Effects of Different Factors on Seed Germination and Seedling Growth in Echinacea purpure

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Abstract. In this experiment, seeds of Echinacea purpure were used as materials to investigate the effects of several factors on the germination and seedling growth. The results showed that 6-BA, SNP and NAA all promoted the germination and seedling growth of E. purpure seeds. The best effects were obtained, when application of soaking treatment for seeds with 5 mg/L NAA before inoculating them onto MS media supplied with 0.1 mg/L IAA, the highest seed germination rate was 86.7%, and the best rooting rate of seedlings was 83.3%. However, seed sprouting and development of seedling were inhibited, when Mn²⁺ was added into mediums.

1. Introduction

Echinacea purpurea L. is native to North America and is a perennial herbaceous plant of Echinacea. It is an ideal natural immune modulator discovered so far. The whole plant can be used as a medicine, and contains various pharmaceutically effective active ingredients such as hydroxyamide, polysaccharide, sesquiterpene, polyacetylene, caffeic acid derivative and cichoric acid and so on. Studies have shown that these extracts of pharmacology from E. purpurea can regulate and enhance the activity of immune cells, resulting in promoting many immune functions, such as fighting infection, accelerating wound healing, and the prevention and treatment of AIDS.

In recent years, due to the frequent occurrence of influenza and respiratory infections, E. purpurea contained immunomodulatory active ingredients had received unprecedented attention. Therefore, the in vitro culture of E. purpurea has also received great attention [1]. At present, the cultivation of E. purpurea adopted the traditional seeding and breeding method. Due to the complex dormancy of E. purpurea seeds and the heterozygosity of genotype caused by cross-pollination, low rate of seed sprouting and difficult collection of coneflower seeds were caused. Moreover, the cultivated population had obvious differences in plant morphology and growth and development process, which were not conducive to the development of field management, quality stability and yield improvement [2]. In addition, because of the geographical range, the germplasm variation, quality and unstable active ingredient content, the further upgrading of the domestic production industry of E. purpurea had been restricted [3].

Therefore, in the case of ensuring high and stable yield of active ingredients, according to the biological characteristics of E. purpurea, the improvement and optimization of E. purpurea varieties to adapt to different climatic conditions and regions had become an urgent problem to be solved. Obtaining a large number of sterile seedling materials was conducive to the subsequent evaluation of
E. purpurea seedlings, which helped to promote in vitro tissue culture, molecular breeding, and unified management. In addition to quickly obtaining a large amount of sterile seedling material in tissue culture of E. purpurea, it’s necessary to increase the germination rate and reduce the pollution rate, and then to make the seedling grow rapidly and well as required. The purpose of this paper was to investigate the effects of different factors on sprouting of seed and development of seedling in E. purpurea, and establish a highly efficient sterile seedling culture method to rapidly produce phenotypically consistent seedlings and gain the in vitro explants for tissue culture. These would provide theoretical support for germplasm conservation, genetic transformation and factory production in coneflower.

2. Materials and Methods

2.1. Source of seeds
Seeds of E. purpurea were sent friendly researcher Yang Yue-sheng who was working at College of Life Sciences in South China Agricultural University.

2.2. Seed Sterilization
Seeds of coneflower were soaked into 75% ethyl alcohol solution for 1 min, and then dipped in 2% solution of chlorinated soda (NaClO) for 20 min for sterilization, conclusively making use of bacteria free water rinsing seeds for 5 times.

2.3. Sterilization of Culture Mediums and Cultivation Conditions
The pH value of culture mediums was adjusted with 1 mol/L NaOH solution to 5.8 - 6.0, and then the medias were sterilized by autoclave sterilizer for 20 min in the condition of 121°C and 0.1 Mpa. After sterilization, medias were moved to culture room under the circumstance of temperature at 25 ± 2°C and light cycle for 12 h/day at 2000 lux..

2.4. Sprouting of Seeds and Development of Seedlings
In order to induce seed germination and seedling growth, the sterile seeds were inoculated on MS media [4] contained 0.1 mg/L IAA (indoleacetic acid) and added different 6-BA (6-benzyladenine) concentrations (0, 0.3, 0.6 and 1 mg/L) and different concentrations of manganese sulfate (Mn²⁺) (0, 0.05%, 0.1% and 0.15%) respectively for 35 days of culture. Moreover, the sterile seeds were soaked into different concentrations of SNP (sodium nitroprusside) (0, 100, 500 and 1000 μmol/L) and different concentrations of NAA (naphthylacetic acid) (0, 5, 10 and 15 mg/L) severally, after that placed them onto MS media contained IAA at the concentration of 0.1 mg/L for 35 days.

2.5. Statistics and analysis of data
Ratio of germination = amount of germinated seeds/number of tested seeds ×100%.
Rooting rate = number of rooted plants/total number of plantlets ×100%.
Average taproot length = length of taproot in each group/total number of cultured seedlings in each group.
Average quantity of primary branch roots = total quantity of primary branch roots in each group/total number of rooting plantlets in each group.

In this study, SPSS statistics 20.0 statistical analysis software was used for variance analysis and Duncan multiple comparison (P ≤ 0.05), and English lowercase letters were used to mark the significance of difference. Different letters indicated that there was a significant difference in inducing effect between treatments.
3. Results

3.1. Influence of Various 6-BA Concentrations on Seed Germination and Seedling Growth in *E. Purpure*

Results of the experiments were shown in Table 1 and Figure 1. From the table it was clear that when
6-BA was adopted at the concentration exceeding 0.3 mg/L, germination rate and quality of seedlings
dropped sharply, and only very tiny and weak buds were generated (Table 1 and Figure 1(A-D)). For
this reason, 0.3 mg/L 6-BA was considered suitable and adopted for facilitating seed germination and
seedling growth (Table 1 and Figure 1B).

Table 1. Results of different concentrations of 6-BA on seed germination and seedling growth in *E. Purpure*

| 6-BA concentrations (mg/L) | Germination rate (%) | Plant height (cm) | Number of leaves |
|----------------------------|----------------------|-------------------|-----------------|
| 0                          | 53.3                 | 1.71±0.35a        | 2.00±0.23c      |
| 0.3                        | 68.8                 | 0.57±0.03b        | 5.83±0.31a      |
| 0.6                        | 62.5                 | 0.36±0.20bc       | 4.00±0.26b      |
| 1                          | 0                    | 0c                | 0d              |

Note: data followed with different lowercase in the same column were significant difference at p value
less than or equal 0.05.

![Figure 1](image1.jpg)

**Figure 1.** Effect of different 6-BA concentrations on seed sprouting and development of seedling in *E. Purpure*

A: 0 mg/L; B: 0.3 mg/L; C: 0.6 mg/L; D: 1 mg/L. (Bars =1 cm)

3.2. Influences of Different Concentrations of Manganese Sulfate (*Mn*²⁺) on Seed Germination and Seedling Growth In *E. Purpure*

We can see from Table 2 and Figure 2 (A-D), it was distinct that all the quantitative value of upgrowth
were visibly reduced by added Mn²⁺ into the sprouting media. So Mn²⁺ was not unfit for promoting
seed germination and seedling growth (Table 2 and Figure 2 A).
Table 2. Effects of various concentrations of Mn^{2+} on seed germination and seedling growth in *E. purpurea*.

| Mn^{2+} concentration (%) | Rate of germination (%) | Plant height (cm) | Number of leaves | Average taproot length (cm) | Number of primary lateral root | Rooting rate (%) |
|---------------------------|-------------------------|------------------|-----------------|---------------------------|-------------------------------|-----------------|
| 0                         | 53.3                    | 1.71±0.35a       | 2.00±0.23a      | 1.81±0.42a                | 2.89±0.56a                   | 33.3            |
| 0.05                      | 10                      | 0.29±0.17ab      | 0.75±0.48b      | 1.15±1.05ab               | 1.50±0.50b                  | 6.7             |
| 0.1                       | 0                       | 0b               | 0c              | 0b                        | 0c                           | 0               |
| 0.15                      | 0                       | 0b               | 0c              | 0b                        | 0c                           | 0               |

Note: data followed with different lowercase in the same column were significant difference at p value less than or equal 0.05.

Figure 2. Effect of various concentrations of Mn^{2+} on seed germination and seedling growth in *E. purpurea*.
A: 0%; B: 0.05%; C: 0.10%; D: 0.15%. (Bars =1 cm)

3.3. Effects of Different Concentrations of SNP on Seed Sprouting and Development of Seedling In *E. Purpurea*

Results of the experiments were summarized in Table 3. From the data it was clear that the SNP concentration at 0 μmol/L was too low, 100 and 500 μmol/L did well almost the same. While when SNP was used at concentration of 1000 μmol/L, the higher rate of germination and the better quality of seedling were gained.

Table 3. Results of various SNP concentrations on seed germination and seedling growth in *E. purpurea*.

| SNP Concentration (μmol/L) | Rate of germination (%) | Plant height (cm) | Number of leaves | Average taproot length (cm) | Number of primary lateral root | Rooting rate (%) |
|---------------------------|-------------------------|------------------|-----------------|---------------------------|-------------------------------|-----------------|
| 0                         | 53.3                    | 1.71±0.35a       | 2.00±0.23b      | 1.81±0.42c                | 2.89±0.56b                   | 33.3            |
| 100                       | 65.6                    | 1.17±0.26a       | 2.18±0.35ab     | 2.52±0.61b               | 4.43±1.59ab                  | 36.7            |
| 500                       | 68.8                    | 1.42±0.30a       | 2.18±0.30ab     | 2.25±0.05bc              | 5.29±0.94ab                  | 43.3            |
| 1000                      | 87.5                    | 1.78±0.34a       | 3.00±0.36a      | 4.26±0.63a               | 6.33±0.73a                   | 63.3            |

Note: data followed with different lowercase in the same column were significant difference at p value less than or equal 0.05.
3.4. Influence of NAA in the Germination Medium on the Culture Response of E. Purpure

Date summarized in Table 4 indicated that NAA supplemented to the germination medium had positive effects, promoting the seed germination and seedling growth significantly. When the concentration of NAA was used at of 5 mg/L, the highest rate of germination and the best quality of seedling were acquired. For plain reason, 5 mg/L was considered the most suitable and adoptable for further experiments.

| NAA concentration (mg/L) | Rate of germination (%) | Plant height (cm) | Number of leaves | Average taproot length (cm) | Number of primary lateral root | Rooting rate (%) |
|--------------------------|-------------------------|-------------------|------------------|-----------------------------|-------------------------------|-----------------|
| 0                        | 53.3                    | 1.71±0.35a        | 2.00±0.23c       | 1.81±0.42b                  | 2.89±0.56c                    | 33.3            |
| 5                        | 86.7                    | 1.41±0.20a        | 5.50±1.19a       | 3.45±0.21a                  | 12.75±2.31a                   | 83.3            |
| 10                       | 73.3                    | 1.62±0.35a        | 5.67±1.20a       | 2.95±0.36a                  | 10.00±3.47a                   | 53.3            |
| 15                       | 63.3                    | 1.11±0.29a        | 3.50±0.65b       | 1.68±0.49b                  | 7.67±1.48ab                   | 46.7            |

Note: data followed with different lowercase in the same column were significant difference at p value less than or equal 0.05.

4. Discussion

As an essential trace element in plants, Mn$^{2+}$ is involved in many metabolic processes of plants, but it is also harmful to plants when the concentration is too high [5]. The results showed that when the Mn$^{2+}$ was directly added into the mediums, the effects of seed germination and seedling growth were significant inhibited with increasing the concentrations of Mn$^{2+}$. This might be due to the excessive concentration of Mn$^{2+}$ added into the mediums.

SNP can release a lot of NO, while NO participates in the regulation of many growth and development processes of plants. As an exogenous donor of NO, SNP has the effects of promoting seed germination and lateral root formation [6]. For gaining better results, it might be necessary to increase the concentration of SNP solution for the treatment of soaking the seeds.

NAA has a dual role in promoting seed germination and seedling growth in plants, depending on the age of the cells, the concentration of NAA, and the type of organ [7]. In the present study, seed soaking with low concentration of NAA promoted the germination percentage and development of seedling in E. purpure.

In terms of tissue culture, the domestic research on the germination percentage and development of seedling in E. purpure is still in its preliminary stage in theory and practice, and a complete system has not yet been established. In the process of the growth of E. purpure seedlings, there are still many problems, such as growth slowly, growing unevenly, and easy browning in the later stage. Therefore, it is hoped that more researchers can further explore E. purpure to establish an efficient sterile seedling culture system and promote the further development of E. purpure in China.

5. Summary

The results showed that the sprouting and seedling development of coneflower seeds were all facilitated by 6-BA, SNP and NAA. While when application of 5 mg/L NAA solution for soaking treatment to seeds before placing them onto MS media contained IAA at the concentration of 0.1 mg/L, the highest seed germination rate (86.7%) and the best rooting rate of seedlings (83.3%) were acquired. However, when Mn$^{2+}$ was added into mediums, seed sprouting and development of seedling were restrained definitively.

6. Acknowledgments

This work were supported by Natural Science Foundation of Guangdong Province (2018A030310057 and 2020A1515011570), Foundation of Education Department of Guangdong Province (2019KTSCX059), Program for Nanhai Youth Scholar Project of Guangdong Ocean University,
Program for Scientific Research Start-up Funds of Guangdong Ocean University (R17023 and R19031), the Project of Science and Technology of Zhanjiang City (2016B01004), the Project for Innovation and Strong School of Department of Education of Guangdong Province (2016KQNCX067), and the College Students Innovation and Entrepreneurship Training Program (CXXL2019184).

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