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OVERCOMING PHYSIOLOGICAL DORMANCY IN *EUCOMMIA ULMOIDES* SEEDS: ROLES OF SCARIFICATION, STRATIFICATION, AND HORMONAL TREATMENTS

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Keywords: Eucommia ulmoides, Seed germination, Physiological dormancy, Scarification, Cold stratification, Gibberellic acid (GA)

Abstract

Eucommia ulmoides is endemic to China and has great development and utilization prospects. Seed dormancy is the main obstacles to the effective propagation of *Eucommia ulmoides* Oliv. The study investigated strategies for breaking dormancy and enhancing germination by evaluating the effects of scarification, cold stratification, gibberellic acid (GA_3), and their combined application at various temperatures. Results showed that the seeds do not have a barrier to water absorption, layers of palisade cells were not found in seeds, the intact seeds germinated at an optimum temperature with a low germination percentage of 5%, scarification can release seed dormancy and stratification and gibberellic acid can also break seed dormancy 15°C was the optimum temperature for seed germination. These findings demonstrated that *E. ulmoides* seeds exhibited physiological dormancy (PD) by scarification and stratification with GA_3 application.

Introduction

Seed germination and dormancy are crucial aspects of seed quality, and comprehending these processes is indispensable for establishing a sound seed production system. These processes are primarily regulated by various physiological mechanisms and environmental factors (Finch-Savage 2010). Bois *et al.* (2006) identified the optimal temperature for maximum germination, while low temperatures could lead to complete inhibition in certain seeds due to embryo mortality. Various studies have elucidated the roles of hormones like auxin and gibberellin in seed vigor and germination (He *et al*. 2020, Lv *et al*. 2021).

The process of scarification, involves weakening or rupturing the seed coat through mechanical or other means, enhances germination rates by increasing water and air permeability and releasing mechanical constraints on embryo germination (Paudyal *et al*. 2021, Nawrot-Chorabik *et al.* 2021). Following seed dispersal, treatments that simulate natural overwintering conditions can release the seeds from dormancy (Chien *et al.* 2011).The range of temperatures usually used for stratification of seeds of some pine species was between 2 and 5°C (Houšková *et al.* 2021), thus allowing germination to occur at temperatures above 15°C (Song *et al.* 2023). Cold stratification is commonly used to break dormancy in water-permeable seeds of many temperate-zone species, especially those whose seeds germinate in the natural habitat in spring. In some species, cold stratification is not very effective in breaking dormancy unless it is preceded by several weeks of warm (≥15°C) moist stratification (Chien *et al.* 2011). Many biochemical and structural changes are known to occur in seeds during cold stratification (Bewley *et al.* 2013)

Eucommia ulmoides Oliv.holds significant medicinal and economic value, yet its seed propagation faces challenges due to deep dormancy (Xu and Bai 1993). Despite efforts involving stratification or dry storage, germination rates remain low (Cheng *et al*. 2013), highlighting the need for further understanding of seed germination and dormancy in this species.

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Therefore, this study aims to investigate the basic characteristics of *E. ulmoides* seeds, identify the optimal germination temperature, and assess the effects of different treatments on seed germination.

Materials and Methods

The *E. ulmoides* seeds were collected from Fengle Avenue, Chuzhou, and subsequently stored at ambient temperature within the Plant Physiology Laboratory of Chuzhou University. Assessment of seed viability was conducted through tetrazolium (TZ) tests (Xue *et al.* 2017). Fresh seeds were subjected to staining with a 0.5% TZ solution at 35°C for a duration of 12 hrs. Each treatment involved two replicates of 100 seeds. Submersion of seeds occurred in 200 ml of 0.5% (w/v) TZ solution within a beaker at 35°C for 12 hrs in darkness. Seeds were subsequently categorized based on staining patterns, ranging from fully stained to completely unstained.

To explore the potential influence of seed coat and endosperm on water absorption, variations in absorption rates were examined among intact seeds, scarified seeds, and embryos. Water absorption curves were generated following the methodology outlined by Li (2020). Each experiment was replicated four times with 30 seeds. Seeds subjected to different treatments, including intact seeds (Fig. 1-CK_1), seeds with fruit coat removed (Fig. 1-CK_2), and seeds with both fruit coat and embryo exposed (Fig. 1-CK_3), were evenly distributed in Petri dishes containing distilled water at room temperature. Regular weighing of seeds were taken at intervals adjusted according to test progression.

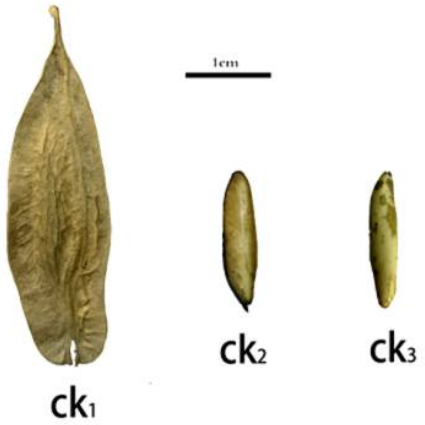

Fig. 1. Photographs of *Eucommia ulmoides* seeds.

Microstructural investigations were carried out using a scanning electron microscope (SEM) Quanta 200 (FEI Ltd, United States), capturing images of various sections such as suture, fracture, and outer surfaces of the endocarp. Samples were coated with a 10-20 nm thick layer of gold.

Eucommia ulmoides seeds were randomly selected, and treated with three methods to evaluate germination. Intact seeds (Fig. 1-CK_1) were soaked at for 7 days，then placed in a germination box covered with moist absorbent cotton. In treatments CK_2 and CK_3, the fruit coat was removed in CK_2 (Fig. 1-CK_2), and the seeds were soaked at room temperature for one day before being placed in a germination box with moist cotton, while in CK_3 (Fig. 1-CK_3), the fruit coat was removed and embryo was further cut to expose the radicle before undergoing the same soaking and germination procedure.

Each treatment involved three replications of 100 seeds. Germination boxes were then incubated overnight at 15, 20 and 25°C. The emergence of a 1 mm radical served as the criterion for seed germination, with germination percentage and rate recorded daily for 30 days (Xue *et al*. 2023).

For physical treatment seeds were randomly selected and treated with various methods (Treatment A-L), such as Treatment A: Intact seeds; B: Removed half of the fruit coat from the radicle end; C: Removed half of the fruit coat from the cotyledon end; D: Removed a small portion of the fruit coat at the radicle end without destroying the endosperm; E: Removed a small portion of fruit coat, endosperm and radicle at the end of radicle; F: Removed fruit coat; G:Removed fruit coat, and cut a small portion of endosperm and radicle; H: Removed a small part of the endosperm at the end of the radicle without destroying the embryo; I: Keep a small part of the endosperm and radicle at the end of the radicle; J: Cutted endosperm at 1/3 of the radicle without damaging the embryo; K: Cutted endosperm at the end of the radicle and L: Isolated embryo. Followed by soaking at room temperature and placement in germination boxes covered with moist absorbent cotton. Each treatment comprised of three replications of 100 seeds. Germination boxes were incubated overnight at 15°C temperatures.

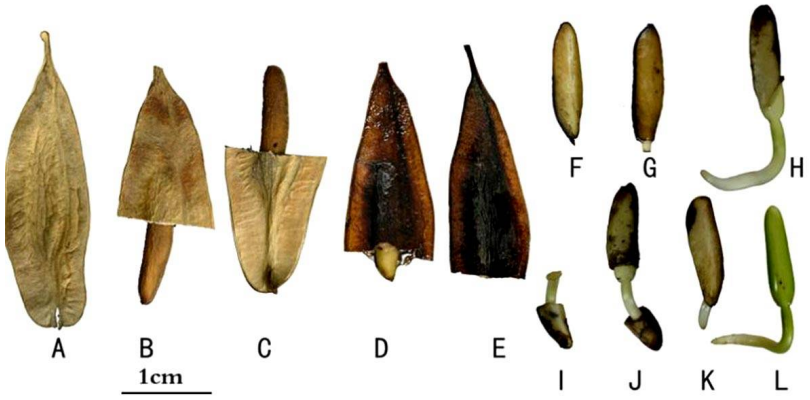

Fig. 2. Photogtaphs of *Eucommia ulmoides* seeds with different physical treatments.

For cold stratification treatment seeds were subjected to 60 days for cold stratification at 4°C, followed by germination assessment at different temperatures. For hormone treatment seeds were randomly selected and soaked for 24 hrs in a 1 g/l GA_3 solution, followed by germination at different temperatures. For combined treatment seeds underwent soaking for 24 hrs in a 1 g/l GA_3 solution, then were placed at 4°C for 60 days. Intact seeds (cold stratification + GA_3) were subsequently germinated at different temperatures.

Data analysis was conducted using SPSS 20, with measures of dispersion analyzed and results compared utilizing the least significant difference method.

Results and Discussion

Tetrazolium tests, indicated that seeds were fully stained dark red, demonstrating a fresh seed viability of 100%. Since water availability is crucial for seed germination, the water absorption capabilities under various seed conditions were examined. After 2 days, the radical moisture content reached 35% in CK_3 seeds and 25% in isolated embryos (CK_2), both were significantly higher than in intact seeds (Fig. 3).

However, the endosperm moisture content across different seed treatments exhibited no significant differences (Fig. 3). It was also observed that all treatments, including intact seeds, absorbed water after soaking, with embryos showing quicker imbibition. These findings suggested that scarification enhanced the water absorption capability of the seeds, potentially promoting seed germination.

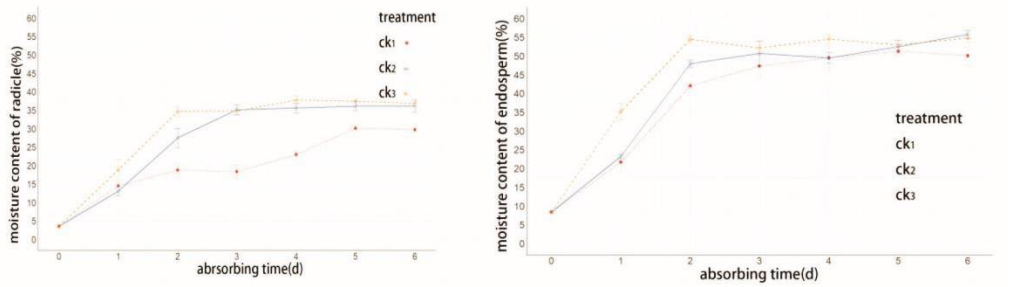

Fig. 3. Water absorption curves of the intact seeds, scarified seeds, and excised embryos of *Eucommia ulmoides*. CK_1: intact seeds, CK_2: the fruit coat removed, CK_3: the fruit coat removed and embryo was cutted to expose the radicle. The percentage of water absorption was expressed as mean ± standard error.

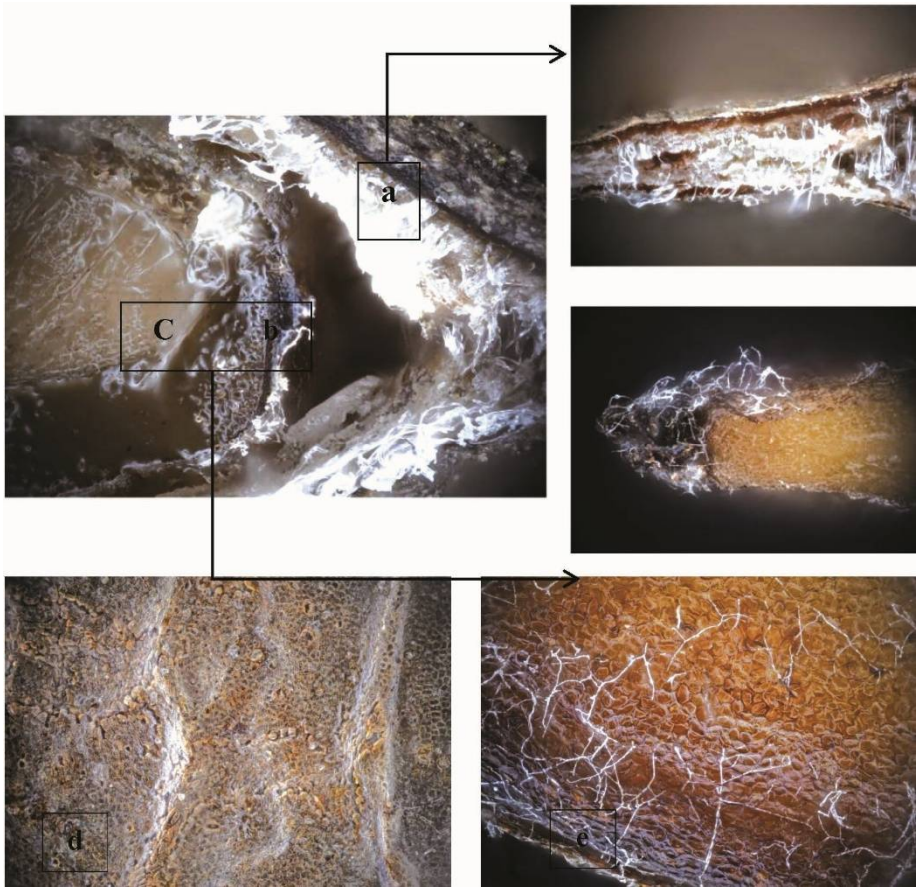

Plate 1. *Eucommia ulmoides* seeds. a: Photographs fruitcoat, b: seed coat, c: endosperm, d-e: endosperm surface layer.

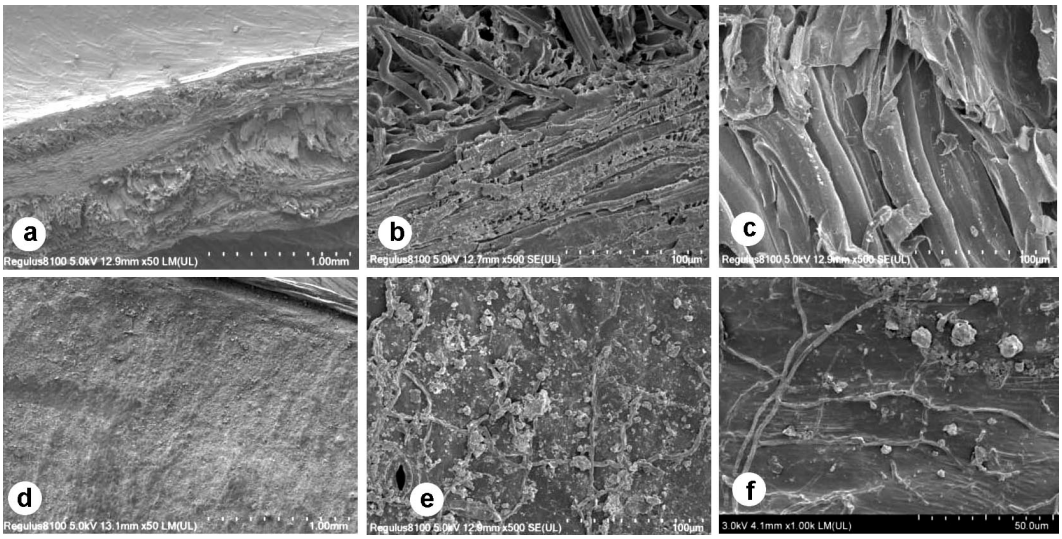

Plate 2. Scanning electron microscope photographs of *Eucommia ulmoides* seeds. a-c:peel section, d-f: endosperm surface layer. a: fruit coat, b: fruit coat, c: fruit coat; d: endosperm surface layer (1 mm), e: endosperm surface layer (100um) and f: endosperm surface layer (50 um).

The morphological structure of *E. ulmoides* seeds was detailed, highlighting the fruit coat (position "a"), which did not exist palisade cell layers, and the seed coat (position "b") characterized by a loose surface arrangement with pores and thin, degraded filaments. The endosperm (position "c") and its surface (position "e") were also examined (Plate 1. SEM results corroborated the absence of palisade cell layers, provided insights into the seed structure (Plate 2).

Fig. 4 illustrated the germination progress of seeds under various treatments and temperatures. Treatment CK_1 exhibited minimal germination across all temperatures, indicating seed dormancy. In contrast, seeds without endocarps (CK_2) germinated more effectively than intact seeds and CK_3, which involved an additional intervention, achieved a germination rate of 95%, effectively breaking seed dormancy.

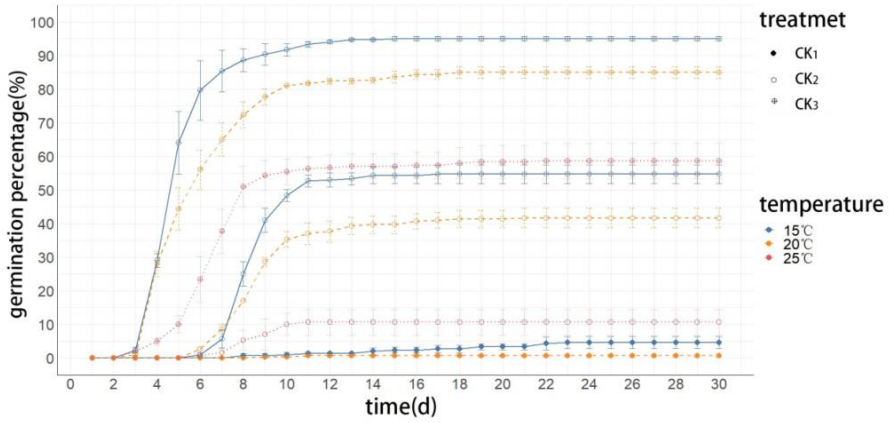

Fig. 4. Germination process of *Eucommia ulmoides* seeds at 15, 20, and 25°C. CK_1: intact seeds, CK_2: fruit coat was removed, CK_3: fruit coat was removed and embryo was cut to expose the radicle.

Further analysis revealed that the germination percentage significantly increased at 15°C (Fig. 4). Fig. 5 confirmed the presence of deep seed dormancy in intact seeds, which germinated at only 5% under optimal conditions. Removal of the seed endocarp was an effective method for overcoming this dormancy. Moreover, seeds treated with CK_3 not only showed the highest germination rate (95%) at 15°C but also maintained a high germination rate (85%) at 20°C.

Seeds from all treatments germinated more effectively at 15°C compared to 20 and 25°C (Figs 4-6). These results collectively suggest that the optimal temperature for germinating *E. ulmoides* seeds was 15°C and the seeds from treatment CK_3 germinated most rapidly.

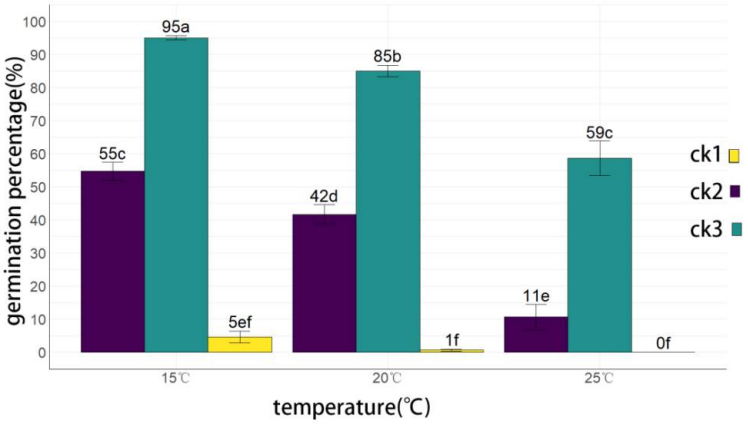

Fig. 5. Germination percentage of *Eucommia ulmoides* seeds at 15, 20, and 25°C. Abbreviations are similar as in Fig. 4.

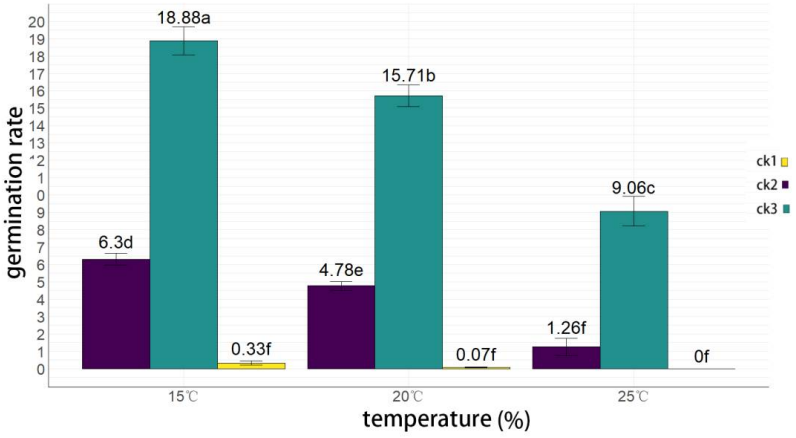

Fig. 6. Germination rate of *Eucommia ulmoides* seeds at 15, 20, and 25°C. Abbreviations are similar as in Fig. 4.

Observations also revealed that whole *Eucommia* seeds rarely germinated (Figs 4-6). However, removing the fruit coat significantly enhanced germination rates, and further increase were noted upon cutting the endosperm. The optimum germination temperature for seeds was identified as 15°C, significantly outperforming the results at 20 and 25°C.

In this study, it was found that only 5% of freshly harvested, intact seeds germinated at their optimum temperature. The viability test for the ungerminated seeds suggested that they were viable but dormant, indicating the presence of seed dormancy. Physical dormancy, results from the presence of palisade cells in the seed coat or fruit covering, imposing a barrier to water absorption (Baskin and Baskin 2004). However, in this study, *E. ulmoides* seeds (Plates 1, 2 and Fig. 4), lacked palisade cells or water absorption barriers, excluding physical dormancy (PY) and combinational dormancy (PY + PD) as forms of dormancy for these seeds. Underdeveloped embryos, indicative of MD or MPD (Finch-Savage 2010), were not observed due to the presence of developed embryos, suggesting physiological dormancy as the dormancy type in *E. ulmoides* seeds.

The study also highlighted that intact seeds (CK_1) showed almost no germination across different temperatures (Fig. 6). Conversely, seeds devoid of endocarps (CK_2) demonstrated enhanced germination, particularly at 15°C, with a germination percentage of 95%, suggesting this as the optimal germination temperature for seeds.

The results indicated that *Eucommia* seeds exhibited physiological dormancy. Removal of the fruit coat alleviated this dormancy to some extent, while concurrent removal of the fruit coat and disruption of the endosperm effectively overcame it. Figs 7-9 illustrated the germination effects of 12 distinct treatments on *E. ulmoides* seeds.

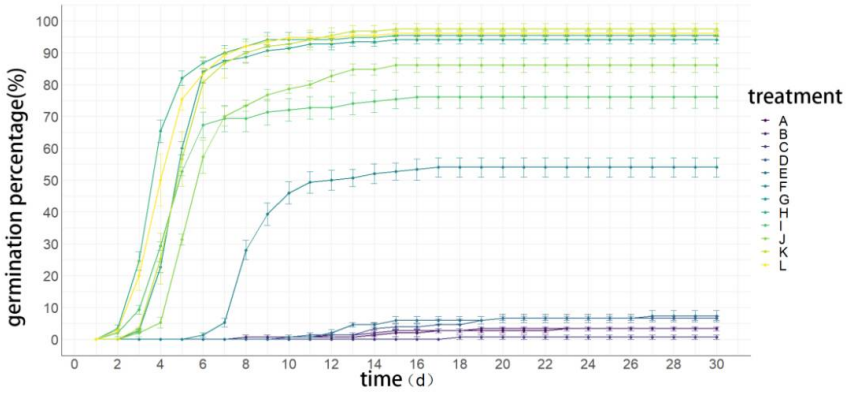

Fig. 7. Germination process of *Eucommia ulmoides* seeds with different treatment at 15°C.

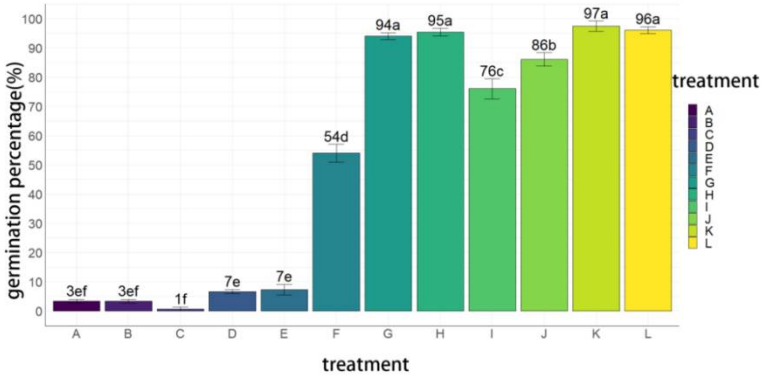

Fig. 8. Germination percentage of *Eucommia ulmoides* seeds with different treatment at 15°C.

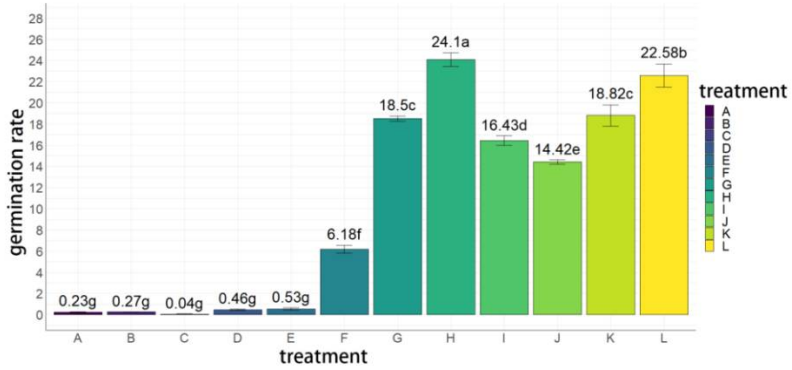

Fig. 9. Germination rate of *Eucommia ulmoides* seeds with different treatment at 15°C.

Treatments preserving the intact fruit coat (A) or partially removing it (B, C, D, and E) resulted in poor germination rates, with fewer than 10% of seeds germinating. However, complete removal of the fruit coat (F) significantly enhanced germination rates to 54%. Further, disrupting the endosperm (G- H, K-L) markedly increased both the percentage of seeds germinating and the rate of germination. The data suggested that the fruit coat and endosperm differentially affect seed germination. The fruit coat impeded germination through lateral pressure exerted by the seed shell, rather than directly obstructing the radicle end. Conversely, the endosperm hindered germination by blocking the radicle end. Cutted endosperm (I- J) reduced this pressure, allowing the seeds to germinate in response.

Scarification emerged as an effective method for breaking seed dormancy in seeds with hard coats. Significantly higher germination rates (94, 95, 97, and 96%) were observed for treatments G, H, K, and L (Figs 7-10), emphasizing the mechanical barrier posed by the seed coat to embryo germination (Deng *et al*. 2021).

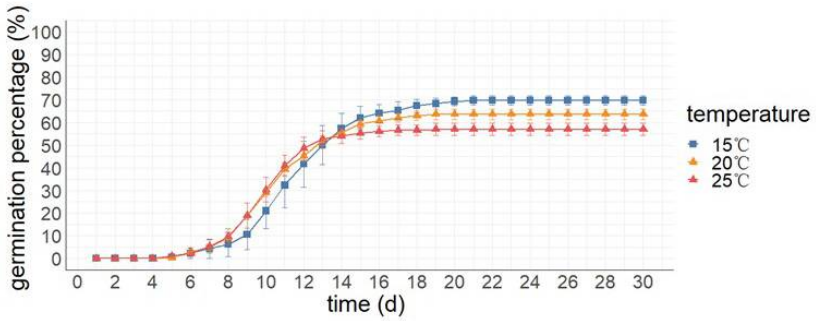

Fig. 10. Germination process of *Eucommia ulmoides* seeds with cold stratification treatment at 15, 20, and 25°C.

Cold stratification partially releaseed seed dormancy, resulting in a germination rate of 70% (Fig. 10). Conversely, treatment with GA_3 for 60 days was not effective in enhancing seed germination, which remained low at 12% (Fig. 11). A combined treatment of cold stratification and GA_3 effectively overcomed seed dormancy, significantly improved germination rates to 82% (Fig. 12). In conclusion, an appropriate temperature, scarification, phytohormone application, and cold moisture stratification were efficacious in breaking seed dormancy and promoting the germination of seeds.

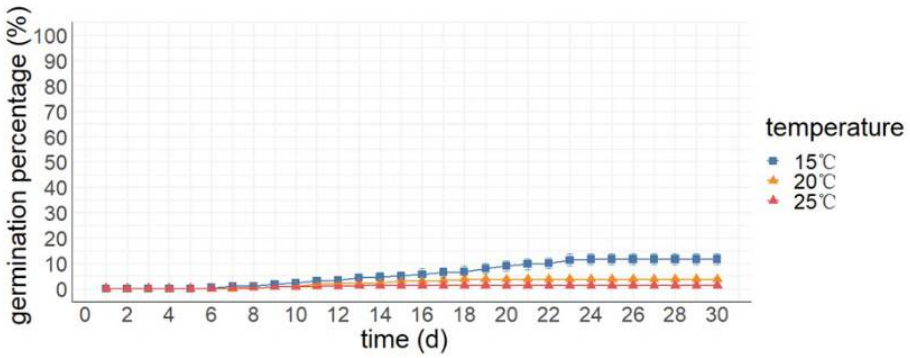

Fig. 11. Germination process of *Eucommia ulmoides* seeds with GA_3 at 15, 20, and 25°C.

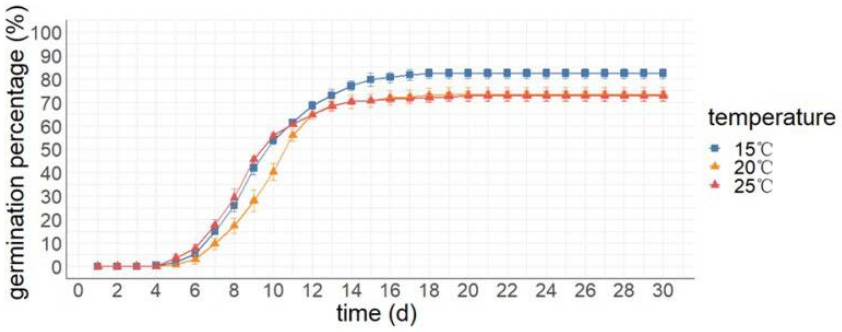

Fig. 12. Germination process of *Eucommia ulmoides* seeds with cold stratification+GA_3 treatment at 15, 20, and 25°C.

Cold stratification could release part of seed dormancy (60% germination). Moreover, in temperate climates, seeds required low temperatures to break dormancy, a physiological mechanism was observed in summer annuals and most temperate perennials (Blandino *et al*. 2022). In natural environments, such a cold stratification requirement could prevent germination during autumn conditions that were only temporarily suitable for seedling establishment, thus coordinating seedling emergence with favorable seasons and preventing frost damage to seedlings (Wang *et al*. 2017). Since seedling emergence and establishment were the most vulnerable life phases for plants, breaking dormancy following winter ensured species survival as a seed (Song *et al*. 2023).

GA_3 treatment in this study did not show good effect on seed germination (8%) (Fig. 11). Combined treatment of GA and ABA during seed germination progress, Liu *et al*. (2024) showed that GA_3 was decreased before germination and increased after germination. ABA increased during water absorption, decreased before germination, and increased again after germination. The hormonal control of dormancy is exerted through the balance between the two most important hormones that regulate it. Abscisic acid (ABA), increased dormancy, and gibberellic acid (GA), reduced it. Their impact was caused by variations in the content, as well as the sensitivity of seeds to them (Bewley and Black 1994).

Cold stratification+GA_3 treatment could release seed dormancy (80% germination) (Fig. 12). The dry-stored *Hydrocharis dubia* seeds treated with 30 days cold stratification treatment with GA_3 showed the highest germination percentage (Xue *et al*. 2023). Stratification treatment and hormone treatment was better than single treatment.

This research established that *E. ulmoides* seeds exhibited physiological dormancy, with scarification, and a combination of cold stratification and gibberellic acid treatment being effective in breaking dormancy. The optimum temperature for seed germination was identified as 15°C.

Acknowledgement

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References

Baskin JM, Baskin CC 2004. A classification system for seed dormancy. Seed Sci. Res. 14: 1-16.

Bewley J and Black M 1994. Seeds: Physiology of Development and Germination. New York, USA, Plenium-Press, New York, USA, pp. 445.

Bewley JD, Bradford KJ, Hilhorst HWM and Nonogaki H 2013. Seeds: Physiology of Development, Germination and Dormancy, 3rd ed. Springer, New York, pp. 392.

Blandino C, Fernández-Pascual E, Newton RJ and Pritchard HW 2022. Regeneration from seed in herbaceous understory of ancient woodlands of temperate Europe. Ann. Bot. **129**: 761-774.

Bois JP, Winkel T, Lhommee J, Rafaillac JP and Rocheteau A 2006. Response of some Andean cultivars of quinua (*Chenopodium quinua* Willd.) to temperature: Effects on germination, phenology, growth and freezing. European J. Agron. **25**: 299-308.

Cheng JF, Tian QJ, Wang SL, He XB and Peng XM 2013. The effect of storage in sand and seeding time on seed germination and seedling growth of *Eucommia ulmoides.* Seed **32**: 93-99.

Chien CT, Chen SY, Chien TY, Baskin JM and Baskin CC 2011. Nondeep simple morphophysiological dormancy in seeds of *Ilex maximowicziana* from northern (subtropical) and southern (tropical) Taiwan. Ecol. Res. **26**: 163-171.

Deng S, Deng Z, Xiaofeng W, Lu H and Xue H 2021. Effects of temperature, scarification, stratification, phytohormones, and after-ripening on the dormancy and germination of *Eucommia ulmoides* Oliv. seeds. Forest **12**: 1593-1605.

Finch-Savage WE and Leubner-Metzger G 2010. Seed dormancy and the control of germination: Tansley review. New Phytol. **171**: 501-523.

He Y, Zhao J, Yang B, Sun S, Peng L and Wang Z 2020. Indole-3-acetate beta-glucosyltransferase OsIAGLU regulates seed vigour through mediating crosstalk between auxin and abscisic acid in rice. Plant Biotech. J. **18**:1933-1945.

Housková K, Klepárník J and Mauer O 2021. How to accelerate the germination of Scots pine and Norway spruce seeds? J. For. Sci. **67**(3): 134-142.

Li T and Min X 2020. Dormancy characteristics and germination requirements of *Phoebe bournei* seed. Sci. Horticul. **260**(2): 108903.

Liu J, Qiu S, Xue T and Yuan Y 2024. Physiology and transcriptome of *Eucommia ulmoides* seeds at different germination stages. Plant Signal. Behav. **19**(1): 1-14.

Lv Y, Pan J, Wang H, Reiter RJ, Li X, Mou Z, Zhang J, Yao Z, Zhao D and Yu D 2021. Melatonin inhibits seed germination~~`n~~ by crosstalk with abscisic acid, gibberellin, and auxin in Arabidopsis. J. Pineal Res. **70**: 1-16. .

Nawrot-Chorabik K, Osmenda M, Słowiński K, Latowski D, Tabor S and Woodward S 2021. Stratification, scarification and application of phytohormones promote dormancy breaking and germination of pelleted Scots pine (*Pinus sylvestris* L.) seeds. Forest **12**: 621-623.

Paudyal SP, Das BD, Paudel N, Paudyal S, Limbu S, Das B, Paudel N and Rahman MM 2021. Effect of mechanical scarification on seed germination of selected weeds occurring in rice fields. J. Agric. Food Environ. **2**: 37-43.

Song Y, Li X, Zhang M, Xia G and Xiong C 2023. Effect of cold stratification on the temperature range for germination of *Pinus koraiensis*. For. Res: **34**: 221-231.

Wang G, Baskin CC, Baskin JM, Yang XJ, Liu GF, Zhang XS, Ye XH and Huang ZY 2017. Timing of seed germination in two alpine herbs on the southeastern Tibetan plateau: the role of seed dormancy and annual dormancy cycling in soil. Plant Soil **421**: 465-476.

Xu BM and Bai KZ 1993. Effects of low temperature on seed germination and seedling establishment of *Eucommia ulmoides*. For. Sci. Technol. **9**: 5-6.

Xue T, Sun Q, Tu J, Liu J and Cui M 2023. Germination and secondary dormancy of *Hydrocharis dubia* (Blume) seed. Bangladesh J. Bot. **52**: 429-435.

Xue TT, Liu J, Shen YB and Liu GQ 2017. Methods for rapid determination of pecan (*Carya illinoensis*) seed viability. Seed Sci. Technol. **45**: 455-464.

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