



Article

Study on Dormant and Germination Characteristics of Chinese Olive (*Canarium album*) Seeds

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Abstract: This study aimed to determine the dormancy type of Chinese olive seeds and improve their germination rate. The water permeability and germination-inhibiting substances of Chinese olive seeds were assessed. Low-temperature stratification and soaking in a GA3 solution were implemented to measure the time lag, initial time, germination rate, and germination potential of the seeds. The findings revealed that the seed coat exhibited poor water permeability, which negatively influenced the germination rate. Additionally, Chinese olive seeds contained substances that inhibited germination. The duration of low-temperature stratification (at 4 ± 1 °C) gradually diminished the dormancy of Chinese olive seeds, resulting in early and rapid germination. The germination rate significantly increased, with the percentage of seed germination rising from 0% to 42.33% within 60 days of stratification. Furthermore, combining low-temperature stratification with different concentrations of GA3 notably enhanced the germination rate. The optimal concentrations of gibberellins for 40 and 60 days of stratification were determined to be 300 and 100 mg/L, respectively. These results indicate that Chinese olive seeds possess non-deep physiological dormancy.

Keywords: *Canarium album*; seed; micromorphology; dormancy; germination; inhibitory substance; low-temperature stratification; gibberellin



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1. Introduction

Canarium album L., commonly known as Chinese olive (also referred to as Gan lan or Qing guo in China), is a fruit tree belonging to the Burseraceae family. It is native to the southeastern region of China and has been introduced to various other Asian tropical and semi-tropical regions [1]. Chinese olives are both medicinal and edible [2], as they are rich in nutrients such as polysaccharides, dietary fiber, vitamin C, and calcium. Due to their nutritional properties, Chinese olives are considered excellent therapeutic fruits [3].

Seed dormancy is a natural physiological process in plant development, serving as a biological adaptation to environmental conditions and seasonal changes. Baskin [4] proposed a classification of seed dormancy into five types: physiological dormancy (PD), morphological dormancy (MD), morphophysiological dormancy (MPD), physical dormancy (PY), and comprehensive dormancy (PY + PD). Within physiological dormancy, there are three levels: deep physiological dormancy, moderate physiological dormancy, and non-deep dormancy. Seed dormancy has a significant impact on crop yield and quality [5,6]. In fruit tree production, failure to relieve seed dormancy in a timely manner often results in low germination rates or alternate year germination, which greatly hinders normal seedling cultivation. Addressing the dormancy of fruit tree seeds has become a prominent topic in current biological and agricultural research. Studies have revealed that seed dormancy is a complex trait influenced by various internal and external factors. Endogenous factors in seed dormancy and germination include plant hormones, nutrients, and enzyme activities

within seeds. External factors, such as temperature, light, and stable environmental conditions, also play a crucial role [7]. Plant hormones like abscisic acid (ABA) and gibberellin (GA) are key players in these processes. GA stimulates amylase synthesis and secretion, promoting germination, while ABA inhibits these processes. Dormant seeds have high ABA and low GA levels [8]. ABA induces and maintains dormancy, while GA counteracts ABA effects and promotes germination [9,10]. Gibberellin helps break physiological dormancy and aids in germination [11,12]. Temperature is a major environmental factor affecting seed dormancy, with low-temperature stratification being a common method to break dormancy [13,14]. For instance, low-temperature layering can reduce rose seed dormancy and improve germination rates [15,16].

The dormancy period of fresh China olive seeds is known to be long, with a germination rate of only 35% for newly harvested seeds [17]. Numerous studies have been conducted on seed dormancy [18,19], revealing varying characteristics among different species. However, there is a lack of research on the dormancy traits of Chinese olive seeds. Our research team has discovered that stratification plays a crucial role in reducing seed germination delay and enhancing overall germination rates [20]. In this experiment, our focus was on studying the water permeability of China olive seeds and the inhibitory effects of extracts from different parts of the seeds. Additionally, we explored the impact of low-temperature stratification and a gibberellin solution with varying concentrations on breaking dormancy and promoting germination of China olive seeds. The findings of this study aim to provide valuable insights for addressing the challenge of low germination rates in the utilization and innovation of the China olive germplasm.

2. Materials and Methods

2.1. Plant Materials

The main *C. album* cultivar ‘Changying’ from Fujian Province, China, was used as the material. The fruits were harvested on 2 December 2021, at the peak of ripeness. Following the harvest, the seeds were extracted, cleaned, and dried. The seed dormancy test was carried out between January 2022 and April 2022. To prepare the seeds for germination tests, they were first sterilized by immersing them in a 2% NaClO solution for 20 min, followed by rinsing with distilled water. The seeds were then soaked in warm water at 30 °C for 48 h before being subjected to further germination test treatments.

Commercial Chinese cabbage (*Brassica rapa* var. *Glabra*) seeds with a purity level of $\geq 97.0\%$, moisture content of $\leq 7.0\%$, and a germination rate of $\geq 85.0\%$ were procured. Chinese cabbage seeds are frequently utilized in experiments to validate seed dormancy inhibitors, given their non-dormant characteristics, high germination rate, straightforward germination process, and short germination cycle [21].

2.2. Observation of Seed Morphology

A total of 30 seeds were randomly selected for the measurement of their vertical and horizontal diameters using a vernier caliper. Additionally, 60 seeds were randomly selected to determine their individual weight using an electronic analysis balance. The surface, longitudinal structure, and seed coat of the seeds were observed using a scanning electron microscope.

2.3. Seed Water Absorption Rate

Twenty untreated intact dry seeds and 20 acid-etched seeds (treated with 98% H₂SO₄ and rinsed with tap water for 24 h) were used for the experiment. The seeds were placed in a beaker containing 100 mL of distilled water and incubated at 25 ± 1 °C. Periodically, the seeds were removed and weighed after drying them with filter paper until their weight remained constant. The intervals for measurements were every 2 h for the first 12 h, every 4 h from 12 to 24 h, and every 8 h from 24 to 48 h. This process was repeated three times. The water absorption rate was calculated using the following formula: Water absorption

rate (%) = $(M_t - M_0)/M_0 \times 100$, where M_t represents the weight of the seed after water absorption and M_0 represents the initial weight of the seed [22].

2.4. Seed Inhibitor Activity Test

2.4.1. Preparation of Crude Extracts from Different Parts of Chinese Olive Seeds

The crude extraction method for each part of the seed using an aqueous solution as the extraction agent was as follows: the seed shell (i.e., inner fruit skin), seed skin, and embryo of fresh Chinese olive seeds were ground with liquid nitrogen. Then, 0.1 g of the ground seed shell, seed skin, and embryo was weighed and placed in a conical flask. Distilled water was added as the extraction solvent, with a solid–liquid ratio of 1:20 (g:mL), and the mixture was extracted at 4 °C for 48 h. Shaking was performed every 6 h during the extraction process. After extraction, the residue was transferred to a centrifuge tube and centrifuged at a speed of 5000 r/min for 5 min at room temperature. The supernatant was then taken to a constant volume of 25 mL, resulting in a 50 mg/mL extraction solution.

The crude extraction method using methanol as the extraction agent for each part of the seed was the same as the aqueous solution extraction method. The only difference was that the extraction agent was changed to methanol. After extraction, the methanol was removed by rotary evaporation. The resulting extract was dissolved in distilled water to make a 25 mL extraction solution with a concentration of 50 mg/mL. All crude extracts of the Chinese olive seed parts were stored at (4 ± 1) °C. Each experiment was repeated three times.

2.4.2. The Inhibitory Effect of Crude Extracts from Different Parts of Chinese Olive Seeds

Plump Chinese cabbage seeds of the same size were selected and rinsed with distilled water. They were then placed in a Petri dish ($\Phi = 9$ cm) with double-layer filter paper. Each dish contained 50 seeds, and this process was repeated three times. To each dish, 5 mL of crude extracts from Chinese olive seeds with concentrations of 100%, 50%, 33%, and 25% was added. Distilled water was used as a blank control. All Petri dishes were cultured in a dark incubator at (25 ± 1) °C, and the number of germinated seeds was counted every 12 h. After 72 h, ten seedlings were randomly selected from each Petri dish, and the length of their roots was measured.

The germination rate was calculated using the following formula [23]:

$$\text{Germination rate (\%)} = n/N \times 100$$

The germination potential was calculated using the following formula [24]:

$$\text{Germination potential (\%)} = n_t/N \times 100$$

The seed germination rate is the percentage of seeds that have germinated by a specific date at the end of the test, compared to the total number of seeds tested [23]. Germination potential, on the other hand, is the percentage of seeds that have germinated in relation to the total number of seeds tested during a specific period of the test [24]. In the formulas provided, 'n' represents the number of seeds germinated at the end of the germination period (72 h), 'N' represents the total number of seeds used for testing, and 'n_t' represents the number of seeds germinated within the specified time frame (24 h).

2.5. Seed Germination Test

Sterilized Chinese olive seeds were soaked in gibberellin solutions of varying concentrations (0, 100 mg/L, 300 mg/L, and 500 mg/L) for 24 h at a temperature of (25 ± 1) °C in an incubation room. Water was used as a control. Subsequently, the gibberellin-treated seeds were mixed with disinfected wet sand containing approximately 60% water content. The wet sand and seeds were mixed at a volume ratio of 1:3, and the sand could be watered by hand. The mixture was then placed in a sand storage area at a low temperature of (4 ± 1) °C, with a sand layer thickness of approximately 10 cm. The sand was replenished

with water and turned daily to maintain moisture and aeration. After 40 and 60 days of low-temperature stratification, 50 seeds from each gibberellin concentration treatment were sown in hole trays filled with a nutrient soil substrate in an incubation chamber at $(25 \pm 1) ^\circ\text{C}$ for germination tests. Daily observations were made to monitor germination.

Seed vigor parameters were evaluated using the germination delay, initial germination time, germination rate, and germination potential. Germination delay refers to the time elapsed before the first seed begins to germinate [25]. Initial germination time corresponds to the number of days it takes for 5% of the seeds to sprout [23]. The calculation formula for the germination rate and germination potential was the same as described in Section 2.4.2.

2.6. Data Analysis

Excel 2010 and Origin 2021 were utilized for counting and graphing the experimental data. One-way ANOVA was conducted using SPSS 26.0 software. The significant difference was assessed through the least significant difference test ($p < 0.05$), and the data were presented as the standard error of the mean.

3. Results

3.1. Morphological Characteristics of Chinese Olive Seeds

Chinese olive seeds, when ripe, are brownish in color and have a fusiform shape with two pointed sides and a rounded middle (Figure 1A). The surface of the seed has both obtuse and acute ribs, with every two obtuse ribs corresponding to a ventricle. It is worth noting that although the seed kernel is usually present in the ventricle of Chinese olive, some ventricles may not contain a seed kernel (Figure 1B). The seed kernel is enveloped by a brown film known as the seed coat, which wraps around the white ripe embryo (Figure 1C). Additionally, the surface of the Chinese olive seed exhibits visible spaces of varying sizes (Figure 1D). Upon examining the seed cross-section, it can be observed that the endocarp of Chinese olive seeds consists of two layers (Figure 1E). The outer layer is thicker and has a rough texture, while the inner layer is thinner and smoother in texture (Figure 1F). The upper epidermis of the seed coat of Chinese olive seeds displays a reticulated ornamentation (Figure 1G), and the subepidermis of the seed coat contains waxes of varying thickness (Figure 1H).

The average single seed weight, longitudinal diameter, and width of Chinese olive seeds were found to be 1.33 g, 32.96 mm, and 10.65 mm, respectively (Figure 2A). The water content of the endocarp (i.e., seed shell), seed coat, and embryo was measured to be 11.4%, 10.85%, and 5.33%, respectively. It was observed that the embryo had a significantly lower water content compared to the seed shell and testa ($p < 0.05$) (Figure 2B).

3.2. Analysis of Seed Water Absorption

The water absorption rate of intact dry seeds and acid-etched 0.5 h seeds exhibited a pattern of a 'rapid rise-slow rise tendency to stabilize' from 0 to 88 h. The fastest water absorption occurred from 0 to 25 h, with the rate of acid-etched seeds being significantly higher than that of intact dry seeds. From 25 to 65 h, the water absorption rate of intact seeds slowed down, while the rate of acid-etched seeds remained higher than that of intact dry seeds. Ultimately, the water absorption rate was 17.06% for intact seeds and 16.79% for acid-etched seeds, which was not statistically significant ($p > 0.05$). In conclusion, it was found that strong acid corrosion increased the permeability of Chinese olive seeds and accelerated the early-stage water uptake rate, but the final water uptake rate did not differ significantly from that of intact dry seeds (Figure 3).

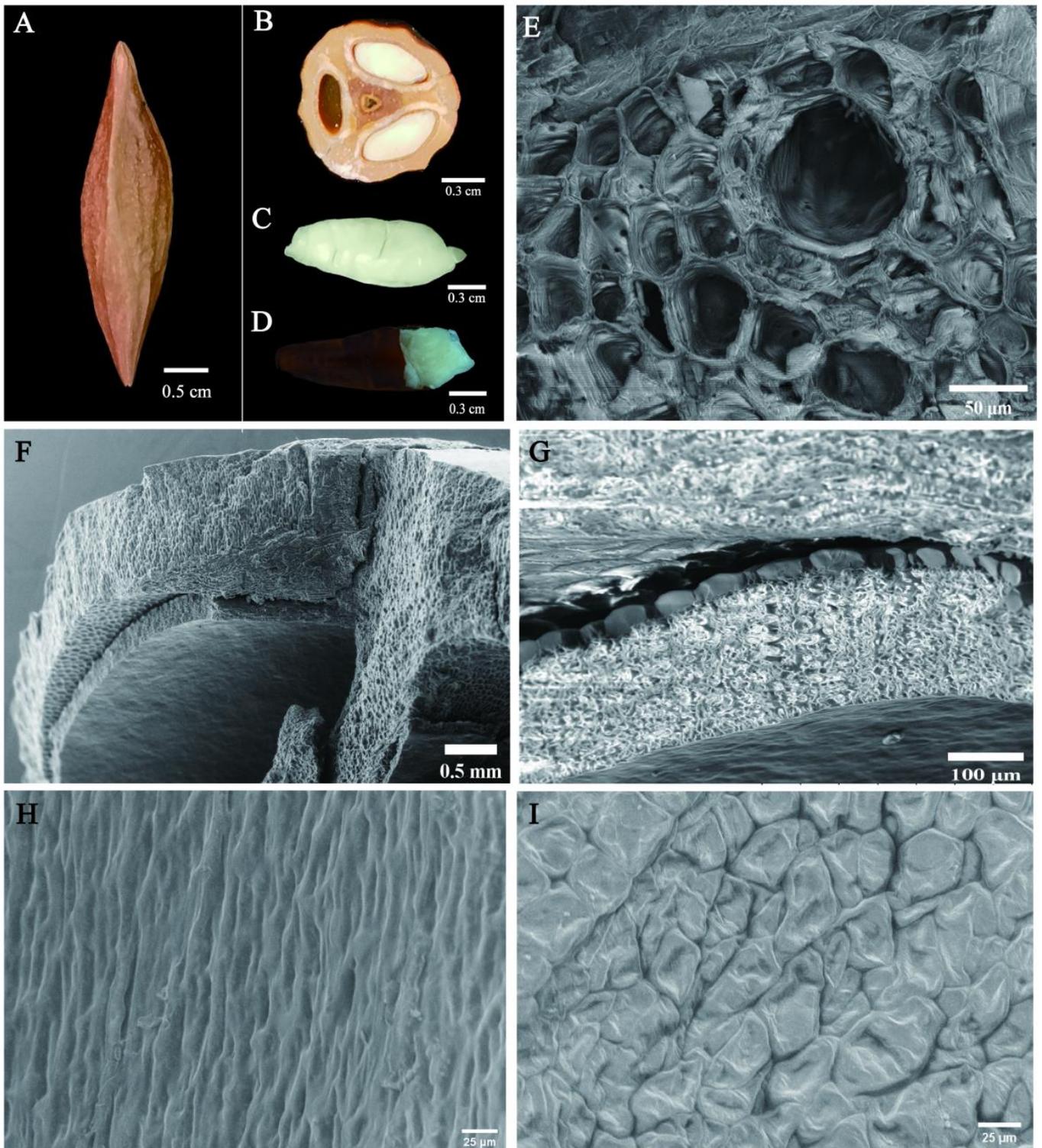


Figure 1. This figure presents the morphological characteristics of Chinese olive seeds. (A) shows complete seeds. (B) depicts a cross-section of a seed. (C) displays a view of a mature embryo. (D) illustrates an embryo with the seed coat. (E) provides a magnified view ($\times 500$) of the inner surface of the fruit coat (testa). (F) shows a cross-section of the endocarp ($\times 30$). (G) offers a closer look ($\times 200$) at the endocarp. (H,I) show the seed coat at higher magnifications ($\times 400$).

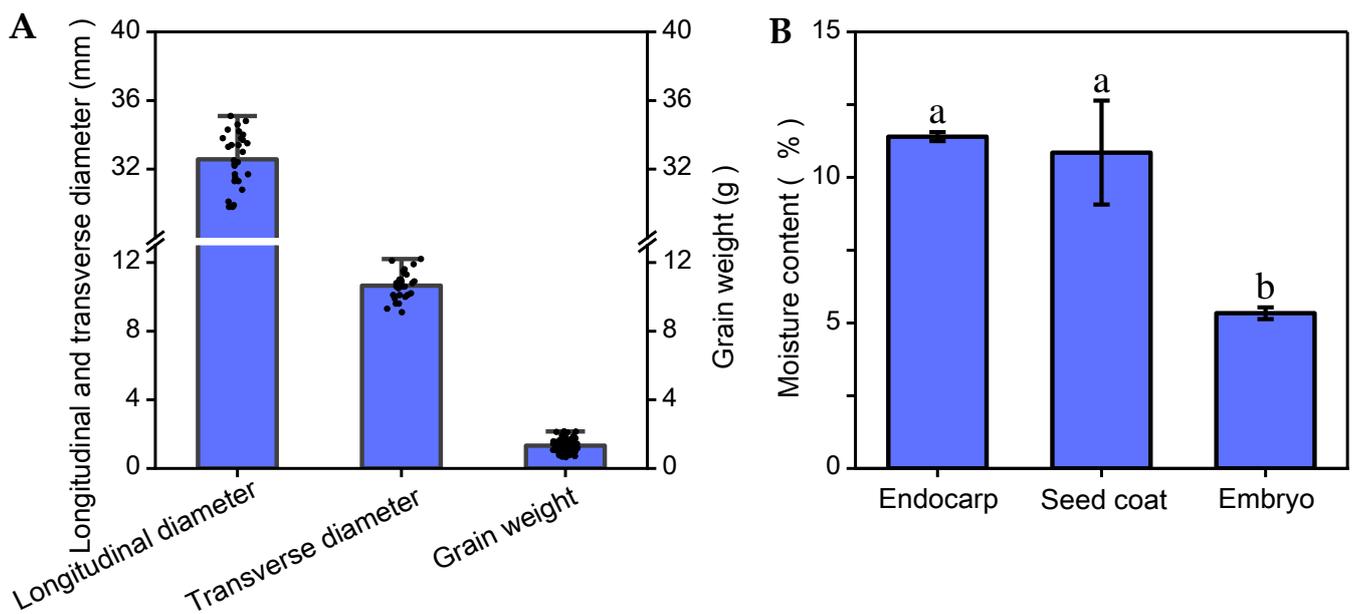


Figure 2. Chinese olive seed morphology and moisture content of various parts of the seed. (A) The vertical and horizontal diameters, as well as the single grain weight of Chinese olive seeds, were measured (mean \pm SE for thirty replicates). (B) The water content of various parts of Chinese olive seeds was also determined (mean \pm SE for three replicates). Different letters indicate significant differences in the means (least significant difference test, $p < 0.05$).

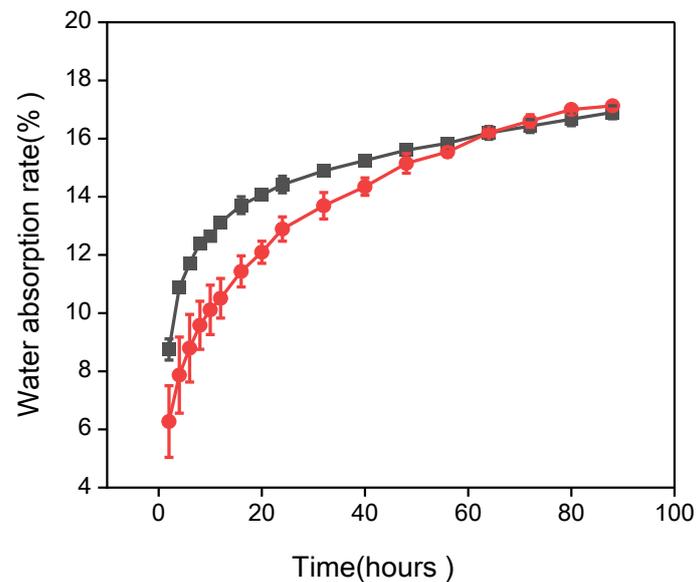


Figure 3. The changes in water absorption rate (mean \pm SE for three replicates) of Chinese olive seeds with or without sulfuric acid corrosion. The seeds with sulfuric acid corrosion are represented by ■, while the seeds without corrosion are represented by ●.

3.3. Effects of Seed Extracts from Different Parts of Chinese Olive on Seed Germination and Radicle Growth of Chinese Cabbage

3.3.1. Effects of Water Extract from Different Parts of Chinese Olive Seeds on Seed Germination and Radicle Growth of Chinese Cabbage

The germination potential and germination rate of Chinese cabbage seeds treated with pure water extracts of the endocarp, seed coat, and embryo were not significantly different from the control group (CK) ($p > 0.05$) (Figure 4A1–C1, A2–C2). However, there were varying degrees of difference in radicle growth. The radicle length of Chinese cabbage

treated with an 100% extract from different parts was lower than that of the control group, and the difference between the 100% extract of the endocarp and embryo and the control group was not significant ($p > 0.05$). The 100% extract of the seed coat was significantly lower than that of the control group ($p < 0.05$). In the concentration range of 50–100%, the inhibitory effect on radicle growth gradually decreased with decreasing concentration. At a concentration of 33%, it had a significant promoting effect on radicle growth. When the concentration was lower than 25%, the inhibition or effect was relieved (Figure 4A3–C3). In summary, the pure aqueous extract of Chinese olive seeds had no significant effect on the germination of Chinese cabbage seeds. However, it did have an effect on radicle growth, which varied at different concentrations.

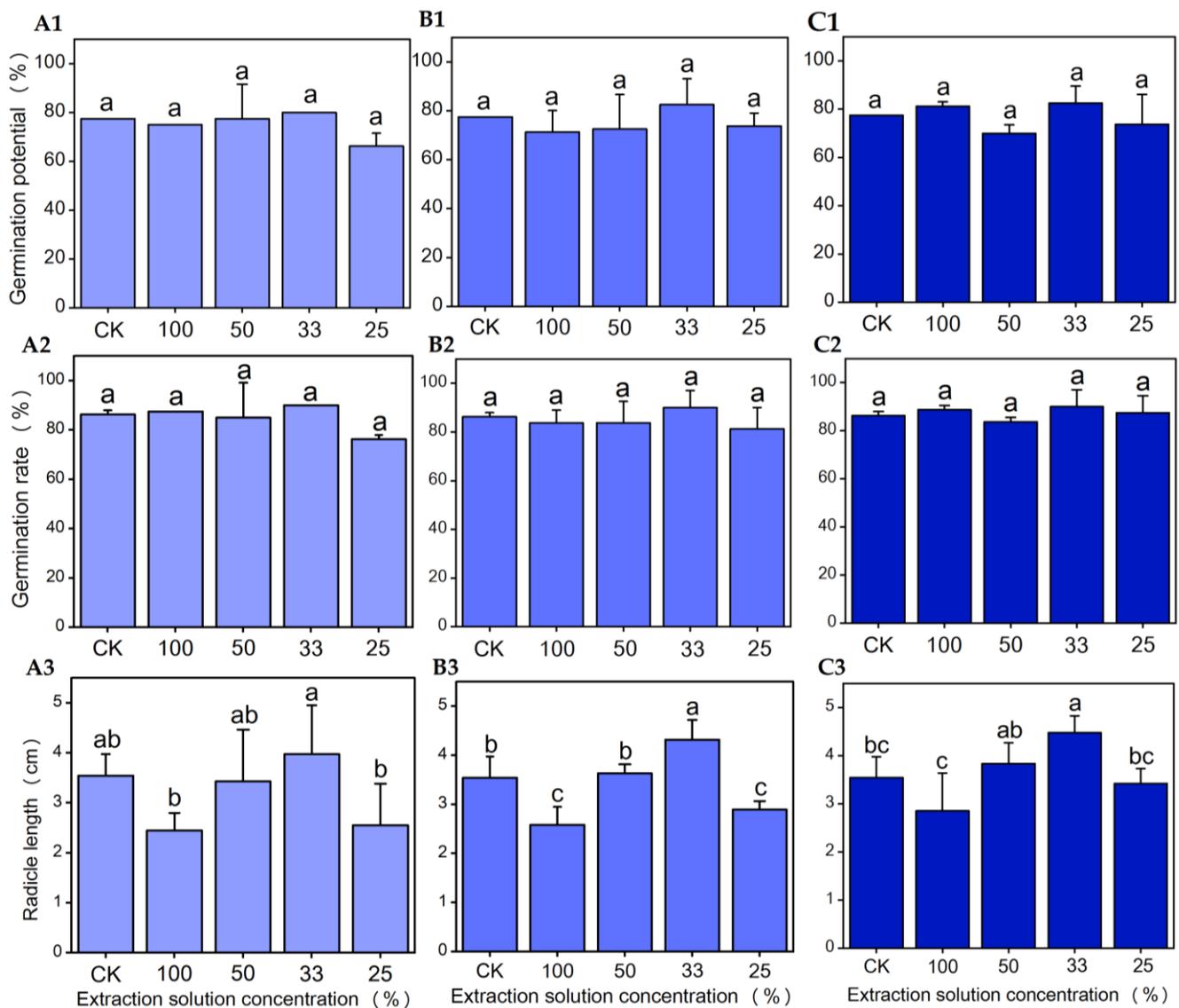


Figure 4. Effects of ultrapure water extracts from different parts of Chinese olive seeds on seed germination and radicle growth of Chinese cabbage. The effects were evaluated based on the endocarp (A), embryo (B), and seed coat (C). The parameters measured included germination potential (1), germination rate (2), and radicle length (3). The extracts were labeled as Extract Liquid (Wes), Extracts diluted 1×, Extracts diluted 2×, and Extracts diluted 3×. Different letters indicate significant differences among means (least significant difference test, $p < 0.05$).

3.3.2. Effects of Methanol Extracts from Different Parts of Chinese Olive Seeds on Seed Germination and Radicle Growth of Chinese Cabbage

This study examined the germination potential and rate of Chinese cabbage seeds treated with methanol extracts from different parts of Chinese olives. The results showed that there were no significant differences in the germination potential and rate when the seeds were treated with different concentrations of the endocarp extract compared to the control group (CK) ($p > 0.05$). However, the germination potential and rate were lower in seeds treated with different concentrations of seed coat extracts compared to the control group. Specifically, the 100% seed coat extract had a significantly lower effect on the germination potential and rate compared to the control group ($p < 0.05$), and the inhibitory effect decreased with decreasing concentration. On the other hand, the 100% embryo extraction solution had a higher effect on the germination potential and rate compared to the control group, with a significantly higher germination rate ($p < 0.05$). The promoting effect decreased as the concentration decreased, and eventually the effect was relieved (Figure 5A1–C1,A2–C2).

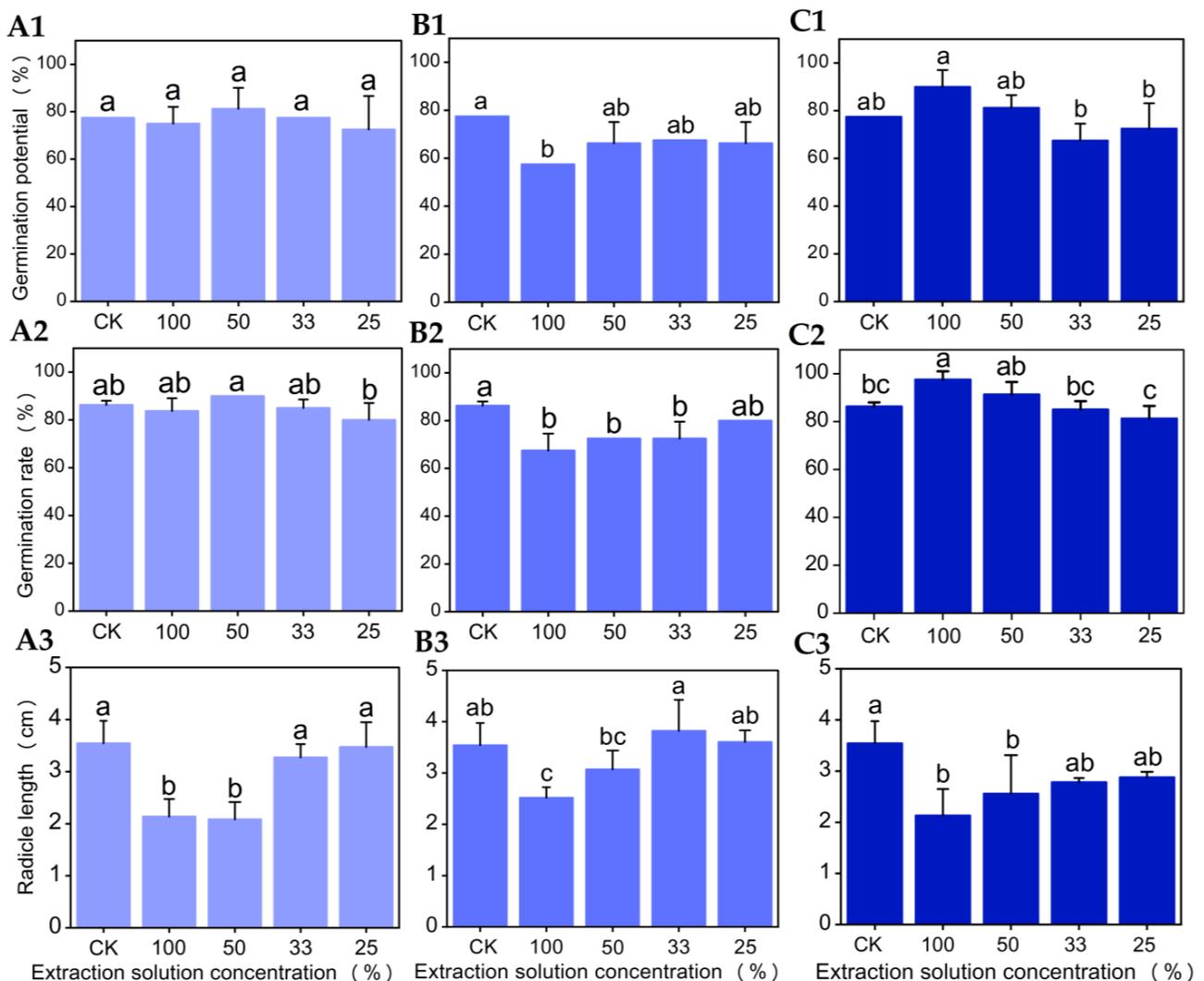


Figure 5. Effects of methanol extracts from different parts of Chinese olive seeds on seed germination and radicle growth of Chinese cabbage. The effects were evaluated based on the endocarp (A), embryo (B), and seed coat (C). The parameters measured included germination potential (1), germination rate (2), and radicle length (3). The extracts were labeled as Extract Liquid (Wes), Extracts diluted 1×, Extracts diluted 2×, and Extracts diluted 3×. Different letters indicate significant differences among means (least significant difference test, $p < 0.05$).

The growth of Chinese cabbage radicles was inhibited by the methanol extract of the Chinese olive seed endocarp, seed coat, and embryo. The radicle length was lower compared to the control group (CK). The 100% extract of each part showed significantly lower radicle growth than the control group ($p < 0.05$). As the concentration decreased, the inhibitory effect of each part extract on radicle growth gradually decreased, eventually leading to relief of inhibition (Figure 5A3–C3).

In conclusion, the methanol extract of each part of Chinese olive seeds had an impact on the germination of Chinese cabbage seeds. The seed coat extract inhibited the germination of Chinese cabbage seeds within a specific concentration range, while the embryo extract promoted germination within a specific concentration range (6.25~50 mg/L). Additionally, both extracts influenced radicle growth, with each part extract inhibiting radicle growth within a specific concentration range.

3.4. Effects of Gibberellin Combined with Low-Temperature Stratification on Seed Germination of Chinese Olive

The results presented in Figure 6 demonstrate that seeds treated with a low temperature for 60 days exhibited a significantly higher germination delay, initial germination time, germination rate, and germination potential compared to seeds treated with a low temperature for 40 days. The germination rate showed a remarkable increase, with the percentage of seed germination rising from 0% to 42.33% within 60 days of stratification. These findings suggest that low-temperature treatment effectively breaks seed dormancy and promotes seed germination. Furthermore, when seeds were subjected to low-temperature treatment for 60 days and soaked in gibberellin solutions with different concentrations for 24 h, a significant improvement in the germination rate was observed. Among the different concentrations tested, 100 mg/L showed the best effect. However, it did not have a positive impact on germination delay, initial germination time, and germination potential. On the other hand, seeds treated with a low temperature for 40 days and soaked in gibberellin solutions with varying concentrations for 24 h exhibited a significantly higher germination delay, initial germination time, germination rate, and germination potential compared to the control group. Among the different concentrations tested, seeds soaked in a gibberellin solution with a concentration of 300 mg/L showed the best effect. The germination delay, initial germination time, germination rate, and germination potential were 42.33%, 48%, 16%, and 2.67% higher, respectively, than those treated with a low temperature for 40 days. In summary, both low-temperature stratification and gibberellin treatment effectively break seed dormancy. The optimal concentrations of gibberellin treatment at 40 and 60 days of low-temperature stratification are 300 mg/L and 100 mg/L, respectively. These findings indicate that the dormancy type of olive seeds is non-deep physiological dormancy.

3.5. Main Effect Analysis of Germination Index of Chinese Olive Seeds Treated with Low-Temperature Stratification and Gibberellin

Table 1 presents the main effects of low-temperature stratification and gibberellin treatment on the germination index of Chinese olive seeds. The results indicated that both low-temperature stratification and gibberellin treatment had significant effects on the germination delay, initial germination, germination rate, and germination potential ($p < 0.05$), suggesting their ability to promote seed germination. Furthermore, the interaction between low-temperature stratification and gibberellin treatment was also found to be significant ($p < 0.05$). The analysis of η^2 revealed that low-temperature stratification has a stronger impact on the seed germination rate. When combined with gibberellin treatment, low-temperature stratification effectively reduces germination delay, enhances initial germination, and improves germination potential.

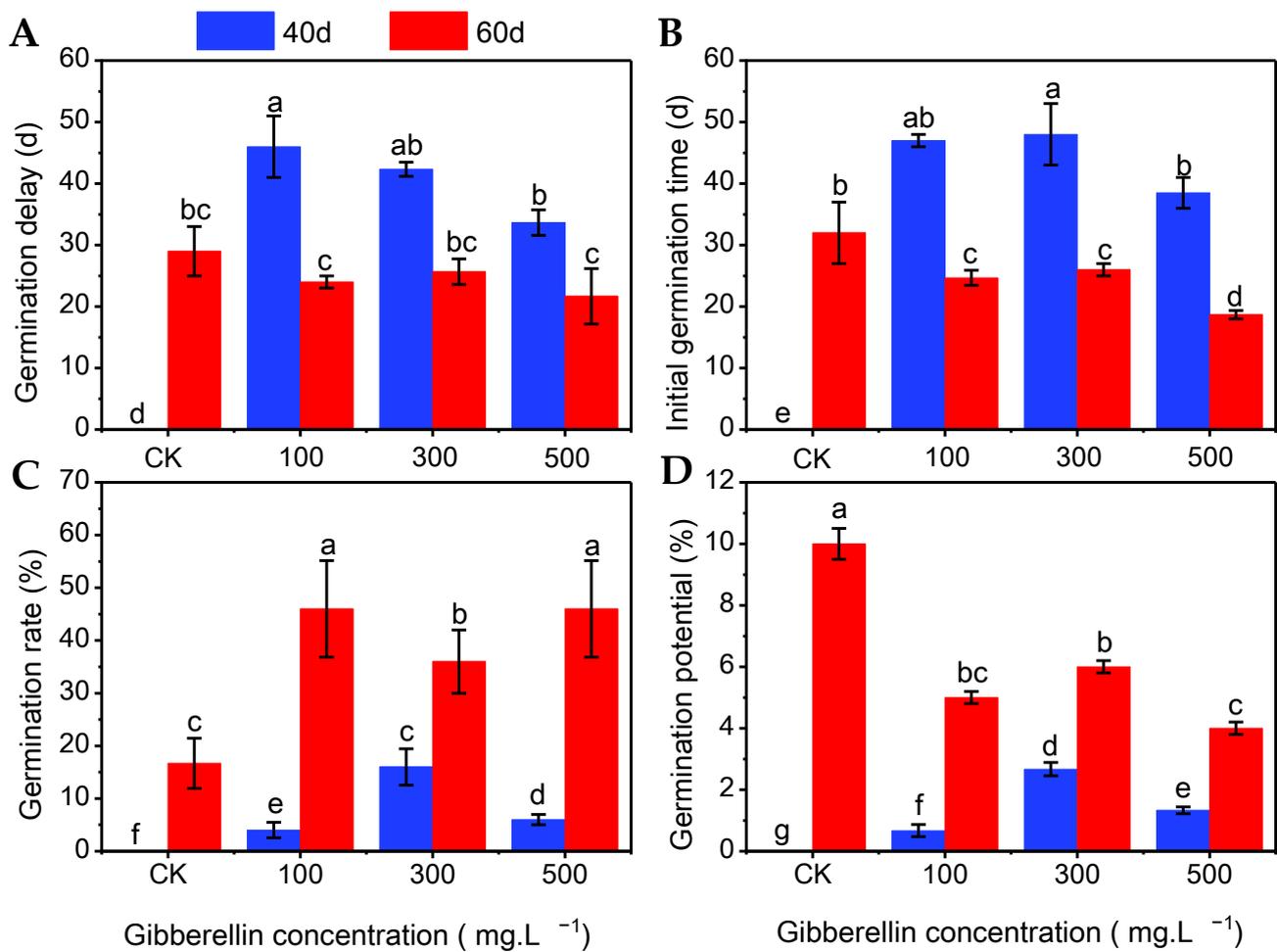


Figure 6. Effects of gibberellin combined with low-temperature stratification on germination delay (A), initial germination time (B), germination rate (C), and germination potential (D) of Chinese olive seeds. The blue and red bars represent 40 and 60 days of cold stratification, respectively. Different letters indicate significant differences among means (least significant difference test, $p < 0.05$). Error bars depict standard errors of the means.

Table 1. Main effect analysis of germination index of Chinese olive seeds treated with low-temperature stratification and gibberellin.

Index	Main Effect of Low-Temperature Stratification (A)			Main Effect of Gibberellin (B)			Interaction (A×B)		
	F	<i>p</i>	η^2	F	<i>p</i>	η^2	F	<i>p</i>	η^2
Germination delay	19.470	0.00	0.549	59.101	0.00	0.917	90.106	0.00	0.944
Initial germination time	524.634	0.00	0.970	755.469	0.00	0.993	1447.751	0.00	0.996
Germination rate	74.318	0.00	0.941	15.600	0.00	0.770	7.600	0.003	0.620
Germination potential	12.944	0.00	0.735	609.961	0.00	0.992	6661.557	0.00	0.993

Note: η^2 values indicate effect values; the larger the effect value, the stronger the relationship between the factor and the dependent variable.

4. Discussion

4.1. Seed Dormancy Characteristics

Seed dispersal is a critical process for plant reproduction in natural environments [5]. In addition to providing protection, the seed coat plays a crucial role in producing protective metabolites and regulating nutrient transport to the embryo [26,27]. Seed physical

dormancy occurs when the covering tissues of the seed impede water entry into the seed. It is believed that the seed coat does not affect normal germination once the seed absorbs 36% of water [28]. Our study investigated the water absorption pattern of Chinese olive seeds with and without sulfuric acid treatment. Both groups displayed similar water absorption trends, reaching a stabilization point after 65 h of water absorption. While there was no significant difference in the water absorption rate between the two groups, the overall absorption rate was only about 17%. This indicates that the seed coat partially restricts water absorption and gas exchange. Scanning electron microscope observations revealed a waxy texture in the seed coat of the Chinese olive, potentially explaining the hindrance in water absorption. These findings suggest that the seed coat may pose mechanical obstacles to seed germination, and the germination rate of seeds can be significantly enhanced with gibberellin treatment. Baskin [4] categorized dormancy caused by mechanical obstacles of the seed coat as physiological dormancy. The presence of endogenous inhibitors, such as abscisic acid, linoleic acid, salicylic acid, and cinnamic acid in plant seeds, can induce seed dormancy [29–32]. Gibberellin and abscisic acid have been found to have an antagonistic effect on seed germination when combined [8,33]. Gibberellin can alleviate seed dormancy and play a crucial role in promoting seed coat rupture [34,35]. The methanol extracts of the endocarp, testa, and embryo of Chinese olive seeds significantly inhibit seed germination and radicle elongation. Conversely, the water extract shows no significant effect. This indicates that the methanol extracts of Chinese olive seeds contain germination inhibitors, although further research is needed to identify the specific components of these inhibitory substances. In conclusion, the physical barrier of the seed coat to the seed embryo and the presence of seed germination inhibitors are the primary factors contributing to the dormancy of Chinese olive seeds, which exhibit physiological dormancy.

4.2. Seed Dormancy Release

Different treatment methods can be employed to overcome seed dormancy based on the specific dormancy types of seeds [36]. Stratification can help soften the seed coat and enhance seed physiological maturity [37]. Gibberellin can counteract the inhibitory effects of certain chemicals on seed germination and facilitate the transformation of seed substances to provide nutrition for seed embryo growth [38]. Temperature also plays a crucial role in seed dormancy and germination [39]. The GA/ABA signaling pathway is recognized as a key regulator of temperature-induced seed germination [40]. Moderate–low temperatures have been shown to reduce dormancy and promote germination in various plant species [41,42]. Research on *Arabidopsis thaliana* indicates that moderate–low temperatures boost gibberellin production and expedite abscisic acid degradation [41,43]. A study on *Saposhnikovia divaricata* reveals that low-temperature stratification at 4 °C increases the levels of soluble sugars, gibberellins, and indole-3-acetic acid in seeds; enhances amylase and α -amylase activity; and reduces starch and abscisic acid content, ultimately facilitating dormant seed germination [44]. In this study, we observed that seeds subjected to 60 days of low-temperature treatment (4 ± 1 °C) exhibited a significantly higher germination time lag, initial germination time, germination rate, and germination potential compared to those treated for 40 days. These findings suggest that low-temperature treatment effectively breaks seed dormancy and promotes seed germination. For seeds with physiological dormancy, the balance between their endogenous ABA and GA₃ contents can be regulated to break seed dormancy [45]. Gibberellin is effective in lifting the physiological dormancy of seeds and promoting seed germination [46]. In this study, it was found that low-temperature treatment combined with gibberellin (300 mg/L) could promote seed germination more effectively. The optimal treatment concentration of gibberellin was 300 mg/L when the days of low-temperature stratification were 40 d, and 100 mg/L when the days were 60 d. This indicates an inverse relationship between the optimal concentration of gibberellin and the stratification time. The germination of Chinese olive seeds after a short period of cold stratification or gibberellin treatment suggests that olive seeds have non-deep physiological dormancy. In contrast, seeds with deep physiological dormancy

are not affected by gibberellin treatments and usually require more than 3 months of cold stratification to promote germination [4,46].

5. Conclusions

The seed coat of Chinese olive seeds has a dense tissue structure and is poorly permeable, which leads to the presence of germination inhibitors and induces dormancy. Low-temperature stratification at $(4 \pm 1) ^\circ\text{C}$ effectively breaks seed dormancy and promotes germination. Comparing 40 days of low-temperature stratification to 60 days, the latter shows a significantly higher germination time lag, initial germination time, germination rate, and germination potential. This indicates that as the duration of low-temperature stratification increases, the dormancy of Chinese olive seeds gradually diminishes, resulting in earlier and faster germination. Gibberellic acid treatment is also effective in lifting seed dormancy and increasing the germination rate. The combination of low-temperature treatment and gibberellic acid yields even better results, further improving the germination of Chinese olive seeds. The optimal concentration of gibberellin for 40 and 60 days of low-temperature stratification is 300 mg/L and 100 mg/L, respectively, suggesting an inverse relationship between stratification time and gibberellin concentration. In conclusion, Chinese olive seeds exhibit non-deep physiological dormancy.

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Data Availability Statement: The data supporting the results of this study are included in the present article.

Conflicts of Interest: The authors declare no conflicts of interest.

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