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GERMINATION AND SECONDARY DORMANCY OF *HYDROCHARIS DUBIA* (BLUME) SEED

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Abstract

The germination of freshly harvested, dry-stored and wet-stored *Hydrocharis dubia* seeds were treated with exogenous GA_3, cold stratification, puncture, different temperatures and combined treatments under controlled laboratory conditions. The results showed that: The optimal temperature for *H. dubia* seed germination is 23℃ and fresh seed has conditional dormancy, which can be released by puncture. Seed germination percentage decreased significantly after dry seed storage and the main reason for the reduction of "seed germination ability" by dry storage was that dry storage. caused secondary dormancy but a reduction of seed vigor. The secondary dormancy is non- deep physiological dormancy, which can be released by cold stratification, seed puncture and combination treatments. The findings provided further insight into how germination strategy contributes to the seed bank formation and species reproduction.

Introduction

Aquatic plants are important aquatic primary producers (Wagner and Oplinger 2017). They also perform several functions in the ecosystem, including sediment uptake and stabilization, absorption and reduction of aquatic nutrients, and provision of valuable habitat for juvenile fish and crabs and other marine and riverine species (Ibelings *et al*. 2007). Aquatic plants have an excellent cleaning effect in eutrophic waters and are attracting increasing attention due to their unique economical advantages, low energy consumption, ease of operation, and benefits in rebuilding and restoring an excellent aquatic ecosystem. However, in recent decades, aquatic plant populations have declined in many places around the world due to eutrophication, pollution, habitat loss, and global warming (Ke and Li 2006, Kauth and Biber 2014). Therefore, restoration and protection of aquatic plant populations are urgently needed. Seed germination and dormancy are critical for restoration and protection. For aquatic plants, some studies focused on seed germination. Increased temperature from 15 to 30℃ had no significant effect on the final germination of *Myriophyllum spicatum* , but enhanced significantly the germination of *Potamogeton malaianus* (Xiao *et al.* 2010). Cold-wet seed storage led to higher and faster germination for *Juncus tenuis, J. ensifolius, Alopecurus aequalis*, but *Carex nebrascensis* seeds and fluctuating temperatures produced the highest germination of the four seeds (Wagner and Oplinger 2017).

Hydrocharis dubia is an aquatic plant with floating leaves. It is a high seed yielding plant. *H. dubia* can be used as fodder, fertilizer, vegetable and ornamental plant. It is also important and effective in purifying water (Zhao *et al.* 2008). However, little is known about the germination and dormancy of *H. dubia* seeds.It has been reported that the germination capacity of *H. dubia* seeds decreases significantly in a dry environment and remains at a high level in a humid environment. It has been suggested that the decreasing germination in a dry environment is due to the reduction of seed vigor and is related to seed moisture and hydrogen peroxide content (Zhao *et al*. 2017). However, the evidence is insufficient because dormancy and inappropriate germination conditions

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can also prevent germination (Baskin and Baskin 2004). And the vigor of dry-stored *H. dubia* seeds was not investigated in this study.

The aim of the present study was to investigate the optimal germination conditions, the type of dormancy, the methods to break dormancy, and the effects of dry storage on dormancy.

Materials and Methods

Ripe fruits of *H. dubia* were harvested in November 2020 from plants growing naturally in Chuzhou Botanical Garden, Anhui Province, China. The fruit is berry-shaped, and each fruit contains hundreds of seeds. The seeds are ellipsoid, with brown coats and hooked spines (Fig. 1). One hundred seeds were weighed, and the fresh weight of one hundred *H. dubia* seed was about 0.5 mg.

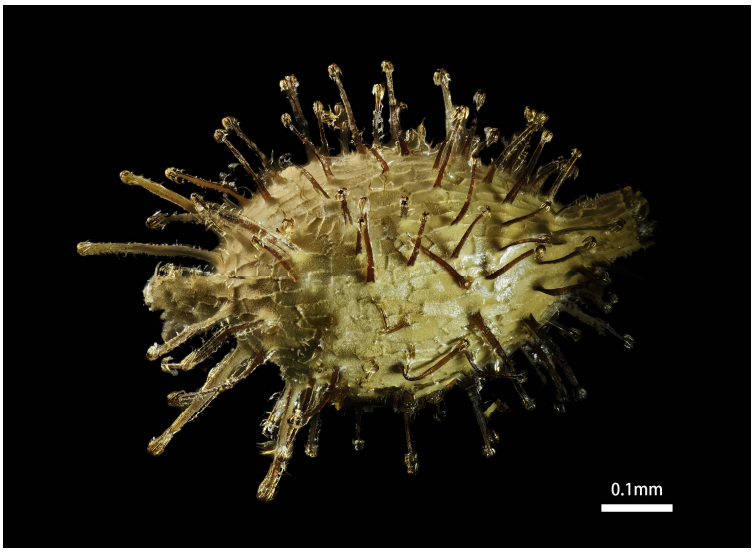

Fig. 1. Appearance of *H. dubia* seed.

Samples were stored under two conditions: (i) seeds were dry and stored at room temperature: seeds were extracted from fruits, washed with distilled water, and then dried naturally in a well-ventilated room and stored in paper bags at room temperature for 7 days and 30 days. (ii) the fruits were stored at a low temperature: the fruits were kept in a box filled with distilled water and stored at 4℃ for 30 and 50 days.

Three replicates of 50 seeds each were placed in glass culture dishes. Germination tests were performed under light at different temperatures. Seeds were kept moist by addingt distilled water to the trays as needed. The emergence of a 2 cm radical was used as a criterion for seed germination. Seed germination was recorded daily for 30 days.

H. dubia seeds were treated with puncture, cold stratification treatment, GA_3, puncture treatment+cold stratification treatment. Seed puncture treatment: the seed coat and embryo of *H. dubia* seeds were pricked with a needle (0.14 mm). Cold stratification treatment: cold stratification treatment is a technique of stimulating seed germination and breaking seed dormancy by exposing them to cold temperatures, mimicking the natural process of cold weather. *H. dubia* seeds were soaked for 48 hrs, then covered with wet filter paper and stored in glass culture dishes at 4°C for

30 and 50 days. GA_3 treatment: GA_3 was dissolved in ethanol to prepare a 0.5 g/l GA_3 solution. For the germination test, 50 ml of the GA_3 solution was added to a Petri dish. Combined treatments: Seed puncture treatment + cold stratification treatment: seeds were punctured at different time points after cold stratification treatment. Combined treatments: GA_3 + cold stratification treatment: seeds were soaked in 0.5 g/l GA_3 solutions at different points in time after cold stratification treatment.

The data were analyzed using SPSS 20.0. The measure of dispersion of the results were analyzed. Results were compared using the least significant difference (multiple comparisons).

Results and Discussion

Two-way analyses ANOVA showed that germination of *H. dubia* was significantly affected by different treatments (Table 1). There were interactive effects between germination temperature and other treatments on germination of *H. dubia* seeds.

Table 2 shows the effect of temperature and treatments on the percentage germination of *H. dubia* seeds. Percent germination of fresh seeds was 1, 88, 81, and 39% at 15, 23, 25, and 30°C, respectively. Dry storage reduced seed germination quite significantly. No seeds germinated after dry storage for 7 and 30 days. Wet storage showed little effect on the germination percentage of *H. dubia* seeds. Puncture treatment enhanced significantly the seed germination. The percentage of germination of fresh seeds and seeds treated by puncture and wet storage was significantly higher at 23°C than at other temperatures.

Table 1. Two-way ANOVA on the effect of different temperatures and other treatments on seed germination of *H. dubia.*

Source	df	Mean square	F-value	Sig.
Other treatments	5	1.387	952.578	***
Temperature	3	0.75	514.986	***
Other treatment * Temperature	15	0.13	88.977	***
Total	72			

***showed significant differences at the 0.01 level.

Table 2. The germination percentage of *H. dubia* seeds with different treatments germinated at 15, 23, 25, and 30 °C.

	Germination Percentage/%					
Temperature (°C)	Fresh seed	Puncture	Dry storage for 7 days	Dry storage for 30 days	Wet storage for 30 days	Wet storage for 50 days
15	1 ± 1^{f}	77 ± 3^{c}	0 ± 0^{f}	0 ± 0^{f}	1 ± 2^{f}	0 ± 0^{f}
23	88 ± 2^{a}	94 ± 2^{a}	0 ± 0^{f}	0 ± 0^{f}	88 ± 4^{a}	89 ± 8^{a}
25	81 ± 4^{b}	89 ± 2^{b}	0 ± 0^{f}	0 ± 0^{f}	53 ± 6^{d}	80 ± 5^{b}
30	39 ± 8^{e}	9 ± 2^{f}	0 ± 0^{f}	0 ± 0^{f}	35 ± 6^{e}	41 ± 5^{e}

The different letters showed significant differences at 0.05 level among treatments as determined by LSD comparison.

Two-way analyses ANOVA showed that germination of *H. dubia* seeds stored dry for 7 days was significantly affected by different germination temperatures and treatments to break dormancy (exogenous GA_3, cold stratification treatment, puncture, and combined treatments) (Table 3). There were interactive effects between germination temperature and dormancy relieving *H. dubia* seed germination.

Table 3. Two-way ANOVA on the effect of different germination temperatures and dormancy relieving treatments on germination of *H. dubia* seed dry stored for 7 d.

Source	df	Mean Square	F-value	Sig.
Treatment	8	0.963	235.444	***
Temperature	2	0.430	105.135	***
Treatment * Temperature	16	0.081	19.761	***
Total	81			

***showed significant differences at the 0.01 level.

Figures 2 to 4 showed that the dry-stored seeds (CK) and the dry-stored seeds treated with GA_3 or puncture did not germinate at 15, 23, and 25℃ (Fig. 2 to 4). At 15℃, the dry-stored seeds treated with puncture after 30 and 50-day cold stratification treatment showed the highest degree of germination (75 and 76%, respectively). The germination percentage was 0% for dry-stored seeds, where as it was 36 % for the seeds subject to cold stratification treatment for 30-day treatment. At 23℃ (Fig. 3), the dry-stored seeds treated with puncture after 50 days cold stratification treatment and 30 days cold stratification treatment $+GA_3$ showed the highest germination percentage (85 and 86%). At 25℃ (Fig. 4), the dry-stored seeds treated with puncture after 30 days of cold stratification treatment had the highest germination percentage (80%). The other treatments also promoted seed germination significantly.

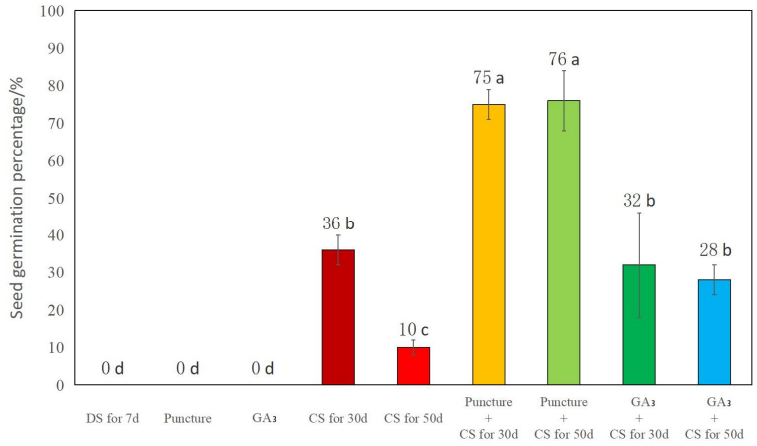

Fig. 2. The germination percentage of *H. dubia* seeds dry stored for 7d with different treatments germinated at 15℃. Vertical bars are standard errors of the mean. The different letters showed significant differences at 0.05 level among treatments as determined by LSD comparison. CK: Control check, seeds dry stored for 7d. CS: Cold stratification treatment.

Many species of aquatic plants exhibit seed dormancy, which protects them from germination under unfavorable environmental conditions (Teltscherová and Hejný 1973). Much is known about the primary dormancy of aquatic plants (Yin *et al*. 2013, Li *et al*. 2015). Table 2 shows the germination percentage at 23℃ is much higher than the other temperatures, suggesting that fresh *H. dubia* seed has conditional dormancy. Seed viability was tested with 1 and 0.5% TTC (2, 3, 5-triphenyltetrazolium chloride) solution. Both whole seeds and punctured seeds were unstained at 30 and 35℃ for five days and examined daily with a stereomicroscope for five days. Meanwhile, some punctured seeds germinated in the TTC solution. Thus, it was not yet known whether ungerminated seeds at 23℃ were caused by dormancy or low seed viability.

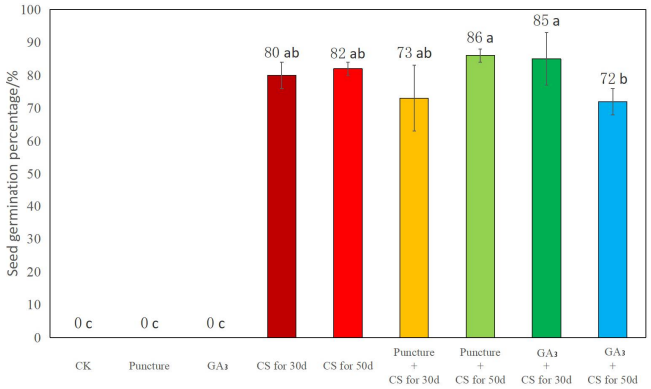

Fig.3. The germination percentage of *H. dubia* seeds dry stored for 7d with different dormancy relieving treatments germinated at 23℃. Vertical bars are standard errors of the mean. The different letters showed significant differences at 0.05 level among treatments as determined by LSD comparison. CK: Control check, seeds dry stored for 7d. CS: Cold stratification treatment.

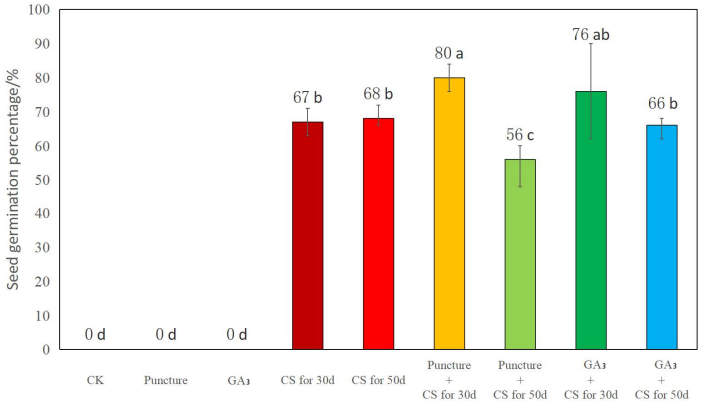

Fig. 4. The germination percentage of H. dubia seeds dry stored for 7d with different dormancy relieving treatments germinated at 25℃. Vertical bars are standard errors of the mean. The different letters showed significant differences at 0.05 level among treatments as determined by LSD comparison. CK: Control check, seeds dry stored for 7d. CS: Cold stratification treatment.

The optimal germination temperature of *H. dubia* seeds was 23℃ (Table 2 and Fig. 3). *H. dubia* seeds mature in November when the ambient temperature was often below 15℃. This avoids seed germination in winter and cold damage. The seed wet storage test simulated the

overwintering process of *H. dubia* seeds. After 30 and 50 days wet storage, the optimal germination temperature of seeds was still 23℃, and the germination percentage was 88% (Table 2). This shows that wet-stored *H. dubia* seeds still have conditional dormancy, which helps the seeds to germinate rapidly in spring when the temperature rises.

At 15, 23, 25, and 30℃ after 7 and 30 days dry seed storage, seeds did not germinate (Table 2). The cold stratification treatment, cold stratification treatment +GA_3, and cold stratification treatment+puncture treatments were able to significantly promote germination of the dry-stored seeds. Moreover, the dry-stored seeds that were punctured after 50 days of cold stratification treatment appeared to have the highest germination percentage (86%) at 23℃ (Fig. 3). Therefore, we considered that the main reason for the reduction of *H. dubia* germination caused by dry storage was not secondary dormancy, but the reduction of seed vigor.

It may help *H. dubia* survive in environments with seasonal drought. The ability of seeds to enter secondary dormancy affects the transmissibility of the seed bank over years (Hawkins 2014). The predisposition of seeds to secondary dormancy varies among genotypes for oilseed rape (Weber *et al*. 2010).

According to Baskin and Baskin (2004) dormancy types, seed dormancy includes physiological dormancy, physical dormancy, morphological dormancy, morphophysiological dormancy, and combined dormancy (Zhao *et al*. 2017). Fresh *H. dubia* seeds germinated well at 23℃, so *H. dubia* seeds have no morphological or morphophysiological dormancy. And the secondary dormancy of *H. dubia* seeds are not deep physiological dormancy, which can be canceled by 30 days cold stratification treatment. Secondary dormancy can be abolished by 30 days cold stratification treatment, so secondary dormancy is probably a non-deep physiological dormancy.

Seed germination and dormancy are controlled by light, temperature, and hormones (Kim *et al*. 2008, Chastain *et al*. 2017, Xue *et al*. 2018). Plant hormones have a great influence on the regulation of seed germination (Muller *et al*. 2006). Furthermore GA_3 is widely known as a regulator of seed germination and dormancy (Graebe *et al*. 2012, Hoang *et al*. 2014). However, GA_3 had no significant effect on the germination of *H. dubia* seeds with secondary dormancy (Figs 2-4).

Puncture treatment is commonly used to make the seed coat permeable and break physical dormancy. In the experiment, the conditional dormancy was well reversed by the puncture treatment (Table 2 and Fig. 2), especially at 15 °C. At 30℃, the seed percentage decreased, but the seed germinated faster (Table 2. This might be due to the fact that the injured embryos are more susceptible to disintegration at high temperatures.

(i) The optimal temperature for germination of *H. dubia* seeds was 23℃, and fresh seed has conditional dormancy that can be induced by the puncture.

(ii) The main reason that dry storage reduced seed "seed germination ability" was that dry storage caused secondary dormancy but reduced seed vigor.

(iii) Secondary dormancy is non-deep physiological dormancy that can be reversed by cold stratification treatment.

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