Effects of different factors on seed germination of *Cassia mimosoides*

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Abstract. In order to improve the germination efficiency of *Cassia mimosoides* seed, the effects of concentrated sulfuric acid, sodium hydroxide, temperature and culture medium on seed germination and seedling growth of *C. mimosoides* were investigated. In this study, the seeds were immersed in water at different temperatures and sodium hydroxide with different concentrations for 10 min, and the seeds were immersed in concentrated sulfuric acid for different periods of time, and then inoculated on media, the effects of the three treatment methods on germination of seeds were studied. It was found that concentrated sulfuric acid and sodium hydroxide had no effect on seed germination of *C. mimosoides*. The seed expansion rate, germination rate and other indexes were enhanced with the increase of temperature of soaking water, and when the water temperature was 80℃, the best result of seed germination was gained in *C. mimosoides*. Compared with MS medium, seed inoculation on 1/2MS medium was more beneficial to the growth and development of germinated seedlings.

1. Introduction

*Cassia mimosoides* Linn. is one of member in the Leguminosae genus [1]. *C. mimosoides* is a very important medicinal plant, which has a very good protective effect on the liver and is in great demand in the market at present [2, 3]. However, it is difficult for *C. mimosoides* seeds to absorb water and germinate under natural conditions because of the hard surface of the seeds, which severely limits the large-scale cultivation and planting of *C. mimosoides*. In order to solve the problem of seed germination difficulty, meet the growing market demand for *C. mimosoides*. Using tissue culture technology in this paper, to explore the different temperature of the water, strong acid, strong alkali, respectively, the impact on the real grass seed germination, to solve the real natural grass breeding cycle for a long time, the problem of low germination rate to provide technical support, at the same time for the real application of herbal active, protecting liver support basic research to provide raw materials.
2. Materials and methods

2.1 Plant material
Seeds of *C. mimosoides* were provided by Dr. Yang Yali of Jiaying University.

2.2 Effects of different temperature treatments on the germination and growth of *C. mimosoides* seeds
The seeds were soaked in water at the temperatures of 25, 40, 60, 80 and 100℃ for 10 min and then inoculated on MS (Murashige and Skoog) [4] and 1/2 MS medium respectively for 20 days of culture.

2.3 Influences of sodium hydroxide (NaOH) at different concentrations on the germination and growth of *C. mimosoides* seeds
The seeds were soaked for 10 min with NaOH at concentrations of 0, 5, 15, 30 and 45 g/L, respectively, and seeded placed into MS medium for 20 days.

2.4 Effects of concentrated sulfuric acid on the germination and growth of *C. mimosoides* seeds
The seeds were soaked in concentrated sulfuric acid (98%) for 0, 5, 10, 20 and 40 min, and then inoculated in MS medium for 20 days of culture.

2.5 Data analysis
All the experiments were repeated three times. SPSS 17.0 software was used for data statistics and significance difference on statistical analysis. Different letters after numbers indicated difference significance (*P*≤0.05).

3. Results

3.1 Effects of different temperature treatments on the germination and growth of *C. mimosoides* seeds in MS medium
It could be seen from Table 1 that water at low temperature soaking treatment has no promoting effect on seed germination of *C. mimosoides*. With the increasing of temperature of water, various indexes such as seed expansion rate, germination rate, length of stem and main root length were improved, and when the soaking water at temperature of 80℃, the best results were gained (Table 1).

| Treatment temperature (℃) | Expansion rate (%) | Germination rate (%) | Length of stem (cm) | Main root length (cm) |
|---------------------------|--------------------|----------------------|---------------------|----------------------|
| 25                        | 0a                 | 0a                   | 0a                  | 0a                   |
| 40                        | 0a                 | 0a                   | 0a                  | 0a                   |
| 60                        | 0.10b              | 0.10a                | 0.60±0.06a          | 0.96±0.13ab          |
| 80                        | 0.97±0.06c         | 0.67±0.49b           | 6.42±0.36c          | 8.69±0.84c           |
| 100                       | 1.00c              | 0.76±0.11b           | 1.79±0.74b          | 2.36±1.66b           |
Figure 1 Effect of immersing *C. mimosoides* seeds with water at different temperatures for 10 min for germination and growth in MS medium.

The seeds were soaked in water at (A) 25, (B) 40, (C) 60, (D) 80 and (E) 100 °C for 10 min and inoculated in MS medium for 20 days of culture.

### 3.2 Germination effect of *C. mimosoides* seeds on 1/2 MS media after different temperature treatment

As shown in Table 2, when the treatment temperature was at 80°C, the germination effect of *C. mimosoides* seeds was the best, and seed expansion rate, the maximum of germination rate, length of stem and main root length were acquired (Table 2).

| Treatment temperature (℃) | Expansion rate (%) | Germination rate (%) | Length of stem (cm) | Main root length (cm) |
|--------------------------|-------------------|---------------------|---------------------|-----------------------|
| 25                       | 0a                | 0a                  | 0a                  | 0a                    |
| 40                       | 0a                | 0a                  | 0a                  | 0a                    |
| 60                       | 0.30±0.05b        | 0.27±0.06b          | 1.81±0.52b          | 2.70±1.06b            |
| 80                       | 1.00c             | 1.00c               | 7.10±0.75c          | 10.37±0.42c           |
| 100                      | 1.00c             | 0.18±0.06b          | 0.60±0.13a          | 1.50±0.78b            |

### 3.3 Effects of different concentrations of NaOH on seed germination of *C. mimosoides*

The results in the Table 3 shown that after soaking in different concentrations of NaOH solution, the *C. mimosoides* seed were inoculated into MS medium at temperature of 25°C and it was found that the seeds of *C. mimosoides* could not germinate normally with soaking in different concentrations of NaOH solution. Therefore, the germination of *C. mimosoides* seeds could not be promoted via soaking the seeds in NaOH solution.

| Concentrations of NaOH (g/L) | Expansion rate (%) | Germination rate (%) | Length of stem (cm) | Main root length (cm) |
|-----------------------------|-------------------|---------------------|---------------------|-----------------------|
| 0                           | 0a                | 0a                  | 0a                  | 0a                    |
3.4 Effects of different dipped periods with concentrated sulfuric acid for seed germination of *C. mimosoides*

As the Table 4 shown that after soaking *C. mimosoides* seeds in different time periods of concentrated sulfuric acid, the seeds were placed into MS medium at temperature of 25°C for 20 days. The results indicated that the seeds of *C. mimosoides* could not germinate after soaking in different time periods of concentrated sulfuric acid. Thus, the germination of *C. mimosoides* seeds could not be facilitated by dipping in different time periods of concentrated sulfuric acid.

| Time (min) | Expansion rate (%) | Germination rate (%) | Length of stem (cm) | Main root length (cm) |
|------------|---------------------|----------------------|---------------------|-----------------------|
| 0          | 0a                  | 0a                   | 0a                  | 0a                    |
| 5          | 0a                  | 0a                   | 0a                  | 0a                    |
| 10         | 0a                  | 0a                   | 0a                  | 0a                    |
| 20         | 0a                  | 0a                   | 0a                  | 0a                    |
| 40         | 0a                  | 0a                   | 0a                  | 0a                    |

4. Discussion

It is hard to absorb water for *C. mimosoides* seeds because of hard shell with wax. Artificial planting often requires abrasive paper to remove the surface wax of *C. mimosoides* seeds, which is laborious and
inefficient in germination. The effects on the germination of two types of *Cassia tora* are obviously promoted by soaking the seeds with warm water (20-80℃) or concentrated sulfuric acid (98%) [5]. In this study, different temperatures of water, concentrated sulfuric acid and sodium hydroxide were used to soak the seeds of *C. mimosoides* to explore the effect on seed germination and growth. The results showed that the concentrated sulfuric acid and sodium hydroxide did not promote the germination of *C. mimosoides* seeds, which might be because the low concentration of sodium hydroxide was not enough to destroy the waxy layer on the surface of *C. mimosoides* seeds, but the embryo of seed might be destroyed by the high concentration of sodium hydroxide and concentrated sulfuric acid. Moreover, soaking the seeds in water at the temperature of 80℃ had a significant effect on seed germination, while most of the seeds did not germinate or grew cotyledons when soaking the seeds in water at 100℃, which might be because the internal structure of the seeds was damaged by overheated water. Some studies have shown that the basic medium can affect plant growth and development [6, 7], and it was found in this study that the growth effect of *C. mimosoides* seeds on MS and 1/2 MS media were different, and our research suggested that the 1/2 MS medium might be more suitable for the germination and growth of *C. mimosoides* seeds. Results of this study provided technical support to solve the problem of low natural reproduction rate and long cycle of germination, and also provided raw material support for basic research on chemical composition and pharmacological action of *C. mimosoides*.

5. Conclusions

The germination efficiency of *C. mimosoides* seed was significantly enhanced, when the seeds were soaked with water at the temperature of 80℃, and the best result of seed germination was gained. Compared with MS medium, seed inoculation on 1/2 MS media was more beneficial to the growth and development of germinated seedlings. However, concentrated sulfuric acid and sodium hydroxide had no effect on seed germination of *C. mimosoides*.

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