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Effects of ultra-dry storage on seed germination and seedling growth of *Handeliondendron bodinieri*

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Highlings

- By reducing the moisture content of the seeds, *Handeliodendron bodinieri* seeds showed a strong tolerance.
- PEG played a protective role in the process of re-wetting ultra-dry seeds.
- Ultra-dry storage could promote *Handeliodendron bodinieri* seedling growth and root development.

Abstract

Handeliodendron bodinieri (H. Lév.) Rehder is a rare, endangered, and therefore, protected tree species native to China. However, there are serious limitations to the effective protection of the species, including a low seed germination-rate and difficult storage due to a high seed oil-content. Here, we evaluated the feasibility of ultra-dry seed storage and its effects on seedling growth. We used the silica gel method to prepare ultra-dry seeds with different moisture contents to find an optimal moisture content range (2.54%–4.77%). Ultra-dry treatment improved storability of *H. bodinieri* seeds. Furthermore, seeds with a moisture content of 4.77% stored at room temperature, and seeds with a moisture content of 3.97% stored at 4 °C yielded the best results. Priming with an appropriate concentration of polyethylene glycol had a certain repairing effect on ultra-dry stored seeds and improved seed vigor, with a two-day priming treatment with 20% polyethylene glycol having the best effect. Finally, compared with sand storage at 4 °C, ultra-dry storage promoted seedling growth and root development; furthermore, it alleviated storage damage to *H. bodinieri* seeds, promoted soluble sugar and soluble protein accumulation, and increased seedling nitrogen, phosphorus, and potassium uptake. Therefore, ultra-dry storage can be effectively used to preserve *H. bodinieri* seeds. Specifically, low-temperature storage of ultra-dry seeds with a moisture content of 3.97% enhanced *H. bodinieri* seed vigor, and seedling growth and development.

Keywords *Handeliodendron bodinieri*; priming treatment seed; ultra-dry treatment
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1 Introduction

The rare and endangered plant species *Handeliodendron bodinieri* (H. Lév.) Rehder (genus, Sapindaceae, Fig. 1) is native to China and only found distributed in karst areas (Fig. 2) including, Guangxi, Guizhou, and Yunnan (Cao et al. 2008; Wang et al. 2008). This woody, fuel-oil plant species is excellent for rock desertification control (Guo et al. 2019a; Guo et al. 2019b; Leng et al. 2020). However, due to its low seed germination rate, its vulnerability to insects, slow growth, shallow root system, and to the destruction of its habitat, it is currently on the verge of extinction and is therefore listed as a national, first-level protection species (He et al. 2012). Moreover, the high oil content of fat seeds is easily hydrolyzed and oxidized, thereby producing many toxic substances, such as malondialdehyde and free fatty acids, which pose a great threat to seed vigor and cause low seed germination-rate (Farhoosh et al. 2009), whereby, they represent serious limitations to the protection of the species. Seeds are the initial concentration of individual plants, and the basis for individual plant growth. Therefore, seed quality determines the strength of the individual to form a population.

Fig. 1. Introduction to *Handeliodendron bodinieri*, including *H.bodinier* (A); Leaf (B); Stamen (C); Pistil (D); Capsule (E); Seeds (F). A–E photo provided by Peng Jian.

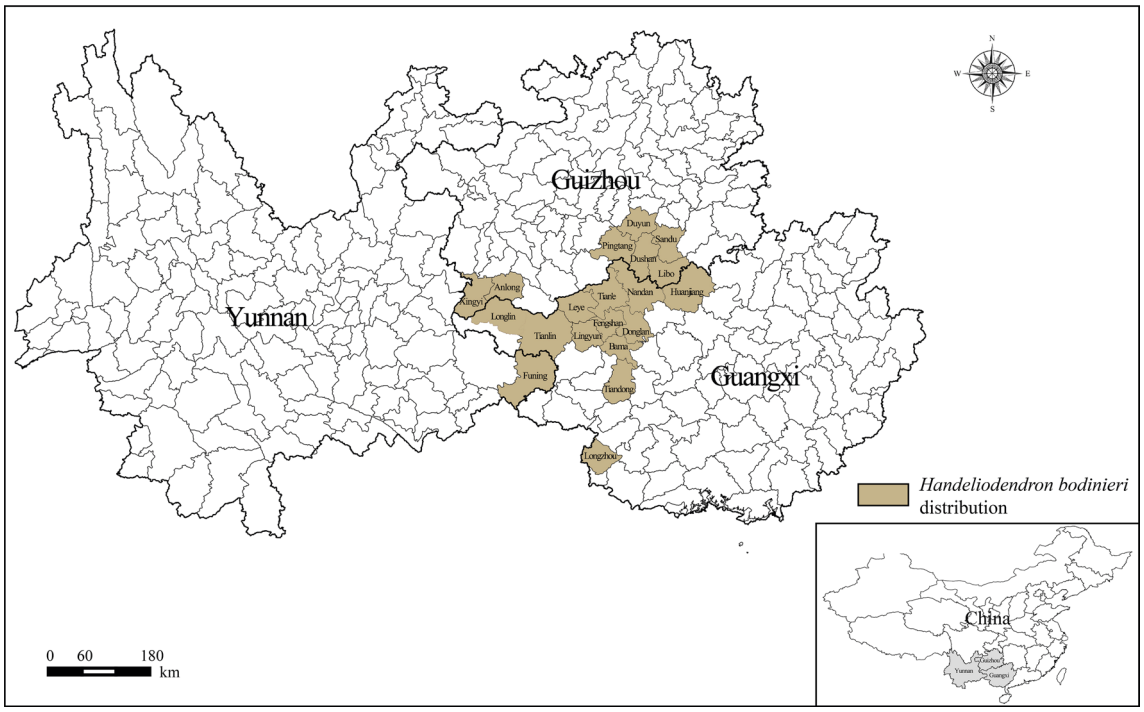

Fig. 2. Distribution of *Handeliodendron bodinieri*, including Funing in Yunnan province; Xingyi, Anlong, Dushan, Pingtang, Libo, Sandu, Duyun in Guizhou province; Longlin, Tianlin, Leye, Lingyun, Tiandong, Longzhou, Tian'e, Fengshan, Nandan, Donglan, Huanjiang, Bama in Guangxi province.

One approach to solving the abovementioned limitations is to use optimized storage methods to preserve seed resources while improving seed germination rate, as well as seedling management and protection (Xie et al. 2020). Furthermore, among the various conservation methods designed for the long-term preservation of endangered species, seed storage has been proposed as one of the most convenient methods (Paunescu 2009; Engelmann 2011). Particularly, ultra-dry seed storage is a method for storing seeds at room or low temperatures, in which seed moisture content (MC) is reduced to an optimal level for safe long-term storage prior to sealing; as such technologies contribute to long-term seed storage without destruction of the integrity of cellular membrane structure and seed functions (Hoekstra et al. 2001), there is an increasing interest in ultra-dry storage technologies in the fields of agriculture and forestry (Yan 2017; Wawrzyniak et al. 2020) that may allow for the maintenance of seed viability, tissue and cell morphology integrity, and stability of the genetic material (Li et al. 2007; Li et al. 2010). Indeed, compared with traditional refrigeration methods, ultra-day seed storage is environmentally friendly, energy-saving, and simple (Ellis et al. 1995; Ellis 1998; Ellis et al. 1998). Therefore, a large number of seed species are currently stored using ultra-dry storage. Particularly, oily seeds such as *Brassica napus* L., *Sesamum indicum* L., *Arachis hypogaea* L., and *Calocedrus macrolepis* Kurz seeds are resistant to dry storage owing to their hydrophobic properties (Ellis et al. 1986; Mira et al. 2019; Zhang et al. 2019). Further, ultra-dry storage has been proposed as an effective and simple technology to improve resource utilization efficiency (Pérez-García et al. 2007; Huo et al. 2015). Seed priming of ultra-dry seeds before germination can protect cell membrane integrity during water absorption, thereby improving seedling viability (Ellis et al. 1990; Van et al. 1994; Taylor et al. 1995; Jiang et al. 2020). Nonetheless, while ultra-dry storage has proved beneficial for seed storage, its effects upon seed germination and seedling growth thereafter, have rarely been studied.

The longevity of stored ultra-dry seeds is affected by MC, storage temperature, storage time, and other factors (Zheng et al. 1998). Ultra-dry stored seeds must not only germinate normally but seedlings must grow and develop normally. However, most studies have only focused on ultra-dry storage of seeds (Ellis et al. 1998; Wang et al. 2005), while there have been no reports on subsequent growth processes, such as seed germination and seedling growth.

Therefore, in this study, we aimed to develop a dry-storage method suitable for seed storage, sowing, and normal seedling growth of *H. bodinieri*. To this end, *H. bodinieri* seeds were dried to different MCs and then stored at room temperature or at 4 °C to find an optimum ultra-dry MC. Further, an optimal seed priming method was established to analyze the effect of ultra-dry storage treatment on seed sowing and seedling development. Finally, we measured various growth indicators including, plant height, stem diameter at ground level, and root length, as well as physiological indicators, such as antioxidant enzyme activity and malondialdehyde (MDA) content in seedlings grown from stored ultra-dry seeds and from seeds stored using the traditional method of wet-sand storage at 4 °C. We hypothesized that proper ultra-dry seed storage would allow adequate storage of *H. bodinieri* seeds and promote normal, healthy, seedling growth upon germination.

2 Materials and methods

2.1 Experimental materials

H. bodinieri seeds were collected in the karst area (24°32′30″–25°06′10″N, 106°08′10″–106°50′35″E) from the end of August to the beginning of September (one-third of the fruit cracked on the infructescence was used as criterion for maturity standard). Over 20 mother trees were selected for seed-harvesting at full fruiting period. Only healthy fruit branches were selected in each individual tree and within them, capsular fruits with a relatively uniform shape, size, appearance, and color were harvested for experimental use. The seed coat and aril were removed, and the seeds were placed in a dry environment at 25 °C. Mean 1000-seed weight and MC were 171.993 g and 9.62%, respectively, while mean seed length and width ranged from 8.70 to 11.30 and from 4.5 to 7.28 mm, respectively. Useless seeds accounted for 11.67% of all seeds harvested. Seedlings were grown in a container (15 × 10.6 × 13 cm) filled with loam soil collected from the Karst area (pH 7.25, total nitrogen: 5.95 g kg^{-1}, total phosphorus: 2.02 g kg^{-1}, total potassium: 0.28 g kg^{-1}, organic matter: 33.07 g kg^{-1}).

2.2 Experimental methods

2.2.1 Seed treatments

Using the silica gel drying method for ultra-dry treatment (Yan 2017), *H. bodinieri* seeds with different MCs (2.54% [mc1], 3.14% [mc2], 3.97% [mc3], and 4.77% [mc4]) were prepared. After the ultra-dry treatment, the seeds were packed in sealed double-layer aluminum foil bags and stored for 160 days either at room temperature (25 °C) or in a refrigerator at 4 °C. Seeds with an MC of 9.62% (mc5) without ultra-dry treatment were used as controls. As for storage in sand at 4 °C, seeds were surface-sterilized by soaking for 30 min in a 0.5% potassium permanganate solution, then rinsed with distilled water, and air-dried to reduce MC to 8.64%. The seeds were placed in three layers in a plastic container previously sterilized with alcohol and then topped with five layers of sand. Finally, the container was closed with a breathable lid and stored for 160 days in a refrigerator at 4 °C.

2.2.2Seed germination experiment

After storage for 160 days, the experimental seeds were soaked in distilled water for 24 h, disinfected by soaking for 15 min in a 0.5% potassium permanganate solution, and rinsed with sterile water. After removing the exopleura, seeds were placed in a petri dish and covered with filter paper. Four replicates, each consisting of 50 seeds, were evaluated for each storage method. Germination was evaluated in an incubator set to a constant temperature of 25 °C and a 12 h light/dark cycle. Water was replenished every day and seed germination was observed. Filter papers were replaced as needed. A seed was considered as germinated once the radicle length exceeded seed diameter. During the germination test, germination rate (*GR*), germination potential (*GP*), germination index (*GI)*, and vitality index (*VI*) were calculated according to formulas reported by Ranal et al. (2006):

$$\text{Germination rate}\,(GR) = \frac{M_1}{M} \tag{1}$$

$$\text{Germination potential}\,(GP) = \frac{M_2}{M} \times 100\% \tag{2}$$

$$\text{Germination index}\,(GI) = \sum \frac{G_t}{D_t} \tag{3}$$

$$\text{Vitality index}\,(VI) = S \times \sum \frac{G_t}{D_t} \tag{4}$$

where, M_1 is the number of germinated seeds, M_2 is the normal number of germinated seeds within the days of germination potential, M is the number of seeds tested, S is the growth of seedlings, G_t is the number of germinated seeds on day t, and D_t is the corresponding number of germination days.

2.2.3 Priming treatment

Seeds with an MC of 4.77% were soaked for 2, 4, or 6 days in 5%, 10%, 15%, 20%, or 25% polyethylene glycol (PEG, molecular weight, 6000) solutions in Petri dishes. Thus, the test comprised 15 treatments, including all possible combinations of PEG concentration and storage times (Table 1). Simultaneously, saturation water vapor-priming was used as a control treatment, in which ultra-dried seeds were hydrated for 48 h in a sealed desiccator containing saturated $CaCl_2$ solutions (relative humidity (RH) of 35%), then for 48 h in a sealed desiccator containing saturated NaCl solutions (RH of 75%), and finally, for another 48 h in a sealed desiccator containing saturated

Table 1. Osmotic adjustment of 15 kinds of PEG (molecular weight, 6000) for *Handeliodendron bodinieri* seeds. Seeds with a moisture content of 4.77% were soaked for 2, 4, or 6 days in 5%, 10%, 15%, 20%, or 25% PEG solutions in Petri dishes. Thus, the test comprised 15 treatments, including all possible combinations of PEG concentration and storage times.

serial number	combination	serial number	combination	serial number	combination
1	5%PEG 2d	6	5%PEG 4d	11	5%PEG 6d
2	10%PEG 2d	7	10%PEG 4d	12	10%PEG 6d
3	15%PEG 2d	8	15%PEG 4d	13	15%PEG 6d
4	20%PEG 2d	9	20%PEG 4d	14	20%PEG 6d
5	25%PEG 2d	10	25%PEG 4d	15	25%PEG 6d

PEG = polyethylene glycol.

water solutions (RH of 100%) at normal atmospheric temperature (25–30 °C) before germination assessment and subsequent experiments. Each treatment was evaluated using 50 seeds and four replicates. Germination was evaluated as described in 2.2.2.

2.2.4 Sowing experiment

The single experimental factor, i.e., storage treatment, was completely randomized. Each treatment was repeated three times using 20 seedlings in each repeat. Optimum MC for ultra-dry seed storage at 25 °C and 4 °C was used. Seeds stored in sand at 4 °C were used as controls. The sowing medium was saturated with water before planting.

2.2.5 Index measurements

Membrane permeability was measured according to the method reported by Blum et al. (1981). Soluble sugar (SS) content was determined using the anthrone method (Irigoyen et al. 1992). Soluble protein (SP) content was determined using Coomassie Brilliant Blue G-250 staining (Bradford 1976). MDA, a product of lipid peroxidation, was measured using the thiobarbituric acid assay described by Sudhakar et al. (2001). Superoxide dismutase (SOD) and peroxidase (POD) activities were measured using the methods reported by Dhindsa (1981).

The growth and physiological indicators were evaluated in one-year old seedlings. Seedling height and stem diameter were measured using a Vernier caliper, a band tape, and a ruler. The root index was measured with a digital scanner (Epson V750 Pro, Epson, Nagano-ken, Japan). Leaf area was measured with a portable leaf area meter (LI-3000C, LI-COR, Lincoln, NE, USA). Chlorophyll was measured using a chlorophyll meter (SPAD-502, Konica Minolta, Tokyo, Japan). Leaf net photosynthetic rate (Pn), stomatal conductance (G_s), and transpiration rate (Tr) in *H. bodinieri* seedling foliage was measured using a portable photosynthesis analyzer (LI-6400, LI-COR, Lincoln, NE, USA). Plant foliar total nitrogen (TN) was measured using a Vario Max CN analyzer (Elementar, Frankfurt, Germany). Plant foliar total K (TK) and total P (TP) were measured after digestion in hydrogen peroxide-sulfuric acid by the Kjeldahl method followed by standard colorimetric assays (O'Neill et al. 1970). SS and SP contents, SOD and POD activities, and MDA content were determined as described above for ultra-dry-stored seeds. Root activity was measured using the triphenyl tetrazolium chloride staining method described by Clemensson-Lindell (1994).

2.2.6 Evaluation of Handeliodendron bodinieri seedling quality after seed ultra-dry storage

The seedling growth-physiological index was used to evaluate seedling quality after the different ultra-dry storage treatments. Plant height, stem diameter at ground level, biomass, rhizome ratio, total root length, total root area, total root volume, leaf area, N, P, K, SOD, POD, MDA, SS, SP, root vitality, net photosynthesis, stomatal conductance, and transpiration were used as indicators of seedling quality. As the importance of each indicator was different, there was a substantial difference in dimension, and each indicator was a continuous variable. Therefore, the continuous nature of the membership function was used for standardization to eliminate the influence of dimension and determine the weight of each indicator in the comprehensive evaluation. Formula (5) was used to calculate indices positively correlated with the quality of *H. bodinieri* seedlings, and formula (6) was used to calculate indices negatively correlated with the quality of *H. bodinieri* seedlings.

$$F(X_i) = (X_{ij} - X_{i\min}) / (X_{i\max} - X_{i\min}) \quad (5)$$

$$F(X_i) = (X_{i\max} - X_{ij}) / (X_{i\max} - X_{i\min}) \quad (6)$$

where, $F(X_i)$ is the membership value of each index, X_{ij} is the observed value of each index, and $X_{i\max}$ and $X_{i\min}$ are the maximum and minimum values of the i^{th} factor, respectively.

Principal component analysis was applied on the membership value to obtain the variance of the common factor of each index and then, the weight of each index was calculated as follows:

$$W_i = [\omega_i F(X_i)]C_i / \sum_{i=1}^{n} C_i \quad (7)$$

where, W_i is the weight of the i^{th} factor, and C_i is the common factor variance of the i^{th} factor.

The fuzzy set weighted synthesis method was used to calculate seedling quality index.

$$\text{Seedling quality index} = \sum_{i=1}^{n} [\omega_i F(X_i)] \quad (8)$$

2.3 Statistical analysis

The effects of ultra-dry storage treatment of *H. bodinieri* seeds on seed storage, seed sprouting, plant growth, plant foliar N, plant foliar P, plant foliar K and plant physiology were evaluated by analysis of variance (ANOVA) in R (http://www.R-project.org/) (Ren et al. 2020a). The assumptions of normality of residuals and homogeneity of variances were assessed for all treatments, and data transformations were applied when appropriate to meet the assumptions (Ren et al. 2021). When main effects were significant, we used pairwise Duncan's tests to determine significant differences among treatments. PCA biplots were generated using the package ggbiplot in R (Ren et al. 2020b).

3 Results

3.1 Effect of ultra-dry storage of seeds with different MCs

3.1.1 Effects of ultra-dry storage of seeds with different MCs on seed physiological indices

Physiological indices showed dynamic changes when seeds with different MCs were stored at room temperature or at 4 °C (Fig. 3). The MDA ($p < 0.05$) content and relative conductivity ($p < 0.05$) increased slowly as the time of storage increased, whereas SS ($p < 0.05$) and SP contents ($p < 0.05$), and SOD ($p < 0.05$) and POD ($p < 0.05$) activities first decreased and then increased. After 160 days of storage at room temperature, SS and SP contents, and SOD and POD activities of mc3 and mc4 seeds were relatively high, whereas the MDA content tended to decrease, which is best for seed storage. The relative conductivity of mc1 seeds was significantly higher than that of mc5 seeds. When stored at 4 °C, seeds with different MCs showed limited differences in the changes of various indices. Relative conductivity increased significantly as MC decreased, and soluble protein contents, SOD and POD activities, and MDA content varied at different time points. After 160 days, SS and SP contents, and POD activity decreased, whereas SOD activity and MDA content increased.

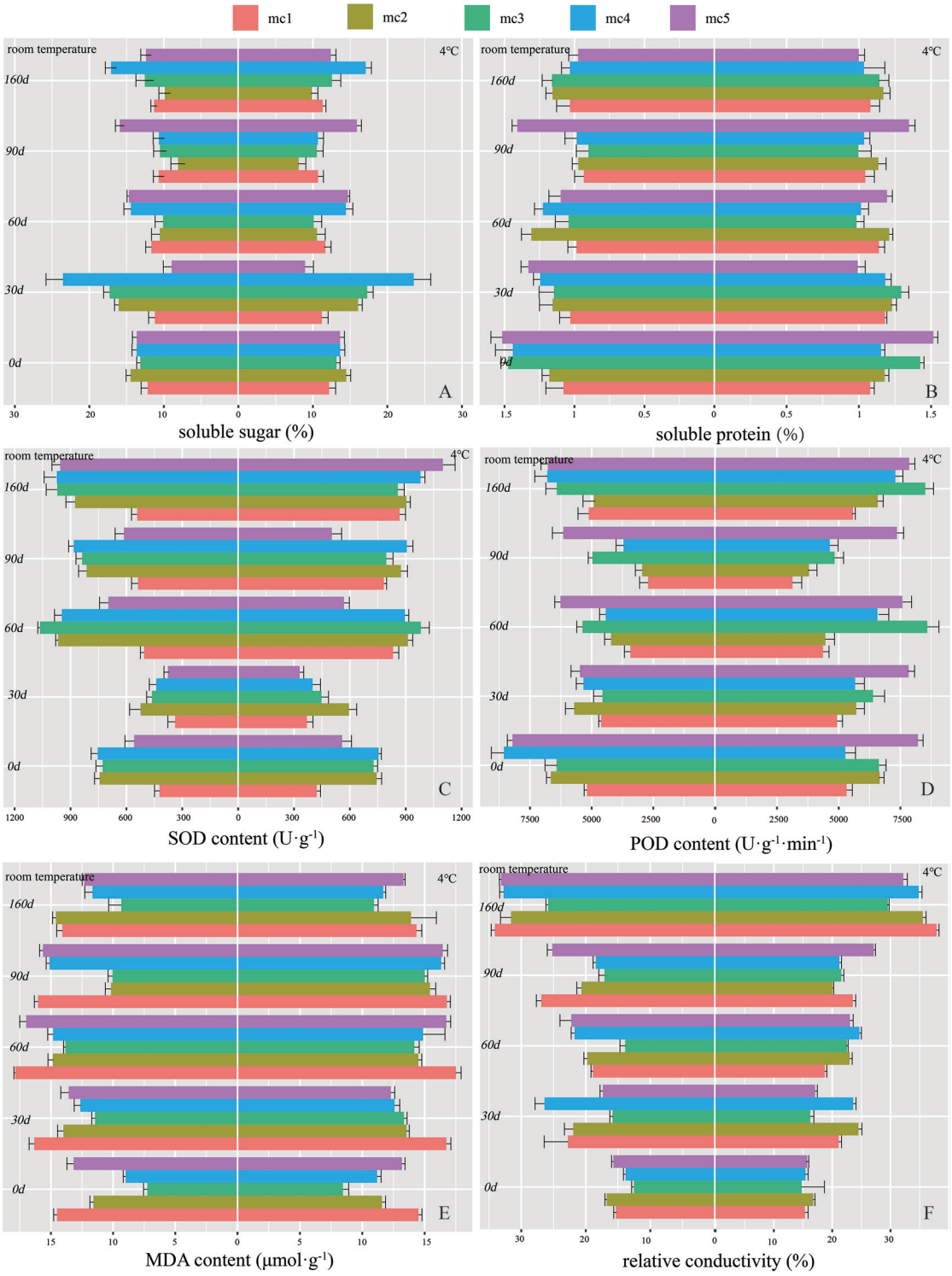

Fig. 3. Physiological analysis of *Handeliodendron bodinieri* seeds after ultra-dry treatment with different moisture content in 4 °C and room temperature. Storage at room temperature, the MDA ($p < 0.05$) content and relative conductivity ($p < 0.05$) increased slowly as the storage time increased, whereas soluble sugar ($p < 0.05$) and soluble protein ($p < 0.05$) contents and SOD ($p < 0.05$) and POD ($p < 0.05$) activities first decreased and then increased. stored at 4 °C, the relative conductivity ($p < 0.05$) increased obviously as the MC decreased, and the soluble sugar ($p < 0.05$) and soluble protein ($p < 0.05$) contents ($p < 0.05$), SOD ($p < 0.05$) and POD ($p < 0.05$) activities, and MDA ($p < 0.05$) content varied at different time points.

3.1.2 Effects of ultra-dry storage of seeds with different MCs on seed germination characteristics

After 160 days of storage at room temperature or at 4 °C (Table 2), ultra-dry-stored seeds with different MCs had significantly different GR, GP, GI, and VI ($p < 0.05$), indicating the MC had a significant effect on these parameters. GR, GP, GI, and VI of *H. bodinieri* seeds were higher at room temperature than at 4 °C. Further, GR, GP, GI, and VI of mc1 seeds were higher than those of mc5 (CK) seeds, and mc4 seeds performed the best. Meanwhile, GR, GP, GI, and VI of *H. bodinieri* seeds kept at 4 °C first increased and then decreased, with mc3 seeds performing best.

3.1.3 Comprehensive evaluation of the effects of ultra-dry storage of seeds with different MCs on germination and physiological indices

Growth and physiological index PCA factors of *H. bodinieri* seeds after ultra-dry storage treatment at room temperature or 4 °C are shown in Fig. 4. As can be seen for storage at room temperature, the first PCA axis (PC1) explained 45.1% of the total variation, while the second (PC2) explained 23.9% (Fig. 4A), for the total cumulative contribution rate of 69%, which indicated the relationship between MC and the various indicators. SOD activity, SS content, GR, GP, GI, and VI were mainly related to PC1, while POD activity was mainly related to PC2. Furthermore, mc1 (–0.26), mc2 (0.15), mc3 (0.32), and mc4 (0.60) treatments were associated with higher principal component scores than the control treatment (–0.80), indicating that ultra-dry storage at room temperature was conducive to adequate seed storage, with mc4 showing the best results.

As for storage at 4 °C, PC1 explained 49.1% of the total variation and PC2 explained 26.5% (Fig. 4B). Thus, the total cumulative contribution rate was as high as 75.6%. SS and SP contents, GR, GP, GI, and VI were mainly related to PC1, while POD activity, MDA content, and relative conductivity were mainly related to PC2. The principal component scores for mc1 (–0.08) and mc4 (–0.71) were higher than those for the control (–0.15), whereas those for mc2 (0.02) and mc3

Table 2. Means and standard errors of germination characteristics of *Handeliodendron bodinieri* seeds at room temperature and 4 °C after storage for 160 days. Germination characteristics of seeds (CK) with a moisture content of 9.62 not recorded are indicated as "n.d."

Moisture content (%)	Germination proportion (%)	Germination potential (%)	Germination index	Vigor index
room temperature				
2.54%	70.45±1.36d	70.45±1.36d	3.00±0.18d	1.15±0.10c
3.14%	80.95±2.00b	80.95±2.00b	3.26±0.07c	1.35±0.03b
3.97%	83.33±1.38b	83.33±1.38b	3.72±0.17b	1.31±0.02b
4.77%	92.50±2.5a	92.50±2.5a	4.53±0.13a	1.93±0.09a
9.62% (CK)	77.27±2.00c	36.36±2.10d	1.94±0.14e	0.66±0.03d
p-value	<0.001	<0.001	<0.001	<0.001
4 °C				
2.54%	68.18±2.09c	22.73±1.55b	2.12±0.08a	0.76±0.11ab
3.14%	73.81±1.91b	19.05±0.76c	1.84±0.04b	0.68±0.03b
3.97%	81.82±2.00a	27.27±0.91a	2.16±0.10a	0.83±0.01a
4.77%	54.76±2.86d	16.67±2.38c	1.09±0.13c	0.37±0.07c
9.62% (CK)	n.d.	n.d.	n.d.	n.d.
p-value	<0.001	<0.001	<0.001	<0.001

Different lowercase letters showed significantly difference at $p < 0.05$. CK = control check.

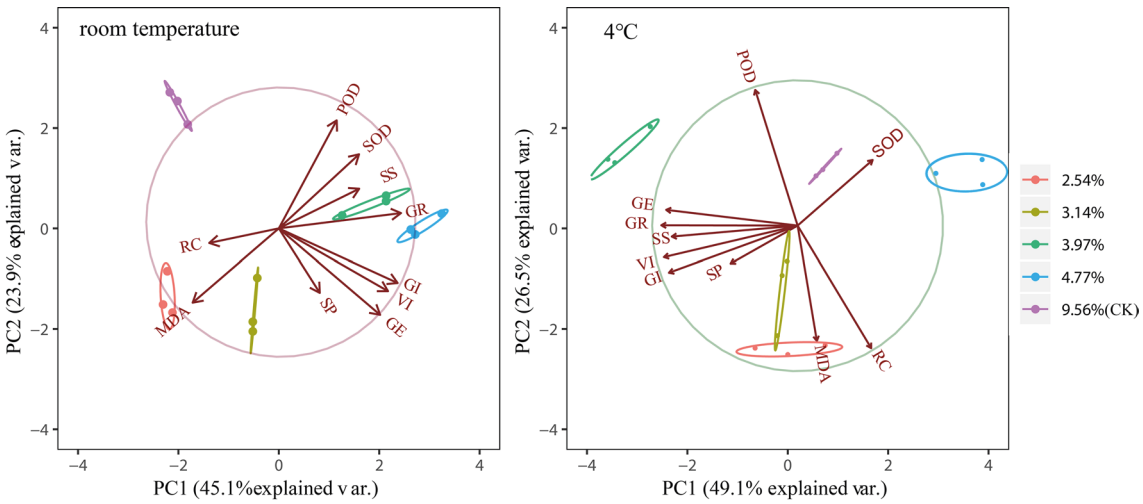

Fig. 4. Physiological index PCA factor of *Handeliodendron bodinieri* seeds after ultra-dry treatment with room temperature(A) and 4 °C(B). In plot A, factor 1 accounted for 45.1% of the variance, factor 2 accounted for 23.9% In plot B, factor 1 accounted for 49.1% of the variance, factor 2 accounted for 26.5%. SOD = superoxide dismutase, POD = peroxidase activity, MDA = malondialdehyde, SS = soluble sugar, SP = soluble protein, RC = relative conductivity, GR = germination rate, GP = germination potential, GI = germination index, VI = vitality index.

(0.92) were higher, indicating that there was little difference between ultra-dry low-temperature storage treatments, with mc3 showing the best results.

3.2 Effect of ultra-dry storage on seed sowing and seedling growth

3.2.1 Priming treatment of stored ultra-dry seeds

The ultra-dry stored *H. bodinieri* seeds showed significant differences in germination rate and germination index after priming ($p<0.05$, Table 3). GR and GI values were higher after the 10 different PEG priming treatments, with 2-day and 4-day treatment times being higher than after seed priming with saturation water vapor. Differences among the 10 PEG treatments were significant ($p<0.05$). GR and GI values after 2 days of PEG treatment were significantly higher than those after priming with saturation water vapor. GR and GI values decreased with increasing PEG-soaking time. As shown in Fig. 5, after two days of treatment, the highest PEG concentration had better results than the low concentration treatments, and at 20% PEG, GR and GI values were highest. After a 4-day priming treatment, low PEG concentrations yielded better results than high concentration treatments, with 10% PEG resulting in the highest GR and GI values. Furthermore, GR and GI values for the seeds after 6 days of treatment were lower than those of saturation water vapor-priming, likely because excessively long soaking times tended to suppress germination. Thus, PEG treatment should not be prolonged excessively.

3.2.2 Effect of ultra-dry storage on the growth of Handeliodendron bodinieri seedlings

No clear effects of ultra-dry treatments were observed on plant height, stem diameter, leaf area, chlorophyll content, total root length, total root surface area, or TN ($p>0.05$), whereas biomass, rhizome ratio, average root diameter, TK, and TP were significantly increased ($p<0.05$). Specifically, the biomass (Fig. 6C), rhizome ratio (Fig. 6F), total root length (Fig. 6G), total root area (Fig. 6H), average root diameter (Fig. 6I), and TP (Fig. 6K) of seedlings grown from ultra-dry stored seeds were higher than those of sand-stored seeds, while those of ultra-dry seeds stored at

Table 3. Means and standard errors of germination percentage and germination index changes of different priming for *Handeliodendron bodinieri* seeds in ultra-dry storage.

serial number	treatment	germination (%)	germination index (GI)
1	5%PEG 2d	83±2.65bcd	3.71±0.25c
2	10%PEG 2d	84±2 bc	3.80±0.19c
3	15%PEG 2d	82.67±1.16cd	3.68±0.11c
4	20%PEG 2d	91.33±1.53a	4.51±0.15a
5	25%PEG 2d	83±2.00bcd	3.71±0.19c
6	5%PEG 4d	78.33±1.53de	3.26±0.15d
7	10%PEG 4d	87.67±1.53ab	4.15±0.15b
8	15%PEG 4d	81.67±2.52cd	3.58±0.24c
9	20%PEG 4d	84±2.00bc	3.80±0.19c
10	25%PEG 4d	76.33±1.53ef	3.07±0.15d
11	5%PEG 6d	70.67±2.08gh	2.53±0.20e
12	10%PEG 6d	59±4.00i	1.58±0.20g
13	15%PEG 6d	67.33±4.04h	2.21±0.37f
14	20%PEG 6d	35.67±4.51l	1.01±0.06h
15	25%PEG 6d	28.33±3.51j	0.88±0.07h
16	SWVP	72.33±1.53fg	2.69±0.15e

Different lowercase letters showed significantly difference at $p < 0.05$. PEG = polyethylene glycol, SWVP = saturated water vapor priming.

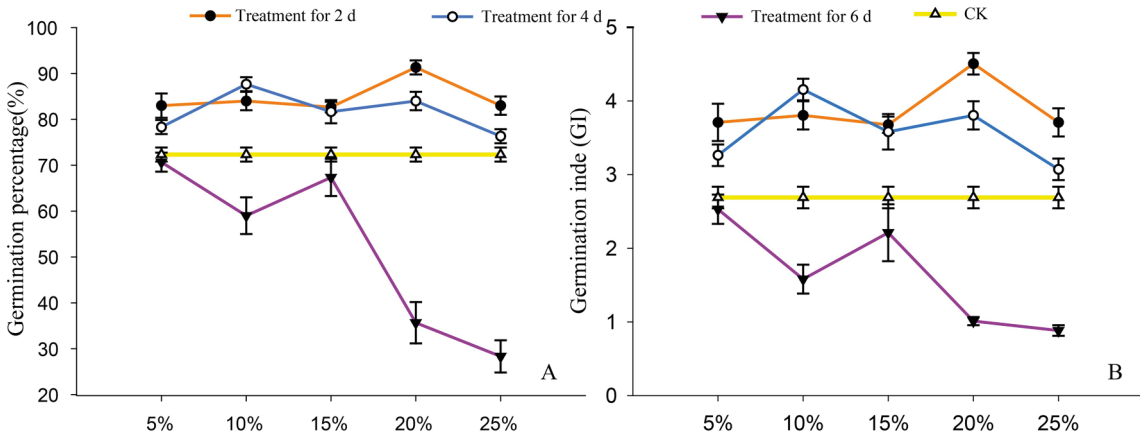

Fig. 5. Germination rate (A) and germination index (B) change of polyethylene glycol treatments (x-axis) for *Handeliodendron bodinieri* seeds. The GR ($p < 0.05$) and GI ($p < 0.05$) were higher after the 10 different PEG priming treatments with treatment times of 2 days and 4 days were higher than after saturated water vapor priming (CK).

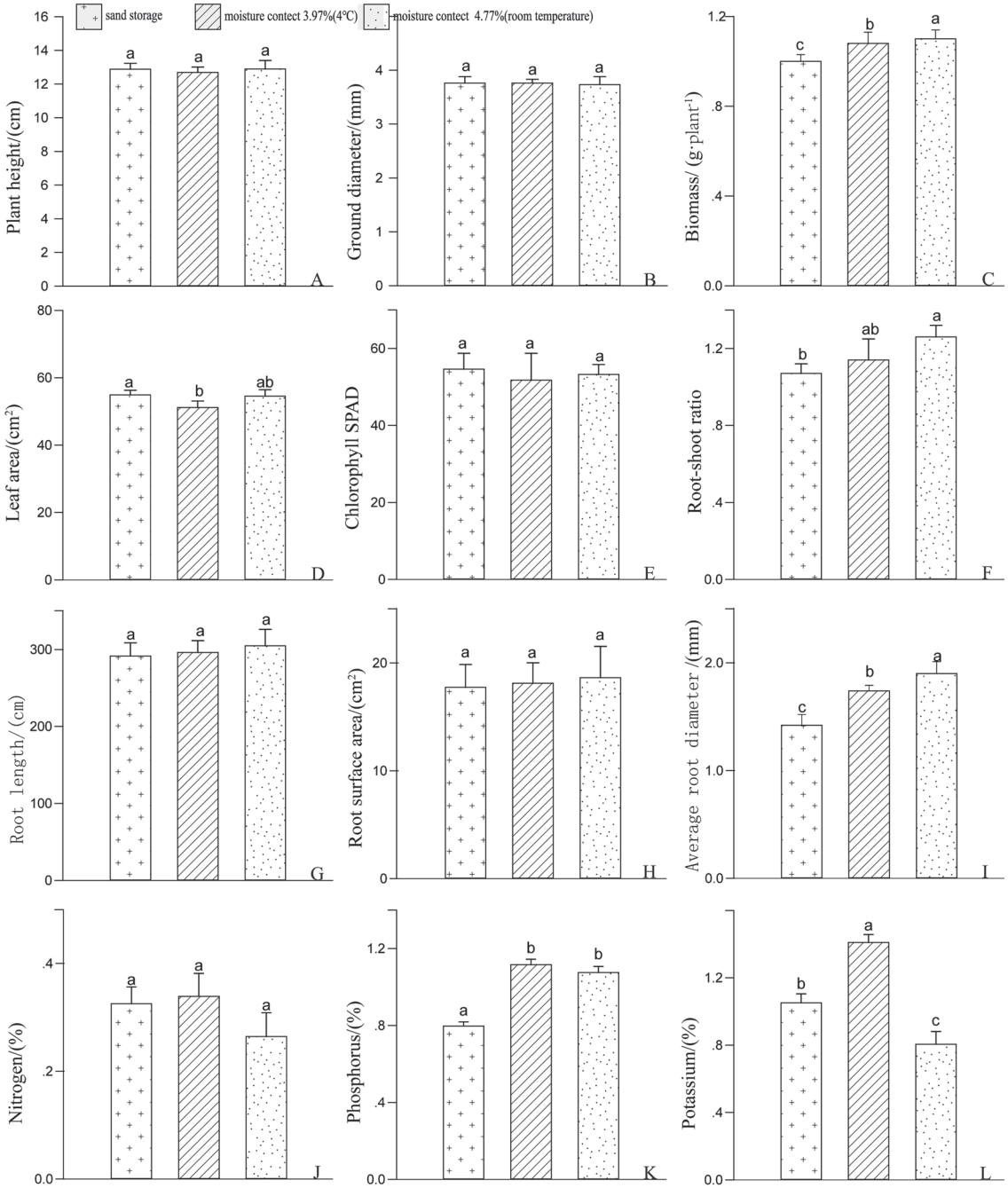

Fig. 6. Effect of ultra-dry storage on plant growth of *Handeliodendron bodinieri* seedlings. The different letter indicate significant differences among different treatments, and the same letter indicates no significant difference ($p < 0.05$ Duncan's).

room temperature were superior to those of ultra-dry seeds stored at 4 °C. Plant height (Fig. 6A) and stem diameter (Fig. 6B) of seedlings from seeds kept under room temperature ultra-dry storage were higher than those of seedlings grown from seeds kept in sand storage (4 °C) and superior to those of seedlings grown from seeds kept under low-temperature ultra-dry storage. TN (Fig. 6J), TK (Fig. 6K), and TP (Fig. 6L) of seedlings grown from low-temperature ultra-dry storage-treated seeds were higher than those of sand-stored seeds. In turn, leaf area (Fig. 6D) and chlorophyll content (Fig. 6E), were slightly lower in seedlings grown from ultra-dry-stored seeds than in those grown from seeds under sand storage, although in this case, the differences were not significant.

Ultra-dry storage treatment of seedlings did not affect plant growth, and proper ultra-dry treatment even promoted plant height, root system development, and biomass formation, which was consistent with previous findings on the performance of *Arachis hypogaea* L. and *Brassica napus* L. (Hong et al. 2005).

As shown in Figs. 7A–C, SOD and POD activities, and MDA content of ultra-dry storage seedlings were consistent, but lower than those of seedlings obtained from sand-stored seeds. This indicated that ultra-dry treatment in a low-temperature environment alleviated the injury suffered by *H. bodinieri* seeds during storage. Seedling SS (Fig. 7D) and SP contents (Fig. 7E) were significantly improved under ultra-dry storage treatment compared to sand storage ($p < 0.05$). The SS content in seedlings from low-temperature ultra-dry storage seeds and the SP content in seedlings from room-temperature ultra-dry storage seeds were superior to those in seedlings grown from sand-stored seeds. As shown in Fig. 7F, ultra-dry treatment had no significant effect on root vitality ($p < 0.05$), nor did it affect seedling root condition. In terms of photosynthesis (Figs. 7G–I), ultra-dry treatment had no significant effect on Pn, Gs, or Tr ($p < 0.05$), and g_s and Tr of seedlings grown from low-temperature ultra-dry-stored seeds were greater to those of seedlings grown from sand-stored seeds, indicating that a proper low temperature during ultra-dry storage was conducive to normal photosynthesis of seedlings.

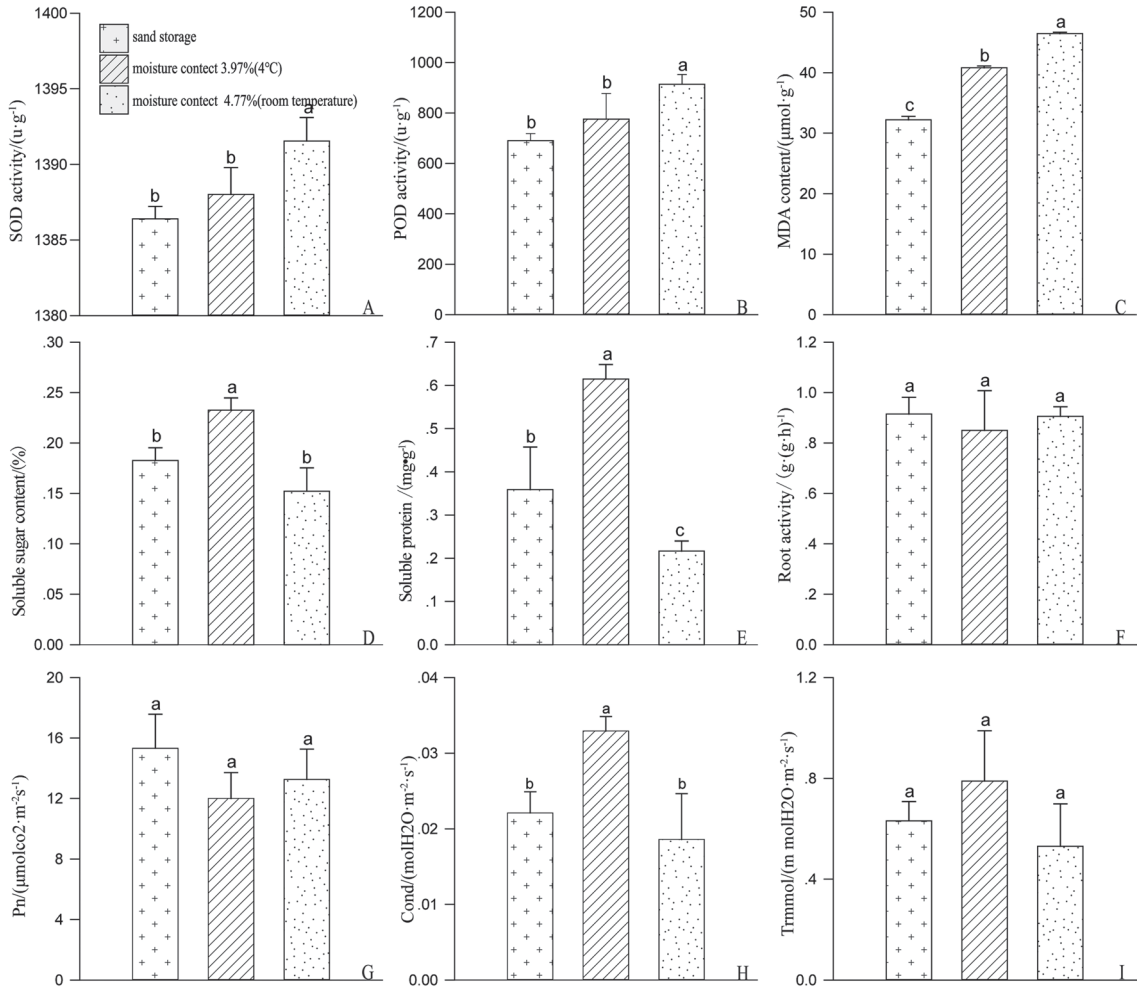

Fig. 7. Effect of ultra-dry storage on plant physiology of *Handeliodendron bodinieri* seedlings. The different letter indicate significant differences among different treatments, and the same letter indicates no significant difference ($p < 0.05$ Duncan's).

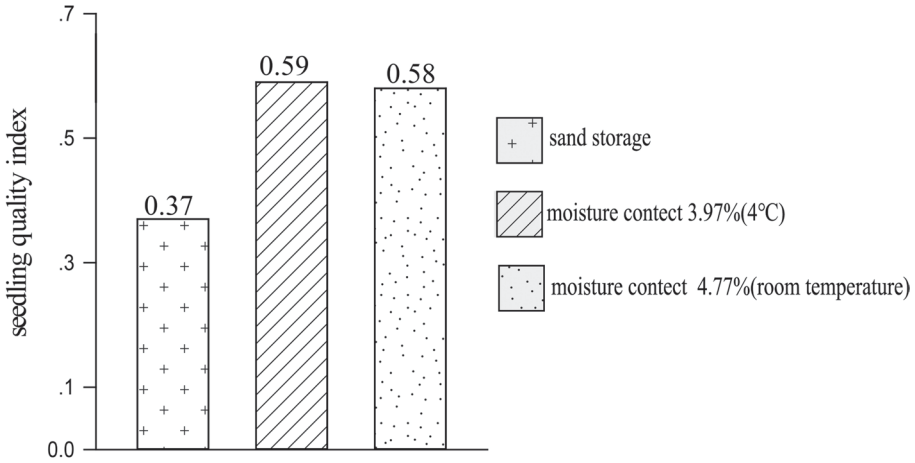

Fig. 8. The seedling quality index of *Handeliodendron bodinieri* seedlings in ultra-dry storage. Seedling quality evaluation score of *H. bodinieri* seedlings from ultra-dry-stored seeds at 4 °C (0.59) and ultra-dry-stored seeds at room temperature (0.58) was significantly higher than that of seedling from sand-stored seeds (0.37).

3.2.3 Comprehensive evaluation of the effects of ultra-dry storage on the quality of Handeliodendron bodinieri seedlings

We used the common factor variance and weight of *Handeliodendron bodinieri* seedlings in ultra-dry storage (Table 4) to obtain a seedling quality index (Fig. 8). Quality evaluation scores for *H. bodinieri* seedlings from ultra-dry-stored seeds at 4 °C (0.59) and at room temperature (0.58) were significantly higher than that for seedlings grown from sand-stored seeds (0.37). The results revealed that the quality of *H. bodinieri* seedlings grown from ultra-dry-stored seeds was significantly higher than that of seedlings grown from sand-stored seeds, while there was no significant difference between quality of seedlings grown from seeds kept under room-temperature and low-temperature ultra-dry storage. These findings demonstrate that proper ultra-dry seed storage improves the quality of *H. bodinieri* seedlings.

Table 4. The common factor variance and weight of the growth physiological index of *Handeliodendron bodinieri* seedlings in ultra-dry storage after planting the ultra-dry storage seeds for one year.

index	Common factor variance	weight	index	Common factor variance	weight
plant height	0.949	0.052	K	0.954	0.053
stem diameter	0.798	0.044	SOD	0.867	0.048
biomass	0.933	0.052	POD	0.939	0.052
Root-shoot ratio	0.894	0.049	MDA	0.99	0.055
total root length	0.94	0.052	SS	0.875	0.048
total root surface area	0.934	0.052	SP	0.96	0.053
Average root diameter	0.898	0.050	RA	0.983	0.054
leaf area	0.908	0.050	Pn	0.714	0.039
N	0.711	0.039	Gs	0.947	0.052
P	0.975	0.054	Tr	0.932	0.051

N = nitrogen, P = phosphorus, K = potassium kalium, SOD = superoxide dismutase, POD = peroxidase activity, MDA = malondialdehyde, SS = soluble sugar, SP = soluble protein, RA = root activity, Pn = net photosynthesis, Gs = stomatal conductance, Tr = trmmol.

4 Discussion

4.1 Ultra-dry storage tolerance of *Handeliodendron bodinieri* seeds

Seeds gradually age and lose their viability, as the seed lifespan is finite (Wawrzyniak et al. 2020). Storage-resistant seeds have a longer lifespan when stored ultra-dry; however, after a certain time, seeds will ultimately begin to age and deteriorate, which will negatively affect seed viability and seedling performance. Thus, to profit from the beneficial effects of ultra-dry storage on seed longevity and to maximize seed shelf life, it is important to find the optimum MC (Vertucci et al. 1993) at which to store ultra-dry seed. The effects of ultra-dry storage on seed vigor and early seedling establishment have to be thoroughly understood in order to produce high-quality seedling stands. Reports on seedling physiology after ultra-dry storage of seeds are scarce and confirm the possible impact on seed quality (Ellis et al. 1980; Ellis et al. 1998; Zhang et al. 2019). There are many factors that affect seed storage tolerance, including endogenous genetic factors, dormancy characteristics, maturity, firmness, MC, composition, and seed vitality. Additionally, external factors, such as storage temperature, moderation, physical factors, and chemical factors also affect seed storage tolerance (Ellis et al. 1980; Walters 1998; Holdsworth et al. 2008). Controlled storage conditions can delay seed deterioration and increase seed storage life. As a case in point, low-temperature storage and ultra-dry storage are effective methods for seed conservation (Harrington 1973; Wang et al. 2018). After *H. bodinieri* seeds were dried and dehydrated, their ultra-dry storage tolerance showed a strong relationship with seed contents of protective substances, cell membrane integrity, protective enzymes activity, and extent of lipid peroxidation. As seed MC decreases during storage, the cytoplasm becomes vitreous under the influence of sugars, whereby cell respiratory metabolism is inhibited and seed damage due to dehydration is reduced (Hendry 1993; Ballesteros et al. 2011). After ultra-dry treatment, the conformation of soluble proteins inside the seed changes, and interactions with other substances in the cell increases protein thermal stability, thereby preventing protein denaturation during dehydration and ensuring the integrity of cell membrane structure (Rajjou et al. 2008). Thus, during ultra-dry storage, favorable changes in soluble sugars within the seed and protein stability guarantee the desiccation tolerance of the seed. The changes in soluble sugar and protein contents during ultra-dry storage observed in our experiments suggested that the desiccation tolerance of *H. bodinieri* seeds was closely related to these contents (Figs. 3A, 3B). MDA is a well-established marker of oxidative stress (López-Fernández et al. 2018). Remarkably, MDA content of *H. bodinieri* seeds (mc1-mc4) was lower than that of the control seeds (mc5), whereas SOD and POD enzyme contents increased, which effectively maintained the corresponding enzyme activity and, consequently, membrane structure integrity.

Our experiments showed that ultra-dry seeds of *H. bodinieri* with an MC of 3.14% to 4.77% showed strong desiccation tolerance, indicating that this MC range is conducive to ultra-dry storage of *H. bodinieri* seeds.

4.2 Germination of ultra-dry stored seed requires seed priming

The effect of ultra-dry storage on seed vigor, tissue and cell morphology, and genetic material stability depends on the level of seed storage tolerance and the specific conditions of storage environment. However, even for storage-tolerant seeds, ultra-dry treatment may cause water to quickly enter the seed in response to a low water potential during seed germination (Ballesteros et al. 2011), which may cause damage to the cell membrane and result in solute leakage into the apoplast (Bewley et al. 2013). It is possible to reduce or eliminate such damage through proper osmotic adjustment and gradual priming before germination (Jisha et al. 2013), such as natural priming, PEG priming,

or saturation water vapor priming, which can improve the quality parameters of aging seeds, such as the germination rate and seedling vigor; furthermore, priming may even restore the original germination rate (Bailly et al. 1998; Murthy et al. 2003).

In this study, natural priming was the most time-consuming priming treatment. On the other hand, compared with saturation water vapor priming (Fig. 5, CK), PEG priming significantly improved germination rate, seedling vigor, and seedling development of the ultra-dry-stored seeds. This may be attributable to the repairing effect of PEG on cells, enhancing antioxidant activity and reducing MDA accumulation (Butler et al. 2009). However, these findings were not a general occurrence, and PEG concentration greatly affected the effect of osmotic adjustment. If improperly used, PEG will not only exert an osmotic adjustment effect, but it may also negatively affect cell membrane repair (Draganic et al. 2012). For example, high concentrations of PEG inhibited the germination of *Lycopersicon esculentum* Mill., *Avena sativa* L., *Castanea mollissima* Blume, and *Betula luminifera* H.J.P.Winkl. seeds (Kester et al. 1997; Xia et al. 2016). This was consistent with the inhibitory effect of 25% PEG priming on the germination of *H. bodinieri* seeds, although such effect by PEG treatment after 6 days of storage was very weak (Fig. 5). Thus, priming of ultra-dry stored seeds can reduce seed damage and promote seed germination. Among the various priming methods evaluated to date, PEG priming reportedly has the best effect (Xia et al. 2016).

4.3 Ultra-dry treatment promoted seed storage feasibility and seedling growth

Importantly, ultra-dry treatment was beneficial for seed storage, and all physiological indices reached stable levels, such as observed herein for ultra-dry storage of *H. bodinieri* seeds (Figs. 3 and 4). Ultra-dry treatment improved germination capacity as well as seedling growth (Table 3 and Fig. 5). Seemingly, ultra-dry storage possibly provides a means to reduce or eliminate the damage caused by seed priming, which regulates the extent of cell water absorption and hydration status, thus stabilizing and synchronizing water absorption by seeds and resulting in successful seedling development (Fig. 5). Ultra-dry storage reportedly had positive effects on seedling growth, photosynthesis, and respiratory rate in oil-, starch-, and some protein-rich seeds, such as *Arachis hypogaea* L., *Sesamum indicum* L., *Oryza sativa* L., *Stylosanthes guianensis* (Aubl.) Sw., *Jatropha curcas* L., and *C. mollissima* seeds (Cui et al. 2014).

Ebone et al. (2019) proposed three stages in the deterioration of stored seeds: in phase I, suppression of the protective mechanism against oxidative damage occurs; in phase II: membrane damage results following lipid peroxidation; and in phase III, seed viability decreases to the point that germination is inhibited. Seeds generally enter phase II of deterioration only after experiencing the phase I, and viable seedlings with suppressed growth are characteristic of phase II. In this study, ultra-dry storage at room temperature or at 4 °C promoted *H. bodinieri* seedling biomass, root system development, and seedling P, consistently with the reported growth pattern of *Xanthoceras sorbifolium* Bunge, which belongs to the same family. However, the ability of the seed to maintain membrane functionality and resist lipid peroxidation depends on its tolerance to ultra-drying (Zhang et al. 2019). In *Fagus sylvatica* L. (Pukacka et al. 2007) and *Populus nigra* L. (Kalemba et al. 2015), germination ability and seedling quality were negatively correlated with seed lipid peroxidation.

During the ultra-drying process, seed MC rapidly decreases, resulting in a series of physiological changes within the seeds that may induce dehydration stress. This dehydration stress is maintained for a certain period of storage and stimulates an increase in protective enzyme activity and protective substances content; however, once the seed reaches a threshold level of dehydration, the content of protective substances decreases (Walters 2015). Nonetheless, cell membrane structure and function in the seed can be maintained through proper priming, thereby reducing seed

damage, likely by removing harmful substances generated during germination and by repairing cell structure (Xia et al. 2016). All these processes require the participation of antioxidant enzymes, the contents of which increase during germination (Demir et al. 2007). Overall, our data indicated that ultra-dry stored *H. bodinieri* seeds did not enter phase II of deterioration.

Compared with ultra-dry stored *H. bodinieri* seeds, antioxidant enzyme activities and MDA content in conventionally stored seeds were not different. The increase in antioxidant enzymes after the sowing of ultra-dry stored seeds may occur to eliminate harmful substances, thus allowing for the observed promotion of normal, healthy seedling growth after ultra-dry storage (Figs. 6 and 7). Further, low-temperature storage was better than room temperature storage, which was more conducive to the accumulation of SS and SP in seedlings, thereby increasing photosynthesis rate and seedling absorption of N, P, and K. This phenomenon has also been reported in Brassicaceae Burnett and legumes (Zhu et al. 2007; Wawrzyniak et al. 2020).

5 Conclusions

This study showed that ultra-dry storage of *H. bodinieri* seeds prevented significant changes in seed viability or seedling vigor; furthermore, seed deterioration due to storage was minimized. Therefore, low-temperature ultra-dry storage was more conducive to seed germination and normal seedling growth thereafter, than room-temperature ultra-dry storage, such that the comprehensive index of seedling growth and the physiological activity were higher in the first case. Ultra-dry treatment was especially beneficial for the storage of *H. bodinieri* seeds with an MC of 3.14%–4.77%, and all physiological indices measured reached a stable level in these seeds. Ultra-dry storage of *H. bodinier* seeds may be used to reduce or eliminate seed damage caused by PEG seed-priming, thus promoting successful seedling emergence. As we confirmed desiccation tolerance of *H. bodneri* seeds, we believe that it is possible to classify the seeds of this species as of the orthodox storage type.

Author contributions

Conceptualization, Z.L. and J.P.; methodology, Z.L.; software, C.X.; validation, T.L.; formal analysis, Z.L.; investigation, S.G.; data curation, C.X.; writing and original draft preparation, C.X.; writing, X.X. and Z.L.; All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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