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ARTICLE

The Effects of Temperature, Light and Moisture on the Seed Germination of *Siphonostegia chinensis* Benth.

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ABSTRACT

To explore the optimum temperature, light intensityand water conditions for seed germination of *Siphonostegia chinensis* Benth.,seed germination experiment were carried out under different temperatures (5/15, 10/20, 15/25, 20/30 ℃), different light intensity (14h light/10h darkness, complete darkness) and different concentrations (0%, 5%, 10%, 15%, 20%) of PEG-6000 solution. In terms of concentration, 5% PEG was regarded as the low level, 10% and 15% as the medium level, and 20% as the high level. The results showed that (1) Germination rate, germination potential, and germination index were increased with the rise of temperature. In addition, seed germination was significantly higher under the dark conditions than that with the 14h light/10h darkness. (2) No seed germination occurred when the temperature was below 10/20 ºC at 14h light/10h darkness. (3) Under 14h light/10h darkness, the germination rate, germination potential and germination index first increased and then decreased with the increase of PEG concentration. The low concentration was more beneficial to the seed germination. (4) Under the condition of complete darkness, the germination rate, germination potential and germination index decline with fluctuation with the increase of PEG concentration. Seed germination of *Siphonostegia chinensis* Benth. was inhibited in high concentration of PEG.

1. Introduction

Light intensity has a great impact on seed germination of plants and seedling growth, which is a key environmental factor on population regeneration of plants. The germination of plant seeds is the adaptive response to specific lighting conditions[1]. According to the different responses to light in the seed germination process, the seeds can be divided into positively photoblastic seeds, negatively photoblastic seeds and non-photoblastic seeds [2.3]. The effect of light on plant seed germination may be three types, such as stimulation, inhibition and no significant effect [4.5]. Therefore, the response of seeds to light can be used as a signal factor to indicate the appropriate environment for germination [6.7]. In addition, temperature and water are two key ecological factors for germination, especially for plants in arid and semi-arid regions [8].

Siphonostegia chinensis Benth, a species of Scrophulariaceae, Siphonostegia, is an annual herb, with the height of 30-80 cm. With an opposite leaf arrangement, flowers are arranged opposite on the upper part of stems and

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branches, being loose racemes[9]. It has two lips on corolla, with the upper lip slightly purple and the lower lip yellow. It is widespread in China, such as dry mountain slopes and grasslands at 800-3400 m above sea level in the northeast China, central China, southern China, northern China, Inner Mongolia, southwest China. It is also found in Japan, South Korea, and Russia. Its entire parts have high medicinal value. Long-term gathering of wild plants leads to a sharp decline in the number of wild plants. The species have disappeared in some regions[9]. Therefore, the study of optimum germination conditions and growth environments of its seeds is of great value and significance. However, current research on *Siphonostegia chinensis* Benth. has focused on the study of chemical composition and the comparison of plant composition in southern and northern regions. Little research has been reported on the optimum germination and screening of growth conditions for the seeds of *Siphonostegia chinensis* Benth. On the basis of the background, the seeds of *Siphonostegia chinensis* Benth. were used as the primary materials in this study. Data of germination under different conditions (temperature, light, and water) were analyzed through filter paper germination method in Petri dishes to provide reference data for the study of optimum growth environment condition of germination, artificial planting, and introduction, thereby enlarging the application range of *Siphonostegia chinensis* Benth. and improving its economic value.

2. Materials and Methods

2.1 Experimental Materials

The experimental materials in this study were obtained from mature seeds of *Siphonostegia chinensis* Benth. in Huo Mountain (36°43′00″ – 36°25′50″ N, 111°47′15″ - 112°2′46″ E), Huozhou, Shanxi, China in October 2017. The mountain is located in the metamorphic zone formed 2.5 billion years ago. It has a warm temperate semi-humid continental monsoon climate. Its main characteristics are hot and less precipitation in summer, cold and dry in winter. The seeds were treated cleanly and placed in a 5 °C freezer for storage [10.11]. Healthy and full seeds with the uniform size were randomly selected as the experimental subjects in the study.

2.2 Experimental Methods

2.2.1 Seed Morphology Observation and Thousand Grain Weight Determination

Healthy and full seeds with the uniform size were obtained to determine the physical properties of seeds, such the thousand grain weight, length, and width. A total of 250 seeds were randomly selected to weigh on an analysis electronic balance with the readability of 0.001 g[12.13]. The experiment was replicated 4 times. The final mean of 4 sets of data was used as the thousand-seed weight value of *Siphonostegia chinensis* Benth.

2.2.2 Different Temperature and Light Treatment

While performing the experiment, 25 seeds were placed evenly in each disposable petri dish with a diameter of 90 mm and two layers of sterilized filter paper. All Petri dishes were numbered and four temperature gradients5/15℃、10/20℃、15/25℃、20/30℃ were set. In the meantime, two light treatments (14h light/10h darkness and complete darkness) were carried out at each temperature, with 4 repetitions per treatment. During the test, 2 mL of distilled water was added to each Petri dish to keep the filter paper moist in the Petri dish. In a set of repeat experiment, 4 Petri dishes were loaded into transparent zip-lock bags [14.15.16]. Petri dishes in the dark treatment were loaded into the opaque aluminum foil bag. All bags were placed in the monitoring-temperature incubators for culture. All the experiments were set to observe and record the germination situation at the same time every day from the 2nd day of the experimental layout. Radicle breaking through 2 mm of the seed coat was regarded as the germination standard [17.18]. If moldy seeds were found, the seeds were picked out with disinfectant tweezers and recorded in time during the test period, avoiding contaminating other seeds [5.19.20]. If seed germination was not seen for 3-4 consecutive days after the germination period, it was regarded as the end of the germination experiment. The vitality of the seeds was determined after the end of the experiment. The remaining seeds were gently pressed with tweezers under the microscope. If the seeds were hard, they were regarded as active species, and vice versa were non-active seeds. Active seedswere continuously cultured under the corresponding light and temperature conditions [21.22]. The experiment was terminated when the seeds were not germinated for two consecutive days. Finally, data were counted and analyzed to obtain the optimum environmental conditions for stimulating germination [23.24.25].

2.2.3 Different Moisture Gradient Treatment

According to the first round of experimental data, the temperature conditions with the highest germination rate and the fastest germination speed were selected. Under conditions of optimum constant temperature, 14h light/10h darkness and complete darkness, Petri disheswere cultured in different concentrations（0%、5%、10%、15%、20%、25%）of PEG-6000 to simulate water treatment.

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After marking the number, the media were cultured in the constant temperature incubator, the germination data are counted at the same time every day. During the test, the media were changed once two days to prevent the change of the medium concentration, seed mildew, or inaccurate experimental results [26.27].

2.3 Determined Indexes

Germination percentage (%) =total number of germinations / number of seeds tested×100%

Germination energy(%)=total number of germination peak / number of seeds tested×100%

Germination index $(GI) = \sum(Gt / Dt)$

Note: *Gt*(daily germination), *Dt*(the corresponding number of days of germination)

2.4 Data Processing

Microsoft Excel was used to perform statistics and calculation for the germination rate, germination potential, and germination index of seeds of *Siphonostegia chinensis* Benth. The variance, standard error and single-variable analysis were conducted with SPSS17.0 software. The significant differences between the factors were compared and the difference map was drawn by Microsoft Excel.

3. Results and Analysis

3.1 Thousand Seeds Weight, The Length and the Width of Seed

Thousand seeds weight 0.077±0.006g, seed length (1.019±0.062cm), seed width0.583±0.037cm.

3.2 The Impact of Light Duration on the Seeds of *Siphonostegia chinensis* Benth. under Different Temperature

The germination rate, germination potential and germination index of the seeds of *Siphonostegia chinensis* Benth. showed obvious difference under the condition of 5/15 °C,10/20 °C,15/25 °C,20/30 °C, 14h light/10h darkness, and complete darkness. Under the condition of 14h light/10h darkness, the seeds of *Siphonostegia chinensis* Benth. were not germinated in the 5/15°Cand 10/20 °Ctemperature range. With the same light, the germination rate, germination potential and germination index increased with the increase of temperature in 15/20 °Cand 20/30 °Ctemperature range. Under different light duration in 15/20 °Cand 20/30 °Ctemperature range, the difference between the germination rate, germination potential and germination index of *Siphonostegia chinensis* Benth. were statistically significant. In addition, the germination rate in the complete darkness was significantly greater than that of 14h light/10h darkness, indicating that high temperature and dark conditions were more suitable for seed germination of *Siphonostegia chinensis* Benth.

3.3 The Impact of Light Duration on the Seeds of *Siphonostegia chinensis* Benth. under Different PEG

Under the light duration of 14h light/10h darkness, the germination rate, germination potential, and germination index of *Siphonostegia chinensis* Benth. increased first and then decreased with the increase of PEG concentration. Among which, each index was the maximum at 5% of PEG concentration; differences of the indexes were statistically significant with 10% and 15% of the PEG concentration. Under the condition of complete darkness, the germination rate, germination potential, and germination index of *Siphonostegia chinensis* Benth. showed a fluctuating trend with the increase of PEG concentration. With the same light condition and 20% of PEG concentration, the seeds of *Siphonostegia chinensis* Benth. were not germinated. It demonstrated the low drought tolerance ability of *Siphonostegia chinensis* Benth.

4. Discussion and Conclusion

Seed germination requires environmental factors such as suitable water, oxygen, temperature or light. The environmental conditions required for different seed germination are various. The effects of different environmental factors diverse from each other, but they are related to each other and affect the life activities of seeds in an integrated way. Different plant seeds have various requirements for temperature conditions. Seedshave active metabolic reactions during germination. Therefore, in a certain temperature range, the germination process stimulates with the increase of temperature, whereas too elevated temperature results in denaturation in some living active substances, such as enzyme degeneration, and then it has a negative effect on germination. The experimental results showed that the germination rate, germination potential, and germination index of *Siphonostegia chinensis* Benth. were on the rise with the increase of temperature. the germination index reached the highest under 20/30 °C, and seed germination indexes of *Siphonostegia chinensis* Benth. under all dark conditions were significantly higher than that of 14h light/10h darkness. Seeds of *Siphonostegia chinensis* Benth. were not germinated at 10/20 °Ctemperature. This result is similar to the study results of Zheng et al. on*Artemisia* sphaerocephala.

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Light is a key factor affecting seed dormancy, which is mainly controlled by the far original red-light absorption photosensitizer (Pfr) and red-light absorption photosensitizer in the seed [28.29]. The effect of light on seed germination is different because of different threshold requirement of original Pfr content to the Pfr/Pr. Quiros et al. proposed that the light cycle demand for the growth of the Lepidium meyenii was not clear. In the origin of Lepidium meyenii, the daytime was less than 13h during the growth, indicating that it may be a short-day or mid-day plant. This study showed that seed germination indexes of *Siphonostegia chinensis* Benth. undercomplete dark conditions were significantly higher than that of 14h light/10h darkness. However, under the condition of drought, the germination rate, germination potential and germination index of *Siphonostegia chinensis* Benth. firstly increased and then decreased at 14h light/10h darkness, and the low concentration was more conducive to the seed germination of *Siphonostegia chinensis* Benth. The germination rate, germination potential, and germination index of *Siphonostegia chinensis* Benth. under all dark conditions showed a fluctuating decline trend, inhibiting the seed germination.

Moisture is an important factor affecting seed germination. Because the seed coat permeability of each species and the water absorption of seed internal components are quite different, seed germination of each species is of great difference in the water potential of the environment, and the minimum moisture required for seed germination of different plants is also various. Some studies showed that the seeds of some species could not germinate when the concentration of PEG-6000 was higher than 15%. Wild chrysanthemum seeds could not germinate when the concentration of PEG-6000 was greater than 20% [30.31.32]. In this study, it showed that the germination rate, germination potential, and germination index of the seeds of *Siphonostegia chinensis* Benth. firstly increased and then decreased with the rise of PEG concentration and 14h light/10h darkness. The low concentration was more conducive to seed germination of *Siphonostegia chinensis* Benth. The germination rate, germination potential, and germination index of *Siphonostegia chinensis* Benth. under all dark conditions showed a fluctuating decline trend with the rise of PEG concentration. Under the high concentration and drought condition, the seed germination was inhibited.

In conclusion, suitable water, temperature, and light are key techniques for seed germination of *Siphonostegia chinensis* Benth. This result provides a scientific reference and an effective way for the protection and rational development and utilization of introduction and cultivation of *Siphonostegia chinensis* Benth. as one of wild Traditional Chinese medicine resources Furthermore, there are diverse factors affecting seed germination and seedling growth of *Siphonostegia chinensis* Benth., which need to further study.

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Supplements

Table1. The impact of light duration on the seeds of *Siphonostegia chinensis* Benth. under different temperature

temperature/℃	Light time/h·d-1	Germination percentage	Germination energy	Germination index
5/15℃	14	0.00±0.00d	0.00±0.00e	0.00±0.00c
10/20℃	14	0.00±0.00d	0.00±0.00e	0.00±0.00c
15/25℃	14	8.25±3.69d	7.00±2.00d	0.26±0.14d
20/30℃	14	28.50±4.73c	15.25±3.60c	0.96±0.16bc
5/15℃	0	0.00±0.00d	0.00±0.00e	0.00±0.00c
10/20℃	0	0.00±0.00d	0.00±0.00e	0.00±0.00c
15/25℃	0	42.00±12.44b	22.00±5.16b	1.24±0.44b
20/30℃	0	67.00±8.87a	40.00±8.64a	2.15±0.36a

Table 2. The impact of light duration on the seeds of *Siphonostegia chinensis* Benth. under different PEG

PEG concentration/%	Light time/h·d^{-1}	Germination percentage	Germination energy	Germination index
0	14	28.50±4.73c	15.25±3.59bcd	0.96±0.16bc
5	14	46.00±15.49b	24.00±9.80b	1.07±0.37b
10	14	39.00±16.13bc	22.00±12.44b	0.98±0.43bc
15	14	11.00±3.83de	7.00±2.00cde	0.28±0.93de
20	14	5.00±3.83e	4.00±3.27de	0.12±0.94e
0	0	67.00±8.87a	40.00±8.64a	2.15±0.36a
5	0	37.00±12.38bc	19.00±8.25bc	0.82±0.29bc
10	0	41.00±11.49bc	19.00±8.25bc	0.95±0.29bc
15	0	26.00±15.49cd	16.00±10.83bcd	0.59±0.37cd
20	0	0.00±0.00e	0.00±0.00e	0.00±0.00e

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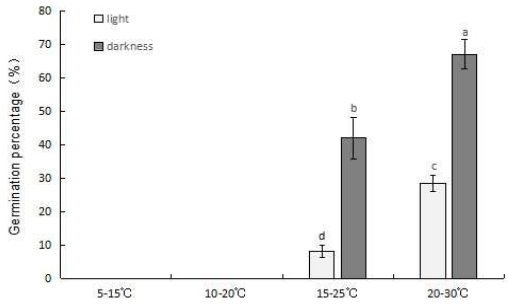

Figure 1. The germination percentage impact of light duration on the seeds of Siuphonostegia chinens is Benth. unedr different temperature.

Note: Each bar represents the mean of three replicates; bars with different lowercase letters are significantly different from each other under various treatments at $p < 0.05$ (Tukey test).

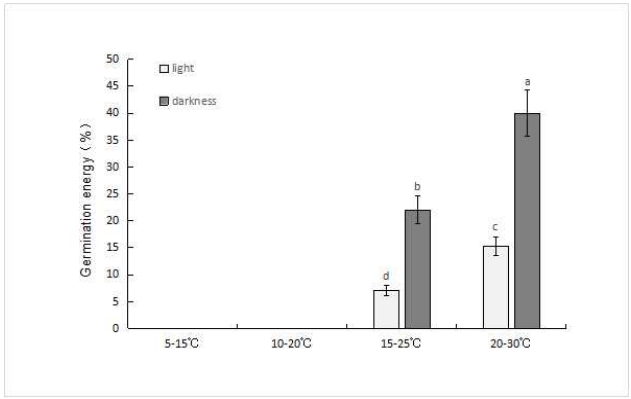

Figure 2. The germination energy impact of light duration on the seeds of Siphonostegia chinens is Benth. under different temperatures.

Note: Each bar represents the mean of three replicates; bars with different lowercase letters are significantly different from each other under various treatments at $p < 0.05$ (Tukey test).

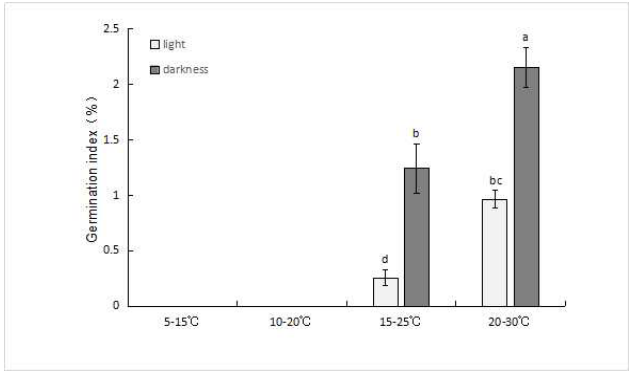

Figure 3. The germination index impact of light duration on the seeds of Siphonostegia chinens is Benth. under different temperatures

Note: Each bar represents the mean of three replicates; bars with different lowercase letters are significantly different from each other under various treatments at $p < 0.05$ (Tukey test).

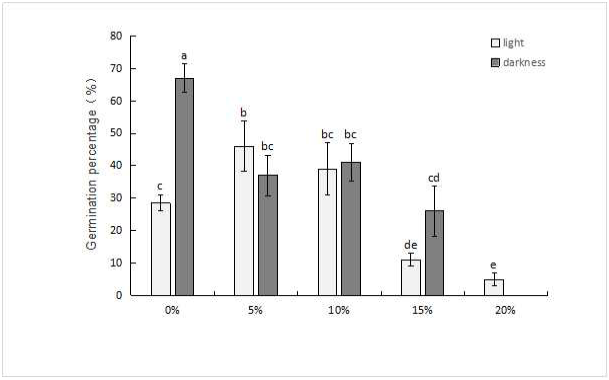

Figure 4. The germination percentage impact of light duration on the seeds of Siphonostegia chinens is Benth. under dfferent PEG

Note: Each bar represents the mean of three replicates; bars with different lowercase letters are significantly different from each other under various treatments at $p < 0.05$ (Tukey test).

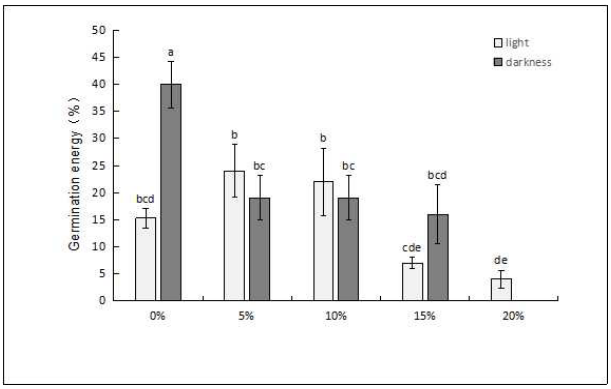

Figure 5. The germination energy impact of light duration on the seeds ofSiphonostegia chinens is Benth. under different PEG

Note: Each bar represents the mean of three replicates; bars with different lowercase letters are significantly different from each other under various treatments at $p < 0.05$ (Tukey test).

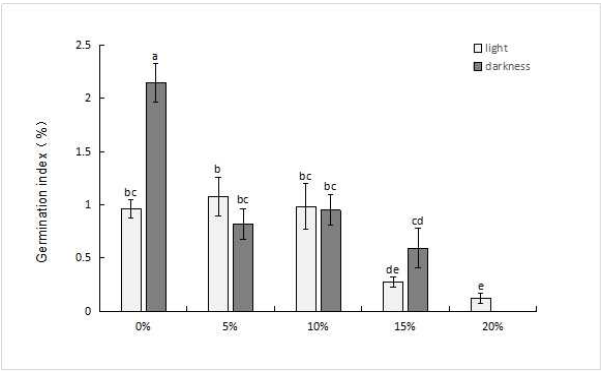

Figure 6. The germination index impact of light duration on the seeds of Siphonostegia chinens is Benth. under different PEG

Note: Each bar represents the mean of three replicates; bars with different lowercase letters are significantly different from each other under various treatments at $p < 0.05$ (Tukey test).

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