Effects of different hormones on germination and callus induction of hemp seeds

BingqingHe, PenglinShen, HongjuanZheng, YiChu, JinqiGuan*, LanLi*

1College of Science & Life, Jiamusi University, Jiamusi City, Heilongjiang Province, 154007, China
2College of Big Data and Software Engineering, Wuzhou University, Wuzhou City, Guangxi Province, 543000, China
*Co-Corresponding author’s e-mail: jmsdx8888@sohu.com; lljmsu@163.com

Abstract. The purpose was to induce and treat hemp seeds and callus, compare the methods to increase the germination rate of hemp seed, obtain suitable disinfectant for hemp seed, and different hormone ratios to promote callus growth. GA3, 6-BA, and IAA were used to induce hemp seed germination, and the effects of different hormones and different concentrations on hemp seed germination were studied. Tissue culture was used to induce hemp seeds to select suitable hemp seeds disinfectant and time; callus was induced by using hemp aseptic seedling leaves. GA3 with a concentration of 20-80mg/L and IAA with a concentration of 20-40mg/L can promote the germination of hemp seeds; the best disinfection method of hemp seeds is: 70% ethanol for 1min + 10% NaClO for 18min; the most the combinations beneficial to callus growth were: 2.0mg/L 6-BA+0.5mg/L NAA. GA3 and low-concentration IAA can promote the germination of hemp seeds; the combination of 70% ethanol and 10% sodium hypochlorite has the best disinfection effect on hemp seeds; 6-BA+NAA can obtain good quality hemp seeds hemp callus.

1. Introduction
Hemp is an annual upright herbaceous plant under the mulberry marijuana family. It has high value and can be used in many fields, including industrial fields such as clothing, paper making; medical fields such as anesthesia and treatment; and military fields. In most cases, hemp seeds cannot be sown in time and need to be stored for a period of time. Long-term storage will reduce the germination rate of hemp seeds. According to research, hemp seeds are stored at room temperature in Heilongjiang province. The germination rate in 2 years will decrease by 5% -15%, and the germination rate in storage for 2-3 years will decrease by 35% -55%, which is very unfavorable to the breeding of hemp. Plant hormones soaked seeds can break their dormant period and promote seed germination. This method can effectively solve the problem of low hemp germination rate after long-term storage. Moreover, hemp is a dioecious and heterogeneous heterogeneous crop. The genetic basis is complex and diverse, and the good traits are difficult to inherit stably. Traditional cultivation methods have difficulties in field selection and long growth cycles. To this end, domestic and foreign experts and scholars continue to study the technology of hemp tissue culture. In 1984, domestic scholars used this technology to obtain green hemp seedlings for the first time. Plant tissue culture refers to the technique of isolating the required organs, tissues, or cells from the plant body, inoculating it on a medium suitable for the growth of the plant under sterile conditions, and obtaining a regenerating intact plant through culture, using this technique can stabilize the good traits of hemp and shorten its growth.
cycle[1,2]. This experiment aims to promote the germination of stored hemp seeds by using different plant growth regulators, then the germinated seeds were cultured in tissue culture, to obtain the hormone concentration to promote the germination of hemp seeds, the appropriate disinfectant and treatment time for hemp seeds, and the best hormone combination to induce callus, and enrich the hemp bioengineering technology breeding system.

2. Experimental materials and methods

2.1. Experimental Materials

2.1.1. Test materials Materials. Hemp seeds, HM-213, courtesy of Heilongjiang Academy of Sciences. Instrument: multifunctional ultra-clean operation table, climate intelligent incubator, LED white light fixture, pressure steam sterilizer. Reagents: gibberellin (GA₃), indole-3-acetic acid (IAA), 6-benzylaminopurine (6-BA), and naphthalene acetic acid (NAA).

2.1.2. Medium. MS culture medium: mother liquor I100mL, mother liquor II10mL, mother liquor III10mL, mother liquor IV10mL, sucrose 30.0g, agar 9.0g, distilled water 870mL, pH 5.8-6.0. ½MS medium: mother liquor I50mL, mother liquor II10mL, mother liquor III10mL, mother liquor IV10mL, sucrose 30.0g, agar 9.0g, distilled water 920mL, pH 5.8-6.0.

2.2. Experimental method

2.2.1. Induce seed germination. Select 480 hemp seeds (30 seeds as a group, a total of 16 groups are set). Hemp seeds that are free of disease and insect pests and have the same fullness are taken out after soaking in clear water for 14 hours. With concentrations of 20mg/L, 40mg/L, 60mg/L, 80mg/L IAA, 6-BA, GA₃ and water were used for soaking for 3h. After flowing water, they were neatly placed in a petri dish (with filter paper to keep the seeds moist) and cultured in a 28°C incubator. The blank was used as a control group (CK) to observe the effect of hormones on seeds. From the day after the seed was placed in the constant temperature incubator, the germination of the seed was continuously observed. Data were recorded from the time of the first seed germination. Germ root breakthrough 1mm as germination[3]. The experiment was repeated 3 times. The data recorded on the 3rd day was used to calculate the germination potential of hemp seeds, and the data on the 7th day was used to calculate the germination rate of the hemp seeds.

\[
D = \frac{T_3}{G} \times 100 \% \quad (1)
\]

\[
T = \frac{T_{\text{total}}}{G} \times 100 \% \quad (2)
\]

Note: \(D\) is the germination potential, \(T_{\text{total}}\) is the germination number of the test seeds, \(T_3\) is the germination number of the test seeds within 3d, \(G\) is the number of test seeds, and \(T\) is the germination rate.

2.2.2. Seed disinfection and access to sterile seedlings. Select the germinated seeds and immerse them in distilled water containing 2-3 drops of surfactant for 15-20 minutes. After soaking, the seeds were wrapped in clean four-layer gauze, rinsed under running water for 20 minutes, and placed in a clean bench. They were respectively treated with 70% ethanol, 10% sodium hypochlorite solution (NaClO), and 0.1% mercury chloride solution (HgCl₂), 10% hydrogen peroxide solution (H₂O₂) for disinfection, see (Table. 1) for specific disinfection methods. Study the effect of different disinfectant and disinfection time combinations on disinfection effect [4]. After the disinfectant treatment, rinse 4-5 times in sterile distilled water to fully remove the residual disinfectant. Dry the seed surface liquid with sterile filter paper and inoculate on 1/2MS solid medium. For each treatment, 40 seeds with basically the same physiological conditions were selected, and the experiment was repeated 3 times. Under the conditions of 24±1°C, 16h light/d, and 8h dark/d, the seeds were germinated, polluted, and sterile seedlings were counted on the 8th day.
Table 1 Screening of disinfecting reagents

| Deal with | Method          | Deal with | Method          | Deal with | Method          |
|----------|----------------|----------|----------------|----------|----------------|
| A1       | ethanol 1min+NaClO | 12min    | ethanol 1min+HgCl2 | 6min     | ethanol 1min+H2O2 | 15min |
| A2       | ethanol 1min+NaClO | 18min    | ethanol 1min+HgCl2 | 8min     | ethanol 1min+HgCl2 | 20min |
| A3       | ethanol 1min+NaClO | 24min    | ethanol 1min+HgCl2 | 10min    | ethanol 1min+HgCl2 | 25min |

2.2.3. Callus induction. Select 7-14 days of hemp aseptic seedlings, use sterilized scissors to cut off the yellow leaves at the base, cut the remaining leaves into small pieces with a size of about 0.5×0.3cm, and have incisions around them. They were inoculated on medium containing different concentrations of plant hormones to induce callus formation [5]. A total of 4 treatments, 10 bottles of each treatment, each medium was inoculated with about 2-3 explants, 3 repeated experiments were carried out, the culture conditions were consistent with the acquisition of sterile vaccines. After the primary culture, callus with good growth condition was selected and subcultured. The subculture frequency was once a week, and the subculture medium was as shown above. Observe at any time and record the appearance and morphological characteristics of the callus. After 7 days of inoculation of hemp leaf explants on the callus-inducing medium, the number of contamination of the explants was counted, and the contamination rate of the explants was calculated. After 20 days, the number of callus growing from the leaves was counted, and the callus induction was calculated rate.

\[
C\% = \frac{E_{Callus}}{E_{total}} \times 100 \% \\
P\% = \frac{E_{fouling}}{E_{total}} \times 100 \% 
\]

Note: \( C\% \) is the callus induction rate, \( P\% \) is the explant contamination rate, \( E_{Callus} \) is the number of explants that produce callus, \( E_{fouling} \) is the number of contaminated explants, and \( E_{total} \) is always the total number of inoculated explants.

2.2.4. Data processing. Use Excel software to process the data and draw according to the processed data.

3. Results and analysis

3.1. Germination data analysis
As shown in Figures 1 and 2, when the concentration of GA3 is between 20-80mg/L, the germination rate and germination potential of hemp are gradually increasing with the increase of concentration. The germination rate and germination potential were significantly higher than the control at a concentration of 80mg/L 20-80mg/L GA3 can promote the germination of hemp seeds. The concentration of 80mg/L GA3 has the best effect and the seed germination rate is the highest. When the concentration of IAA was 20-40mg/L, the germination rate of hemp seeds increased with increasing concentration, and the concentration was between 60-80mg/L, and the germination potential and germination rate showed a downward trend. It can be seen that low concentration of IAA can promote hemp seed germination. 4 concentration gradients of 6-BA significantly inhibited the germination of hemp seeds. As the concentration increased, the inhibitory effect became more obvious. At a concentration of 60 mg/L, hemp seeds were completely inhibited from germinating. 6-BA was not suitable as A hormone that promotes the germination of hemp seeds.
3.2. Effects of different treatment time on seed germination and aseptic seedling growth

Observing the seed germination process and analyzing the data processing, we can see that under the same conditions, the disinfection effect of the combination disinfectant at different times is significantly different. The combination of A1, A2, A3 and B1, B2, and B3 all have strong bactericidal effects on seeds, but from the plant growth status, it can be seen that 0.1% HgCl₂ has irreversible damage to seeds. The reaction time of 70% ethanol remains unchanged, and the pollution rate of 10% NaClO disinfection shows a trend of decreasing first and then increasing with time. The disinfection effect is the best at 18 minutes, and the pollution rate is 20.0%; with the 0.1% HgCl₂ reaction time with the prolongation, the pollution rate of hemp seeds gradually decreased, and the pollution rates were 37.5%, 32.0%, and 25.0% at 6min, 8min, and 10min respectively; 10%H₂O₂ had no obvious effect on disinfection of hemp seeds for 15-25min, and the infection rate Higher. As shown in Table 2, A2 is the best combination for hemp seed disinfection: 70% ethanol for 1 min + 10% NaClO for 18 min.

| Deal with | A1 | A2 | A3 | B1 | B2 | B3 | C1 | C2 | C3 |
|-----------|----|----|----|----|----|----|----|----|----|
| Pollution rate | 27.5% | 20.0% | 30.0% | 37.5% | 32.0% | 25.0% | 65.0% | 57.5% | 45.0% |
| Plant growth status | Good | Good | General | Good | General | Worse | Good | Good | General |

3.3. Effects of different hormone combinations on the induction of hemp callus

Different hormone combinations and different concentrations will affect the formation of callus and its color and morphology. 6-BA is the cytokinin selected in this experiment. The auxin used in combination is NAA and IAA. As shown in Table 3, the combination of 6-BA and NAA has the highest induction rate of callus. The optimal hormone ratio is 2.0mg/L 6-BA+0.5mg/L NAA. The color of callus is pale yellow and transparent, and the texture is relatively loose, good growth. 6-BA and IAA are used together. When the IAA concentration is 0.2mg/L, the callus is yellow-green, the structure is compact, and browning is easy to occur. When the IAA concentration is 2mg/L, the callus color is pale yellow, and the structure is more loose. This shows that when the concentration of IAA is between 0.2mg/L and 2mg/L, IAA can promote the formation of callus. With the increase of IAA concentration, the quality of the callus induced gradually becomes better.

4. Conclusion and discussion

4.1. Effects of different hormones on seed germination rate
Both GA3 and IAA are endogenous auxins that are ubiquitous in plants. They have different contents in different parts of the plant. Different contents have different effects on plants and have duality on plant growth. At low concentrations, it can promote seed germination and break the dormancy of seeds. At high concentrations, the osmotic pressure increases, the water absorption of seeds weakens, and the seed germination is suppressed [6]. GA3 promotes crop growth and development and increases crop yield. 20-80mg/L GA3 can promote hemp seed germination. The optimal concentration is 80mg/L. The effect of GA3 concentration greater than 80mg/L can be explored in the future.

4.2. Effects of different disinfection methods on the growth status of sterile vaccines
In the process of plant tissue culture, the necessary condition for establishing plant regeneration is disinfection of explants. The best disinfection method is different due to the large difference between the internal and external microorganisms of different plants and different materials under natural conditions [7]. Ethanol with high permeability can sterilize the explants for 30-60s, which can enter the bacteria carried by the explants, solidify the protein in the body, combine with other disinfectants to denature the protein in the bacteria, and enhance the overall disinfection effect. The experimental result is that 70% ethanol for 1min+10% NaClO for 18min has the lowest pollution rate, but HgCl2 was more toxic, and the longer the disinfection time was likely to cause the growth period of sterile seedlings prolonged and poor physiological state, the mortality of hemp seeds is gradually increasing, and it is not good for tissue culture and transformation. NaClO is less harmful to the seeds, which can reduce the pollution rate and ensure the survival rate of the seeds. In order to avoid the damage caused by disinfection to the explants, the appropriate disinfectant, the concentration of the disinfectant and the disinfection time should be selected during the disinfection process of the explants.

| Hormone combination | Callus induction rate | Callus growth |
|---------------------|-----------------------|---------------|
| 2.0mg/L6-BA+0.5mg/LNAA | 86.65% | Light yellow, loose structure |
| 2.0mg/L6-BA+2.0mg/LIAA | 83.33% | Light yellow with loose structure |
| 2.0mg/L6-BA+1.0mg/LIAA | 72.67% | Yellow-green, compact structure |
| 2.0mg/L6-BA+0.2mg/LIAA | 61.00% | Yellow-green, compact structure |

4.3. Effects of different hormone combinations on callus induction
In the process of plant in vitro culture, in order to obtain good quality callus, it is necessary to find the hormone suitable for the plant and the most suitable ratio of the hormone [9]. In the experiment of hemp leaf callus induction, the effects of different concentrations of growth regulators are obviously different. The appropriate concentrations of auxin NAA and IAA have a positive effect on callus induction. Cytokinin 6-BA and the above two all kinds of auxins can be used to form callus.

Acknowledgments
Heilongjiang Provincial Department of Education Basic Research Project, China, (2017-KYYWF-0577); China Scientific and technological innovation projects in Jiamusi University, Heilongjiang Province, College students: 201910222010.

References
[1] Rybczynski, J.J. (1997) Plant regeneration from highly embryogenic callus, cell suspension and protoplast cultures of Trifoliumfragiferum. J. Sci. Plant Cell, Tissue and Organ Culture, 51: 159-170.
[2] Leifert, C., Pryce, S., Lumsden P.J. & Waites, W M. (1992) Effect of medium acidity on growth and rooting of different plant species growing in vitro. J. Sci. Plant Cell, Tissue and Organ Culture, 30: 171-179.
[3] Seo, M., Nambara, E., Choi, G. & Yamaguchi, S. (2009) Interaction of light and hormone signals in germinating seeds. J. Sci. Plant molecular biology, 69: 463-471.
[4] Da Silva, J.A.T. (2003) Chrysanthemum: advances in tissue culture, cryopreservation, postharvest technology, genetics and transgenic biotechnology. J. Sci. Biotechnology advances, 21: 715-766.

[5] Ranalli, P. (2004) Current status and future scenarios of hemp breeding. J. Sci. Euphytica, 140: 121-131.

[6] Richards, D.E., King, K.E., Ait-Ali, T. & Harberd, N.P. (2001) How gibberellin regulates plant growth and development: a molecular genetic analysis of gibberellin signaling. J. Sci. Annual review of plant biology, 52: 67-88.

[7] Özgen, M., Türet, M., Özcan, S. & Sancak, C. (1996) Callus induction and plant regeneration from immature and mature embryos of winter durum wheat genotypes. J. Sci. Plant Breeding, 115: 455-458.