Postprint: Response of Seed Germination of Different Xanthoceras sorbifolium Varieties to Low-Temperature Storage

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Abstract

To investigate the dormancy and germination mechanisms of seeds from different *Xanthoceras sorbifolium* cultivars, this study selected seeds of four cultivars ('Putong' (PT), 'Qihong' (QH), 'Wofeng' (WF), and 'Woshi' (WS)) for storage treatments at -20 ℃ over different durations (30, 60, 90, 120, and 150 d). Germination indices were measured at each stage, and seed internal contents and hormone levels were analyzed before storage, after storage, and during a 7-day germination period. The results demonstrated: (1) Storage at -20 ℃ significantly improved germination quality in small-seeded cultivars (PT and QH), with the 60-day treatment being optimal, achieving germination rates of 48.3% and 58.3%, respectively; however, the effects on large-seeded cultivars WF and WS were inferior to those of the former two. (2) During 60-day storage at -20 ℃ and 7-day moist sand germination, oil content and seed coat thickness decreased significantly across all four cultivars, with substantial reductions occurring between days 3–7 (except for WS). Kernel moisture content increased rapidly during days 1–3 of germination, then gradually increased to peak on day 7, while kernel starch and soluble sugar contents accumulated significantly at days 3–4. (3) Low-temperature storage elevated the GA/ABA and tHor/ABA ratios (where tHor = IAA + GA + ZR + iPA) in small-seeded cultivars, thereby promoting oil degradation and seed coat thinning to break dormancy. In conclusion, large seed size, thick and hard seed coat, and physiological after-ripening constitute the primary causes of dormancy in *Xanthoceras sorbifolium*, which exhibits combinational dormancy. Low-temperature storage effectively breaks dormancy and enhances germination rates, with greater efficacy in small-seeded cultivars. Large-seeded cultivars such as 'Woshi' can achieve improved germination (38.7%) through extended low-temperature storage (150 d). Low-temperature storage combined with moist sand germination represents a rapid and convenient method for promoting *Xanthoceras sorbifolium* seed germination. This

study provides valuable insights for the promotion of superior cultivars and research on seed dormancy-release mechanisms.

Full Text

Preamble

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Title: Responses of Seed Germination to Low Temperature Storage in Different Cultivars of *Xanthoceras sorbifolium*

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Abstract: To study the seed dormancy and germination mechanism of *Xanthoceras sorbifolium*, seeds from four cultivars ('Putong' (PT), 'Qihong' (QH), 'Wofeng' (WF), and 'Woshi' (WS)) were stored at -20 ℃ for different periods (30, 60, 90, 120, 150 d). The seed germination indexes were determined for each treatment, and changes in reserve substances and hormone content were analyzed across three stages: before storage, after storage, and during 7 days of germination. The results were as follows: (1) Cold storage significantly promoted the germination rate and germination potential of small seeds (PT, QH). The optimal treatment was cold storage for 60 days, with germination rates reaching 48.3% and 58.3%, respectively. The effect on large seeds (WF and WS) was lower than that on small seeds. (2) The kernel oil content and seed shell thickness (SST) in seeds of all four cultivars decreased significantly during cold storage and germination, particularly during days 3-7 of germination (except for WS). Kernel water content increased rapidly within 1-3 days of germination, then slowly increased to a peak on day 7. Kernel starch and soluble sugar contents accumulated significantly on days 3-4 of germination. (3) Low-temperature storage increased the GA/ABA and tHor/ABA ratios (where tHor = IAA+GA+ZR+iPA) in small-seed cultivars, thereby promoting oil degradation and seed shell thinning to break dormancy. In conclusion, the main factors causing dormancy in *Xanthoceras sorbifolium* seeds are large seed size, thick and hard seed coats, and physiological after-ripening, indicating a comprehensive dormancy type. Low-temperature storage can break seed dormancy and improve germination rates, with better effects on small-seed cultivars than large-seed ones. For large-seed cultivars like 'Woshi', extending the low-temperature storage duration to 150 d can increase the germination rate to 38.7%. Low-temperature storage combined with wet sand germination is a rapid and simple method to promote *Xanthoceras sorbifolium* seed germination. This study provides a reference for the promotion of superior varieties and research on seed

dormancy-breaking mechanisms in *Xanthoceras sorbifolium*.

Keywords: *Xanthoceras sorbifolium* cultivars, cold storage, seed germination, reserve substance, hormone, breaking dormancy mechanism

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1. Materials and Methods

1.1 Experimental Materials

The experimental materials were obtained from the *Xanthoceras sorbifolium* germplasm resource nursery of Shandong Woqi Agricultural Development Co., Ltd. Four cultivars were selected: 'Putong' (PT), 'Qihong' (QH), 'Wofeng' (WF), and 'Woshi' (WS). The 'Putong' cultivar is the original variety in the germplasm resource nursery. 'Qihong' (ornamental type) features pink to purplish-red petals and serves as both an ornamental and oil-use variety. 'Wofeng' (high-yield type) is a multi-flower, multi-fruit variety with white petals that turn purplish-red at the base and produces abundant fruit. 'Woshi' (oil-use type) has white petals with yellow bases, exhibits clustered fruiting at terminal and lateral buds, demonstrates strong fruiting ability, and shows drought resistance and strong adaptability.

In December 2021, plump, undamaged, pest-free seeds from each cultivar were selected for -20 ℃ storage treatment (preliminary experiments comparing sand storage, -20 ℃ storage, PEG, ABT, hot water, and other methods showed -20 ℃ storage to be the most effective). Seeds were randomly placed in perforated self-sealing bags and stored in a -20 ℃ freezer for five durations: 30, 60, 90, 120, and 150 d. Each cultivar had 300 seeds per time period (100 seeds per bag, with three replications), with room temperature storage at approximately 25 ℃ for 60 d serving as the control.

1.2 Seed Germination Assessment

Sterilized sand was mixed with water and placed in germination boxes. When the sand could form a ball when squeezed but would crumble when released, seeds from both cold storage and room temperature storage were evenly mixed into the wet sand and covered with a 3 cm layer of wet sand (100 seeds per

box). Germination was conducted at approximately 25 ℃ room temperature. During the experiment, seeds were regularly turned to maintain aeration and sprayed with water to maintain consistent moisture. After germination began, the number of germinated seeds was observed and recorded daily until germination ended at 30 d.

Germination rate (%) = (Total number of germinated seeds / Total number of tested seeds) × 100% (based on 30 d statistics). Germination potential (%) = (Number of germinated seeds at peak germination (day 15) / Total number of tested seeds) × 100%. Germination index (Gi) = Σ(Gi/Dt), where Gi represents the number of germinated seeds at different times and Dt represents the germination time (calculated for the first 30 d in this study). Germinated seeds were transplanted into non-woven bags containing a substrate mixture of peat:perlite:vermiculite (2:1:1) and cultivated for one month to record seedling survival rates and observe growth conditions.

1.3 Measurement of Seed Traits and Physiological Indices

Concurrent with germination testing, an additional three replicates of 300 seeds per cultivar were subjected to the same 60 d storage and germination treatments for determination of oil, hormones, and other internal substances. Seeds from both cold storage and room temperature storage were divided into six time periods for sampling: before storage (Stage I), end of storage (wet sand germination day 0) (Stage II), wet sand germination day 1 (Stage III), wet sand germination day 3 (Stage IV), wet sand germination day 4 (Stage V), and wet sand germination day 7 (seed germination stage) (Stage VI). Measured indices included seed coat thickness, seed thickness (measured with vernier calipers), single-grain weight, and physiological indices such as water content, oil content, soluble sugar, starch, soluble protein, and five hormones (IAA, GA, ZR, iPA, ABA). Single-grain weight was measured only at Stage I, while the five hormones were measured only at Stages I, II, and VI.

Water content was determined using the oven-drying method: seeds were periodically removed from wet sand, washed clean, surface moisture was blotted with paper towels, seeds were cracked with pliers, and kernels were weighed for fresh weight (M1), then dried in a 60 ℃ oven to constant weight for dry weight (M2). Kernel water content (%) = (M1-M2)/M1 × 100. Seed coats were measured for thickness using vernier calipers, with 50 replicates per treatment and averages calculated.

Soluble sugar and starch (anthrone method), soluble protein (Coomassie brilliant blue method), and oil content (Soxhlet extraction method) were determined following Wang's method (2017). Plant hormone content was measured using enzyme-linked immunosorbent assay (ELISA, Wu et al., 1988). Plant hormones were measured using fresh seeds (before oven-drying), while oil, soluble sugar, starch, and soluble protein were measured using oven-dried seeds and calculated as percentage content. All indices were measured with three replicates

per cultivar per time period.

1.4 Data Statistics and Analysis

Data are presented as mean ± standard deviation (SD). All data were analyzed using SPSS 23.0 software for one-way ANOVA and Duncan's multiple range tests. Prism and R software were used for graphing.

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2. Results

2.1 Comparison of Seed Phenotypes Among *Xanthoceras sorbifolium* Cultivars

Seed phenotypes of the four cultivars were measured before storage. As shown in [Figure 1: see original paper], WF and WS cultivars belong to the large-seed category, with seed thicknesses of 1.19 cm and 1.21 cm and single-grain weights of 1.22 g and 1.36 g ($P < 0.05$), respectively, both significantly higher than PT and QH cultivars (seed thicknesses of 1.09 cm and 1.08 cm; single-grain weights of 0.95 g and 0.99 g, belonging to the small-seed category).

2.2 Effects of Cold Storage Duration on Seed Germination Characteristics

Seeds of the four *Xanthoceras sorbifolium* cultivars treated with -20 ℃ cold storage for 30, 60, 90, 120, and 150 d and room temperature storage for 60 d (control, CK) were germinated in wet sand at room temperature. Changes in germination indices are shown in [Figure 2: see original paper]. As indicated in Figure 2A, the average initial germination days for room temperature-stored seeds of the four cultivars were 7.3, 8.3, 9.0, and 8.3 d, respectively. After cold storage at all five time periods, germination time was advanced for all cultivars. The shortest initial germination time for PT cultivar was 5.3 d at 60 d, 2 d earlier than the control. QH cultivar showed initial germination times of 6.0 d and 5.6 d at 60 d and 150 d of cold storage, respectively, 2.3 d and 2.7 d earlier than the control. WF cultivar had its shortest initial germination time of 6.3 d at 150 d, while WS cultivar showed its shortest germination time of 5.7 d at 120 d. Overall, 60-150 d of cold storage was appropriate, advancing initial germination by approximately 2 d for all cultivars.

As shown in Figures 2B-D, after room temperature storage, large-seed cultivars WF and WS had germination rates of 25.3%, while small-seed cultivars PT and QH had germination rates of 33.3% and 41.3%, respectively, with the latter showing higher germination potential and germination index ($P < 0.05$). Cold storage treatment promoted seed germination, and although the three indices remained higher in small seeds than in large seeds, the trends differed. During 0-60 d of cold storage, PT, QH, and WF cultivars showed increasing trends in

all three indices, peaking at 60 d with significant differences from room temperature storage ($P < 0.05$). After 60 d, these indices began to decline significantly, gradually stabilizing between 90-150 d (with no significant differences among these three time periods for PT and WF). QH cultivar showed a rebound to the 60 d level at 150 d. WS cultivar showed no significant changes in the three indices compared to the control during 0-120 d ($P > 0.05$), but increases occurred after 120 d, with values at 150 d significantly higher than the control ($P < 0.05$). The ranking of the three indices from highest to lowest was QH > PT > WF > WS (with WS > WF at 150 d cold storage). PT, QH, and WF cultivars all showed maximum values at 60 d cold storage, with QH achieving the highest germination rate of 58.3%. Compared to their respective room temperature storage, germination rates increased by 45.3%, 41.1%, and 32.9%; germination potential increased by 45.8%, 36.7%, and 28.1%; and germination index increased by 78.8%, 58.7%, and 51.0%. WS cultivar at 150 d cold storage showed increases of 52.7%, 64.0%, and 66.0% in germination rate, germination potential, and germination index, respectively, compared to room temperature storage (Figures 2B, C, D). After transplanting germinated seeds and cultivating for one month, WS cultivar showed the highest seedling survival rate and produced relatively robust seedlings.

2.3 Dynamic Changes in Seed Coat Thickness and Kernel Contents Under Cold Storage

The effects of low-temperature cold storage and room temperature storage on seed coat thickness, water content, and kernel contents of the four *Xanthoceras sorbifolium* cultivars are shown in [Figure 3: see original paper] through [Figure 5: see original paper]. As shown in Figure 3A, kernel water content among the four cultivars ranged from 4.1% to 5.8% before storage. After 60 d of cold storage, kernel water content decreased to 1.7%-2.1% across all cultivars, while room temperature-stored kernels showed even greater decreases to 1.3%-1.9%, with QH and PT cultivars having higher water content than WF and WS ($P < 0.05$). When seeds from both storage treatments were placed directly in wet sand for germination, kernel water content showed an S-shaped trend: a rapid (linear) increase during 0-3 d, an inflection point at 3-4 d marking the transition to a slow phase, and a plateau during 4-7 d. Differences between cold and room temperature storage mainly occurred on germination days 1 and 7 (when radicles began to emerge). On day 1, room temperature-stored seeds showed significantly higher water content than cold-stored seeds, with increases of 21.3%, 32.6%, 21.9%, and 10.2% across the four cultivars ($P < 0.05$, non-significant for WS), indicating faster water absorption in room temperature-stored seeds during early germination. On day 3, QH cultivar still showed lower water content under cold storage, but the other three cultivars had values approaching those of room temperature storage. By day 7, all cultivars except PT showed significantly higher water content than cold-stored seeds, with increases of 8.3%, 7.4%, and 8.5%.

Regarding seed coat thickness, WS cultivar had the thickest coats before storage, followed by WF, QH, and PT with the thinnest. After 60 d of storage (Stage II), both treatments showed reduced coat thickness. Cold storage decreased coat thickness by 6.0%, 3.0%, 16.0%, and 4.0% across the four cultivars compared to harvest time, while room temperature storage caused greater reductions. During germination, room temperature-stored seeds showed no significant changes in coat thickness except for a slight decrease on day 7 (non-significant). In contrast, cold-stored seeds showed gradual decreases in coat thickness during germination, with reductions of 17.0%, 14.0%, 10.0%, and 5.0% on day 7 compared to day 0 ($P < 0.05$). Moreover, cold-stored seeds had higher coat thickness than room temperature-stored seeds during early germination (1-3 d), but by day 7, PT and QH cultivars showed lower thickness than room temperature-stored seeds (decreases of 5.7% and 4.9%), while WF and WS remained higher. This demonstrates that thick seed coats contribute to stronger dormancy in large-seed cultivars (Figure 3B).

Before storage, kernel oil content varied significantly among the four cultivars, ranking WS > WF > PT > QH, with large-seed WS showing the highest oil content, 7.3% higher than the lowest QH ($P < 0.05$). After cold storage, oil content decreased in all cultivars by 4.5%, 4.75%, 5.1%, and 2.6%, respectively, compared to before storage. Room temperature-stored seeds showed greater decreases of 8.9%, 6.5%, 7.9%, and 6.2% ($P < 0.05$). During germination (0-7 d), the two storage methods showed different declining patterns. Room temperature-stored seeds showed the greatest decrease during 0-1 d, with QH and WF decreasing by 7.4% and 5.7% on day 1, followed by a gradual decline. Cold-stored seeds showed significant decreases during two stages: 0-1 d (QH, WF) and 4-7 d (PT, WF) or 3-7 d (QH), with QH decreasing by 7.6% between days 3-7 and WF decreasing by 4.5% between days 4-7 ($P < 0.05$, Figure 4A).

Soluble protein content in cold-stored seeds decreased at the end of storage, fluctuated somewhat during germination (0-4 d), but overall showed no significant changes, returning to pre-storage levels by day 7. Room temperature-stored seeds showed significantly lower soluble protein content after storage ($P < 0.05$), with little change during germination but values significantly lower than cold-stored seeds at all stages. On day 7, the four cultivars showed decreases of 19.0%, 16.5%, 16.4%, and 17.4% compared to cold-stored seeds ($P < 0.05$, Figure 4B).

Starch content decreased from before storage to the end of cold storage, then gradually increased during germination, peaking on day 4 (day 3 for QH) before declining to the lowest level on day 7 when radicles began to break through the seed coat. At the end of cold storage (day 0), starch content had decreased by 19.0%, 15.0%, 33.0%, and 17% compared to before storage ($P < 0.05$). By day 4 of germination, starch content had increased by 70.0%, 44.6% (QH on day 3), 122.0%, and 102.0% compared to the end of cold storage, followed by a sharp decline. On day 7, PT, QH, and WF cultivars showed starch content lower than at day 0, while WS remained higher than both cold-stored day

0 and room temperature day 7. After room temperature storage (Stage II), starch content did not show significant decreases compared to before storage, with QH and WF even showing significant increases. During germination under room temperature, PT showed fluctuating starch content during 0-3 d, QH showed a significant decrease, but both gradually increased during 3-7 d; WF remained essentially at day 0 levels with little change; WS showed a gradual increase during 0-4 d followed by a decline on day 7 ($P < 0.05$). On day 7, cold-stored seeds of PT, QH, and WF showed significantly lower starch content than room temperature-stored seeds, with reductions of 68.3%, 49.2%, and 32.2%, respectively.

Soluble sugar content showed greater variation than starch. Cold storage significantly reduced soluble sugar content, with decreases of 29.0%, 54.0%, and 37.0% at the end of storage compared to before storage (except WS). During 0-4 d of germination, soluble sugar content increased substantially, then fell back to day 0 levels by day 7, with all cultivars peaking on day 4 at increases of 103.4%, 165.3%, 70.5%, and 63.3% compared to the end of cold storage. Room temperature-stored seeds showed much greater decreases in soluble sugar content than cold-stored seeds (except PT). During germination, room temperature-treated seeds showed fluctuating soluble sugar content, with no significant differences from day 0 (except PT, which was significantly higher on day 7 than day 1). Compared to room temperature storage, cold-stored seeds showed 53.1%, 63.2%, 25.8%, and 70.55% higher soluble sugar content on day 4, but 28.7%, 39.5%, and 16.6% lower content on day 7 for PT, QH, and WF ($P < 0.05$).

2.4 Effects of Cold Storage on Endogenous Hormone Content

Before storage (Stage I), GA content was highest in WS, followed by PT, and lowest in QH. After cold storage (Stage II, day 0), GA content decreased in three cultivars but increased by 32.0% in QH. On day 7 of germination (Stage VI), GA content in PT and QH increased significantly compared to Stage II by 84.0% and 24.0%, respectively, while WF and WS increased by only 5.0% and 12.0% (Figure 6A).

ABA content before storage ranked QH > PT > WS > WF across the four cultivars. After 60 d of cold storage, ABA content decreased in PT, increased slightly (non-significantly) in QH ($P > 0.05$), and increased by 30.0% and 14.0% in WF and WS, respectively. On day 7 of germination, ABA content decreased dramatically in all four cultivars by 48.0%, 30.0%, 24.0%, and 39.0% compared to Stage II (Figure 6B).

Before storage (Stage I), IAA content was highest in PT and lowest in WF. After 60 d of cold storage, IAA content decreased by 25.0% and 19.0% in PT and QH, respectively, while increasing by 20.0% and 10.0% in WF and WS. By day 7 of germination, PT and QH showed significant increases of 31.0% and 21.0% compared to Stage II, while WS decreased by 18.0% (Figure 6C).

ZR (zeatin riboside) and iPA (isopentenyl adenosine) contents in QH cultivar showed an initial increase followed by a decrease across the three stages, increasing after low-temperature storage then decreasing by day 7 of germination. The other three cultivars showed the opposite pattern: decreasing after cold storage then recovering to levels similar to harvest time by day 7 of germination (Figures 6D-E).

The GA/ABA ratio in QH cultivar increased by 23.5% after cold storage compared to before storage (Stage I), while the other three cultivars showed significantly lower ratios, with PT, WF, and WS decreasing by 29.1%, 26.0%, and 22.4%, respectively. On day 7 of germination, the GA/ABA ratio was significantly higher than in the previous two periods, with the four cultivars showing increases of 213.9%, 87.2%, 80.3%, and 81.1% compared to Stage II (Figure 6F).

The IAA/ABA ratio at the end of cold storage was lower than before storage (Stage I) in all four cultivars, with decreases of 15.6%, 24.3%, 8.0%, and 3.5%, respectively, reaching significant levels in PT, QH, and WF ($P < 0.05$). By day 7 of germination, the IAA/ABA ratio increased significantly compared to Stage II by 127.1%, 83.4%, 68.6%, and 52.1% across the four cultivars (Figure 6G).

The tHor/ABA ratio (where tHor = IAA+GA+ZR+iPA) decreased significantly at the end of cold storage compared to Stage I by 18.6%, 8.6%, 6.9%, and 5.2% ($P < 0.05$). By day 7 of germination, this ratio increased dramatically, being significantly higher than the previous two stages, with particularly large increases compared to the end of cold storage of 146%, 75.4%, 77.0%, and 81.1% across the four cultivars (Figure 6H).

2.5 Correlations Among Indices Under Cold Storage

Correlations among indices at various stages are shown in [Figure 8: see original paper]. Closely correlated indices included GA, ABA, GA/ABA, tHor/ABA, kernel water content, kernel oil content, and seed coat thickness. The GA/ABA and tHor/ABA ratios showed significant negative correlations with ABA and oil content (across all four cultivars), seed coat thickness (PT, QH, WS), and starch (PT, QH), but significant positive correlations with GA and water content (all four cultivars) and starch (WF, WS) ($P < 0.01$ or $P < 0.05$). Seed coat thickness showed significant or highly significant negative correlations with water content across all four cultivars, significant positive correlations with oil content, and significant positive correlations with starch in PT and QH ($P < 0.01$ or $P < 0.05$). Oil content in PT and QH showed significant positive correlations with starch ($P < 0.05$).

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3. Discussion and Conclusion

Current classifications identify five types of seed dormancy: physiological dormancy, morphological dormancy, morphophysiological dormancy, physical dor-

mancy, and combinational dormancy (Baskin et al., 2005). Physical dormancy results from seed size, seed coat hardness, and thickness affecting water permeability (Schutte et al., 2014; Rodrigues-Junior et al., 2018). Season and seed size determine dormancy degree, with larger seeds of the same species typically showing higher germination rates (Rubio de et al., 2017; Liyanage & Ooi, 2018). However, for *Xanthoceras sorbifolium* seeds with hard and thick coats, germination rate differs from these findings. In this study, small-seed cultivars PT and QH showed higher germination rates, while large-seed cultivars WF and WS showed lower rates, likely related to seed coat thickness and hardness in large seeds. WF and WS seeds were difficult to crack with pliers, with thick, hard coats representing one reason for low germination rates in large seeds—indicating stronger physical dormancy in large seeds than in small seeds.

Physiological dormancy arises from germination inhibitors or physiological after-ripening that suppress radicle emergence (Baskin et al., 2014). In this study, oil content in seeds of the four cultivars ranged from 57% to 62%, suggesting that physiological after-ripening may be another cause of physiological dormancy. After low-temperature storage, contents of oil, protein, starch, and soluble sugar in *Xanthoceras sorbifolium* kernels decreased, but the former two decreased less than under room temperature storage, while starch and soluble sugar decreased more. This indicates that low-temperature storage can reduce metabolic intensity and decrease consumption and degradation of seed reserves (Da Silva et al., 2018). These results are consistent with findings in *Libidibia ferrea* seeds stored at room temperature for 6 months, which showed decreased glucose and amino acid content, while these substances changed little at -18 ℃ (Bragante et al., 2018).

The transition from dormancy to germination involves mobilization of stored substances such as starch, protein, and oil (Vondrakova et al., 2020). Studies show that protein and crude fat mobilize rapidly during *Pinus tabulaeformis* seed germination, being the first reserves utilized (Chen & Shen, 2010). Peanut seed oil content also declines rapidly during germination (Wang et al., 2017). In this study, *Xanthoceras sorbifolium* seed oil content gradually decreased during germination, soluble sugar increased dramatically during days 3-4, and starch content also increased during days 1-4, indicating that oil degradation occurs first, gradually converting to starch and soluble sugar to supply radicle and plumule elongation. This is consistent with Jin et al. (2015) regarding oil reduction during *Xanthoceras sorbifolium* seed germination. Similar patterns of these internal substance changes occur during germination of dormant seeds in *Phoebe hui* and *Phoebe sheareri* (Zhang et al., 2022; Liu et al., 2023).

Seed germination is a water absorption-induced process of gradually increasing respiration and metabolism. This study found that low-temperature storage reduced consumption of seed internal substances while also altering seed coat characteristics. After cold storage, all cultivars showed higher seed coat thickness than room temperature-stored seeds. This difference resulted in lower water absorption during 0-3 d of wet sand germination compared to room tempera-

ture seeds, but higher absorption during 4-7 d. This suggests that cold storage brings seed coat thickness to a specific state that alters mechanical strength and water permeability, allowing seeds to undergo a gradual rewarming process during water absorption in wet sand, which prevents physical damage to kernels from excessive swelling in the early stage. Additionally, cold storage may enhance seed antioxidant capacity, while room temperature storage leads to reactive oxygen species accumulation, seed deterioration, and viability loss. For example, H_2O_2 content peaks after imbibition in room temperature-stored seeds (Bicalho et al., 2016), indicating oxidative stress that may damage kernels—a particularly important consideration for small-seed PT and QH cultivars in this study. These results align with findings that peanut seeds undergo fission after one year of storage and that teak seeds show higher initial water absorption rates with hot water treatment (Zhang et al., 2018; Ling et al., 2018), and correspond with results showing that mixed sand moisture-preserving cold storage significantly improves germination rates in *Corydalis saxicola* seeds (Pan et al., 2022). Further research is needed on physiological changes and reactive oxygen species metabolism during water absorption in cold-stored seeds.

Seed dormancy and germination are primarily determined by the GA/ABA ratio, with high ratios favoring germination and low ratios favoring dormancy (Zhang et al., 2022). Appropriate low-temperature storage can promote degradation of inhibitory substances such as ABA and oil, stimulate synthesis of growth hormone GA, complete after-ripening, and promote germination (Wu & Shen, 2021; Scepanovic et al., 2022). In this study, after cold storage, PT seeds showed significant ABA decrease, while QH showed increased GA content and GA/ABA ratio higher than before storage, indicating inherent differences in maturity and dormancy degree among *Xanthoceras sorbifolium* cultivars. Small-seed QH and PT cultivars had higher maturity; cold storage enabled rapid ABA decline (PT) or increased GA content (QH), gradually breaking dormancy—consistent with changes during dormancy release in *Ginkgo biloba* and *Tilia miqueliana* seeds (Jia et al., 2020; Wu & Shen, 2021). In contrast, due to large size and abundant internal substances, WF and WS showed no ABA decrease after low-temperature storage, and their GA/ABA ratio increased less than in small seeds, resulting in less pronounced germination improvement. This study also found that GA/ABA and tHor/ABA ratios increased substantially on day 7 of wet sand germination (when radicles broke through the seed coat), indicating that cold storage combined with wet sand germination better promotes seed germination.

In summary, *Xanthoceras sorbifolium* seed dormancy is comprehensive, resulting from three main factors: (1) large seed size; (2) thick, hard seed coats; and (3) physiological after-ripening. The four cultivars can be divided into two categories: small-seed 'Qihong' and 'Putong' with high germination rates, and large-seed 'Wofeng' and 'Woshi' with low germination rates. Cold storage at -20 ℃ for 60 d significantly improved germination rates of small-seed cultivars, advancing germination time, with 'Qihong' and 'Putong' reaching 58.3% and 48.3% germination rates, respectively. Large-seed 'Woshi' required extended

cold storage to 150 d for better results, achieving 38.7% germination. Small-seed cultivars, having lower oil content and thinner coats, showed reduced ABA content or increased GA/ABA ratio after low-temperature storage, thereby promoting oil degradation and seed coat thinning to break dormancy. Large-seed WS, with high oil content and thick coats, showed only partial dormancy release after low-temperature storage, maintaining relatively high oil content, coat thickness, and ABA content, resulting in lower germination rates. Low-temperature storage combined with wet sand germination is a rapid and simple method to promote *Xanthoceras sorbifolium* seed germination.

References

BASKIN JM, BASKIN CC, 2014. What kind of seed dormancy might palms have? [J]. Seed Sci Res, 24 (1): 17-22.

BASKIN CC, BASKIN JM, 2005. Underdeveloped embryos in dwarf seeds and implications for assignment to dormancy class [J]. Seed Sci Res, 15 (4): 357-360.

BICALHO EM, MOTOIKE SY, BORGES EEDE et al., 2016. Enzyme activity and reserve mobilization during Macaw palm (*Acrocomia aculeata*) seed germination [J]. Acta Bot Bras, 30 (3): 437–444.

BRAGANTE RB, HELL AF, SILVA JPN, et al., 2018. Physiological and metabolic responses of immature and mature seeds of *Libidibia ferrea* (Mart. ex Tul.) L. P. Queiroz) under contrasting storage temperatures [J]. Braz J Bot, 41 (1): 43–55.

CHEN LP, SHEN YB, 2010. Material metabolism of *Pinus tabulaeformis* seeds during initial germinating stage [J]. J Beijing For Univ, 32(2): 69-73.

GRAEBER K, NAKABAYASHI K, MIATTON E, et al., 2012. Molecular mechanisms of seed dormancy [J]. Plant Cell Environ, 35 (10): 1769-1786.

Da Silva TL, Gomes HT, Scherwinski-Pereira JE, et al., 2017. Designing ex-situ conservation strategies for seeds storage of *Piper aduncum* and *P. hispidinervum* through cryopreservation and low-temperature techniques [J]. J Forest Res, 22 (6): 380-385.

JIA Z, ZHAO B, IU S, et al., 2020. Embryo transcriptome and miRNA analyses reveal the regulatory network of seed dormancy in *Ginkgo biloba* [J]. Tree Physiol, 41 (4): 1247-1263.

JIN XH, LI X, LI YD, et al., 2015. The research on transformations of main inclusions and changes of related enzyme activity during seed germination of *Xanthoceras sorbifolia* Bunge [J]. Agric Sci J Yanbian Univ, 37 (2): 102-106.

LING LF, LI LF, YANG WJ, et al., 2018. Preliminary analysis of the quality and water absorption characteristic of *Tectona grandis* seeds [J]. Seed, 37(3):122-125.

LIU CX, CHEN ZX, YANG AJ, et al., 2020. A study on seeds dormancy relief based on stratification on *Xanthoceras soebifolia* Bunge[J]. J Weifang Univ, 20 (6): 1-6.

LIU M, GAO HD, GAO Y, et al., 2023. Study on the physiological and biochemical changes of *Phoebe sheareri* seed during its dormancy breaking [J]. J Nanjing For Univ (Nat Sci Ed), 47 (2):9-17.

LIYANAGE GS, OOI MKJ, 2018. Seed size-mediated dormancy thresholds: a case for the selective pressure of fire on physically dormant species [J]. Biol J Linn Soc, 123 (1): 135-43.

PAN YL, GUO LF, WANG XG, et al., 2022. Study on seed germination characteristics of *Corydalis saxicola* [J]. Guihaia, 2022-11-19.

PEI YX, CAO J, DU KB, et al., 2020. Effects of storage temperature on seed storability of *Liquidambar formosana*[J]. For Sci Res, 33 (5): 55-60.

RORDRIGUES-JUNIOR AG, Caroline MA, BASKIN CC, et al., 2018. Why large seeds with physical dormancy become nondormant earlier than small ones [J]. PLOS ONE, 13 (8): e0202038.

SCEPANOVIC M, KOSCAK L, PISMAROVIC L, SOSTARCIC V, 2022. Stimulation of germination of freshly collected and cold-stored seeds of *Ambrosia artemisiifolia* L.[J]. Plants, 11 (14): 1888.

SCHUTTE BJ, DAVIS AS, PEINADO SAJ et al., 2014. Seed-coat thickness data clarify seed size-seed bank persistence trade-offs in *Abutilon theophrasti* (Malvaceae)[J]. Seed Sci Res, 24 (2): 119-131.

SONG MH, LIANG BY, WANG RU et al., 2021. Effects of different treatments on seedling emergence rate and seedling growth of *Xanthoceras sorbifolium* Bunge [J]. J W Chin For Sci, 50 (4): 41-45, 59.

RUBIO de C, RAFAEL W, CHARLES G, et al., 2017. Global biogeography of seed dormancy is determined by seasonality and seed size: a case study in the legumes [J]. New Phytol, 214 (4).

VONDRAKOVA Z, PESEK B, MALBECK J, et al., 2020. Dormancy breaking in *Fagus sylvatica* seeds is linked to formation of abscisic acid-glucosyl ester [J]. New Forest, 51 (4): 671–688.

WANG, X, ZHENG, YQ, SU SC, et al., 2019. Discovery and profiling of microRNAs at the critical period of sex differentiation in *Xanthoceras sorbifolium* Bunge [J]. Forests, 10 (12): 1141.

WANG ZL, HUI M, SHI XQ, et al., 2022. Characteristics of the seed germination and seedlings of six grape varieties (*V. vinifera*) [J]. Plants, 11(4): 479.

WANG S G, 2017. Experiment course of plant physiology[M]. Beijing: Science Press.

WANG Y, LIU T, HE XY, et al., 2017. Oil bodies microstructure observation of peanut seeds at different developmental stages [J]. J Henan Agric Univ, 51(6):775-780.

WU S R, CHEN W F, ZHOU X, 1988. Enzyme linked immunosorbent assay for endogenous plant hormones. Plant physiol commun, (5): 53-57.

WU Y, SHEN YB, 2021. Sulfuric acid and gibberellic acid (GA3) treatment combined with exposure to cold temperature modulates seed proteins during breaking of dormancy to germination in *Tilia miqueliana* [J]. Protein J, 40 (6): 940-954.

XU HM, GUO L, MA RJ. 2022. Effects of cold storage on seed germination and seedling growth of peach rootstock cultivar Nemaguard [J]. Jiangsu J Agric Sci, 38 (1): 200-206.

YU HY, FAN SQ, BI QX, et al., 2017. Seed morphology, oil content and fatty acid composition variability assessment production [J]. Ind Crop Prod, 97(3): 425-430. in yellowhorn (*Xanthoceras sorbifolium* Bunge) germplasm for optimum biodiesel

ZHANG J, LIU J, ZANG XW, et al., 2018. Research of seed germination ability and physiological change of peanut under different storage method[J]. J Agric Sci Technol, 20 (6):19-27.

ZHANG H, QIU Y, JI Y, et al., 2022. Melatonin promotes seed germination via regulation of ABA signaling under low temperature stress in cucumber[J]. J Plant Growth Regul, 42 (6): 2232-2245.

ZHANG Q, SU BL, JIN H, et al., 2014. Fast germination and seedling of *Xanthoceras sorbifolia*[J]. J NE For Univ, 42 (9): 161-163.

ZHANG XY, YAN X, LI TH, et al., 2022. Physiological responses of seed dormancy and germination to cold stratification in *Phoebe hui* Cheng ex Yang [J]. Plant Sci J, 40 (3):398-407.

ZHU F, AOY, HIRST PM, et al., 2022. Suitable pollen source for the improvement of fruit and seed traits in *Xanthoceras sorbifolium*[J]. Ind Crop Prod, 182 (8): 114858.

Note: Figure translations are in progress. See original paper for figures.