Optimization of Aseptic Germination System of Seeds in Soybean (Glycine max L.)

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Abstract. The results in the process of the optimization of aseptic germination system of seeds in soybean were found as follows. Using the method of chlorine sterilization to sterilize soybean seeds for 2-4 hours were found to get the best results and found to be suitable for the growth of soybean seeds and obtainment of the best quality explants. Relative to other varieties, the soybean variety of Mao-Dou No. 5 was found to gain the best germination effects, and the localization way of seed inoculation and the light conditions had no significant effects on the germination of seeds of Glycine max.

1. Introduction

Glycine max L. is an important economic crop, so increasing the yield and optimizing the quality of soybean have become the focus of attention. Because conventional breeding techniques are affected by factors such as the incompatibility of species hybridization and linkage of undesirable genetic traits, the introduction of many other unfavorable genes at the same time as the introduction of excellent genes from other species is difficult to further utilize [1]. Therefore, people began to explore new methods of soybean breeding.

The use of transgenic technology to transfer anti-bacterial, disease-resistant and insect-resistant genes into soybean may be one of the effective ways to increase the yield and improve the quality of soybean. Meanwhile, a good soybean regeneration system can provide a suitable receptor system for soybean genetic transformation. Therefore, the establishment of a good tissue culture regeneration system, especially a good seed sterile germination system, can provide a good source of genetic transformation receptor for soybean genetic transformation, which is the prerequisite for successful genetic transformation of soybean.

The optimization of seed aseptic germination system in soybean is the key and premise of soybean genetic transformation research. The aseptic germination effect of soybean seeds is directly related to the quantity and quality of the obtained sterile explants, which seriously affects the result of subsequent tissue culture procedure. Although there have been some reports on the sterile germination of soybean, there are poor experimental reproducibilities, and the quality of the obtained explants is poor, which seriously affects the subsequent experiments. It is still the main problem faced by the soybean genetic transformation receptor system, and further research is needed.
2. Materials and Methods

2.1. Source of Seeds
The soybean varieties of named Huachun No. 2, Huachun No. 3, and Maodou No. 5 were provided by Professor Hai Nian from the Agricultural College of South China Agricultural University.

2.2. Preparation of Mediums and Condition of Culture
The basic medium is hormone-free MSB5 medium (MS salts plus B5 vitamins) [2]. All mediums were regulated pH value to 5.8-6.0 by adding 1 mol/L NaOH. 100 mg/L inositol, 7 g/L agar, 300 mg/L peptone and 25 g/L sucrose were added into the mediums, which were placed in a high pressure steam sterilizer, sterilized at a temperature of 121°C and 0.1 MPa for 15 mim. All mediums were placed at 25 ± 2°C with light cycle of 12 h/day around 2000 lux.

2.3. Sterilization of Soybean Seeds
There were three sterilization methods applied to soybean seeds, chlorine (Cl₂) [3] (soybean seeds were sterilized with chlorine for different time (2, 4 and 6 h), 0.1% mercuric chloride (HgCl₂) (the soybean seeds were dipped into 0.1% HgCl₂ solution for ten minutes), and 1% sodium hypochlorite (NaClO) (the soybean seeds were dipped into 0.1% NaClO for ten minutes). Soybean seeds were sterilized for different time (2, 4 and 6 h), and then rinsed the seeds 5 times with sterile distilled water.

2.4. Sterile Germination of Soybean Seeds
The sterilized soybean seeds were inoculated into MSB5 medium with different concentrations of BA (0, 1 and 2 mg/L) for 5 days of culture.

2.5. Data Analysis.
All the data from the experimental results were statistically analyzed using SPSS17.0 software and significant difference among average amount was tested by Duncan multiple comparison at the level of P≤0.05. Germination percentage of seeds (%) = (amount of gemmative soybean seeds / sum of used soybean seeds) × 100%. Rate of pollution (%) = (number of contaminated soybean seeds / sum of used soybean seeds) × 100%. Germination rate of grade A (%) = (number of soybean germination seedlings with hypocotyl more than 1 cm / number of germinated soybean seeds) × 100%. Germination rate of grade B (%) = (number of soybean germination seedlings with hypocotyl less than 1 cm / number of germinated soybean seeds) × 100%. Mean of raw weight increased for every seedling = (sum raw weight of seedlings after disposed - sum raw weight of seedlings before treatment) / sum number of seedlings inoculated.

3. Results

3.1. Influence of Different Sterilization Time on Soybean Seeds with Chlorine
Soybean seeds were treated using chlorine with various sterilization time before being cultured. Results of the experiments were summarized in Table 1. The results shown clearly that 6 hours was too long, 2 and 4 hours did well almost the same. For plain reason, 2-4 hours were considered more suitable and adopted for sterilization on soybean seeds with chlorine.

Table 1. Results of sterilizing time of chlorine on germination of soybean seeds

| Sterilization time (h) | Germination rate (%) | Rate of grade A (%) | Germination rate of grade B (%) | Rate of pollution (%) |
|-----------------------|----------------------|---------------------|--------------------------------|----------------------|
| 2                     | 86.12a               | 89.70a              | 10.30b                         | 14.26a               |
| 4                     | 78.89a               | 82.86a              | 17.14b                         | 20.00a               |
| 6                     | 66.11b               | 72.30b              | 27.70a                         | 18.89a               |

Note: the sterilized huachun No. 2 soybean seeds were inoculated on MSB5 medium without hormone, and the statistical data were obtained after 5 days of culture. Data followed with different letters in the same column were significantly different at the level of P≤0.05 level.
3.2. Influence of Different Sterilizing Methods on Germination of Soybean Seeds

Soybean seeds were treated with various sterilization methods before being cultured (Table 2). From the results shown in Table 2, it was clear that the effect of soybean seeds sprouting was affected by the way of sterilization observably. Chlorine was much more effective for the sterilization of soybean seeds and more conducive to germination of soybean seeds (Table 2).

Table 2. Comparison of three sterilization methods

| Sterilization methods | Germination rate (%) | Germination rate of grade A (%) | Germination rate of grade B (%) | Rate of pollution (%) |
|-----------------------|----------------------|-------------------------------|-------------------------------|----------------------|
| Cl₂                   | 93.00a               | 87.76a                        | 12.24b                       | 2.00b                |
| 0.1 % HgCl₂           | 58.00b               | 30.35b                        | 69.65a                       | 10.67b               |
| 1 % NaClO             | 48.00b               | 23.63b                        | 76.37a                       | 26.33a               |

Note: the sterilized huachun No. 2 soybean seeds were inoculated on MSB5 medium without hormone, and the statistical data were obtained after 5 days of culture. Data followed with different letters in the same column were significantly different at the level of P≤0.05 level.

3.3. Effects of Different Light Conditions on Seed Germination of Soybean

Date summarized in Table 3 indicated that light conditions didn’t play an obvious role on germination of soybean seeds. The germination rate of seeds and the quality of germinated seeds were not affected by light conditions significantly (Table 3).

Table 3. Results of light conditions on germination of soybean seeds

| Light conditions   | Germination rate (%) | Germination rate of grade A (%) | Germination rate of grade B (%) |
|--------------------|----------------------|-------------------------------|-------------------------------|
| 2 d dark+3 d light | 98.33a               | 86.29a                        | 13.71a                       |
| 5 d light          | 100a                 | 96.67a                        | 3.33a                        |
| 5 d dark           | 96.67a               | 92.93a                        | 7.07a                        |

Note: the sterilized huachun No. 2 soybean seeds were inoculated on MSB5 medium with 1mg/L BA, and the statistical data were obtained after 5 days of culture. Data followed with different letters in the same column were significantly different at the level of P≤0.05 level.

3.4. Effects of Inoculation Way on Soybean Seeds Sprouting

The effects of inoculation way on seeds sprouting was also conducted. Results of the experiments were summarized in Table 4. From the data it was clear that the way of inoculation had no significant effects on seeds germination.

Table 4. Results of light conditions on germination of soybean seeds

| Way of inoculation | Germination rate (%) | Germination rate of grade A (%) | Germination rate of grade B (%) |
|-------------------|----------------------|-------------------------------|-------------------------------|
| A                 | 98.33a               | 86.30a                        | 13.70a                       |
| B                 | 93.33a               | 80.00a                        | 20.00a                       |
| C                 | 100.00a              | 83.33a                        | 16.67a                       |

Note: the sterilized huachun No. 2 soybean seeds were inoculated on MSB5 medium with 1mg/L BA, and the statistical data were obtained after 5 days of culture. Data followed with different letters in the same column were significantly different at the level of P≤0.05 level. A: the umbilicus was placed downward; B: the umbilicus was placed upward; C: the umbilicus was placed horizontally.

3.5. Comparison of Germination Effects of Soybean Seeds Among Different Genotypes

Furthermore, the effects of genotype on the germination were tested among three varieties (Table 5). The results indicated that the germination was much more effective for the variety of Mao-Dou No. 5 (Table 5).
Table 5. Comparison of the effects on culture responses of treatment among three varieties

| Genotypes     | Germination rate (%) | Germination rate of grade A (%) | Germination rate of grade B (%) |
|---------------|----------------------|---------------------------------|---------------------------------|
| Maodou No. 5  | 100a                 | 97.00a                          | 3.00b                           |
| Huachun No. 3 | 90.00ab              | 83.00ab                         | 17.00ab                         |
| Huachun No. 2 | 83.33b               | 73.00b                          | 27.00a                          |

Note: the sterilized soybean seeds were inoculated on MSB5 medium, and the statistical data were obtained after 5 days of culture. Data followed with different letters in the same column were significantly different at the level of P≤0.05 level.

4. Discussion

Cl2 has a strong role in killing microorganisms. The research results showed that good sterilizing effect for soybean seeds could be obtained via using chlorine [4]. In this research, the sterilization effectiveness and germination effect of soybean seeds with three kinds of sterilization methods were compared subsequently, and it was found that the chlorine had the best effect and was suitable for sterilization of soybean seeds. Cl2 has the function of surface sterilization, and can also penetrate into the gaps of seed coats, so the sterilization effect of it will be more thorough. However, HgCl2 and NaClO solution may enter the seed coats through the swelling effect of the seeds, which will cause certain damage and injury for the seeds, affecting the germination of seeds. Therefore, sterilization of soybean seeds is preferably carried out by Cl2.

The results suggested that the seeds of Gypsophila davurica could germinate in the presence or absence of light [5]. Our findings suggested that there was no significant difference in the germination effect of soybean seeds under the three light conditions of culture.

The growth and development of seedlings were affected by the way of inoculation and placement of seeds to mediums in Juglans regia [6]. However, the results of this experiment shown that the inoculation orientation of seeds on mediums has no significant effect on the germination in soybean. This might be the result of different plants having different developmental characteristics.

Some reports found that the physiological and biochemical characteristics of different genotype of seeds in maize (Zea mays) had some differences during germination [7]. The results of this experiment shown that the germination effects of different genotypes of soybean seeds were different, which might be determined by the genetic characteristics of the seeds themselves.

5. Summary

The very good results of germination effects on soybean seeds was gained successfully, when the sterilization method of Cl2 was applied to sterilizing for 2-4 hours. Moreover, the soybean variety of Mao-Dou No. 5 was found to obtain the best germination effects. However, the localization way of seeds inoculation to mediums and the light conditions both had no obvious affect on the germination of seeds in soybean.

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7. References

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