ABRATES

Journal of Seed Science

ISSN 2317-1545
www.abrates.org.br/revista

ARTICLE

Journal of Seed Science, v.45, e202345018, 2023

http://dx.doi.org/10.1590/2317-1545v45270437

Dormancy and germination characteristics of *Tarenaya hassleriana* (Cleomaceae) seeds

Zhao Ren-Fei[1,2,3], Shen Xue-Yang[1,2,3], Rong Zi-Han[1,2,3], Mou Jiao-Lin[1,2,3], Xu Li[1,2,3], Deng Zhi-Jun[1,2,3]*

ABSTRACT: Elucidating the physiological and ecological mechanisms of seed dormancy and germination is of great significance for species conservation and the application of plant resources. Based on Baskin and Baskin's classification system for seed dormancy, the cause of dormancy in *Tarenaya hassleriana* (Cleomaceae) seeds was studied using alternating temperature, cold moist stratification, dry storage, and GA_3 soaking treatment. The results indicated that fresh mature *T. hassleriana* seeds had a combinational dormancy, including a physical dormancy and a type 2 non-deep physiological dormancy, and were photoblastic, with an optimal germination temperature of 35°C. In addition, fresh mature *T. hassleriana* seeds may be efficiently released from dormancy and promoted to germinate by an alternating temperature of 20 °C/30 °C, cold moist stratification, and cold moist stratification following dry storage. Furthermore, GA_3 soaking treatment could also promote dormancy release and subsequent germination at 35 °C, and dry storage treatment could promote dormancy release and subsequent germination at 5–15 °C. These results also suggested that there were complex cross-talks among phytohormone, osmotic potential, and the temperature signaling regulatory pathways during dormancy release and germination of *T. hassleriana* seeds, which deserve further study.

Index terms: alternating temperature, cold moist stratification, dry storage, GA_3 soaking.

RESUMO: Elucidar os mecanismos fisiológicos e ecológicos de dormência e germinação de sementes é de grande importância para a conservação e uso de espécies vegetais. Baseando-se no sistema de classificação de Baskin e Baskin para dormência de sementes, neste trabalho foram estudadas as causas da dormência em sementes de *Tarenaya hassleriana* (Cleomaceae), utilizando-se temperaturas alternadas, estratificação úmida fria, armazenamento seco e tratamento de imersão em GA_3. Os resultados indicaram que as sementes maduras frescas de T. hassleriana tinham dormência combinada, incluindo dormência física e dormência fisiológica não profunda tipo 2, e eram fotoblásticas, com temperatura ótima de germinação de 35 °C. Além disso, sementes maduras frescas de *T. hassleriana* podem ter a dormência eficientemente quebrada e germinar por temperatura alternada de 20 °C/30 °C, estratificação úmida fria e estratificação úmida fria após armazenamento seco. Além disso, o tratamento de imersão com GA_3 também pode quebrar a dormência e subsequente germinação a 35 °C, e o tratamento de armazenamento a seco pode quebrar a dormência e subsequente germinação a 5–15 °C. Esses resultados também sugerem que houve interações complexas entre o fitohormônio, o potencial osmótico e as vias regulatórias de sinalização de temperatura durante a liberação de dormência e germinação de sementes de *T. hassleriana*, que merecem um estudo mais aprofundado.

Termos para indexação: temperaturas alternadas, estratificação fria e úmida, armazenamento a seco, imersão em GA_3.

***Corresponding author**
dengzhijun@hbmzu.edu.cn

Received: 12/15/2022.
Accepted: 04/13/2023.

[1]Hubei Key Laboratory of Biologic Resources Protection and Utilization (Hubei Minzu University), Enshi, Hubei Province, 445000, China.

[2]Research Center for Germplasm Engineering of Characteristic Plant Resources in Enshi Prefecture (Hubei Minzu University), Enshi, Hubei Province, 445000, China.

[3]The Plant Germplasm Resources Laboratory, School of Forestry and Horticulture, Hubei Minzu University, Enshi, Hubei Province, 445000, China.

INTRODUCTION

Seed dormancy to an evolutionary behavior that causes the viable seed not to germinate for a certain time even under ideal conditions (Baskin and Baskin, 2004). It is an adaptive trait that helps a seed of a particular species survive harsh environments, and seed germination and seedling emergence occur at the proper timing (Xu et al., 2014). Seed dormancy is also a typical quantitative genetic trait that involves a large number of genes and is controlled by both the parent plant and the environment (Baskin and Baskin, 2004); it may be one of the most complicated traits in genetic qualities of plants (Sohindji et al., 2020). For the foregoing reasons, it has been difficult to systematically and properly comprehend the mechanisms of seed dormancy and germination to date, necessitating the accumulation of more study data on seed dormancy and germination in more species.

Although seed dormancy favors the survival and propagation of wild species as an evolutionary trait, it also frequently has negative effects on agricultural production, such as vivipary and untidy seedling (Bewley et al., 2013). Therefore, it is of great theoretical and practical significance to explore the cause of seed dormancy and realize the artificial regulation of seed dormancy and germination.

The equilibrium between the physical–chemical inhibition of embryo growth by the tissues (pericarp, seed coat, and endosperm) around the embryo and the growth potential of the embryo controls seed dormancy and germination (Bewley et al., 2013; Nonogaki, 2014). Based on the causes of seed dormancy, Baskin and Baskin divided seed dormancy into five main classes using Nikolaeva's dormancy categorization scheme, i.e., physical dormancy (PY), physiological dormancy (PD), morphological dormancy (MD), morphophysiological dormancy (MPD), and combinational dormancy (PY+PD) (Baskin and Baskin, 2004, 2014, 2021; Nautiyal et al., 2023). Among them, PY is caused by the impermeability of the seed coat (or pericarp), PD is caused by the insufficiency of growth potential for the embryo, MD is caused by the underdeveloped embryos in the mature fruits, MPD has both MD and PD components, and combinational dormancy has both PY and PD components (PY + PD). Furthermore, some dormancy classes can be further divided into various dormancy levels, and some dormancy levels can also be further divided into several dormancy types. Determination of water permeability of the seed coat (or pericarp); observation of the developmental state of the embryo within the seed; culture of an excised embryo; and a germination test after GA_3, dry storage, and stratification treatment can be used to comprehensively analyze the causes of seed dormancy (Baskin et al., 2006; Deng et al., 2010).

There are 17 genera and 150 species in the family Cleomaceae worldwide, which are distributed over tropical and subtropical regions, and five genera and five species in China (Wu etal., 2008). To date, seed dormancy and germination have been reported in only *Tarenaya hassleriana* (Ye et al., 2012; Shi, 2016) and *Gynandropsis gynandra* (Ochuodho and Modi, 2007; Muasya et al., 2009; Nemahunguni et al., 2020) in Cleomaceae, but the causes of the seed dormancy could not be accurately determined from these studies. Previous studies have shown that *G. gynandra* seeds are photoblastic, and their dormancy release and germination could significantly be promoted by treatments, such as GA_3 soaking, cold moist stratification for 2 weeks, and smoke-water soaking (Ochuodho and Modi, 2007; Muasya et al., 2009; Nemahunguni et al., 2020).

Tarenaya hassleriana, an annual herb from the family Cleomaceae, is native to tropical America (Flora of China, 1999). There are a total of 33 plant species in the genus *Tarenaya*, and there is only *T. hassleriana* in China (Wu et al., 2008). It has high ornamental values, an excellent antipollution ability, and is also a nectar source and a medicinal plant (Zhang et al., 2015). *T. hassleriana* is propagated by seeds in production, and dormancy always results in delayed germination and a poor germination rate (Ye et al., 2012; Shi, 2016). A temperature difference of over 10 °C (Shi, 2016) and GA_3 soaking treatment (Ye et al., 2012) have been demonstrated to hasten the germination process and increase the germination rate of *T. hassleriana* seeds. Exploring the causes of seed dormancy in *T. hassleriana* not only provides the necessary basic data for the theoretical research on the phylogenetic and physiological mechanisms of seed dormancy and germination traits but also promotes the horticultural breeding, production, and promotion of this species, which has both theoretical and practical significance.

MATERIAL AND METHODS

Seeds: *T. hassleriana* seeds were collected from at least 50 plants growing in the nursery in the Heilongjiang Forest Botanical Garden in Harbin, China in October 2012. First, the collected seeds were naturally dried indoors for one week and then used in subsequent experiments. *Determination of the moisture content of the seed and 1000-seed weight*: Based on the International Seed Testing Protocol (ISTA, 1999), the moisture content of the seed was determined gravimetrically in four replicates of 30 seeds each. The seed moisture content was 13.63 ± 0.55%, and the 1000-seed weight is 2.23 ± 0.02 g. The 1000-seed weight was determined from four replicates of 1000 seeds each using the method of Song et al. (2005).

Determination of the water uptake by seeds: Using the method of Deng et al. (2010), intact and scarified fresh and dry-stored (8 months at 20 °C) seeds were sown on two pieces of filter paper moistened with 5 mL of distilled water in 9-cm-diameter Petri dishes and then incubated at 25 °C in a daily photoperiod of 12 h light/12 h dark (photosynthetic photon flux density is 121 $\mu mol.m^{-2}.s^{-1}$). At regular intervals, the incubated seeds were gently dried with absorbent filter paper and then weighed on an electronic balance (accuracy of 0.0001 g), until the seed mass no longer increased significantly. Each treatment of 30 seeds was replicated four times. The percentage water uptake was calculated using the following formula:

$$\text{Percentage water uptake (\%)} = (W_t\text{-}W_0)/W_0 \times 100$$

where: W_t represents the mass of the seeds after a given interval of imbibition (g), and W_0 represents the mass of the seeds before imbibition (g), i.e., the initial seed mass.

Germination test: Four replicates of 50 seeds were sown on two pieces of filter paper moistened with distilled water in 9-cm-diameter Petri dishes under given temperature and light conditions (darkness or alternating light). The darkness conditions were created by tightly wrapping the Petri dishes with aluminium foil, and the photoperiod was 12 h light/12 h dark per day (photosynthetic photon flux density is 121 ± 2 $\mu mol.m^{-2}.s^{-1}$). The germinated seeds were counted at around 20:30 every night using a green light for lighting. The germination tests ended after 30 days of incubation. Visible radicle protrusion was used as a germination criterion.

Alternating temperature treatment: Similar to the germination tests, fresh mature seeds were incubated under two alternating temperature conditions of 15 °C/25 °C and 20 °C/30 °C, and the control group was synchronously incubated at 35 °C in alternating light with 12 h dark/12 h light per day. The light and dark times of the alternating light were daily synchronous with the high- and low-temperature times of the alternating temperature. After 30 days of incubation, the experiments ended.

Dry storage treatment: The fresh mature seeds, well mixed with an equivalent volume of dry perlite, were placed into black polyethylene bags and sealed and then kept at 20 °C for a dry storage treatment. After a dry storage treatment for 8 months, the germination tests were conducted at 5, 10, 15, 20, 25, 30, and 40 °C under darkness or alternating light conditions.

GA_3 treatment: A certain amount of fresh mature seeds was randomly sampled and then soaked in 0.2, 0.4, 0.6, 0.8, and 1 $mmol.L^{-1}$ GA_3 solutions. After 24, 48, and 72 h of soaking, the seeds were taken out and washed under running water and then subjected to a germination test at 15, 25, and 35 °C under alternating light conditions. After incubation for 30 days, the initial germination rates were calculated. The seeds that did not germinate were transferred to 35 °C for an additional 30-day incubation, and then, the final accumulative germination rates were calculated.

Cold moist stratification treatment: Fresh mature seeds were evenly mixed with moist perlite (with a water content of 62.84 ± 0.23%) in a 1:3 volume ratio and then sealed in black polyethylene bags for a cold moist stratification treatment at 4 °C. After 1, 2, 3, 4, and 5 weeks of stratification, the seeds were sampled to test the germination at 5, 10, 15, 20, 25, 30, and 40 °C in alternating light.

GA_3 soaking treatment after dry storage: The seeds that had been dry-stored at 20 °C for 8 months were soaked in GA_3 solutions at concentrations of 0.2, 0.4, 0.6, 0.8, and 1 $mmol.L^{-1}$ at 25 °C. After 24 h, the GA_3 solutions were removed

and the seeds were washed under running water, and then, a germination test was conducted at 15, 25, and 35 °C in alternating light. After incubation for 30 days, the initial germination rates were calculated, and then, the seeds that did not germinate under several temperatures were transferred to 35 °C for an additional 30-day incubation, and the final accumulative germination rates were calculated.

Cold moist stratification treatment after dry storage: Dry-stored (8 months at 20 °C) seeds were mixed with moist perlite in a 1:3 volume ratio and then sealed in black polyethylene bags for a cold moist stratification treatment at 4 °C. After 1, 2, 3, 4, and 5 weeks of stratification, the seeds were sampled to test germination at 5, 10, 15, 20, 25, 30, 35, and 40 °C in alternating light.

Data analysis: All statistical analyses and graphing were done using the R software (R i386 3.5.2). The data on the germination of fresh and dry-stored seeds treated with GA_3 solutions were analyzed using multifactor ANOVA, while other germination data and the water uptake data were analyzed using one-way ANOVA, followed by the Student–Newman–Keuls multiple comparisons test (P = 0.05). To stabilize the variances, all percentage data were arcsine-transformed before the statistical analysis. All statistics are presented as mean ± standard error (SE) in the text.

RESULTS

Under darkness, no seeds germinated under 5–40 °C constant temperature regimes. Under the light, only very few seeds germinated at 20, 30, and 35 °C, and the final average germination rates after a 30-day incubation were all less than 5% (The results are not presented in graphs or tables). These results indicate that the fresh mature *T. hassleriana* seeds have dormancy and were likely photoblastic.

Seed coat integrity significantly affected seed water uptake ($P < 0.05$), but dry storage treatment did not (Figure 1). The rapid water uptake period (phase I) of fresh intact seeds is 0–8 h, followed by the water uptake plateau period (phase II), while phase I of the fresh scarified seeds is 0–48 h, followed by phase II. The water uptake of the intact and scarified dry-stored seeds was similar to that of the intact and scarified fresh seeds. Furthermore, regardless of the fresh or dry-stored seeds, the water uptake percentages of the scarified seeds were significantly higher than those of the intact seeds ($P < 0.05$).

Compared to the control group (35°C), the 20 °C/30 °C alternating temperature treatment significantly promoted the dormancy release and germination of *T. hassleriana* seeds ($P < 0.05$), and the final average germination rates were more than 90%, regardless of alternating light or darkness; the final average germination rate in alternating light was significantly higher than that in darkness ($P < 0.05$; Figure 2). However, the final average germination rate under 15 °C/25 °C alternating temperature treatment was not significantly different from that of the control group ($P > 0.05$), being less than 5% (Figure 2).

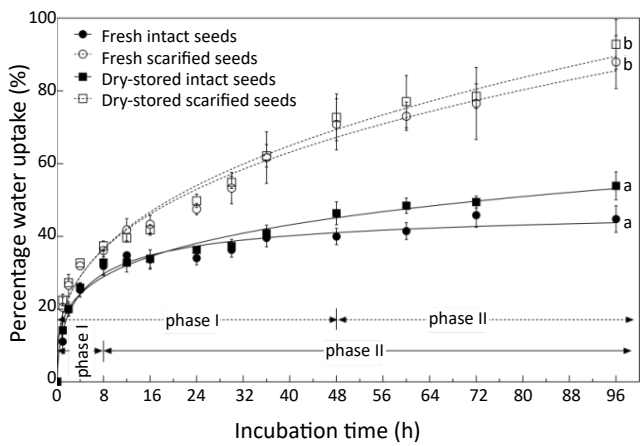

Figure 1. Water uptake curves of *Tarenaya hassleria*na seeds. Data marked with the same lowercase letters had no significant differences among each other (P = 0.05).

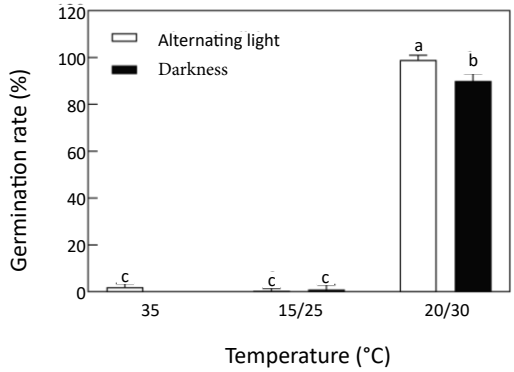

Figure 2. Effects of alternating temperature treatments on dormancy release and germination of fresh mature *Tarenaya hassleriana* seeds. The dark and light times of the alternating light were synchronous with the low- and high-temperature times of the alternating temperature treatments.Data marked with the same lowercase letters had no significant differences among each other (P = 0.05).

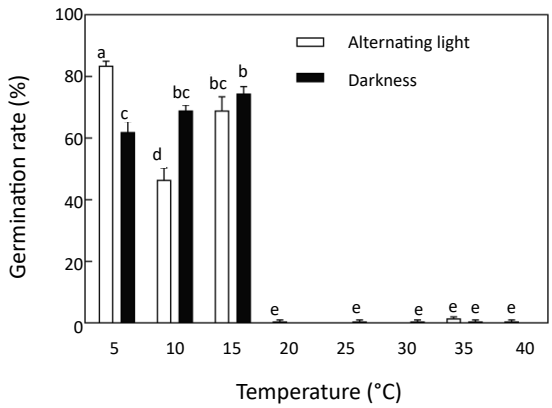

Figure 3. Effects of dry storage treatments on dormancy release and germination of fresh mature *Tarenaya hassleriana* seeds. Data marked with the same lowercase letters had no significant differences among each other (P = 0.05).

The germination rates of the dry-stored seeds increased significantly at 5, 10, and 15 °C, but not at 20-40 °C, regardless of the alternating light or darkness (Figure 3). From 5 °C to 15 °C, the germination rates in darkness rose with the rising temperature, while the germination rates in alternating light presented a changing trend of falling first and then rising (Figure 3).

The control group seeds soaked in distilled water for 24, 48, and 72 h barely germinated after 30 days of initial incubation at 15, 25, and 35 °C (Figure 4). After the first 30-day initial incubation, the ungerminated seeds were subsequently transferred to 35 °C or consecutively kept at 35 °C for another 30-day incubation. From the results, there were significant differences between the final cumulative germination rates following a 60-day incubation (P < 0.05). Specifically, the seeds initially incubated at 25 °C and 35 °C and subsequently transferred to 35 °C or consecutively kept at 35 °C still barely germinated (Figures 4D–I), while the final cumulative germination rates of the seeds initially incubated at 15 °C and then transferred to 35°C increased significantly and increased with extended soaking time (Figures 4A–C).

For the treatments wherein the seeds were first incubated at 15 °C for 30 days after GA_3 soaking treatment and then transferred to 35 °C for another 30-day incubation, the first 30-day initial germination rates at 15 °C were significantly promoted by the 24- and 48-h GA_3 soaking treatments compared to the control groups (P < 0.05, Figures

5A–B), whereas the 72-h GA_3 soaking treatments had no significant effect on the first 30-day initial germination rates at 15 °C ($P > 0.05$, Figure 4C). Specifically, for the 24-h GA_3 soaking treatments, only 0.6–1.0 mmol.L^{-1} GA_3 soaking treatment significantly increased the first 30-day initial germination rates; however, the initial germination rates were all less than 10% ($P < 0.05$, Figure 4A); in contrast, for the 48-h GA_3 soaking treatments, 0.2–1.0 mmol.L^{-1} GA_3 soaking treatments significantly increased the first 30-day initial germination rates; however, the initial germination

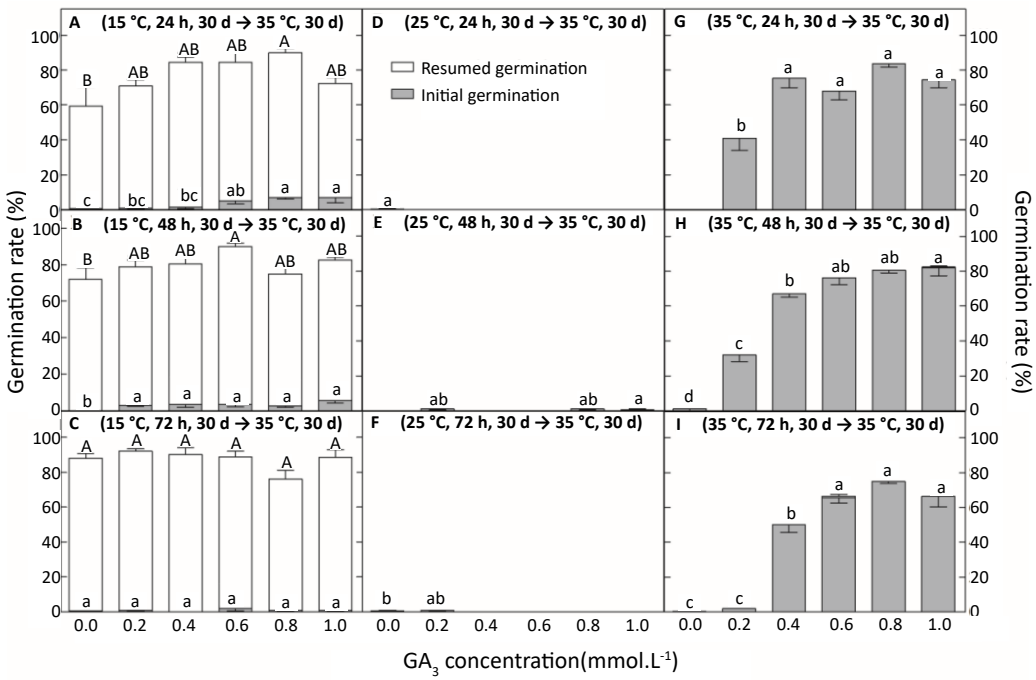

Figure 4. Effects of GA_3 soaking treatments on dormancy release and germination of fresh mature *Tarenaya hassleriana* seeds. "15 °C, 24 h, 30 d → 35 °C, 30 d" in Figure 4A means that the seeds were first soaked in GA_3 solutions for 24 h, followed by incubation at 15°C under alternating light conditions for 30 days for an initial germination test, and then transferred for another 30-day incubation at 35°C under alternating light. Panel captions in Figures 4B–I are similarly understood. The final cumulative germination rates are equal to the initial germination rates plus the resumed germination rates. There is no significant difference among the initial germination rates marked with the same lowercase letters and among the final cumulative germination rates marked with the same uppercase letters in the same figure ($P = 0.05$).

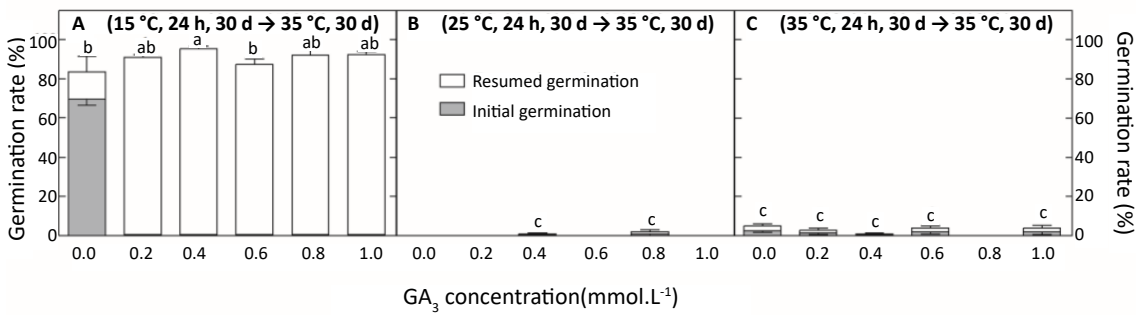

Figure 5. Effects of GA_3 soaking treatments after a dry storage treatment on dormancy release and germination of *Tarenaya hassleriana* seeds. Data marked with the same lowercase letters had no significant differences among each other ($P = 0.05$).

rates were also all less than 10% ($P < 0.05$, Figure 4B). After the seeds subjected to GA_3 soaking treatment were first incubated at 15 °C for 30 days and then transferred to 35 °C for another 30-day incubation, the final cumulative germination rates increased significantly for all GA_3 soaking treatments (Figures 4A–C). Among them, only the final cumulative germination rates of seeds soaked in 0.8 mmol.L^{-1} GA_3 solutions were significantly higher than those of the control groups in the 24-h GA_3 soaking treatments, reaching 90.0 ± 1.8% ($P < 0.05$, Figure 4A); only the final cumulative germination rates of seeds soaked in 0.6 mmol.L^{-1} GA_3 solution were significantly higher than those of the control groups in the 48-h GA_3 soaking treatments, reaching 90.0 ± 1.6% ($P < 0.05$, Figure 4B). The final cumulative germination rates of all concentrations of GA_3 soaking treatments were not significantly different from those of the control groups in the 72-h GA_3 soaking treatments ($P > 0.05$, Figure 4C).

For the treatments wherein the seeds were first incubated at 25 °C for 30 days after GA_3 soaking treatment and subsequently transferred to 35 °C for another 30-day incubation, both the first 30-day initial germination rates and the final cumulative germination rates were almost zero, regardless of the concentration of the GA_3 solution (Figures 4D–F).

Compared to the control groups, the GA_3 soaking treatments showed a significant promotive effect on the first 30-day initial germination under 35 °C, except for the 72-h 0.2 mmol.L^{-1} GA_3 soaking treatments ($P < 0.05$), while during the second 30-day incubation under 35 °C, there was no new increase in the final cumulative germination rates (Figures 4G–I). In addition, with the extended soaking time, the first 30-day initial germination rates showed a trend of falling, especially the 0.2 and 0.4 mmol.L^{-1} GA_3 soaking treatments (Figures 4G–I).

For the cold moist stratification of fresh seeds, the seed germination rates under various constant temperatures gradually increased along with extended stratification time, and the temperature ranges within which seeds could germinate gradually expanded from high to low temperatures (Table 1). In addition, the seeds stratified for 3 weeks achieved the highest germination rate of 94.0 ± 2.8% under 35 °C (Table 1).

The seeds that were dry-stored for 8 months germinated only at 5–15 °C, of which the highest germination rate of 83.5 ± 2.4% was achieved at 5 °C, while almost no germination occurred at 20–35 °C (Table 1). A cold moist stratification treatment after an 8-month dry storage treatment had a significant effect on the dormancy release and germination of

Table 1. Effects of cold moist stratification on dormancy release and germination of fresh and dry-stored *Tarenaya hassleriana* seeds.

Fresh/Dry-stored seeds	Stratification time (w)	Germination rate (%)						
		5 °C	10 °C	15 °C	20 °C	25 °C	30 °C	35 °C
Fresh seeds	0	0^{bA}	0^{bA}	2.0 ± 6.9^{cA}	0^{eA}	0^{eA}	0^{eA}	0.5 ± 4.1^{cA}
	1	0^{bA}	0^{bA}	0.5 ± 4.1^{cA}	0.5 ± 4.1^{eA}	5.5 ± 9.3^{dA}	2.0 ± 5.0^{dA}	2.0 ± 5.0^{cA}
	2	0^{bC}	0.5 ± 4.1^{bC}	2.5 ± 7.5^{cC}	0^{eC}	24.5 ± 4.5^{cB}	30.0 ± 4.0^{cB}	62.5 ± 5.2^{bA}
	3	0^{bF}	0^{bF}	0.5 ± 4.1^{cF}	8.0 ± 4.0^{dD}	70.5 ± 4.4^{bB}	57.5 ± 5.4^{bC}	94.0 ± 2.8^{aA}
	4	0.5 ± 4.1^{bE}	0^{bE}	23.0 ± 3.6^{bD}	38.5 ± 7.9^{cC}	70.0 ± 2.7^{aB}	77.0 ± 1.8^{bB}	91.5 ± 3.1^{aA}
	5	0.5 ± 4.1^{bE}	0.5 ± 4.1^{bE}	14.5 ± 10.0^{bD}	51.5 ± 7.7^{bC}	42.0 ± 2.5^{aC}	66.5 ± 2.7^{aB}	92.5 ± 3.7^{aA}
	6	8.0 ± 5.7^{aD}	10.0 ± 2.9^{aD}	68.5 ± 4.3^{aBC}	79.5 ± 5.9^{aB}	57.5 ± 4.4^{aC}	80.0 ± 4.7^{aB}	94.0 ± 6.1^{aA}
dry-stored seeds	0	83.5 ± 2.4^{aA}	46.5 ± 4.5^{aC}	69.0 ± 5.6^{aB}	0.5 ± 4.1^{dD}	0^{dD}	0^{fD}	1.5 ± 4.1^{fD}
	1	0.5 ± 4.1^{cB}	0^{dB}	1.0 ± 4.7^{dB}	0^{dB}	10.0 ± 5.9^{cA}	11.0 ± 7.0^{eA}	17.0 ± 5.2^{eA}
	2	0.5 ± 4.1^{cE}	0.5 ± 4.1^{dE}	22.0 ± 1.9^{cCD}	34.0 ± 2.6^{B}	29.5 ± 1.2^{bBC}	20.0 ± 2.6^{dD}	60.0 ± 5.8^{dA}
	3	25.5 ± 7.9^{bBC}	0.5 ± 4.1^{dD}	29.0 ± 3.7^{cBC}	18.5 ± 5.9^{cC}	36.5 ± 1.1^{bB}	36.0 ± 4.1^{cB}	86.0 ± 3.5^{bA}
	4	21.5 ± 5.4^{bC}	7.0 ± 6.0^{cD}	24.0 ± 6.3^{cC}	23.5 ± 4.6^{cC}	26.0 ± 6.4^{bC}	53.5 ± 1.7^{bB}	75.0 ± 1.7^{cA}
	5	90.0 ± 11.5^{aA}	20.5 ± 5.0^{bD}	55.0 ± 5.6^{bC}	82.5 ± 5.6^{aAB}	64.5 ± 7.6^{aBC}	74.5 ± 6.3^{aBC}	95.0 ± 3.6^{aA}

Note: Dry-stored seeds mean that dry seeds are sealed and stored for 8 months at 20 °C. There is no significant difference among data marked with the same superscript lowercase letters in the same column and the same superscript uppercase letters in the same row for the germination rates of stratified fresh and dry-stored seeds ($P = 0.05$).

T. hassleriana seeds ($P < 0.05$, Table 1). Concretely, the cold moist stratification treatment promoted germination of the dry-stored seeds at 20–35 °C but seemed to have inhibited that at 5–15 °C (Table 1).

For the 24-h distilled water and 0.2–1.0 mmol.L^{-1} GA_3 soaking treatments, only the seeds subjected to 24-h distilled water soaking treatments achieved a high initial germination rate of 69.0 ± 5.6% after the first 30-day incubation at 15 °C, while there was almost no germination in other treatments (Figure 5). When the seeds that were first incubated for 30 days at 15, 25, and 35 °C after a GA_3 soaking treatment were subsequently transferred to 35 °C or consecutively kept at 35 °C for another 30-day incubation, only seeds that were first incubated for 30 days at 15 °C had a large increase in the final accumulative germination rates, regardless of the concentrations of the GA_3 solutions for soaking, and the final accumulative germination rates were more than 80% (Figure 5A).

DISCUSSION

The initial germination test results indicated that the fresh mature *T. hassleriana* seeds are dormant and are likely to be photoblastic and that the optimal germination temperature is likely to be 35 °C.

PY is caused by one or more layers of water-impermeable palisade tissue cells in the seed coat or pericarp (Geneve et al., 2018; Nautiyal et al., 2023). Under natural conditions, high temperature, massively fluctuating temperature, fire, drying, freezing, and thawing, and going through the digestive tracts of animals are effective in releasing PY (Baskin and Baskin, 2014). It has been reported that PY in some seeds may be released by dry storage treatment (Baskin and Baskin, 2014). In this study, the water uptake rate and the percentage of scarified seeds were significantly higher than those of intact seeds, regardless of being fresh mature or dry-stored seeds (Figure 1), suggesting that both fresh mature and dry-stored *T. hassleriana* seeds have a PY, which also well explains why warm water soaking treatment can promote germination of *T. hassleriana* seeds in practical production (Shi, 2016).

The modified hormone balance theory of seed dormancy suggests that embryo-produced ABA induces dormancy during seed development and that GA promotes the germination of nondormant seeds (Baskin and Baskin, 2004; Mimi et al., 2023). During seed germination in *Arabidopsis* and potato, ABA and GA were antagonistic to each other in reducing and increasing embryo growth potential (Ableyet al., 2021). In the seed dormancy classification system of Baskin and Baskin (2004), the response of seed germination to GA treatment was also used as an important criterion for determining the level of PD, that is, GA promotes germination of all non-deep physiological dormant seeds and some intermediate physiological dormant seeds but not of deep physiological dormant seeds (Baskin and Baskin, 2014; Baek et al., 2021). In the present study, we found that the GA_3 soaking treatment significantly promoted the germination of *T. hassleriana* seeds at 35 °C, with the highest germination rate of approximately 85%, while there was either no germination or a very low germination rate (less than 10%) at 25 °C and 15 °C (Figures 4G–I), being similar to the findings of Ye et al. (2012) and suggesting that fresh mature *T. hassleriana* seeds have a non-deep PD or an intermediate PD and that the optimal germination temperature is 35 °C.

In the seed dormancy classification system of Baskin and Baskin (2021), the response of seed germination to dry storage treatment is also one of the indicators of the level of PD, that is, dry storage treatment can promote germination of some seeds with non-deep PD and shorten the time of cold moist stratification treatment of seeds with intermediate PD but has no effect on seeds with deep PD (Baskin and Baskin, 2014; Oh et al., 2021). In this study, the dry storage treatment for 8 months at 20 °C significantly promoted the germination of *T. hassleriana* seeds at 5–15 °C but extended the time of the subsequent cold moist stratification treatment (Figure 3, Table 1). Therefore, *T. hassleriana* seeds should have non-deep PD, and there seems to be a complex interplay between dry storage treatment and cold moist stratification treatment.

Based on the changing pattern of the temperature ranges within which seeds can germinate with the gradual seed dormancy release, non-deep PD can be further subdivided into six dormancy types, i.e., types 1–6 (Baskin and Baskin, 2021). In this study, the temperature ranges within which *T. hassleriana* seeds could germinate presented a

changing pattern extending from high to low temperatures along with the gradual dormancy release under cold moist stratification treatments, and the seeds stratified for 3 weeks achieved the highest germination rate of 94.0 ± 2.8% at 35 °C (Table 1). According to the seed dormancy classification system of Baskin and Baskin (2021), fresh mature *T. hassleriana* seeds should have a type 2 non-deep PD and an optimal germination temperature of 35 °C. The high optimal germination temperature of *T. hassleriana* seeds is because of its tropical American origin, supporting the view that seed dormancy and germination behaviour are evolutionarily adaptive traits.

Alternating temperatures generally promote seed germination more than constant temperatures (Baskin and Baskin, 2004; Forti et al., 2020). An alternating temperature treatment can increase the level of germination-promoting phytohormones and decrease the level of germination-inhibiting phytohormones (Huarte et al., 2014; Özden et al., 2021). Compared to the initial germination of freshly harvested *T. hassleriana* seeds at constant temperature , the 20 °C/30 °C alternating temperature treatment could effectively release seed dormancy and promote germination, regardless of alternating light or darkness, whereas the germination rate in alternating light was significantly higher than that in darkness ($P < 0.05$), being more than 90%, while the 15°C/25°C alternating temperature treatment did not show a significant effect on seed germination (Figure 2). These results is similar to those in *G. gynandra* seeds also from the family Cleomaceae (Mashamaite et al., 2022). The physiological mechanisms by which alternating temperature treatments promote seed dormancy release and germination deserve further study, and *T. hassleriana* seeds are the ideal subjects of study.

Effects of GA_3 soaking, dry storage, cold moist stratification, and incubation temperature on seed dormancy release and germination involve the regulation of seed dormancy and germination by phytohormone, osmotic potential, and the temperature signalling transduction pathways, whereas the interrelationship between these three signal transduction pathways is still unclear to date. In this study, the first interesting phenomenon is that GA_3 soaking treatments basically could promote dormancy release and germination of fresh mature *T. hassleriana* seeds at 35 °C (Figures 4G–I), while dry storage treatments seemed to deepen the seed dormancy at 35 °C, which is perhaps because dry storage treatment reduced the sensitivity of seeds to GA_3, so that GA_3 soaking treatments hardly played any role in the dormancy release and germination of dry-stored seeds (Figure 5). A similar phenomenon was also observed in the study on *Arabidopsis* seeds (Basbouss-Serhal et al., 2016). The second interesting phenomenon is that, regardless of being soaked with distilled water or GA_3 solution, the seeds that were first incubated for 30 days at 15 °C had a fairly high final cumulative germination rate after being transferred to 35°C for another 30-day incubation (Figures 4A–C), whereas both the initial germination rates of seeds first incubated for 30 days at 25 °C after a soaking treatment with distilled water or GA_3 solution and the final cumulative germination rates after being transferred to 35 °C for another 30-day incubation were almost zero (Figures 4D–F). This seems to imply a complex cross-talk between the temperature and phytohormone signalling regulatory pathways during seed dormancy release and germination. The third interesting phenomenon is that seeds dry-stored for 8 months at 20 °C had a high germination rate only at low temperatures of 5–15 °C, with an average germination rate of 46.5–83.5%, while almost no germination occurred at high temperatures of 20–35 °C, and the cold moist stratification treatment after a dry storage treatment greatly facilitated seed dormancy release and germination at various incubation temperatures—in other words, the dry storage treatment shortened the time of the cold moist stratification treatment required for seed dormancy release and germination (Table 1). These results suggest that there are complex cross-talks among the phytohormone, osmotic potential, and temperature signalling regulatory pathways during seed dormancy release and germination, which deserve further in-depth study.

CONCLUSIONS

Fresh mature *T. hassleriana* seeds have a combinational dormancy, including a PY and a type 2 non-deep PD, and are photoblastic, with an optimal germination temperature of 35 °C. Fresh mature *T. hassleriana* seeds may be efficiently released from dormancy and promoted to germinate by an alternating temperature of 20 °C/30 °C, cold

moist stratification, and cold moist stratification following dry storage. In addition, GA_3 soaking treatment could also promote dormancy release and germination at 35 °C, and dry storage treatment could promote dormancy release and germination at 5–15 °C. These results also suggest that there are complex cross-talks among phytohormone, osmotic potential, and the temperature signalling regulatory pathways during dormancy release and germination of *T. hassleriana* seeds, which deserve further study.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (31860073, 81303169) and the Project of Opening Fund for the Year 2022 of Hubei Key Laboratory for the Protection and Utilization of Biological Resources (PT012212). We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

REFERENCES

ABLEY, K.; FORMOSA-JORDAN, P.; P, TAVARES, H.; CHAN, E.Y.T.; AFSHARINAFAR, M.; LEYSER, O.; LOCKE, J.C.W. An ABA-GA bistable switch can account for natural variation in the variability of *Arabidopsis* seed germination time. *Elife*, v.10, e59485, 2021. https://doi.org/10.7554/eLife.59485.

BAEK, S.G.; IM, J.H.; KWAK, M.J.; PARK, C.H.; LEE, M.H.; NA, C.S.; WOO, S.Y. Non-deep physiological dormancy in seed and germination requirements of *Lysimachia coreana* Nakai. *Horticulturae*, v.7, n.11, p.490, 2021. https://doi.org/10.3390/horticulturae7110490

BASBOUSS-SERHAL, I.; LEYMARIE, J.; BAILLY, C. Fluctuation of *Arabidopsis* seed dormancy with relative humidity and temperature during dry-storage. *Journal of Experimental Botany*, v.67, n.1, p.119-130, 2016. https://doi.org/10.1093/jxb/erv439

BASKIN, C.C.; BASKIN, J.M. *Seeds: ecology, biogeography, and evolution of dormancy and germination*. 2nd ed. San Diego, USA: Academic Press, 2014. 1586p.

BASKIN, C.C.; THOMPSON, K.; BASKIN, J.M. Mistakes in germination ecology and how to avoid them. *Seed Science Research*, v.16, n.3, p.165-168, 2006. https://doi.org/10.1079/SSR2006247

BASKIN, J.M.; BASKIN, C.C. A classification system for seed dormancy. *Seed science research*, v.14, n.1, p.1-16, 2004. https://doi.org/10.1079/SSR2003150

BASKIN, J.M.; BASKIN, C.C. The great diversity in kinds of seed dormancy: a revision of the Nikolaeva–Baskin classification system for primary seed dormancy. *Seed Science Research*, v.31, n.4, p.249-277, 2021. https://doi.org/10.1017/S096025852100026X

BEWLEY, J.D.; BRADFORD, K.J.; HILHORST, H.W.M.; NONOGAKI, H. *Seeds: physiology of development, germination and dormancy*. 3th ed. New York: Springer Science and Busines Media, 2013. 392p.

DENG, Z.J.; CHENG, H.Y.; SONG, S.Q. Effects of temperature, scarification,dry storage, stratification,phytohormone and light on dormancy-breaking and germination of *Cotinus coggygria* var. *cinerea* (Anacardiaceae) seeds. *Seed Science and Technology*, v.38, n.3, p.572-584, 2010. https://doi.org/10.15258/sst.2010.38.3.05

EDITORIAL BOARD OF FLORA OF CHINA, Chinese Academy of Sciences. *Flora of China*. Beijing: Science press, 1999. 534p.

FORTI, C.; OTTOBRINO, V.; BASSOLINO, L.; TOPPION, L.; ROTION, G.L.; PAGANO, A.; MACOVEI, A.; BALESTRAZZI, A. Molecular dynamics of pre-germinative metabolism in primed eggplant (*Solanum melongena* L.) seeds. *Horticulture research*, v.7, n.1, p.12, 2020. https://doi.org/10.1038/s41438-020-0310-8

GENEVE, R.L.; BASKIN, C.C.; BASKIN, J.M.; GEHAN JAYASURIYA, K.M.G.; GAMA-ARACHCHIGE, N.S. Functional morpho-anatomy of water-gap complexes in physically dormant seed. *Seed Science Research*, v.28, n.3, p.186-191, 2018. https://doi.org/10.1017/S0960258518000089

HUARTE, H.R.; LUNA, V.; PAGANO, E.A.; ZAVALA, J.A.; BENECH-ARNOLD, R.L. Fluctuating temperatures terminate dormancy in Cynara cardunculus seeds by turning off ABA synthesis and reducing ABA signalling, but not stimulating GA synthesis or signalling. *Seed Science Research*, v.24, n.2, p.79-89, 2014. https://doi.org/10.1017/S0960258514000051

ISTA. *International Rules for Seed Testing Association*, No.Suppl. Zurich, 1999. p.47-50.

MASHAMAITE, C.V.; MANYEVERE, A.; CHAKAUYA, E. *Cleome gynandra*: A wonder climate-smart plant for nutritional security for millions in semi-arid areas. *Frontiers in Plant Science*, v.13, 2022. https://doi.org/10.3389/fpls.2022.1003080

MIMI, C.O.; SOUSA, M.C.; CORRÊA, P.L.C.; DE-LA-CRUZ-CHACÓN, I.; BOARO, C.S.F.; FERREIRA, G. Impact of GA_3 on sugar and lipid degradation during *Annona×atemoya* Mabb. seed germination. *Horticulturae*, v.9, n.3, p.388, 2023. https://doi.org/10.3390/horticulturae9030388

MUASYA, R.M.; SIMIYU, J.N.; MUUI, C.W.; RAO, N.K.; DULLOO, M.E.; GOHOLE, L.S. Overcoming seed dormancy in *Cleome gynandra* L. to Improve Germination. *Seed Technology*, v.31, n.2, p.134-143, 2009. https://www.jstor.org/stable/23433309

NAUTIYAL, P.C.; SIVASUBRAMANIAM, K.; DADLANI, M. Seed dormancy and regulation of germination. In: Dadlani, M.; Yadava, D.K. (Eds) *Seed Science and Technology*. Singapore: Springer, 2023. p.39-66. https://doi.org/10.1007/978-981-19-5888-5_3

NEMAHUNGUNI, N.K.; GUPTA, S.; KULKARNI, M.G.; FINNIE, J.F.; STADEN, J.V. The effect of biostimulants and light wavelengths on the physiology of *Cleome gynandra* seeds. *Plant Growth Regulation*, v.90, n.3, p.467-474, 2020. https://link.springer.com/article/10.1007/s10725-019-00546-7

NONOGAKI, H. Seed dormancy and germination — Emerging mechanisms and new hypotheses. *Frontiers in Plant Science*, v.5, p.233, 2014. https://doi.org/10.3389/fpls.2014.00233

OCHUODHO, J.O.; MODI, A.T. Light-induced transient dormancy in *Cleome gynandra* L. seeds. *African Journal of Agricultural Research*, v.2, n.11, p.587-591, 2007. https://www.webofscience.com/wos/woscc/full-record/WOS:000259933100007

OH, H.J.; SHIN, U.S.; LEE, S.Y.; KIM, S.Y.; JEONG M.J. Non-deep physiological dormancy in seeds of *Euphorbia jolkinii* Boiss. native to Korea. *Journal of Ecology and Environment,* v.45, p.20, 2021. https://doi.org/10.1186/s41610-021-00194-x

ÖZDEN, E.; LIGHT, M.E.; DEMIR, I. Alternating temperatures increase germination and emergence in relation to endogenous hormones and enzyme activities in aubergine seeds. *South African Journal of Botany*, v.139, P.130-139, 2021. https://doi.org/10.1016/j.sajb.2021.02.015

SHI, J.W. Introduction and application of Drunken Butterfly in Ji'an area. *Contemporary Horticulture*, v.22, p.119-120, 2016. https://doi.org/10.14051/j.cnki.xdyy.2016.22.096

SOHINDJI, F.S.; SOGBOHOSSSOU, D.E.O.; ZOHOUNGBOGBO, H.P.F.; HOUDEGBE, C.A.; ACHIGAN-DAKO, E.G. Understanding molecular mechanisms of seed dormancy for improved germination in traditional leafy vegetables: An overview. *Agronomy*, v.10, n.1, 57, 2020. https://doi.org/10.3390/agronomy10010057

SONG, S.Q.; CHENG, H.Y.; LONG, C.L.; JIANG, X.C. *Seed Biology Research Guide*, Beijing: Science press, 2005. 192p.

WU, Z.Y.; RAVEN, P.H.; HONG, D.H. *Flora of China*. Beijing: Science press and St. Louis: Missouri Botanical Garden Press, 2008. p.429-432. https://www.cabdirect.org/cabdirect/abstract/20093249776

XU, J.; LI, W.L.; ZHANG, C.;LIU, W.; DU, G.Z. Variation in seed germination of 134 common species on the eastern Tibetan Plateau: phylogenetic, life history and environmental correlates. *Plos one*, v.9, n.6, e98601, 2014. https://doi.org/10.1371/journal.pone.0098601

YE, J.X.; ZHANG, G.C.; YANG, S.; YU, Z.D.; QIAO, J.L. The Effect of GA3 and temperature on the germination of *Cleome spinosa* Jacq seeds. *Seed*, v.31, n.8, p.84-85, 2012. https://doi.org/10.16590/j.cnki.1001-4705.2012.08.076

ZHANG, K.; WU, Z.S.; HOU, M. Cultivation and application of *Tarenaya hassleriana*. *Contemporary Horticulture*, n.16, p.43, 2015. https://doi.org/10.14051/j.cnki.xdyy.2015.16.038