

Optimization of Seed Germination in Snow Lotus (*Saussurea involucre*): Synergistic Effects of PEG-GA 3 -IBA Treatments and Peat-Vermiculite Substrates

Zeyi Luo

College of Food and Pharmaceutical Science, Xinjiang Agricultural University, China

Shengjun Ma

wlmqmsj@xjau.edu.cn

College of Food and Pharmaceutical Science, Xinjiang Agricultural University, China

Bingbing Sun

College of Food and Pharmaceutical Science, Xinjiang Agricultural University, China

Xinjie Zhang

College of Food and Pharmaceutical Science, Xinjiang Agricultural University, China

Song Jiang

College of Food and Pharmaceutical Science, Xinjiang Agricultural University, China

Research Article

Keywords: *Saussurea involucre*, Seed germination, Pot experiment, Chemical treatment, Cultivation substrate

Posted Date: May 11th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-6339962/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Optimization of Seed Germination in Snow Lotus (*Saussurea involucre*): Synergistic Effects of PEG-GA₃-IBA Treatments and Peat-Vermiculite Substrates

Zeyi Luo^{1,2}, Shengjun Ma^{1,2*}, Bingbing Sun^{1,2}, Xinjie Zhang^{1,2}, Song Jiang^{1,2}

*Corresponding author: Shengjun Ma, email: wlmqmsj@xjau.edu.cn

¹College of Food and Pharmaceutical Science, Xinjiang Agricultural University, Urumqi 830052, China

²Modern Industrial College of Chinese Herbal Medicine, Xinjiang Agricultural University, Urumqi 830052, China

Abstract:

Background: *Saussurea involucre* (*S. involucre*), a rare medicinal plant of the Asteraceae family, primarily grows in high-altitude cliffs (~3000 m) in Xinjiang, China. Its dried aerial parts exhibit pharmacological properties such as anti-inflammatory, antioxidant, and antitumor effects. However, wild populations have sharply declined due to environmental changes and human activities, leading to its classification as a nationally protected species (Class II). Artificial cultivation is critical for conservation, but challenges persist: seeds exhibit strong dormancy, low viability, and a short lifespan, coupled with strict environmental requirements (e.g., altitude, temperature), resulting in poor germination rates (<10%). Previous studies focused on single-factor interventions (e.g., gibberellic acid (GA₃)) or substrate optimization but neglected synergistic effects and comprehensive protocols. This study innovatively combines polyethylene glycol (PEG), GA₃, and indole-3-butyric acid (IBA) treatments with peat-vermiculite substrates, aiming to establish an optimized protocol for enhancing seed germination and seedling resilience, thereby supporting scalable cultivation.

Results: Compared with those of the control group, the germination potential increased by 34.1%, the germination rate improved by 26.6%, the germination index rose by 26.67%, the vigor index increased by 94.7%, the length of the roots increased by 257.4%, the activities of three antioxidant enzymes (SOD, POD, and CAT) increased, and the malondialdehyde (MDA) content decreased.

Conclusions: When a 50% peat soil + 50% vermiculite substrate was used and seeds were soaked with a combination of 25 g/L PEG 4000, 150 mg/L GA₃, and 10 mg/L IBA, *S. involucre* seeds presented optimal comprehensive performance in terms of seed vigor indices and physiological indicators. Under these conditions, the seeds presented greater germination capacity, improved growth potential, and greater stress tolerance. These experimental results provide technical references for the artificial cultivation of *S. involucre* seeds.

Keywords: *Saussurea involucre*; Seed germination; Pot experiment; Chemical treatment; Cultivation substrate

* Corresponding author:
Shengjun Ma
wlmqmsj@xjau.edu.cn

29 *S. involucrata* is a plant of the genus *Saussurea* in the Asteraceae family. Its medicinal part is the dried above-ground part. It mainly grows on steep
 30 cliffs or in ice-moraine crevices at an altitude of approximately 3000 m in the Tianshan Mountains, Altai Mountains, Kunlun Mountains and other areas in
 31 Xinjiang, China [1, 2]. Research indicates that *S. involucrata* has anti-inflammatory, antioxidative, antitumor, analgesic, antifatigue, antiaging, antihypoxic,
 32 blood lipid reducing, neuroprotective, and immunomodulatory effects [2–4]. It possesses unique pharmacological effects and medicinal value.

33 Owing to environmental changes and the influence of human factors, the quantity of *S. involucrata* resources has decreased sharply. The Fourth National
 34 Census of Chinese Medicinal Resources in China revealed that the distribution area of *S. involucrata* in Xinjiang is less than 700,000 hectares. In the "List
 35 of Wild Plants under State Key Protection" issued in 2021, *S. involucrata* was classified as a Class II protected wild plant, and it was also included in the
 36 "Red Book of Chinese Medicinal Plants" published in 2022 [5, 6]. As a national third-level endangered species explicitly prohibited from being dug in
 37 China, the great medicinal value of *S. involucrata* cannot be fully utilized.

38 Artificial cultivation is an effective way to protect the resources of *S. involucrata*. However, owing to its special growth location, *S. involucrata* has
 39 high requirements for altitude, soil, temperature, moisture, etc., during artificial cultivation. There are certain difficulties in the propagation of *S. involucrata*
 40 seeds. Its own characteristics, such as seed dormancy, low seed viability, and short seed lifespan, as well as environmental characteristics such as soil,
 41 contribute to its low germination rate. In this study, germination experiments and pot experiments were performed to explore the effects of different chemical
 42 seed-soaking treatments and cultivation substrates on the germination of *S. involucrata* seeds.

43 **2. Materials and methods**

44 *2.1. Materials and chemicals*

45 Seeds of *S. involucrata* were collected from a meadow on the sunny slope of a high mountain near Tianshan Glacier No. 1 (43° 06' N, 87° 15' E) in
 46 Xinjiang, China, at an altitude of approximately 3500 m in June 2021. The seeds were kindly provided by the Forestry and Grassland Bureau of Urumqi
 47 County. *S. involucrata* seeds were identified by Associate Professor Shengjun Ma, College of Food Science and Pharmacy, Xinjiang Agricultural University.
 48 River sand soil was purchased from the flower market in Saybagh district, Urumqi, China.

49 Ethanol (absolute, 99.5%) ($\text{CH}_3\text{CH}_2\text{OH}$) and sodium dihydrogen phosphate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$) were purchased from Tianjin Zhiyuan Chemical Reagent
 50 Co., Ltd. (China). Methionine ($\text{C}_5\text{H}_{11}\text{O}_2\text{NS}$) and gibberellin ($\text{C}_{19}\text{H}_{22}\text{O}_6$) were purchased from Shanghai Blue Seasons Technology Development Co., Ltd.
 51 (China). Anhydrous calcium chloride (CaCl_2) was purchased from Xilong Science Co., Ltd. (China). Light calcium carbonate (CaCO_3) was purchased from
 52 Tianjin Shengao Chemical Reagent Co., Ltd. (China). Polyethylene glycol 4000 ($\text{HO}(\text{CH}_2\text{CH}_2\text{O})_n\text{H}$), choline chloride ($\text{C}_5\text{H}_{13}\text{Cl}_2\text{N}$), L-methionine
 53 ($\text{C}_5\text{H}_{11}\text{NO}_2\text{S}$) and 6-benzyladenine ($\text{C}_{12}\text{H}_{11}\text{N}_5$) were purchased from Shanghai Maclin Biochemical Technology Co., Ltd. (China). NaClO and disodium
 54 hydrogen phosphate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) were purchased from Tianjin Xinbote Chemical Co., Ltd. (China). Indole-3-acetic acid ($\text{C}_{10}\text{H}_9\text{NO}_2$) and indole-3-
 55 butyric acid ($\text{C}_{12}\text{H}_{13}\text{NO}_2$) were purchased from Goldclon (Beijing) Biotechnology Co., Ltd. (China). A catalase assay kit was purchased from Wuhan Pyeast
 56 Bioengineering Co., Ltd. (China). Thirty percent hydrogen peroxide (H_2O_2) was purchased from Chengdu Colon Chemical Co., Ltd. (China). Riboflavin
 57 ($\text{C}_{17}\text{H}_{20}\text{N}_4\text{O}_6$) was purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Sodium EDTA dihydrate ($\text{C}_{10}\text{H}_{14}\text{N}_2\text{O}_8\text{Na}_2 \cdot \text{H}_2\text{O}$) and trichloroacetic acid
 58 ($\text{C}_2\text{HCl}_3\text{O}_2$) were purchased from Tianjin Yongsheng Fine Chemical Co., Ltd. (China). Nitroterazolium blue chloride ($\text{C}_{40}\text{H}_{30}\text{Cl}_2\text{N}_{10}\text{O}_6$) and guaiacol
 59 ($\text{C}_7\text{H}_8\text{O}_2$) were purchased from Shanghai Yuanye Biotechnology Co., Ltd. (China). 2-Mercaptopyrimidine-4,6-diol ($\text{C}_4\text{H}_4\text{N}_2\text{O}_2\text{S}$) was purchased from
 60 Shanghai Bide Pharmaceutical Technology Co., Ltd. (China). Bromothymol blue ($\text{C}_{27}\text{H}_{28}\text{Br}_2\text{O}_5\text{S}$) was purchased from Tianjin Tianxin Fine Chemical
 61 Development Center.

2.2.1. Different growing media

S. involucrata seeds of uniform size were selected and soaked in a 200 mg/L gibberellin (GA₃) solution for 48 h. The seeds were treated with 70% alcohol for 30 s and then rinsed 3 times with sterile water. Next, the seeds were disinfected with a sodium hypochlorite solution (containing $\geq 2\%$ active chlorine) for 10 min and rinsed thoroughly. Thirty treated seeds were randomly selected and sown in different cultivation substrates (Table 1) in uniform-sized Petri dishes with a diameter of 9 cm. The sowing depth was 0.8 cm. Each treatment had 3 replicates, and a germination experiment was conducted in a constant-temperature environment. A fixed amount of water was added daily to ensure consistent substrate humidity. The germination period was 15 d, the temperature was 20 $\pm$ 1°C, and there was 14 hours of illumination. In accordance with the methods of 2.3, various seed vigor indices of *S. involucrata* were statistically analyzed, comprehensively evaluated, and the three best-performing test substrates were screened out.

Table 1 Numbers and ratios of test substrates

Code	Growing medium composition and proportion (V/V)
A	River Sand (100%)
B	Vermiculite (100%)
C	Perlite (100%)
D	Peat soil (100%)
E	River Sand (50%) + Vermiculite (50%)
F	River Sand (50%) + Perlite (50%)
G	River Sand (50%) + Peat soil (50%)
H	Vermiculite (50%) + Perlite (50%)
I	Vermiculite (50%) + Peat soil (50%)
J	Perlite (50%) + Peat soil (50%)
K	River Sand (33.4%) + Vermiculite (33.3%) + Perlite (33.3%)
L	River Sand (33.4%) + Vermiculite (33.3%) + Peat soil (33.3%)
M	River Sand (33.4%) + Perlite (33.3%) + Peat soil (33.4%)
M	Vermiculite (33.3%) + Perlite (33.3%) + Peat soil (33.4%)
O	River Sand (25%) + Vermiculite (25%) + Perlite (25%) + Peat soil (25%)

On the basis of the results of the germination experiment, several groups of suitable cultivation substrates were further screened for the pot experiment, with 3 replicates for each treatment. The pretreatment of seeds and substrates, the constant-temperature environmental conditions, and the standard of quantitative water supplementation were the same as those used in the germination experiment. One hundred milliliters of plant nutrient mixture was added every three days to supplement the trace elements in the substrate and ensure the healthy growth of *S. involucrata* seedlings. In accordance with the methods of 2.3, 2.4 and 2.5, the MDA content, chlorophyll content, and activities of three antioxidant enzymes, CAT, SOD, and POD, were measured and calculated once a week after the seedlings had grown for 40 days. These indicators were used as the criteria for evaluating the growth quality of the seedlings.

2.2.2. Different chemical treatments

In accordance with the International Rules for Seed Testing and [7], on the basis of preliminary experiments, the paper-towel germination method was used to investigate the optimal chemical treatments for the germination of *S. involucrata* seeds.

First, a single-factor experiment was carried out. Different seed-soaking treatments were set up. Sterile water (control group), 15 mmol/L CaCl₂, 35 g/L PEG 4000, 200 mg/L GA₃, 20 mg/L IBA, 2 mg/L IAA, 100 mg/L CCC, and 50 mg/L CTK were used to soak the seeds of *S. involucrata* for 48 h. After the soaking treatment, the seeds were first treated with 70% alcohol for 30 s and then rinsed 3 times with sterile water. The seeds were subsequently disinfected with sodium hypochlorite solution (containing $\geq 2\%$ active chlorine) for 10 min and then rinsed 3–4 times with sterile water. The treated seeds were placed in sterile Petri dishes with two layers of filter paper, a certain amount of sterile water was added, and then they were placed in an artificial incubator for a 15-day germination experiment (daytime: 28°C, 16-hour light period, light intensity of 1700 lx; nighttime: 26°C, 8-hour dark period without light). Each treatment included 3 Petri dishes, and each dish contained 30 seeds. In accordance with the methods described in section 2.3, various seed vigor indices of *S. involucrata* were statistically analyzed. The three best-performing treatment reagents were comprehensively evaluated and screened.

On the basis of the results of the single-factor experiment, the L9(3⁴) orthogonal array was selected, and the orthogonal experiment was carried out according to the design in Table 1 to investigate the optimal combined seed-soaking treatment.

Table 2 Factors and levels for the orthogonal experiment

Level	Factor		
	PEG 4000(A)	GA ₃ (B)	IBA(C)
1	25 g/L	150 mg/L	10 mg/L
2	30 g/L	200 mg/L	15 mg/L
3	35 g/L	250 mg/L	20 mg/L

On the basis of the paper-towel germination test, the optimal seed-soaking reagents selected from the two previous experiments were used for the soil-based pot experiment. Five treatment groups were set up, namely, the sterile water group (control group), the 35 g/L PEG 4000 group, the 200 mg/L GA₃ group, the 20 mg/L IBA group, and the 25 g/L PEG 4000 + 150 mg/L GA₃+10 mg/L IBA group (combined treatment group). Each group had 3 replicates. Fifty *S. involucrata* seeds with a consistent appearance and plump grains were randomly selected from each pot. After being soaked in different reagents for 48 h, the seeds were evenly spread in sandy soil. The sowing depth was 0.8 cm, the humidity was maintained at 50%, and natural light was provided. The samples were collected after 40 days. In accordance with the methods of 2.3, 2.4, and 2.5, the activities of three antioxidant enzymes, CAT, SOD, and POD, and the MDA content were measured and calculated once a week after the seedlings had grown for 40 days. These indicators were used as the criteria for evaluating the growth quality of the seedlings.

2.3. Determination of seed vigor

2.3.1. Germination rate and germination potential

In accordance with the methods of Kunal Adhikary et al. [8], the germination of *S. involucrata* seeds was observed and recorded daily. The germination standard is determined by the emergence of the plumule from the soil. The overall germination period was 20 d. The number of germinated seeds was determined each day, and the germination rate and germination potential of the seeds were calculated according to the following formulas:

$$\text{Germination rate(\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of test seeds}} \times 100\%$$

$$\text{Germination potential(\%)} = \frac{\text{Number of seeds germinated within 7 days}}{\text{Total number of test seeds}} \times 100\%$$

2.3.2. Germination index and seed vigor index

Referring to and improving the methods of Alok Kumar Singh, Mingye Zhang et al. [9, 10], the germination index and seed vigor index were calculated. After 20 days, germination was completed, and the germination index and vigor index of *S. involucrata* seeds were calculated via the following formulas:

110
$$\text{Germination Index (GI)} = \sum \frac{GT}{DT}$$

111 where GT represents the number of germinated seeds within T days and DT represents the corresponding number of germination days.

112
$$\text{Seed Vigor Index (SVI)} = \text{GI} \times S$$

113 where S represents the average fresh weight of the seedlings (cm).

114 2.3.3. Root length, fresh weight and dry weight

115 The methods of Miłosz Rutkowski et al. were modified [11].

116 Root length: Five days after germination, on the 25th day after sowing, 3 seedlings from each of the 3 sowing depths were randomly selected, the root
117 lengths of the *S. involucrata* seedlings were measured with a Vernier caliper, the average value for each group was calculated, and the measurement data
118 were accurate to 0.01 cm.

119 Fresh weight and dry weight: After the water was blotted with filter paper, the fresh weights of the *S. involucrata* seedlings were measured with an
120 electronic balance. The seedlings were placed in an oven at 105°C for 30 minutes and then dried in an oven at 70°C for 48 h until the weight was constant.
121 The dry weights of the *S. involucrata* seedlings were then determined with an electronic balance, and the measurement data were accurate to 0.01 mg.

122 2.4. Determination of antioxidant enzyme activity

123 2.4.1. Superoxide dismutase (SOD) activity

124 The determination of SOD enzyme activity in *S. involucrata* seedlings refers to and improves upon the methods of Charles Beauchamp and Fridovich
125 [12].

126 *S. involucrata* seedling leaves (0.5 g) were placed in a precooled mortar, 0.05 M sodium phosphate buffer (PBS) with a pH of 7.8 was added, and the
127 samples were ground. The volume of the grinding liquid was increased to 10 mL, and this mixture constituted the extract. The extract was poured into a
128 centrifuge tube and centrifuged at 4°C and 8000 rpm for 15 min. The supernatant was the crude enzyme mixture.

129 Then, 1.5 mL of 50 mM sodium phosphate buffer (PBS), 0.3 mL of 130 mM methionine solution, 0.3 mL of 750 μM nitroblue tetrazolium solution,
130 0.3 mL of 100 μM disodium ethylenediaminetetraacetate solution, 0.3 mL of 20 μM riboflavin solution and 0.2 mL of distilled water were added to a 5-mL
131 centrifuge tube, and 0.1 mL of the crude enzyme mixture was added. Each treatment was performed in triplicate. The mixture was placed under 4000 lux
132 illumination for 20 min and stored in the dark. A set of solution treatments without the enzyme solution was prepared under illumination as the control, and
133 another set of solution treatments without the enzyme solution was placed in the dark for zero adjustment. The absorbance value was measured at a
134 wavelength of 560 nm, and the SOD activity was calculated according to the following formula.

135
$$\text{SOD activity (U} \cdot \text{g}^{-1} \text{FW)} = \frac{(A_{CK} - A_E) \times V}{0.5 \times A_{CK} \times W \times V_t}$$

136 SOD activity is expressed as enzyme units per gram of fresh weight (U·g⁻¹FW); A_{CK}: the absorbance of the light-illuminated control tube; A_E: the
137 absorbance of the sample tube; V: the total volume of the extract (mL); V_t: the amount of the crude enzyme mixture used for determination (mL); W: the
138 fresh weight of the sample (g).

139 2.4.2. Peroxidase (POD) activity

140 The methods of Manoranjan Kar and Dinabandhu Mishra [13] were improved.

141 Fifty milliliters of 50 mM sodium phosphate buffer (pH=6.0) was prepared, and 28 μL of guaiacol was added. After dissolution, 19 μL of 30% H₂O₂
142 solution was added, and the mixture was mixed to prepare the reaction mixture. A total of 3.9 mL of the reaction mixture and 0.1 mL of the crude enzyme

143 mixture in the 2.4.1 were added to a centrifuge tube and shaken well. Each treatment was performed in triplicate. The time was recorded well, the absorbance
 144 value was measured at a wavelength of 470 nm every minute, and a mixture of 3.9 mL of the reaction mixture and 0.1 mL of sodium phosphate buffer was
 145 used for zero adjustment. The POD activity is calculated according to the following formula.

$$146 \quad \text{POD activity (U} \cdot \text{g}^{-1} \cdot \text{min}^{-1} \text{FW)} = \frac{\Delta \text{OD}_{470} \times V_t}{W \times 0.01 \times t \times V_s}$$

147 ΔOD_{470} represents the change in absorbance during the reaction time; V_t represents the total volume of the extracted enzyme mixture (mL); FW
 148 represents the fresh weight of the *S. involucrata* sample (g); V_s represents the volume of the enzyme mixture used for the determination (mL); and t represents
 149 the reaction time (min).

150 2.4.3. Catalase (CAT) activity

151 The determination of CAT activity refers to and improves upon the methods of Hugo Aebi [14].

152 The reaction mixture was prepared by mixing 60 mL of 0.1 M hydrogen peroxide solution and 240 mL of 0.1 M sodium phosphate buffer (pH=6)
 153 evenly. To determine the activity of catalase (CAT), 2.9 mL of the reaction mixture was added to a 5-mL centrifuge tube, and 0.1 mL of the crude enzyme
 154 mixture in 2.4.1 was added. Each treatment was performed in triplicate. Sodium phosphate buffer was used as the blank control, the change in the OD_{240}
 155 was measured every 30 s, and the data were recorded and counted. The CAT activity was subsequently calculated.

$$156 \quad \text{CAT activity (U} \cdot \text{g}^{-1} \cdot \text{min}^{-1} \text{FW)} = \frac{\Delta \text{OD}_{240} \times V_t}{W \times V_s \times 0.01 \times t}$$

157 ΔOD_{240} : the change in absorbance per unit time; V_t : the total volume of the sample-extracted enzyme solution (mL); W: the fresh weight of the *S.*
 158 *involutrata* sample (g); V_s : the volume of the enzyme mixture used for the determination (mL); t: the reaction time (min).

159 2.5. Malondialdehyde (MDA) content

160 Referring to and improving the methods of Christophe Bailly et al. [15].

161 *S. involucrata* seedling leaves (0.5 g) were removed, 5 mL of 10% (m/V) trichloroacetic acid was added, and the mixture was ground. This is the total
 162 extract. The mixture was centrifuged at 8000 rpm for 10 min, the supernatant was removed, an equal volume of 0.6% thiobarbituric acid solution was added,
 163 and the mixture was mixed to make the reaction mixture. The samples were boiled in a boiling-water bath at 100°C for 30 min, cooled, and then centrifuged
 164 at 8000 rpm for 10 min. The absorbance values of the supernatants were measured at 450, 532, and 600 nm. The malondialdehyde (MDA) content was
 165 subsequently calculated.

$$166 \quad \text{MDA content (mmol} \cdot \text{g}^{-1} \text{FW)} = \frac{[6.452 \times (\text{OD}_{532} - \text{OD}_{600}) - 0.599 \times \text{OD}_{450}] \times V_t \times V_f}{V_s \times \text{FW}}$$

167 OD_{532} is the absorbance at 532 nm; OD_{600} is the absorbance at 600 nm; OD_{450} is the absorbance at 450 nm; V_t is the total volume of the extract (mL);
 168 V_f is the volume of the reaction mixture; V_s is the volume of the extract used for the determination (mL); FW is the fresh weight of the leaves (g).

169 2.6. Data analysis

170 Excel software was used for data organization, GraphPad Prism 10 software was used to create bar charts with error bars, SPSS Statistics 27 software
 171 was used for variance analysis, and OriginPro 2025 software was used to create correlation analysis heatmaps.

3. Results

3.1. Germination response to different growing media

3.1.1. Analysis of seed vigor indicators

As shown in Table 3, the cultivation substrates with relatively high overall seed vigor indices include A (river sand), B (vermiculite), E (river sand: vermiculite=1 : 1), F (river sand: perlite=1 : 1), H (vermiculite: perlite=1 : 1) and I (vermiculite: peat soil=1 : 1). Among them, A, E and H have 6 relatively high indices, B has 5, F has 4 and I has 3. However, in the actual cultivation process, due to the poor soil moisture retention of B, F and H, substrates A, E and I were selected for further 40-day pot experiments.

Table 3 Seed Vigor Indices of *S. involucrata* under Different Cultivation Substrates

Cod e	Germination rate (%)	Germination potential (%)	Germination index	Seed vigor index	Fresh weight (mg)	Dry weight (mg)	Root length (cm)
A	36.67±16.67 ^{ab}	31.11±9.62 ^{ab}	13.5679±4.3866 ^a	524.02±177.79 ^a	38.43±1.76 ^{abcd}	6.7±0.56 ^a	2.12±1.1370 ^{def}
B	27.78±18.36 ^{abcde}	24.44±16.78 ^{abcd}	11.2597±7.8081 ^a b	332.23±229.91 ^a b	29.37±0.7 ^{cd}	3.47±0.55 ^{de}	3.25±1.2030 ^{abcd}
C	26.67±6.67 ^{abcde}	16.67±0 ^{bcde}	6.1725±2.0409 ^{bc} d	187.08±47.71 ^{bc} d	30.8±2.35 ^{bcd}	3.3±1.08 ^{de}	2.36±1.1597 ^{bcde}
D	17.78±3.85 ^{de}	8.89±3.85 ^{de}	3.4476±1.2596 ^{cd}	124.15±49.14 ^{cd}	35.77±1.06 ^{abcd}	3±0.7 ^{ef}	1.28±0.5805 ^{ef}
E	24.44±5.09 ^{abcde}	21.11±1.92 ^{abcde}	9.0238±1.3015 ^{ab} c	340.64±70.63 ^{abc}	37.53±2.45 ^{abcd}	4±0.3 ^{bcd}	3.54±1.2651 ^{abc}
F	38.89±5.09 ^a	34.44±12.62 ^a	14.2722±3.9880 ^a	391.42±188.39 ^a	27.33±8.62 ^d	4.67±0.86 ^b	0.86±0.3406 ^f
G	26.67±3.33 ^{abcde}	15.56±1.92 ^{bcde}	5.2259±0.9807 ^{cd}	240.41±63.31 ^{cd}	45.67±6.51 ^a	3.8±0.44 ^{bcde}	2.28±1.1555 ^{cde}
H	35.56±10.18 ^{abc}	27.78±18.36 ^{abc}	11.7864±6.3376 ^a b	513.94±307.6 ^{ab}	42±13 ^{ab}	3.9±0.8 ^{bcde}	3.60±1.5606 ^{ab}
I	30±3.33 ^{abcd}	11.11±1.92 ^{cde}	4.8162±0.9272 ^{cd}	210.77±48.51 ^{cd}	44.7±12.45 ^a	3.97±0.4 ^{bcd}	2.86±1.4699 ^{abcd}
J	18.89±6.94 ^{cde}	13.33±8.82 ^{bcde}	5.0092±2.5844 ^{cd}	145.89±74.29 ^{cd}	29.3±0.95 ^{cd}	2.13±0.25 ^f	2.56±1.2422 ^{abcd} e
K	14.44±8.39 ^{de}	14.44±8.39 ^{bcde}	4.7995±2.9119 ^{cd}	149.24±86.43 ^{cd}	31.57±1.16 ^{bcd}	3.5±0.3 ^{de}	3.22±1.0545 ^{abcd}
L	18.89±3.85 ^{cde}	11.11±10.72 ^{cde}	3.3959±2.1085 ^{cd}	132.67±82.11 ^{cd}	39.1±1.85 ^{abcd}	3.63±0.42 ^{cde}	2.00±1.3928 ^{def}
M	12.22±8.39 ^e	4.44±1.92 ^e	1.8474±1.0599 ^d	69.78±37.43 ^d	38.47±1.82 ^{abcd}	3.53±0.67 ^{cde}	1.42±0.5404 ^{ef}
N	15.56±6.94 ^{de}	10±5.77 ^{de}	3.7478±2.1319 ^{cd}	128.42±34.04 ^{cd}	38.13±10.53 ^{abc} d	4.47±0.55 ^{bc}	2.46±1.6712 ^{bcde}
O	20±10 ^{bcde}	14.44±5.09 ^{bcde}	4.4275±2.04302 ^c d	176.08±75.66 ^{cd}	40.23±1.56 ^{abc}	3.83±0.31 ^{bcd} e	3.77±1.2785 ^a

*The data in the above table are expressed as the means ± standard deviations. Lowercase letters indicate significant differences among different cultivation substrates for the same seed vigor index ($p < 0.05$).

3.1.2. Antioxidant enzyme activity of seedlings and malondialdehyde (MDA) content of seedlings

As shown in Figure 1A, the SOD activity of *S. involucrata* seedlings generally tended to increase first but then decrease. Moreover, the order of SOD activity under different cultivation substrates varied across different weeks. At the 1st week, there was no significant difference in the SOD activity of *S. involucrata* seedlings cultivated on the three substrates ($p < 0.05$). At the 2nd week, the SOD activity of *S. involucrata* seedlings cultivated in the river sand substrate was the highest and was significantly different from that of the other substrates ($p < 0.05$). At the 3rd week, the SOD activity of the *S. involucrata* seedlings cultivated in the river sand + vermiculite substrate was the highest. At the 4th and 5th weeks, the SOD activity of *S. involucrata* seedlings cultivated in the peat soil + vermiculite substrate was the highest. Although the SOD activity of *S. involucrata* seedlings cultivated in the peat soil + vermiculite substrate was not the highest in the early stage of cultivation, over time, its activity tended to increase and gradually became the optimal substrate.

As shown in Figure 1B, the POD activity of *S. involucrata* seedlings generally tended to increase gradually. At the 1st week, the POD activity of *S. involucrata* seedlings cultivated in the river sand substrate was the highest and was significantly different from that of the other substrates ($p < 0.05$). At the 2nd week, there was no significant difference in the POD activity of *S. involucrata* seedlings cultivated on the three substrates. At the 3rd, 4th, and 5th weeks, the POD activity of *S. involucrata* seedlings cultivated in the peat soil + vermiculite substrate was the highest. At the 4th and 5th weeks, there was a significant difference in activity between the peat soil + vermiculite group and the other groups ($p < 0.05$). Overall, over time, the activity of the peat soil + vermiculite group gradually increased and became the optimal treatment group.

As shown in Figure 1C, the CAT activity of *S. involucrata* seedlings generally tended to increase gradually. With respect to the week fixed, at all weeks, the CAT activity of *S. involucrata* seedlings cultivated in the peat soil + vermiculite substrate was the highest and was significantly different from that of the other groups ($p < 0.05$). In terms of the CAT activity level, this treatment was the optimal treatment group.

As shown in Figure 1D, the MDA contents of the three treatment substrates were different at different weeks. Among them, at the 1st and 3rd weeks, the MDA content of *S. involucrata* seedlings in the river sand group was the lowest and was significantly different from that of the other groups ($p < 0.05$). At the 2nd, 4th, and 5th weeks, the MDA content of *S. involucrata* seedlings in the peat soil + vermiculite group was the lowest and was significantly different from that in the other groups ($p < 0.05$). Overall, when the substrate was river sand or peat soil + vermiculite, the MDA content of *S. involucrata* seedlings was relatively low.

In conclusion, over time, the activities of the three enzymes in *S. involucrata* seedlings in the peat soil + vermiculite group gradually increased, and the MDA content gradually decreased, gradually becoming the optimal group. When *S. involucrata* was cultivated in peat soil + vermiculite substrate, its antioxidant activity and stress resistance were the greatest.

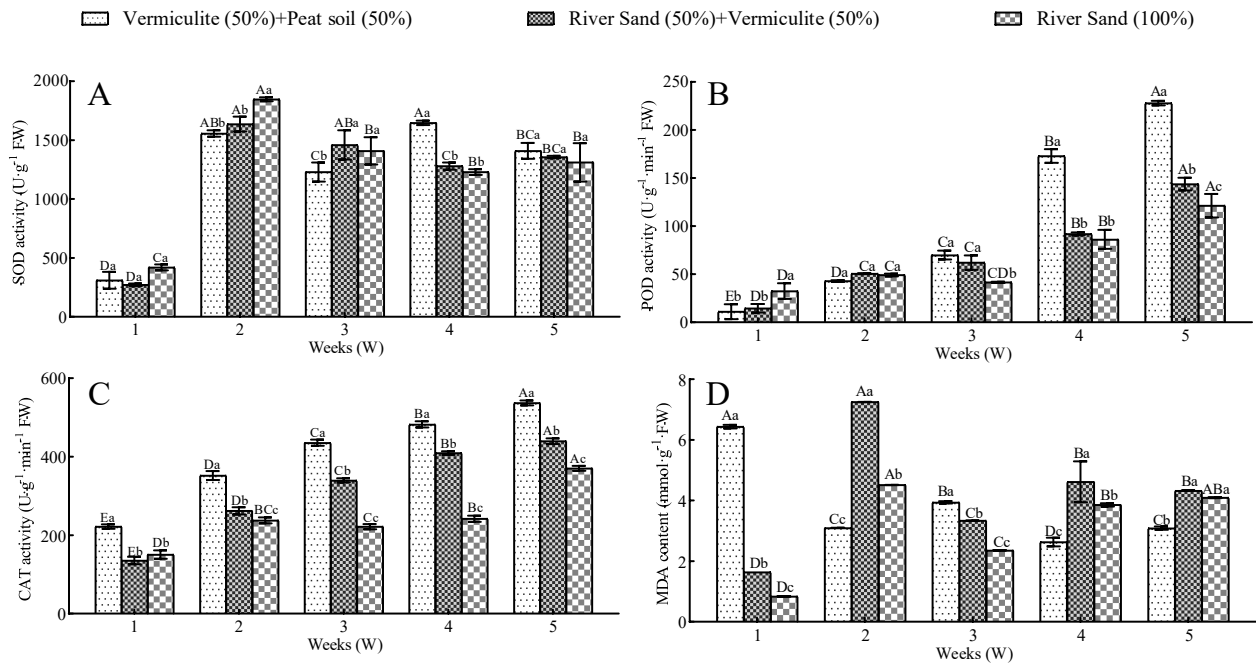


Figure 1 Effects of different cultivation substrates on SOD (A), POD (B), and CAT (C) enzyme activities and the MDA content (D) in *S. involucrata* seedling leaves.

*Data are presented as the means $\pm$ standard deviations of 3 replicates per sample. Different capital letters indicate significant differences ($p < 0.05$) among weeks for the same substrate; different lowercase letters indicate significant differences ($p < 0.05$) among substrates in the same week.

3.1.3. Correlation analysis of parameters

Figure 2 revealed that the germination rate of *S. involucrata* was extremely significantly negatively correlated with root length and the MDA content ($p < 0.001$) and extremely significantly positively correlated with SOD activity ($p < 0.001$). The germination potential was extremely significantly positively correlated with the germination index, seed vigor index, and dry weight ($p < 0.001$) and extremely significantly negatively correlated with POD and CAT activities ($p < 0.001$). The germination index was extremely significantly positively correlated with the seed vigor index and dry weight ($p < 0.001$) and extremely significantly negatively correlated with POD and CAT activities ($p < 0.001$). The seed vigor index was extremely significantly positively correlated with dry weight ($p < 0.001$) and extremely significantly negatively correlated with POD and CAT activities ($p < 0.001$). Dry weight was extremely significantly negatively correlated with POD and CAT activities ($p < 0.001$). Root length was extremely significantly negatively correlated with SOD activity ($p < 0.001$) and extremely significantly positively correlated with the MDA content ($p < 0.001$). SOD activity was extremely significantly negatively correlated with the MDA content ($p < 0.001$). POD activity was extremely significantly positively correlated with CAT activity ($p < 0.001$).

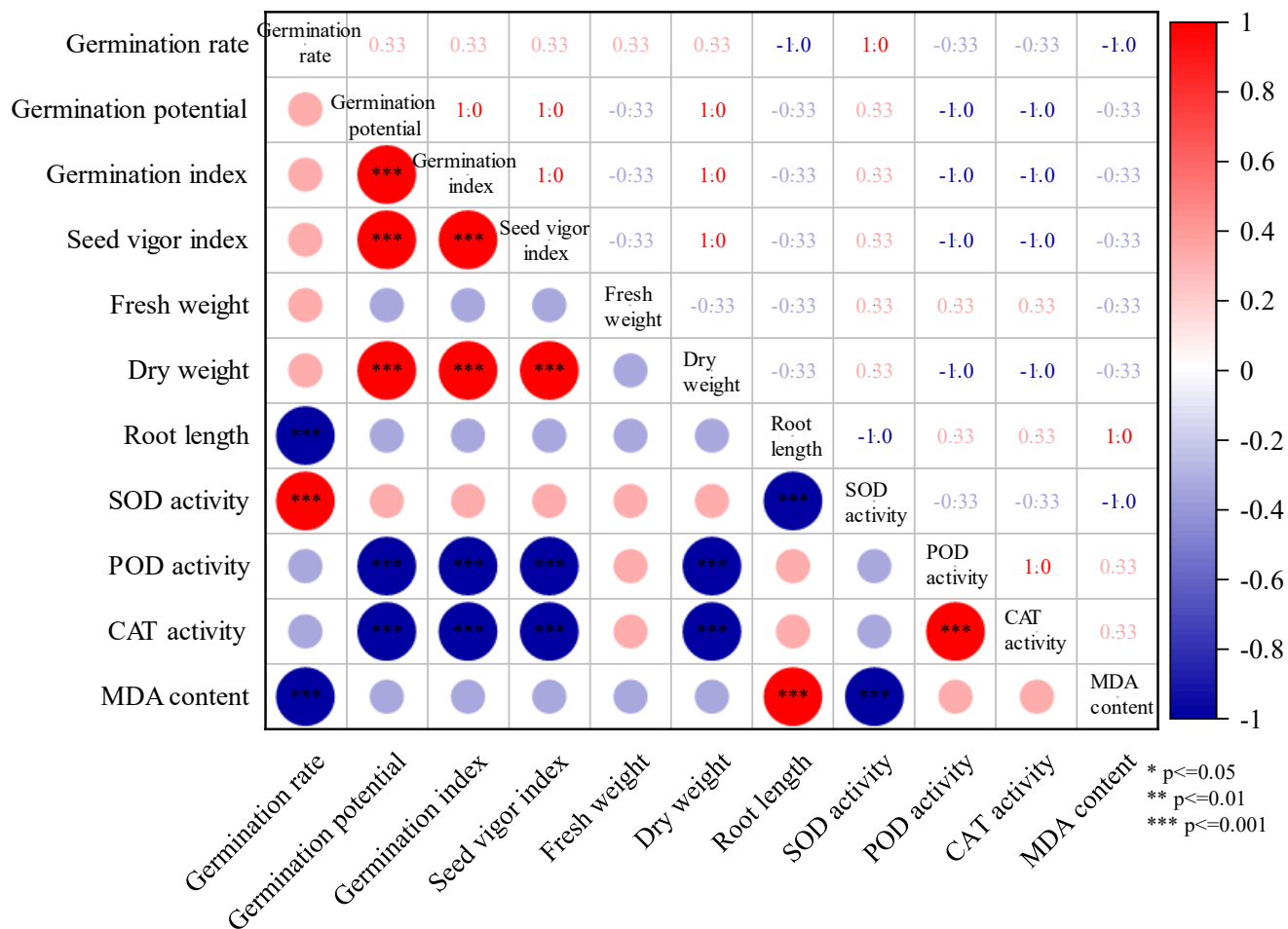


Figure 2 Correlation analysis diagram of *S. involucrata* seed germination indices at different sowing depths (Kendall rank correlation coefficient)

3.2. Germination response to different chemical treatments

3.2.1. Analysis of seed vigor indicators

The results of the single-factor experiment are shown in Table 4. The treatment with the highest germination potential and germination rate was 35 g/L PEG 4000. The treatments with the highest germination indices were 15 mmol/L CaCl₂, 35 g/L PEG 4000, 200 mg/L GA₃, and 20 mg/L IBA. The treatments with the best vigor index and fresh weight were 200 mg/L GA₃, followed by 35 g/L PEG 4000 and 20 mg/L IBA. The treatments with the best dry weight were 15 mmol/L CaCl₂, 35 g/L PEG 4000, 200 mg/L GA₃, 20 mg/L IBA, 2 mg/L IAA, and 50 mg/L CTK. The treatment with the longest root length was 20 mg/L IBA. Overall, among the seed vigor indices, the treatments with the highest indices were 35 g/L PEG 4000, 200 mg/L GA₃, and 20 mg/L IBA. Compared with those of the control group, their germination indices increased by 53.23%, 44.57%, and 43.84%, respectively, and their vigor indices increased by 116.40%, 233.62%, and 133.09%, respectively, compared with those of the control group; these differences were significant ($p < 0.05$). Therefore, the three soaking treatments with 35 g/L PEG 4000, 200 mg/L GA₃, and 20 mg/L IBA had the greatest effects on promoting the germination of *S. involucrata* seeds.

241 Table 4 Results of the single-factor experiments in the paper germination test

Chemical treatment	Germination potential (%)	Germination Rate (%)	Germination index	Seed vigor index	Fresh weight (mg)	Dry weight (mg)	Root length (cm)
15 mmol/L CaCl ₂	56.67±28.89 ^{bc}	75.00±0.00 ^b	9.10±0.27 ^{abc}	394.80±13.16 ^{bcd}	43.40±0.79 ^f	3.20±0.30 ^{bc}	2.18±0.08 ^c
35 g/L PEG 4000	76.67±5.77 ^a	93.33±2.89 ^a	10.45±0.54 ^a	485.16±31.38 ^{bc}	46.40±0.72 ^e	4.17±0.95 ^{ab}	0.91±0.12 ^f
200 mg/L GA ₃	58.33±28.89 ^b	70.00±5.00 ^b	9.86±1.22 ^{ab}	747.98±101.25 ^a	75.80±1.87 ^a	3.90±0.53 ^{abc}	2.11±0.1 ^c
20 mg/L IBA	53.33±7.64 ^{bc}	70.00±10.00 ^b	9.81±1.80 ^{ab}	522.59±95.67 ^b	53.30±0.62 ^d	3.50±0.46 ^{abc}	3.33±0.04 ^a
2 mg/L IAA	45.00±5.00 ^c	70.00±5.00 ^b	6.47±1.30 ^c	364.91±67.4 ^{cde}	56.50±1.11 ^c	4.00±0.66 ^{abc}	2.41±0.09 ^b
100 mg/L CCC	50.00±0.00 ^{bc}	73.33±7.63 ^b	7.41±2.07 ^{bc}	309.15±88.91 ^{de}	41.70±1.06 ^f	3.20±0.60 ^{bc}	1.72±0.07 ^d
50 mg/L CTK	48.33±16.07 ^{bc}	68.33±20.21 ^b	6.81±2.51 ^c	423.37±161 ^{bcd}	61.90±1.35 ^b	4.40±0.44 ^a	0.71±0.15 ^e
CK	50.00±5.00 ^{bc}	65.00±5.00 ^b	6.82±0.30 ^c	224.2±7.33 ^e	32.9±0.69 ^g	3.00±0.10 ^c	1.51±0.07 ^e

242 *The data in the table are presented as the means ± standard deviations. Different lowercase letters denote the significance of the same seed vigor index
 243 under different temperature and humidity treatments ($p < 0.05$).

244 On the basis of the single-factor experiment, three reagents, PEG 4000, GA₃, and IBA, were selected for seed soaking in combination. Three
 245 concentration levels were chosen for orthogonal optimization. The results are shown in Table 5.

246 Table 5 Results of the orthogonal experiment

Number	Factor				Germination potential (%)	Germination rate (%)	Germination index	Seed vigor index	Root length (cm)
	A (PEG 4000 treatment)	B (GA ₃ treatment)	C (IBA treatment)	D					
1	1	1	1	1	55.00	76.67	7.6103	372.18	3.3667
2	1	2	2	2	45.00	61.67	6.1524	257.66	2.2333
3	1	3	3	3	26.67	41.67	4.0341	179.83	2.5000
4	2	1	3	2	38.33	53.33	6.0897	224.38	2.8667
5	2	2	1	3	50.00	68.33	6.2675	321.99	2.8000
6	2	3	2	1	40.00	56.67	5.1818	136.09	1.3333
7	3	1	2	3	38.33	53.33	5.0230	188.09	3.7333
8	3	2	3	1	33.33	48.33	3.5468	121.77	2.5000
9	3	3	1	2	43.33	58.33	6.1492	184.55	2.6000
K ₁	205.29%	192.01%	210.13%						
K ₂	215.31%	193.04%	206.07%						
K ₃	197.70%	233.25%	202.10%						
R	17.61%	41.25%	8.04%						

247 According to the intuitive analysis of Table 5, the order of influence of the three factors on the germination of *S. involucrata* seeds was GA₃ treatment >
 248 PEG 4000 treatment > IBA treatment. Under the conditions of PEG 4000, GA₃, and IBA concentrations of 25 g/L, 150 mg/L, and 10 mg/L, respectively,

the germination potential, germination rate, and vigor index all reached their highest values. Compared with those in the control group, the germination potential increased by 34.1%, the germination rate increased by 26.6%, the germination index increased by 26.67%, the vigor index increased by 94.7%, and the root length increased by 257.4%, which was significantly greater than the optimal conditions for a single factor.

Table 6 Analysis of variance table

Factor	Sum of squares of deviations	Degrees of freedom	Mean square	F Ratio	Significance
PEG 4000	50308.943	2	25154.471	10.871	0.001
GA ₃	42688.279	2	21344.140	9.224	0.001
IBA	71894.364	2	35947.182	15.535	0.000
Error	46279.293	20	2313.965		

The analysis of variance table in Table 6 shows that the significance of all three factors is less than 0.05, which is similar to the intuitive analysis result in Table 5.

3.2.2. Antioxidant enzyme activity and malondialdehyde (MDA) content of the seedlings

As shown in Figure 3A, over time, the SOD activity of *S. involucrata* seedlings generally tended to first increase, then decrease, and then increase again. During the 1st and 2nd weeks of growth, the SOD activity was the highest in the treatment with 25 g/L PEG 4000 + 150 mg/L GA₃ + 10 mg/L IBA (combined treatment); during the 3rd and 4th weeks of growth, the enzyme activity was the highest in the blank control group, and the SOD activities of all the treatment groups were lower than those of the blank group; during the 5th week of growth, the SOD activity was the highest in the 20 mg/L IBA treatment group. There were significant differences ($p < 0.05$) between the treatment group with the highest SOD activity each week and the other treatment groups in the same week.

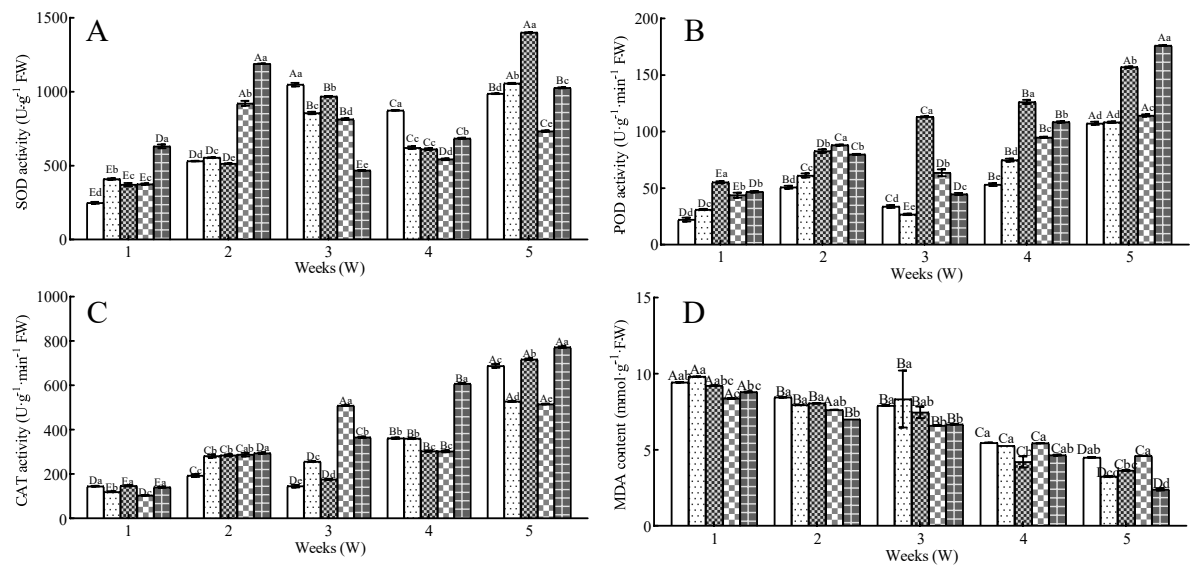
As shown in Figure 3B, over time, the POD activity of *S. involucrata* seedlings generally tended to increase. During the 1st, 3rd, and 4th weeks of growth, the POD activity was the highest in the 200 mg/L GA₃ treatment; during the 2nd week of growth, the POD activity was the highest in the 20 mg/L IBA treatment; and during the 5th week of growth, the POD activity was the highest in the combined treatment. There were significant differences ($p < 0.05$) between the treatment group with the highest POD activity each week and the other treatment groups in the same week.

As shown in Figure 3C, over time, the CAT activity of *S. involucrata* seedlings generally tended to increase. During the 1st, 2nd, 4th, and 5th weeks of growth, the CAT activity was the highest in the combined treatment. Among them, during the 4th and 5th weeks, there were significant differences ($p < 0.05$) in CAT activity between the highest treatment and the other treatments; during the 3rd week of growth, the CAT activity was the highest in the 20 mg/L IBA treatment, and there were significant differences ($p < 0.05$) between it and the other treatments.

As shown in Figure 3D, over time, the MDA content of *S. involucrata* seedlings generally tended to decrease. During the 1st week, the MDA contents in the 200 mg/L GA₃ treatment, 20 mg/L IBA treatment, and combined treatment groups were the lowest, and there were no significant differences ($p < 0.05$) among them. During the 2nd week, the MDA contents in the 20 mg/L IBA treatment and combined treatment groups were the lowest, and there were no significant differences ($p < 0.05$) between them. During the 3rd week, the MDA contents in the 200 mg/L GA₃ treatment, 20 mg/L IBA treatment, and combined treatment groups were the lowest, and there were no significant differences ($p < 0.05$) among them. During the 4th week, the MDA contents in the 200 mg/L GA₃ treatment and combined treatment groups were the lowest, and there were no significant differences ($p < 0.05$) between them. During the 5th week, the MDA contents in the 35 g/L PEG 4000 treatment and combined treatment groups were the lowest, and there were no significant differences ($p < 0.05$) between them.

279 In conclusion, although the activities of the three enzymes were not always the highest in the combined treatment group, the MDA content was always
 280 the lowest among all the treatment groups. This means that, from the perspective of the antioxidant process, the antioxidant enzyme activity of *S. involucrata*
 281 was not the highest, but in terms of the antioxidant results, its ability to scavenge peroxides was the strongest, the degree of cell damage was the lightest,
 282 the organism's health state was the best, the stress resistance was the strongest, and finally, the protective effect on the plant was the strongest. Therefore, in
 283 general, it is better to choose the combined treatment for *S. involucrata* seeds.

□ CK ▨ 35 g/L PEG 4000 ▩ 200 mg/L GA₃ ▤ 20 mg/L IBA ▦ 25 g/L PEG 4000+ 150 mg/L GA₃+ 10 mg/L IBA



284
 285 Figure 3 Effects of different chemical treatments on *S. involucrata* seedling leaf SOD (A), POD (B), and CAT (C) activities and the MDA (D) content. The
 286 data are presented as the means $\pm$ standard deviations of 3 replicates per sample. Capital letters indicate significant differences among weeks for the same
 287 reagent ($p < 0.05$), and lowercase letters indicate significant differences among reagents in the same week ($p < 0.05$).

288 3.2.3. Correlation analysis of parameters

289 As shown in Figure 4, there was a significant positive correlation between the germination rate and the germination potential ($p < 0.05$), an extremely
 290 significant positive correlation between the seed vigor index and the fresh weight ($p < 0.01$), a significant positive correlation between the fresh weight and
 291 POD activity ($p < 0.05$), a significant positive correlation between the dry weight and CAT activity ($p < 0.05$), and a significant negative correlation between
 292 the CAT activity and the MDA content ($p < 0.05$).

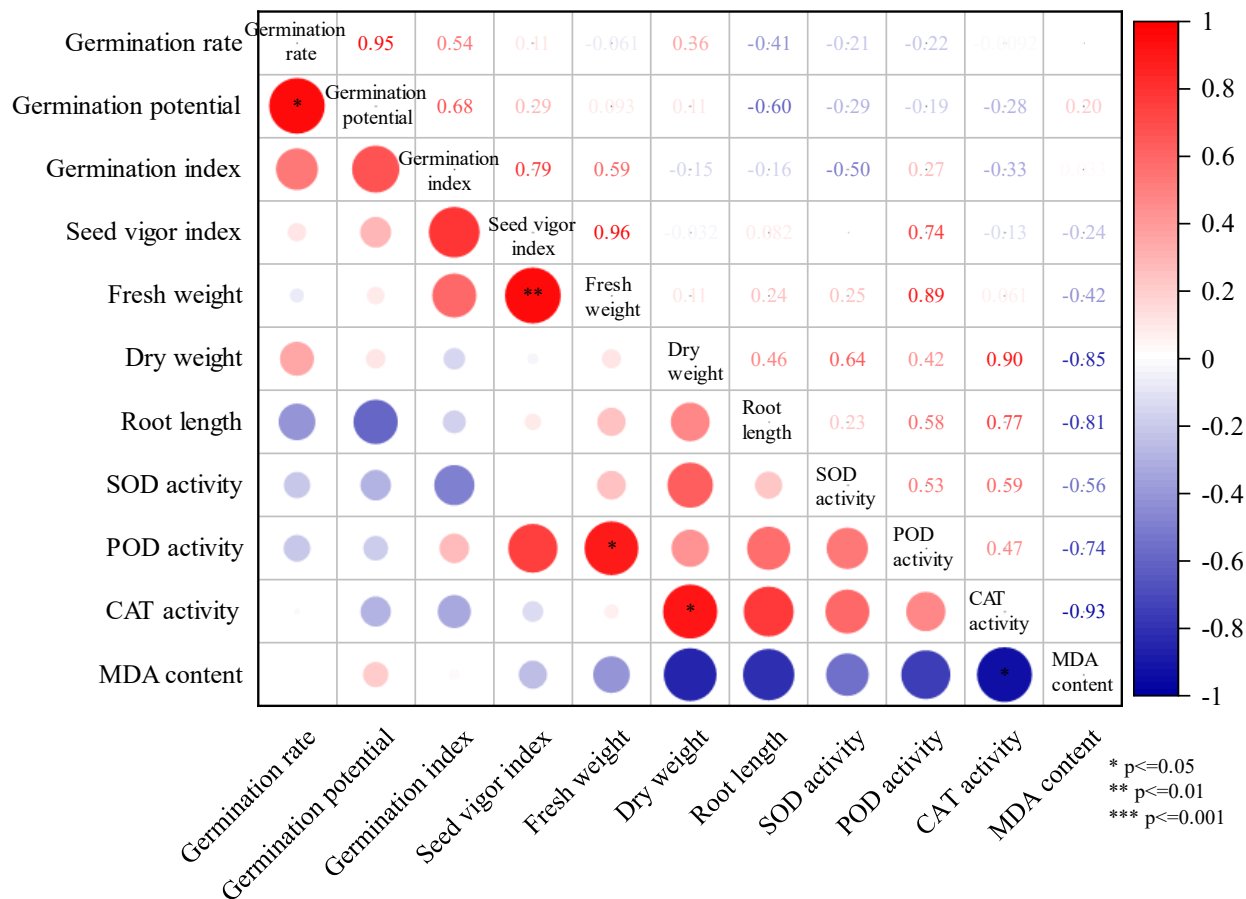


Figure 4 Correlation analysis diagram of *S. involucrata* seed germination indices under different chemical treatments (Pearson coefficient)

4. Discussion

This study aimed to address the technical problems associated with large-scale artificial cultivation of the endangered medicinal plant *S. involucrata* in the Compositae family. The effects of different chemical seed-soaking treatments and different cultivation substrates on the germination of *S. involucrata* seeds were explored. The results revealed that when seeds were soaked with a combination of three chemical reagents, 25 g/L PEG 4000 + 150 mg/L GA₃+10 mg/L IBA, and peat soil (50%) + vermiculite (50%) was used as the cultivation substrate for *S. involucrata*, all growth indicators of *S. involucrata* were comprehensively optimized, meeting the experimental expectations.

In this study, the purpose of chemical treatment was to break the dormancy of *S. involucrata* seeds. Combining the literature and the results of this paper, the type of seed dormancy was discussed. In the Baskin & Baskin classification system, seed dormancy can be divided into physiological dormancy (PD), morphological dormancy (MD), morphophysiological dormancy (MPD), physical dormancy (PY), and combinational dormancy (PY + PD) [16, 17]. Common methods used to relieve physiological dormancy include soaking seeds with chemical agents and soaking them in water [18]. Physiological dormancy includes nondeep physiological dormancy, moderate physiological dormancy, and deep physiological dormancy. In addition to deep physiological dormancy, the other two types of dormancy can be disrupted by hormones such as gibberellin [16]. For nondeeply physiologically dormant species, such as *Taraxacum officinale* and *Helianthus tuberosus* in the Compositae family, gibberellin can promote seed germination [19, 20]. In this study, *S. involucrata* seeds were treated with chemical agents and water, and their germination performance was good, indicating that this treatment was effective in breaking

309 physiological dormancy. This result implies that the dormancy type of *S. involucrata* seeds is likely nondeep physiological dormancy. This hypothesis can
310 provide certain theoretical insights for subsequent ways to improve the germination rate of *S. involucrata* seeds by breaking seed dormancy.

311 *S. involucrata* grows on cliffs at an altitude of approximately 3000 m. Because of its special location, exploring its optimal growth environment is
312 equally important. Relevant research has been reported. For example, Chen et al. [21] studied the optimal sowing depth of the soil, and the results revealed
313 that a sowing depth of 1.0 or 0.8 cm was more suitable for the germination of *S. involucrata* seeds. The cultivation substrate is an important factor leading
314 to the environmental dormancy of *S. involucrata* seeds, yet relevant research is rare. Yue et al. [22] studied another Compositae plant, chrysanthemum. The
315 results revealed that different cultivation substrates significantly affected the growth of the three chrysanthemum varieties. 'Huihuang', 'Bordeaux Red', and
316 'Huang Furong' grew best in the substrate formulas of peat, peat:perlite:leaf mold:rice husk with a volume ratio of 6:2:1:1, and peat:perlite with a volume
317 ratio of 4:1, respectively. This result highlights the importance of peat soil for the growth of chrysanthemum, which is similar to the results of this study.

318 There are various methods used to promote seed germination, and multiple factors affect plant growth. This paper optimized only the aspects of
319 chemical seed-soaking and cultivation substrates, which is a shortcoming of this paper. *S. involucrata* grows in special areas with high altitudes, high
320 intensities, and low oxygen contents [23]. Research should further explore its cultivation in view of its special growth environment, laying a solid foundation
321 for subsequent large-scale artificial cultivation and market entry.

322 **5. Conclusion**

323 This study explored the optimal chemical reagents for seed soaking and the best cultivation substrates for the germination and growth of *S. involucrata*
324 seeds through seed germination tests and pot experiments. The results revealed that when seeds were soaked with a combination of three chemical reagents,
325 25 g/L PEG 4000 + 150 mg/L GA₃ + 10 mg/L IBA, and peat soil (50%) + vermiculite (50%) was used as the cultivation substrate for *S. involucrata*, the
326 seed vigor indices and physiological indices of *S. involucrata* seed germination presented the best comprehensive performance. Under these conditions, *S.*
327 *involucrata* seeds have high germination ability, good growth potential, and strong stress tolerance. The experimental results can provide certain technical
328 references for the artificial cultivation of *S. involucrata* seeds.

329 **Acknowledgments**

330 This study was funded and supervised by Professor Shengjun Ma.

331 **Author contributions**

332 Shengjun Ma provided guidance for the entire writing process, Zeyi Luo and Bingbing Sun were responsible for the writing and translation, and Xinjie
333 Zhang and Song Jiang were responsible for the experiment.

334 **Declaration of competing interests**

335 The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work
336 reported in this paper.

337 **Funding**

338 This study was not supported by any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

339

340

341

342

343

344
345

346

347

348

349

350

351
352
353
354
355
356
357
358
359
360
361
362
363
364
365

Data availability

Not applicable.

Declarations

Ethics approval and consent to participate

The seeds of *Saussurea involucrata* used in this study were kindly provided by the Forestry and Grassland Bureau of Urumqi County, Xinjiang, China.
All materials were obtained in compliance with institutional and national regulations governing the collection and use of endangered plant species.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Clinical trial number

Not applicable.

References

1. National Pharmacopoeia Commission. Pharmacopoeia of the People’s Republic of China. Beijing: China Medical Science Press; 2020.

368 2. Gong GW, Huang J, Yang Y, Qi BH, Han GY, Zheng YZ, et al. *Saussureae involucratae* herba (snow lotus): Review of chemical compositions and
369 pharmacological properties. *Front Pharmacol.* 2019;10:1549.

370 3. Yang L, Lyu H, Yiming AYXMGL, Xu XZ, Ma CL, Tu S, et al. Integrated metabolism, network pharmacology, and pharmacokinetics to explore the
371 exposure differences of the pharmacodynamic material basis *in vivo* caused by different extraction methods for *Saussurea involucrata*. *J*
372 *Ethnopharmacol.* 2022;298:115648.

373 4. Kumar J, Pundir M. Phytochemistry and pharmacology of *Saussurea* genus (*Saussurea lappa*, *Saussurea costus*, *Saussurea obvallata*, *Saussurea*
374 *involucrata*). *Mater Today Proc.* 2022;56:1173–81.

375 5. Huang LQ, Zhang BG, Qin HN. Red List of Chinese Medicinal Plants. Beijing: Science and Technology Press; 2022.

376 6. National Forestry and Grassland Administration, Ministry of Agriculture and Rural Affairs. List of National Key Protected Wild Plants. 2021.

377 7. Ista. International Rules for Seed Testing. 2024.

378 8. Adhikary K, Mondal T, Majumder J, Chowdhuri TK, Mukherjee S, Maherukh K. Examine the impact of green-synthesized nanomaterials on the
379 germination rates and seedling characteristics of african marigold (*Tagetes erecta* L. var. Pusa *narangi ganda* and pusa *basanti ganda*). *Heliyon.*
380 2025;11:e42319.

381 9. Singh AK, Singh JK, Kumar R, Sharma P, Kumar NM, Singh BK, et al. Various presowing treatments for enhancing *Melia dubia* (cav.) seed germination,
382 seedling development, and vigor index. *Trees, Forests People.* 2024;17:100629.

383 10. Zhang MY, Liu SC, An Y, Zhang DJ, Qi Q, Jiang M, et al. Re-watering solution facilitates seed germination and seedling growth of *Carex schmidtii*:
384 Implication for species re-introduction in degraded semi-arid wetlands. *J Environ Manage.* 2024;362:121286.

385 11. Rutkowski M, Krzemińska-Fiedorowicz L, Khachatryan K, Khachatryan G, Kalisz A, Sękara A. Impact of silver nanoparticles in alginate gels on seed
386 germination, growth and stress biochemical parameters of cucumber seedlings. *Plant Stress.* 2024;12:100491.

387 12. Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal Biochem.* 1971;44:276–87.

388 13. Kar M, Mishra D. Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence 1. *Plant Physiol.* 1976;57:315–9.

389 14. Aebi H. [13] catalase *in vitro*. In: *Methods in Enzymology.* Academic Press; 1984. p. 121–6.

390 15. Bailly C, Benamar A, Corbineau F, Come D. Changes in malondialdehyde content and in superoxide dismutase, catalase and glutathione reductase
391 activities in sunflower seeds as related to deterioration during accelerated aging. *Physiol Plant.* 1996;97:104–10.

392 16. Baskin JM, Baskin CC. A classification system for seed dormancy. *Seed Sci Res.* 2004;14:1–16.

393 17. Baskin CC, Baskin JM. Chapter 3 - types of seed dormancy. In: Baskin CC, Baskin JM, editors. *Seeds.* San Diego: Academic Press; 1998. p. 27–47.

- 394 18. Liu MQ, Xu YW, Liu YY, Wei SC. Research Advances in Seed Dormancy and Germination Characteristics of Medicinal Plants. *J Tradit Chin Vet Med*.
395 2023;42:47-51 (in Chinese).
- 396 19. Li G, Zhang J, Wu YF, Chen LF, Chen R. Study on Dormancy Characteristics of *Taraxacum mongolicum*. *Seed*. 2017;36:42-45 (in Chinese).
- 397 20. Kantar M, Betts K, Hulke BS, Stupar RM, Wyse D. Breaking tuber dormancy in *helianthus tuberosus* L. and interspecific hybrids of *helianthus annuus*
398 L. × *helianthus tuberosus*. *Hortscience*. 2012;47:1342–6.
- 399 21. Chen YR, Nurbolat, Liu M, Wang XJ, Yin LK. Effects of Sowing Depth on Seed Germination of *Saussurea involucrata*. *J Plant Resour Environ*.
400 2013;22:115-117 (in Chinese).
- 401 22. Yue LR, Ni XY, Liu MY, Zhou YW. Effects of Different Cultivation Substrates on the Growth of Potted Chrysanthemums. *J Zhejiang Agric Sci*.
402 2024;36:2521-2534 (in Chinese).
- 403 23. Jing TR, Xu SY, Ni YF, Zhang YJ, Guo B, Wei YH. Research Advances in Biotechnology of *Saussurea involucrata*. *Genom Appl Biol*. 2016;35:2201-
404 2210 (in Chinese).