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Effects of Different Treatments on Seed Germination of *Bupleurum chinensis*

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Abstract: In order to solve the problem of low emergence rate of *Bupleurum chinense* seeds, and screen out the best germination conditions, the seeds of *Bupleurum chinense* L. were treated with different soaking time, germination temperature and exogenous regulating substances to improve the germination rate. The results showed that the germination rate of fresh water at 12 h was significantly higher than that at 8 h and 36 h; The germination rate of seeds treated with constant temperature 15 ℃ and variable temperature 25 ℃/15 ℃ (day/night) was significantly higher than that of other temperature treatments. Seed soaking time and culture temperature also influenced seed germination start day and peak day. Different concentrations of regulatory substances could promote seeds germination. The germination rate of seeds induced by 0.50 mg/L 6-BA was the highest, the start day and peak day were 4 days and 5 days earlier than control check (CK) respectively.

Key words: *Bupleurum chinense* L., soaking time, culture temperature, exogenous regulator, germination rate.

1. Introduction

Bupleurum chinense is a valuable traditional Chinese medicine, belonging to plants of the family umbelliferae. It has the effect of soothing the liver and raising the Yang of the liver [1]. Due to the rapid growth of market demand and rising prices of *Bupleurum chinense*, excessive mining of wild *Bupleurum* has resulted in depletion of natural resources. Relying on digging wild *Bupleurum* is no longer enough to meet the market demand. Therefore, it is urgent to study the technology of artificial planting system of *Bupleurum chinense*. However, there are some problems in the production of *Bupleurum chinense*, the seed germination rate was only 30% and 40% in the current year; the germination time was long, etc. [2, 3], which brings difficulties to artificial cultivation.

The germination status of *Bupleurum chinense* seeds is related to the inhibition of endogenous substances in seed coat on the one hand [4], on the other hand, to the budding environment and the induction of exogenous substances. ZHOU et al. [5] showed that soaking the seeds of *Bupleurum chinense* with clear water was beneficial to the extraction of endogenous inhibitors and to the improvement of germination rate. YANG et al. [6] considered that there were significant differences in the response of different *Bupleurum* germplasm to germination temperature and seed germination performance at the optimum germination temperature. DENG et al. [7] studied the effect of insecticide treatment on germinating rate of different germplasm *Bupleurum chinense*, ZHOU et al. [8] also studied the effects of different exogenous substances on seed vigor of *Bupleurum chinensis*. In this experiment, the seeds of *Bupleurum chinensis* in Shexian County were used as materials. The effects of different soaking time, culture temperature and exogenous regulator on seed

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germination were studied, which provided theoretical basis for artificial cultivation.

2. Materials and Methods

2.1 Materials

The seeds of *Bupleurum chinense* are provided by a China Herbal Medicine Cultivation Company.

2.2 Preprocessing

The experiment was carried out in the horticultural laboratory of Hebei University of Engineering. Plump seeds with the same size were selected and soaked in distilled water for 2 minutes, stirring and floating off the seeds which were empty, the selected seeds were disinfected with 0.5% KM_nO_4 for 30 minutes, then rinsed with distilled water for 3 times. Petri dishes and filter paper were sterilized with autoclaving, two layers of wet filter paper were placed on the top and bottom of the petri dishes, spraying water into the dishes regularly to keep the seed germination process in high moisture.

2.3 Treatments

The following treatments are germinated in the biological incubator, germination was recorded every day with 3 replicates and 100 seeds per dish.

(1) Soaking time: 8 h, 12 h, 24 h, 36 h;

(2) Germination temperature: 5 ℃, 20 ℃, 25 ℃/15 ℃ (day/night), 30 ℃;

(3) Exogenous regulatory substance: GA_3 (Gibberellin A_3), 6-BA (6-Benzylaminopurine), SNP (sodium nitropmsside) distilled water as control (Table 1).

2.4 Sampling

Start day: the first seed germinating days; Peak day: the day when the number of germinated seeds increased the most; Germination potential: germinating total number/seed number × 100 on the first 15 days; Germination rate: number of germinated seeds/number of tested seeds × 100 on the first 40 days; Germination criteria: on the basis of the extension of the germ at the rhizome [9].

2.5 Data Analysis

SPSS 19.0 and Excel 2003 software were used for data processing and statistical analysis.

3. Results

3.1 Effect of Soaking Time on Seed Germination of Bupleurum Chinensis

Table 2 shows that different soaking time has no significant effect on seed initiation day and peak day of *Bupleurum chinense*. But different seed soaking time showed significant difference on seed germination rate and germination potential. The germination rate at 12 h and 24 h's seed-soaking was significantly higher than that at 8 h and 36 h's, but the difference between 12 h and 24 h's seed-soaking is not significant. The germination rate and germinative force of seed

Table 1 Different concentrations of exogenous regulatory substances.

Exogenous regulatory substance	Concentration (mg/L)			
GA_3	0	10	50	100
6-BA	0	0.10	0.50	1.00
SNP	0	50	125	250

Table 2 Effect of soaking time on seed germination characteristics of *Bupleurum chinense.*

Soaking time (h)	Start day (d)	Peak day (d)	Germination percentage (%)	Germinative force (%)
8	10	17	34.67b	19.33ab
12	9	17	49.00a	23.33a
24	9	16	45.00a	22.67a
36	9	18	31.33b	16.00b

soaking for 12 hours were the highest, which were 49% and 23.33%, respectively.

3.2 Effect of Culture Temperature on Seed Germination of Bupleurum Chinense

Table 3 shows that different temperatures have a certain effect on seed germination of *Bupleurum chinensis*. At 25 ℃/15 ℃ (day/night), the highest germination rate of *Bupleurum chinense* seeds was 59.33%, and the start and peak days were advanced. The germination rate and germination potential of *Bupleurum chinense* seeds were significantly increased at 15 ℃, 73.41% and 57.20% higher than that at 30 ℃, respectively. With the increase of temperature, the germination rate and germination potential showed a decreasing trend, and the start day and peak day were delayed.

3.3 Effect of GA_3 on Seed Germination of Bupleurum Chinensis

Different concentrations of GA_3 can promote seed germination, as shown in Table 4. With the increase of GA_3 concentration, seed germination rate increased at the beginning and then decreased. The germination rate of 50 mg/L GA_3 was 66%, which was significantly higher than that of other treatments, and increased by 90.37% compared with CK; The germination rate of 10 mg/L GA_3 treatment was increased 59.59% compared with CK; There was no significant difference between 100 mg/L and CK. The seed germination potential of each treatment was significantly higher than that of CK. The germination potential of 50 mg/L GA_3 seeds was 31.33%, and there was no significant difference among different treatments. The germination start day and peak day of 50 mg/L GA_3 were 3 days and 4 days earlier than CK, respectively, 100 mg/L GA_3 had no significant effect on seed germination initiation day and peak day.

3.4 Effect of 6-BA on Seed Germination of Bupleurum Chinensis

According to Table 5, the germination rate of *Bupleurum chinense* seeds was significantly increased by different concentrations of 6-BA. The germination rate of 0.50 mg/L 6-BA treatment was significantly higher than that of other treatments, and the germination potential was 100.95% and 115.57% higher than that of CK, respectively; the peak day was 4 days earlier than that of CK, and the peak day was 5 days earlier than that of CK. However, the germination potential and germination rate of 1.00 mg/L 6-BA were significantly lower than that of other concentration treatments. Compared with other treatments, the peak day of germination was delayed, which was 1 day earlier than that of CK.

Table 3 Effect of culture temperature on seed germination characteristics of *Bupleurum chinense.*

Temperature (℃)	Start day (d)	Peak day (d)	Germination percent (%)	Germinative force (%)
15	9	16	54.33a	25.67ab
20	10	17	34.67b	19.33bc
25/15	8	15	59.33a	32.33a
30	11	18	31.33b	16.33c

Table 4 Effects of different GA_3 on seed germination index of *Bupleurum chinense.*

GA_3 concentrations (mg/L)	Start day (d)	Peak day (d)	Germination percentage (d)	Germinative force (d)
CK (0)	8	15	34.67c	19.33b
10	8	15	55.33b	29.33a
50	7	13	66.00a	31.33a
100	9	16	43.67c	27.67a

Table 5 Effects of 6-BA on seed germination of *Bupleurum chinensis.*

6-BA concentrations (mg/L)	Start day (d)	Peak day (d)	Germination percentage (%)	Germinative force (%)
CK (0)	10	17	34.67c	19.33c
0.10	7	15	57.00b	33.00b
0.50	6	12	69.67a	41.67a
1.00	9	16	55.67b	26.00b

Table 6 Effect of SNP on seed germination of *Bupleurum chinensis.*

SNP concentrations (mg/L)	Start day (d)	Peak day (d)	Germination percentage (%)	Germinative force (%)
CK (0)	10	17	34.67c	19.33c
50	7	15	62.33a	25.00ab
125	8	15	57.33ab	28.33a
250	9	15	51.67b	23.00ab

3.5 Effect of SNP on Seed Germination of Bupleurum Chinensis

Table 6 shows that different concentrations of SNP can promote the germination of *Bupleurum chinense* seeds. The germination rate of 50 mg/L SPN was the highest, which was 79.78% higher than that of CK. There was no significant difference in germination rate between 125 mg/L and 250 mg/L SNP, but they were significantly higher than CK, and increased by 65.36%, 49.03% compared with CK. And 50 mg/L SPN 3 days earlier than CK start day, and there was no significant difference in germination peak day among treatments.

4. Conclusions

Soaking seeds with clear water, temperature management and pesticide treatment had significant effects on breaking seed dormancy, shortening start-up day and peak day, accelerating germination efficiency and improving uniformity and consistency of seedling emergence.

(1) The germination rate of the seeds of *Bupleurum chinensis* was significantly increased after a period of soaking in water. The germination rate of seed after soaking 12 h, 24 h in this experiment was higher than that of CK, and the seed germination rate was inhibited after soaking 36 h. It may be because the seed soaking time was too long, the substance exudated out and consumed too much, resulting in the impregnation harm [10];

(2) The seed was treated with variable temperature in the suitable temperature range, which was helpful to break the seed dormancy. The germination rate of seeds was the highest at 25 ℃/15 ℃ (day/night) and 15 ℃ under constant temperature;

(3) Various insecticides had effects on seed germination of *Bupleurum chinense* under different concentrations. Fifty mg/L GA_3 and 0.50 mg/L 6-BA significantly promoted seed germination, among which 0.50 mg/L 6-BA treatment had the highest germination rate, reaching 69.67%. When the concentration of GA_3 and 6-BA increased to 100 mg/L and 1.00 mg/L, the seed germination was inhibited to some extent.

In summary, after being soaked with water for 12 h, the seed of *Bupleurum chinense* was treated with the concentration of 0.50 mg/L in 6-BA. It was the best solution to germinate the seeds of *Bupleurum chinense* at 25 ℃/15 ℃ (day/night) temperature.

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