Influence of Illumination Time and Soil Moisture on Seed Germination and Seedling Establishment of *Magnolia sprengeri* Pamp.

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Abstract. This study investigated the effects of illumination time and soil moisture on seed germination and seedling establishment of *Magnolia sprengeri* Pamp. to improve the seed germination percentage and seedling survival percentage of *M. sprengeri*. It is of great significance for rapid propagation, seedling regeneration, field management, and artificial high-efficiency cultivation of *M. sprengeri*. In this study, the seeds of natural *M. sprengeri* populations from original habitat were used as test materials. Seed germination and seedling establishment of *M. sprengeri* were performed under different illumination time and soil moisture treatments in artificial climate incubator. The study found that there were significant differences among various key parameters related to seed germination and seedling establishment under different treatments ($P < 0.05$). Germination percentage, germination potential, germination index, vigor index, germination rate, plant height, number of leaves, base diameter, taproot length, number of lateral roots, maximum lateral root length, single plant leaf area, fresh weight, and seedling survival rate reached the maximum at continuous illumination and 13% of soil moisture, respectively. By the integrative evaluation for the influence of these two factors on seed germination and seedling establishment, soil moisture is a dominant factor affecting seed germination and seedling establishment, whereas illumination is an important promoting factor for seedling establishment of *M. sprengeri*. Continuous illumination and 13% of soil moisture content is suitable for seed germination and seedling establishment of *M. sprengeri*.

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Magnolia sprengeri Pamp. (*M. sprengeri* var. *sprengeri*, the northern variety of *M. sprengeri*) is a deciduous arbor of the genus *Magnolia* in *Magnoliaceae* (Kang and Ejder, 2011; Song and Liu, 2019). In China, it mainly distributed in Henan province, Gansu province, Hubei province, Shaanxi province at the altitude of 1300–2400 m (Yang et al., 2015). *M. sprengeri* is an important plant resource and is regarded as one of the endangered plants in Gansu Province. Its flower buds commonly substituted for *Magnolia biondii* Pamp. or *Magnolia denudata* Desv. as medicine that is called as ‘Xinyi’, which can treat influenza and headache (Shi et al., 2014). Additionally, *M. sprengeri* is also an excellent garden tree species because of the elegant flowers and straight tree trunk.

Seed germination and seedling establishment occupied an important position in the whole life history of plant, which is the interaction result caused by the internal and external factors (Wang, 2006). Seeds can launch the nutrient reserves and start life activities when they are stimulated by external signals such as suitable temperature, light, water, and oxygen in the environment. However, seeds are generally prone to death at this stage. Seed germination and seedling establishment are critical and complicated processes in the growth and development of plants, which directly affect the spatial distribution and reproductive evolution of plant population (Vijai Anand et al., 2020). Illumination time and soil moisture are important environmental factors regulating seed life activities (Yan et al., 2011). Seed germination and seedling establishment of most of plant depend on light. Light intensity, light quality, and light duration can be sensed by light receptors and affect the growth and development of plant, such as plant phytochrome directly involved in seed germination induction process. Light can break seed dormancy and is regarded as one of the signal molecules of seed germination (Liu et al., 2019). On the other hand, the growth and development of plants cannot be separated from water. Plant roots are water-oriented, and water is an essential external environmental factor for seed germination (Wang, 2018). Therefore, environmental factors such as light and water can be regulated to promote seed germination and seedling establishment, which is beneficial to plant reconstruction.

Seed germination and seedling establishment of plant is influenced by many factors such as physiological characteristics, harvest time, treatment methods, sowing time, and environmental factors (Yang and Kong, 2003). In particular, *M. sprengeri* seeds possess deep dormancy characteristic and postripening phenomenon (Shen, 2007a). Also, postripening period is very long. Therefore, the *M. sprengeri* has low seed vigor and long germination time as well as long seedling emergence time under natural environment. Improvement of the seed germination rate and seedling establishment can be helpful to effectively promote the high-value innovative development and utilization of *M. sprengeri* resources. Previous research focused on the promotion and application of *M. sprengeri* (He, 2010a; Peng and Chen, 2018), grafting-seedling cultivation (He, 2008a), cutting-seedling cultivation (Shen, 2007b), container-seedling cultivation (He, 2008b), and seed-propagation-seedling cultivation (He, 2010b; Lan et al., 1998; Liang et al., 2014), which provides the theoretical foundations for the artificial propagation of *M. sprengeri*. However, only a few studies on the influence of environmental factors on the seed germination and seedling establishment of *M. sprengeri* were available. The author had conducted a detailed study on the effects of temperature and sowing depth on seed germination and seedling establishment of *M. sprengeri* (Zhang et al., 2020), and the results indicated that temperature and sowing depth

had important effects on seed germination and seedling establishment of *M. sprengeri*. Based on the previous research, the present study was conducted to fully understand the influence of environmental factors on the seed germination and seedling establishment of *M. sprengeri*. In this study, seeds of natural *M. sprengeri* communities grew in Shennongjia Forestry District in Hubei Province were collected as the test materials. Seed germination and seedling establishment experiments were conducted in the artificial climate incubator by setting two environmental factors including light time and soil moisture. The optimal light time and soil moisture for seed germination and seedling establishment of *M. sprengeri* will be revealed through the determination and comparison of key indicators such as germination percentage (GPe) and seedling survival number. The present study aims to improve the seed GPe and seedling survival percentage of *M. sprengeri*, which could provide a new theoretical reference for the rapid propagation, seedling regeneration, field management, and artificial high-efficiency cultivation of *M. sprengeri*.

Materials and Methods

Seed materials. Seeds of *M. sprengeri* were collected from the natural *M. sprengeri* communities (lat. 31°26'40.76"N, long. 110°06'44.07"E) on 21 Sept. 2018 in Xiejiapo, Dajiuhe Town, Shennongjia Forest District, Hubei Province of China. In detail, reddish-brown follicles were harvested, and were dried in the shade. Seed testas and arils were removed. Also, the seeds with red arils were mixed with fine river sand, and the arils were rubbed off by hand. The plump and healthy seeds were screened out for subsequent application. In this study, the thousand-grain weight of *M. sprengeri* seeds was 134.13 g, and the longitudinal diameter and transverse diameter were 10.8 mm and 10.1 mm, respectively. The seed germination substrate was sampled from the 0–60 cm soil layer where the natural *M. sprengeri* communities grow.

Seed treatment. *M. sprengeri* seeds were treated using stratification germination forcing method (Shen, 2007a). On 5 Nov. 2018, seeds were put into the 40-cm-deep cellar and were checked every 2 weeks to prevent quality change of the seeds. The seeds were taken out for next experiments after the germination forcing was successful on 6 Mar. 2019.

Seed germination experiment. The seed germination experiment was carried out in the artificial climate incubator by setting different light time and soil moisture treatments. In the illumination experiment, the qualitative filter paper was put into a petri dish as a germination bed. Different treatments were performed in triplicate (90 seeds per treatment). The experimental data were observed and recorded every 24 h when the seeds germinated (about 10 d after sowing) (NFSSTC, 1999; Zhang, 2012). The seed germination judgment criteria were as follows in this study: the radicle length is 1 mm out of the

seedcoat and the hypocotyl length is 5 mm out of the soil surface. The germination experiment stopped when there was no seed germination observed for 14 consecutive days. Light and soil moisture settings were as follows.

Light settings. *M. sprengeri* seeds were cultured under the continuous light, continuous darkness, and fluctuating light time (16/8 h day/night) condition at 25 °C. Light was provided by stick-type light-emitting diode (stick-LED) lamps (Panasonic Co., Ltd., Beijing, China), which emitted most of their light radially (Xia et al., 2016). The light intensity was 4000lx (Lu et al., 2013). The petri dish quality was weighed every 24 h because of the water lost by evaporation, and then water was replenished to keep constant weight.

Soil substrate moisture setting. The soil collected from the sampling site was screened by 1 mm pore size. The soil was dried in an oven at 105 °C for 48 h, which aimed to kill other plant seeds in the soil. The dried soil mixed with vermiculite according to the ratio of 1:2, and then high temperature disinfection was conducted. Prepared soil (200 g) was placed into the culture box, and soil moisture was set as 5%, 7%, 8%, 10%, 12%, 13%, 15%, and 20% with distilled water, respectively. *M. sprengeri* seeds were sown and covered by soil (germination substrate) with a depth of 1 cm. The seeds of *M. sprengeri* were cultured under the continuous light condition at 25 °C. During the culture period, the water was replenished every day to keep the soil moisture constant.

Seedling establishment experiment. The experiment of seedling establishment was performed for 110 d. Thirty seedlings with the same growth status and the first pair of true leaves were reasonably selected from the abovementioned two treatments for seedling establishment experiment. The experimental conditions of seedling establishment were the same as that of the seed germination experiment.

Determination and calculation of key parameters of seed germination. The key parameters of seed germination included GPe, germination index (GI), germinating potentialities (GPs), germination rate (GR), and vigor index (VI).

GPe is a proportion of the germinated seeds number and the total tested seeds, which can reflect the potential of seeds forming seedlings. GPe was calculated by Eq. [1].

$$\text{GPe} = \left(\frac{\text{number of germinated seeds}}{\text{total amounts of tested seeds}} \right) \times 100\% \quad [1]$$

GI is an index that can reflect the seed germination uniformity. GI was calculated by Eq. [2].

$$\text{GI} = \sum (Gt/Dt) \quad [2]$$

where *Gt* refers to the number of seed germination on the *t* day and *Dt* refers to the corresponding seed germination days.

GPs refers to the GPe when the seed germination reaches the peak. GPs was calculated

by Eq. [3].

$$\text{GPs} = \left(\frac{\text{number of germinated seeds at the germination peak}}{\text{number of all seeds tested}} \right) \times 100\% \quad [3]$$

GR can reflect the seed germination speed. Higher GR means the seed germination speed will be faster (Yan et al., 2011; Zhang et al., 2020). GR was calculated by Eq. [4].

$$\text{GR} = \sum 100Gi/nti \quad [4]$$

where *n* is the number of seeds used in each treatment and *Gi* is the number of germinated seeds in *ti* (*ti* = 0, 1, 2, 3) days.

VI can be used to measure the seed growth and growth uniformity (Tian et al., 2014). In this study, the VI calculation method can be expressed as Eq. [5].

$$\text{VI} = \text{seed germination percentage} \times (\text{seedling root length} + \text{seedling stem length}) \quad [5]$$

Determination and calculation of key parameters of seedling formation. The key parameters of seedling formation included the number of survival seedlings, number of lateral root (NLR), leaf number (LN), the tap root length (TRL), maximum of lateral root (MLR), seedling height (SH), basal stem diameter (BSD), leaf area per seedling (LAPS), and fresh weight of seedling (FW). The digital vernier caliper was employed to measure the TRL value, MLR value, SH value, and BSD value, respectively. The LAPS value was measured by Li-3100 leaf area tester. The FW was measured by electronic balance (1/10000). All parameters were observed and recorded every 5 d. The seedlings survival percentage was calculated by Eq. [6] (Yu et al., 2018).

$$\text{Seedling survival percentage} = \left(\frac{\text{number of survival seedlings}}{\text{initial seedlings number}} \right) \times 100\% \quad [6]$$

Statistical analysis. Excel 2010 was used for data statistics, and all data were expressed by mean ± SD (*n* = 3). SPSS25.0 software was used to analyze the data by one-way ANOVA and multiple comparisons were finished by Duncan method (*P* < 0.05).

Results

Differentiation in seed germination parameters of *M. sprengeri* under different light conditions. There were significant differences in seed germination parameters of *M. sprengeri* under different light conditions (*P* < 0.05) (Fig. 1). The seed GPe, germination potential, GI, VI, and GR of *M. sprengeri* under continuous light were significantly higher than those of 16 h/8 h light–dark alternating group and dark treatment group (*P* < 0.05), and their values were 63.41%, 53.18%, 16.37, 0.47, and 0.71, respectively. All the parameters in the light–dark alternating group were significantly higher than those in the dark treatment group (*P* < 0.05). Under the

Fig. 1. Effects of illumination time on the seed germination of *Magnolia sprengeri*. Different lowercases indicate significant differences among different treatments ($P < 0.05$).

condition of continuous darkness, all the seed germination parameters values of *M. sprengeri* were the minimum. These resulting data indicated that seed germination of *M. sprengeri* was sensitive to light, and the increase of light time could be beneficial to seed germination of *M. sprengeri*.

Differentiation of seedling formation parameters of *M. sprengeri* under different light conditions. There were significant differences in seedling formation parameters of *M. sprengeri* under different light conditions ($P < 0.05$) (Table 1). Table 1 showed that the seedling height (12.29 cm), LN (2.75), BSD (1.91 mm), taproot length (11.58 cm), lateral root number (21.54), MLR length (5.22 cm), LAPS (31.24 cm²), FW (2.68 g), and seedling survival percentage (57.35%) in continuous light treatment group were significantly higher than those in continuous dark and day/night (16 h/8 h) fluctuation treatment group ($P < 0.05$). For example, compared with the continuous dark treatment group, these indexes increased by 38.89%, 22.18%, 50.26%, 55.01%, 52.00%, 55.75%, 50.86%, 61.19%, and 62.25%, respectively.

Differentiation of seed germination parameters of *M. sprengeri* under different soil moisture. Plant growth is inseparable from water, and soil moisture can directly affect seed germination. The seed germination parameters of *M. sprengeri* were significantly different under different soil moisture content treatments ($P < 0.05$) (Fig. 2). The seed GPe, germination potential, GI, VI, and GR of *M. sprengeri* increased at first and then decreased with the increase of soil moisture and reached the maximum at 13% of the soil moisture. Their values were 65.49%, 62.35%, 20.12, 0.74, and 0.86, respectively, which were significantly higher than any other treatment group ($P < 0.05$). The VI value (0.14) and GI value (6.35) is the lowest at 5% of the soil moisture. Therefore, *M. sprengeri* seed germination is not tolerant to drought, and seed germination is more sensitive to soil moisture, meaning that the appropriate increase of soil moisture is conducive to seed germination.

Differentiation of seedling formation parameters of *M. sprengeri* under different soil moisture. There were significant differences in seedling formation parameters of

M. sprengeri under different soil moisture (Table 2). Table 2 showed that the seedling height, LN, BSD, taproot length, lateral root number, MLR length, LAPS, FW, and seedling survival rate of *M. sprengeri* were the minimum at which the soil moisture was 5%. The maximum values of these parameters were observed at 13% of the soil moisture, their values were 17.23 cm, 4.14, 2.74 mm, 18.21 cm, 32.65, 8.45 cm, 43.54 cm², 3.95 g, and 78.53%, respectively, increased by 51.54%, 72.46%, 61.31%, 65.02%, 73.51%, 58.34%, 69.02%, 43.04%, and 62.33% compared with the 5% of soil moisture treatment group with the lowest, respectively. In addition, the SH, MLR length, FW, and seedling survival rate of *M. sprengeri* in 13% of soil moisture treatment group were significantly higher than those in other treatments ($P < 0.05$). When the soil moisture was 13%, the number of leaves, taproot length, and lateral root number were different from those of 12% of soil moisture treatment (not significant), but significantly higher than those of other treatments ($P < 0.05$). When the soil moisture was 13%, the BSD was different

Table 1. Effects of illumination on the seedling establishment of *Magnolia sprengeri*.

Illumination treatment	Continuous light	Continuous dark	Alternating light (16 h) and dark (8 h)
Seedling height (cm)	12.29 ± 1.23 a	7.51 ± 1.11 c	9.12 ± 0.95 b
Leaf number	2.7 ± 0.365 a	2.14 ± 0.25 b	1.85 ± 0.47 bc
Basal stem diameter (mm)	1.91 ± 0.21 a	0.95 ± 0.23 b	1.16 ± 0.17 b
Taproot length (cm)	11.58 ± 1.55 a	5.21 ± 0.94 c	9.34 ± 1.13 b
Number of lateral root	21.54 ± 1.56 a	10.34 ± 1.12 c	18.48 ± 1.39 b
Maximum of lateral root length (cm)	5.22 ± 0.65 a	2.31 ± 0.32 c	3.57 ± 0.14 b
Leaf area per seedling (cm ²)	31.24 ± 2.36 a	15.35 ± 1.54 c	22.21 ± 1.11 b
Fresh weight (g)	2.68 ± 0.55 a	1.04 ± 0.25 b	1.59 ± 0.42 b
Seedling survival rate (%)	57.35 ± 2.23 a	21.65 ± 1.24 c	34.67 ± 2.51 b

Different lowercases mean significant differences among different treatments ($P < 0.05$).

from that of 15% of soil moisture (not significant), but significantly higher than that of the other treatments ($P < 0.05$). When the soil moisture was 13%, the LAPS was different from that of 12% and 15% of soil moisture treatment groups (not significant), but was significantly higher than that of the other treatments ($P < 0.05$).

Discussion

Effects of light time on seed germination and seedling formation of M. sprengeri. Plant regulates seed germination and seedling formation by sensing the changes of photoperiod and light quality, and light is an important

regulating factor in the process of plant growth and development. Light induces a series of physiological and biochemical reactions by affecting the conversion of photosensitive pigments Pr and Pfr, which is beneficial to the seed germination and seedling growth of plants especially woody plants (Lu et al., 2013; Yan et al., 2011). Lin et al. (2009) studied the effect of light time on the *Magnolia officinalis* seedlings, and the results showed that the light time could significantly affect the height and ground diameter of *M. officinalis* seedlings. Also, the height and ground diameter of *M. officinalis* seedlings could be significantly increased with the direct light time extension. Shu et al. (2010) studied the average

germination rate of *M. officinalis* seeds was 50.8% under light and 43.2% under total darkness. There was a very significant difference in the seed germination rate of *M. officinalis* under light and darkness conditions ($P < 0.01$). In this study, *M. sprengeri* seed germination and seedling formation were sensitive to light, and continuous light significantly affected the seed germination and seedling formation of *M. sprengeri* ($P < 0.05$), which was consistent with the results of Li and Ma (2003) on *Quercus liaotungensis* in the field environment. The key parameters reflecting the seed germination of *M. sprengeri* such as the GPe, GPs, GI, and the key parameters reflecting seedling formation, such as SH, LN, and taproot length under

Fig. 2. Effects of soil moisture on the seed germination of *Magnolia sprengeri*. Different lowercases indicate significant differences among different treatments ($P < 0.05$).

Table 2. Effects of soil moisture on the seedling establishment of *Magnolia sprengeri*.

Soil moisture (%)	5	7	8	10	12	13	15	20
Seedling height (cm)	8.35 ± 0.32 d	9.12 ± 0.36 c	10.34 ± 0.65 c	13.65 ± 0.75 b	14.87 ± 0.64 b	17.23 ± 0.98 a	13.02 ± 0.12 bc	9.64 ± 0.68 c
Leaf number	1.14 ± 0.23 d	1.36 ± 0.25 d	2.31 ± 0.24 c	3.06 ± 0.24 b	3.76 ± 0.14 ab	4.14 ± 0.52 a	3.01 ± 0.36 b	1.25 ± 0.34 d
Basal stem diameter (mm)	1.06 ± 0.23 c	1.21 ± 0.36 c	1.66 ± 0.24 bc	1.85 ± 0.54 b	2.13 ± 0.67 b	2.74 ± 0.21 a	2.21 ± 0.25 ab	1.47 ± 0.31 bc
Taproot length (cm)	6.37 ± 0.58 d	8.54 ± 0.57 c	10.36 ± 0.45 c	13.54 ± 0.56 b	17.14 ± 0.61 a	18.21 ± 0.58 a	15.34 ± 0.34 b	11.75 ± 0.31 bc
Number of lateral root	8.65 ± 0.56 d	11.25 ± 0.57 cd	15.67 ± 0.84 c	20.34 ± 1.02 b	28.36 ± 1.32 a	32.65 ± 1.01 a	25.34 ± 0.87 b	21.79 ± 0.65 b
Maximum of lateral root length (cm)	3.52 ± 0.56 d	4.36 ± 0.67 d	5.17 ± 0.68 c	6.58 ± 0.69 b	7.02 ± 0.76 b	8.45 ± 0.65 a	6.39 ± 0.35 b	5.42 ± 0.65 c
Leaf area per seedling (cm ²)	13.49 ± 0.68 e	18.37 ± 0.69 d	28.98 ± 0.61 c	37.58 ± 0.74 b	40.35 ± 0.85 a	43.54 ± 0.58 a	39.68 ± 0.65 ab	27.35 ± 0.61 c
Fresh weight (g)	2.25 ± 0.34 b	2.67 ± 0.45 b	3.15 ± 0.24 a	3.37 ± 0.65 a	3.58 ± 0.35 a	3.95 ± 0.55 a	3.45 ± 0.23 a	2.69 ± 0.36 b
Seedling survival	29.58 ± 0.68 e	38.36 ± 0.87 e	46.87 ± 0.46 d	54.85 ± 0.98 c	66.36 ± 1.01 b	78.53 ± 1.26 a	67.58 ± 0.86 b	43.26 ± 0.45 d

Different lowercases mean significant differences among different treatments ($P < 0.05$).

the continuous light conditions, increased by 43.84%, 42.52%, 42.70%, 38.89%, 22.18%, and 55.01% compared with the continuous dark treatment group, respectively.

Light time and light intensity can directly affect plant photosynthesis, and enhance plant energy storage and organic substances accumulation, which could accelerate plant seed germination and seedling growth process (Wang et al., 2017). Therefore, the appropriate increase of light time is beneficial to promote the seed germination and seedling formation of *M. sprengeri*.

Effects of soil moisture on seed germination and seedling formation of *M. sprengeri*. Soil water maintains plant metabolism, nutrient absorption, and synthesis and transformation of organic substances. Soil water content directly affects the growth and development of plants (Guan, 2019). In this study, the results showed that soil moisture could significantly affect the seed germination and seedling formation of *M. sprengeri* based on the experiment of eight soil moisture gradients treatments ($P < 0.05$). The important indicators of seed germination including GPe, GPs, and VI, and the important indicators of seedling formation including seedling height, LN, and taproot length, reached the maximum when the soil moisture was 13%, increased by 62.80%, 71.11%, 68.44%, 51.53%, 72.46%, and 65.02% than the initial group (5% of soil moisture), respectively. In the seed germination stage, the seed expands by absorbing water in soil, the oxygen permeates into seed and seed respiration strengthens. At the same time, water can promote the seed protoplast state change from gel to sol, which can enhance physiological activity and accelerate seed germination. Furthermore, in the seedling establishment stage, the absorption of water and water-soluble minerals by the epidermis stimulated the secretion of secondary metabolites in the roots because of water tropism of plant roots, which increased the number of root hairs, enhanced root life activities, and promoted the growth of main root and lateral root (Chen et al., 2019). High soil moisture leads to hypoxia of soil environment, which resulted in weak plant respiration and incomplete oxidation of organic matters. A large number of harmful substances such as lactic

acid or alcohol are naturally produced. Simultaneously, plant has poor stress resistance, and plant growth is thus inhibited by high soil moisture (Fang et al., 2015). By contrary, plants cannot complete normal physiological and metabolic activities under low water content, resulting in the hysteresis of plant growth and development. These may be the reasons why too high or too low soil moisture affects plant seed germination and seedling formation. Obviously, the appropriate soil moisture can provide a suitable growth environment for the plant root, which is thus beneficial to the seed germination and seedling establishment of *M. sprengeri*. In this study, 13% of soil moisture content was a suitable environmental condition for seed germination and seedling formation of *M. sprengeri*, which was consistent with the findings in *Thermopsis lanceolata* and *Sophora flavescens* described by Wang et al. (2011). Guan (2019) also found a similar result, viz., the survival percentage of *Platycladus orientalis* seedlings was above 96.8% under the soil moisture at 12.1% to 14.74%.

It is a complex process from the seed germination by water absorption to a complete plant individual. There are many factors affecting plant seed germination and seedling formation. The life activity procedure of seed germination is initiated by the interaction of internal and external factors (Guan et al., 2020), but its related mechanism has been not clear until now. It needs to be further studied by combining different disciplines such as molecular biology, plant physiology, genomics, and proteomics. In conclusion, illumination time and soil moisture have an important impact on the seed germination and seedling formation of *M. sprengeri*. Continuous light and 13% of soil moisture content is suitable for seed germination and seedling establishment of *M. sprengeri*.

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