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RELATIONSHIP BETWEEN SEED MATURITY AND SEED DORMANCY IN *ACER BUERGERIANUM* MIQ.

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Abstract

Seeds are fundamental to plant survival and dispersal, yet their development and germination are influenced by various internal and external influences. There is little known about seed maturity and seed dormancy in *Acer buergerianum*. So, we investigated the relationship between seed maturity and seed dormancy through assessments of seed moisture content and various germination tests. The results showed that: 1. The water content in *A. buergerianum* seeds decreases as they mature. 2. Elevated seed maturity is linked to increased dormancy depth. 3. *A. buergerianum* seeds exhibit physiological dormancy. 4. Stratification can effectively release dormancy in *A. buergerianum* seeds. Understanding the dynamics between seed water content, maturity, and dormancy is pivotal for seed germination and seed propagation in Acer species.

Introduction

In plant reproductive biology, the correlation between seed maturity and dormancy is crucial. Environmental factors, such as temperature, soil, nutrients, light, water, humidity, air, and pollutants, substantially influence seed dormancy and germination, alongside other developmental stages (Yang and Li 2017; Ali *et al.* 2021). Seed maturity, including the developmental stage and ripeness of the embryo and endosperm, is important for seed quality and viability (Qanmber 2019; Faiza 2019). It defines nutrient reserves and developmental integrity, essential for germination. Seed dormancy, a natural adaptive mechanism, prevents untimely germination, ensuring survival under unfavourable conditions. The relationship between seed maturity and dormancy, complex and species-specific, requires further exploration (Bareke 2018), particularly in *Acer palmatum* seeds.

The genus *Acer* exhibits a diverse range of seed dormancy levels. Particularly, *A. saccharinum* seeds are non-dormant (Suszka and Tomasewska 1971), in contrast to *A. ginnala's* deep dormancy (Dumbroff and Webb, 1970). Acer's dormant seeds fall into two categories: embryo dormancy, where isolated embryos remain non-germinative, and testa-imposed dormancy, characterised by immediate germination following testa removal (Pinfield and Dungey 1985). However, this distinction is often ambiguous, with many Acer species displaying traits of both dormancy types. Research on various *Acer* species, including *A. saccharum*, *A. ginnala*, *A. pensylvanicum*, and *A. pseudoplatanus*, demonstrates this overlap (Webb and Dumbroff 1969; Dumbroff and Webb 1970; Wilson *et al.* 1979; Pinfield *et al.* 1987). Moreover, Thomas *et al.* (1973) identified embryo dormancy in *A. pseudoplatanus*, typically classified as testa-imposed dormant, suggesting that variations in dormancy are attributed to differences in seed developmental stages and the timing of fruit dispersal (Gleiser *et al.* 2015).

This study examined seed maturity and dormancy in *A. palmatum*, a deciduous tree renowned for its ornamental foliage. Given the significance of seed germination for propagation, we investigated how *A. palmatum*'s variable seed dormancy affects germination and growth. The

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present research evaluated seed maturity at various stages and analyzed dormancy characteristics using diverse methods. Understanding the interplay between seed maturity and dormancy in *A. buergerianum* will inform optimal practices for seed collection, storage, and germination, enhancing propagation of this valuable species.

Materials and Methods

Fresh *Acer buergerianum* fruits were collected at Chuzhou University in Chuzhou, Anhui Province, China. Different seed lots were collected on August 15th (Seed Lot 1), August 30th (Seed Lot 2), September 15th (Seed Lot 3), September 30th (Seed Lot 4), October 15th (Seed Lot 5), and October 30th (Seed Lot 6) in 2022. The seeds were selected and stored at room temperature in the physiological laboratory of Chuzhou University.

Tetrazolium test: For each seed lots of *A. buergerianum*, 100 seeds were immersed in 200 mL 0.5% (w/v) TZ solution in a 500 mL glass beaker at 35°C in darkness for staining. Staining patterns were evaluated after 24h. Tetrazolium test method for seeds were provided (AOSA, 2010) and the International Seed Testing Association (ISTA, 2003).

Moisture content test: For each seed lots of *A. buergerianum*, the seed husks were removed, the seed kernels were chopped and mixed evenly, and 3× 5g of seed kernels were collected using a constant temperature drying method (drying at 103°C for 24 h) to determine the water content of the samples (Xue *et al.* 2023).`

Water absorption test: 3*20g *A. buergerianum* seeds of different seed lots were selected. The wings were removed and the dry weight was measured and recorded using an electronic scale. Then, the seeds were placed in a culture dish with water for water absorption. After 24 hours, seeds were taken out, water on the surface of seeds was wiped off with a tissue, and the weight was measured and recorded.

Seed germination test: To test for germination of *A. buergerianum*, seeds were selected from each seed lot. The seeds were soaked in water for 24 hours, then sown in three replicates of 100 whole seeds and embryos each onto cotton beds. These were then placed in an incubator set to different temperatures (5, 15, 25°C). Throughout the experiment, the cotton and sand beds were kept moist by adding distilled water as needed, with great care taken to ensure adequate hydration. The emergence of a 2 cm radical was used as a criterion for seed germination. Seed germination was recorded daily for 30 days (Xue *et al.* 2023).

Other germination treatments: Seed of *A. buergerianum* germinated at optimum temperature with other different treatment: a. Seed germinated at dark; b. Seed germinated at light; c. seed germinated in 0.5g/L GA3; broken seed (the seed coat were punctured).

Stratification treatment test: For the stratification of *A. buergerianum* seeds (Seed lot 6), sands were washed with tap water to remove dust and sterilized at 121°C for 30 minutes. The seeds were sterilized in a 1% (v/v) sodium hypochlorite solution for 15 minutes, followed by three washes with sterilized water. The seeds were then mixed with moist sands in closed plastic bags, with a sands to water ratio of 1:10 (w/w). After standing for 10 minutes, excess water in the bottom of the bags was removed. The seeds were then placed in closed plastic bags and stored in darkness at different temperatures (4°C and room temperature) for 0, 30, or 90 days.

Statistical analyses of the data of germination were performed by SPSS 20.0 for Windows. The results were subjected to an analysis of variance to detect differences between the means and the means compared using least significant difference (multiple comparisons).

Results and Discussion

In this study, we investigated the variability in harvest times and maturity levels across different seed lots of *A. buergerianum* Seed lot 1, harvested first, showed the lowest maturity level, whilst seed lot 6, harvested last, exhibited the highest. Viability of seeds across all lots was confirmed through Tetrazolium tests. Moreover, water absorption tests indicated that seeds from each lot were capable of absorbing water. An anticipated decrease in water content was observed across the seed lots (Fig.1). Initially, seed lots 1 and 2 had higher water contents (10.3% and 10.1%, respectively), which decreased progressively to 9.7% in seed lot 3, and further to 9.3% and 9.1% in subsequent lots. The final seed lot, seed lot 6, showed a significant reduction in water content to 8.8%. This trend highlights the crucial relationship between decreasing water content and increasing seed maturity, a vital aspect in understanding seed physiology and germination behaviour (Rondanini 2007). As seeds mature, they undergo physiological and biochemical modifications, including a decrease in water content and an increase in dry weight, preparing them for germination and growth (Dussert *et al.* 2000).

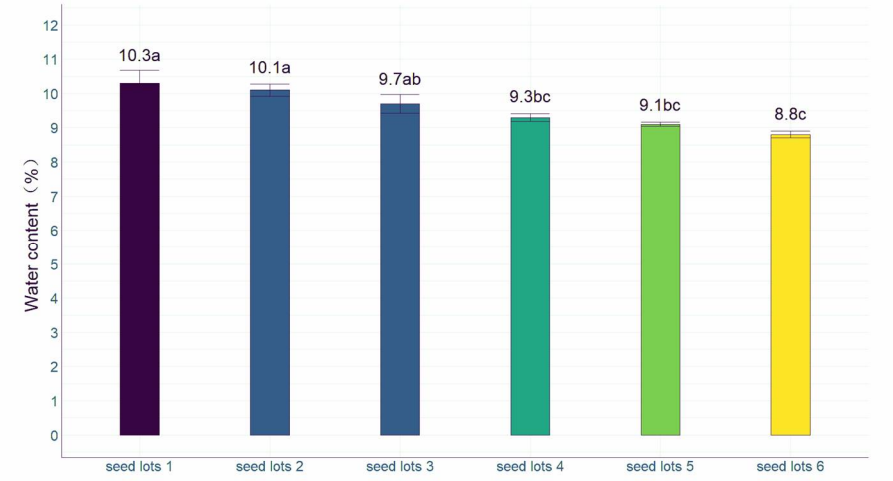

Fig.1. Water contents of different seed lots. (Vertical bars are standard errors of the mean. The different letters showed significant differences at 0.05 level among treatments as determined by LSD comparison and Duncan's Multiple Range Test.).

The relationship between seed water content and germination potential is complex and species-specific (Menendez *et al.* 2019). In some plant species, a certain level of seed water content is necessary for germination to occur. Seeds with excessively low water content may experience dormancy or have reduced germination rates. However, in *A. buergerianum* seed, Seeds (seed lot 1 and 2) generally have better germination rates when their water content is higher. Understanding the optimal water content range for germination in a particular species is important for successful seed propagation and conservation efforts.

Two-way ANOVA was used to analyse the germination rate of *A. buergerianum* seeds across different seed lots and treatments at varying temperatures (5, 15, and 25℃). The results indicated significant impacts of seed lot variability and treatment on germination rates at all temperatures. Moreover, interactive effects were observed between the seed lots and treatments.

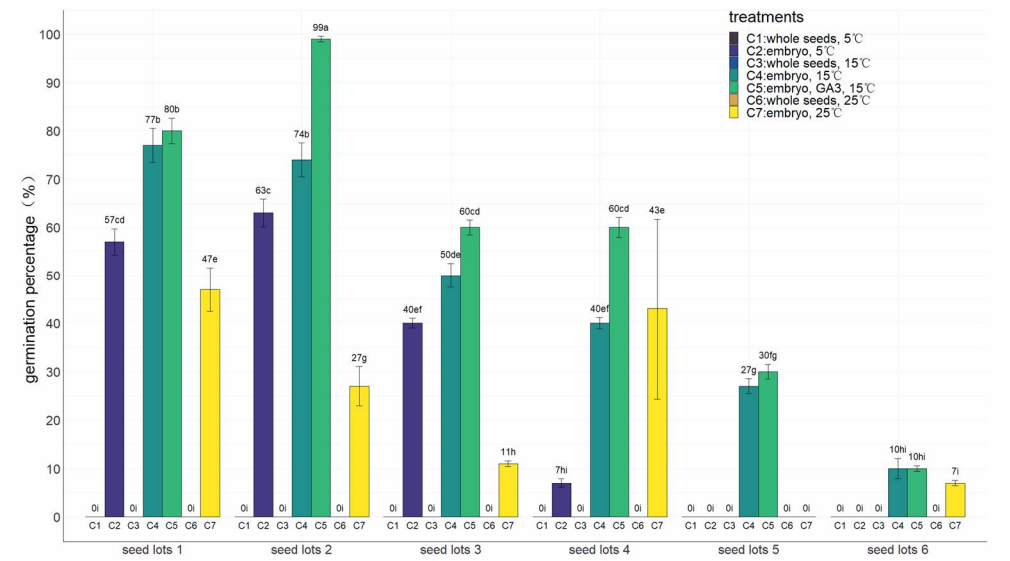

Fig. 2. Germination percentage of *A. buergerianum* seeds with different seed lots germinated at 5, 15 and 25°C. (Notes: Vertical bars are standard errors of the mean. The different letters showed significant differences at 0.05 level among treatments as determined by LSD comparison.)

At 5°C, no whole seeds germinated . The germination rate commenced high for embryos in seed lots 1 and 2 (57% and 63%, respectively), but significantly declined in lots 3 and 4 (40% and 7%, respectively), with no germination in lots 5 and 6. A similar pattern was observed at 15°C, albeit with higher initial germination rates (77% for lot 1 and 74% for lot 2). The rate gradually decreased across the subsequent lots, with lot 6 showing only 10% germination. No whole seeds germinated at 15°C either. At 25°C, no whole seeds germinated and the germination rate of embryos across different seed lots decreased (Fig. 2).

These observations illustrate a trend where later-harvested seeds exhibited deeper dormancy. The optimal germination temperature for *A. buergerianum* seeds was identified as 15°C (Fig. 2). Previous research in the genus Acer, including works by Webb (1972, 1973), Pinfield and Dungey (1985), and Gleiser (2015), has primarily focused on aspects of seed dormancy and germination inhibitors. Tang (2021) noted a non-deep physiological dormancy in *A. cinnamomifolium* seeds. However, there is limited knowledge regarding seed maturity and dormancy in *A. buergerianum*.

The correlation between seed maturity and dormancy in *A. buergerianum* is complex. Generally, a negative correlation exists between seed maturity and germination potential. Our findings suggest that seeds harvested earlier tend to have lower dormancy levels. Seed maturity, defined as the developmental stage at which a seed is physiologically ready for germination, is intricately linked with the breaking of dormancy (Faiza *et al.* 2019). The results from Figures 2 reinforce the observation that the optimal germination temperature for A. buergerianum seeds is 15°C. For seed lot 1, seed with treatment (Embryo+ dark) germinated 66%, and embryo treated with light germinated 77%, and embryo treated with light and GA_3 germinated 80%. Meanwhile, for all seed lots, the data suggested light and GA_3 can improve seed (embryo) germination rate. But there was no whole seed germinated with the treatments, including: Whole seed+dark, Whole seed+light, Whole seed+light+GA_3. And, there was no seed germinated with the treatments, including: Broken seed+light, Broken seed+light+GA_3, too. There was no obvious effect on

germination promotion when the small cut was made, and the seeds were quickly moulded. GA_3 had no significant effect on whole seed germination (no seed germination), but had significant effect on embryo germination (seed lot 2 germinated 99%).

To better understand and overcome seed dormancy in *A. buergerianum* seeds, research on breaking seed dormancy is essential. This research focuses on investigating various methods to promote germination in dormant seeds. For example, light, scarification, or the application of growth regulators (GA_3) are used to break dormancy in *A. buergerianum* seeds. But, as tab.1 showed, there was no whole seed germinated with the treatments, including: Whole seed+dark, Whole seed+light, Whole seed+light+GA_3. And, there was no seed germinated with the treatments, including: Broken seed+light, Broken seed+light+GA_3, too. And, GA_3 had no significant effect on whole seed germination (no seed germination), but had significant effect on embryo germination (seed lot 2 germinated 99%). *A. buergerianum* seeds have shallow embryo dormancy and can be broken by hormone treatments. Whole *A. buergerianum* seeds have deep dormancy, and breaking their dormancy is difficult.

Table 1. The germination percentage of *A. buergerianum* seeds with six treatments germinated at optimum temperature (15°C).

	Germination rate/%					
	Seed lots					
Treatments	1	2	3	4	5	6
Whole seed+dark	0	0	0	0	0	0
Whole seed+light	0	0	0	0	0	0
Whole seed+light+GA_3	0	0	0	0	0	0
Embryo+ dark	60±6	54±4	30±1	18±3	9±4	1±2
Embryo+light	77±6	74±6	50±4	40±2	27±3	10±4
Embryo+light+GA_3	80±5	99±1	60±3	60±4	30±3	10±1
Broken seed+light	0	0	0	0	0	0
Broken seed+light+GA_3	0	0	0	0	0	0

Fig. 3 showed that with the increase of stratification time, different stratification methods could promote the breaking of seed dormancy and seed germination rate. Fig. 3 also showed that after 30 days of stratification, changed temperature stratification has broken the dormancy of seed embryos, and the germination rate of seed embryos at 15℃ is 93%. As shown in Fig. 3, some whole seeds began to germinate after 30 days of stratification, and the germination rate was higher at 5℃ (the best germination rate is 46% for cold stratification). After 90 days of stratification, seeds germinated and the radicle broke through the seed coat, the germination rate of cold stratification was 91%, the germination rate of changed temperature stratification was 83%, and the seed dormancy was relieved. *A. buergerianum* seeds exhibit dormancy when fully mature (seed lot 6, freshly harvested seeds), requiring additional factors or treatments to overcome dormancy and initiate germination. After treatment of stratification, seed dormancy released (seed germinated 83 and 91% during 90d stratification).

The characteristics of seed dormancy in *A. buergerianum* seeds further contribute to the complexity of this relationship. Dormancy in these seeds may be influenced by various factors, such as physiological dormancy, physical dormancy (e.g., hard seed coat), or even embryo dormancy. Each type of dormancy mechanism requires specific conditions or treatments to break

dormancy and promote germination (Baskin and Baskin 2004). According to Baskin and Baskin (2004) dormancy types, seed dormancy includes physiological dormancy, physical dormancy,

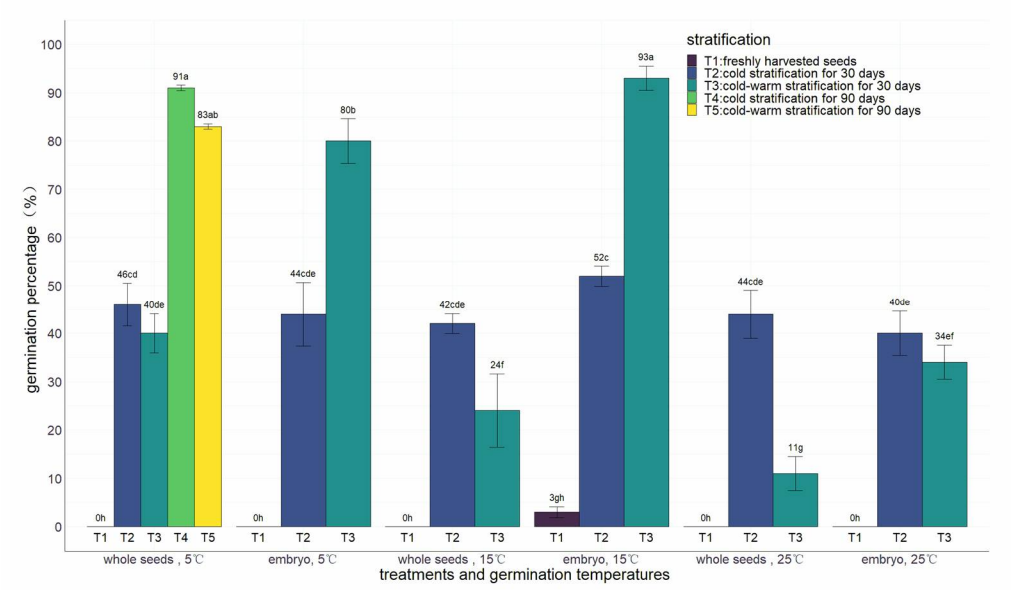

Fig. 3. The germination percentage of A. buergerianum seeds with different treatments at different temperature. (Notes: Vertical bars are standard errors of the mean.)

morphological dormancy, morphophysiological dormancy, and combined dormancy (Xue *et al.* 2023). *A. buergerianum* seeds have permeable seed coats, and their embryos are well-formed, so they are not physical dormancy, morphological dormancy, morphophysiological dormancy, and combined dormancy. *A. buergerianum* seeds are physiological dormancy. And seed dormancy can be released by stratification.

1) As *A. buergerianum* seeds reach maturity, their water content progressively diminishes.

2) A greater degree of seed maturity correlates with a more profound state of dormancy, thereby rendering the breaking of this dormancy a challenging task.

3) However, *A. buergerianum* seeds exhibit a relatively shallow embryo dormancy, which can be effectively overcome with hormonal treatments. The seeds possess a permeable coat, and their embryonic morphology remains intact, categorising them as experiencing physiological dormancy.

4) Moreover, stratification has been shown to be an efficacious method for releasing seed dormancy in *A. buergerianum*.

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