue culture, ascorbic acid (Vc) is commonly used as an antioxidant to prevent browning, but it can also promote the growth of buds [11]. It’s found that the regeneration rate of adventitious buds could be increased at the right amount addition of DA-6, SN and Vc in mediums.

Studies have reported that SNP could significantly induce the differentiation of adventitious buds in tea (Camellia sinensis) [12]. Our results found that the addition of an appropriate amount of SNP in the medium containing cytokinins significantly induced the regeneration of adventitious buds, and the inducing effects were more prominent than the effects of using only cytokinins. SNPs as exogenous NO donors could significantly facilitate adventitious buds regeneration, probably because cytokinins could rapidly stimulate the production of NO [13].

This study aims to provide a method for the regeneration and rapid propagation of adventitious buds in E. purpurea. It is hoped that more researchers will optimize the regeneration system of E. purpurea, which will make the largest development and utilization of the ornamental and medicinal value of E. purpurea.

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