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Effects of Different Temperature Treatments on Breaking Dormancy of *Pyrus pyrifolia* Cultivar 'Pearl pear' Seeds

Jialiang Kan[1,2], Yufeng Zhang[1,3], Yutong Ye[1], Hui Li[1], Chunxiao Liu[1], Jinxing Wang[1], Jin Lin[1], Yeqing Ying[2], Youhong Chang[1], Fuliang Cao[2,3]* and Xiaogang Li[1]*

[1]*Institute of Pomology, Jiangsu Key Laboratory for Horticultural Crop Genetic Improvement, Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu, China*

[2]*State Key Laboratory of Subtropical Silviculture, Zhejiang A&F University, Lin"an, Zhejiang, China*

[3]*Co-Innovation Center for Sustainable Forestry in Southern China, Nanjing Forestry University, Nanjing, Jiangsu, China*

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***Corresponding author:** State Key Laboratory of Subtropical Silviculture, Zhejiang A&F University, Lin"an, Zhejiang, China & Co-Innovation Center for Sustainable Forestry in Southern China, Nanjing Forestry University, Nanjing, Jiangsu, China

Xiaogang Li, Co-Innovation Center for Sustainable Forestry in Southern China, Nanjing Forestry University, Nanjing, Jiangsu, China

Abstract

Temperature is a key factor that influences the breaking of pear seed dormancy. The seeds of *Pyrus pyrifolia* (sand pear) cultivar, 'Pearl Pear' were used to investigate the effects of temperature on seed dormancy breaking. The seeds were cultured at stable temperatures of 4, 8, and 12°C and variable temperatures of 8-4-8 and 4-8-12°C. According to the results, significant differences were observed in seed germination and growth parameters (germination, rooting, and cotyledon expansion rates) after exposure to a variable temperature treatment of 4-8-12°C. Cotyledon expansion rate of 'Pearl pear' seeds treated at 4-8-12°C was the highest and seedlings grew well after germination. The 4-8-12°C variable temperature treatment can be used as an optimum temperature treatment for breaking the dormancy of 'Pearl pear' seeds. This study could provide a practical and theoretical framework for breeding pears and other fruit trees, forest trees, as well as the production of seedlings.

Keywords: *Pyrus pyrifolia*; Seed dormancy; Tempetature; Germination; Grow

Introduction

Pear (*Pyrus spp.*) is one of the most commercially important fruit trees and is widely cultivated in temperate regions of the world [1]. The area under pear tree cultivation continues to expand due to a high planting efficiency, which facilitates agricultural planting structure adjustment [2]. Pears are rich genetic resources with more than 2000 pear varieties. The main cultivated pear varieties are divided into five categories: White, Sand, Qiuzi, Xinjiang, and Western pears [3]. Sand pear is mainly cultivated in the middle and lower reaches of Huai River, Yangtze River, and South China Sea. As a crucial germplasm resource, sand pear is not only economically beneficial, but also an important parental material for breeding new pear varieties [4].

Pyrus pyrifolia cultivar 'Pearl pear', which is an early maturing sand pear variety bred by the Shanghai Academy of Agricultural Sciences in China, is an interspecific hybrid variety. The female parent is sand pear variety 'Yakumo' from Japan, whereas the male parent is an early maturing pear variety 'Beurre Giffard' from France [5]. 'Pearl pear' matures early and the fruit develops in 73 days. The maturity period of the pear in Nanjing, China, is from mid to late June. 'Pearl pear' has a small fruit, with a single fruit weighing up to 90 g and containing only 10.2% of the soluble solids. Heterosis typically occurs in pear and parents with rich genetic diversity are more likely to obtain ideal offspring [6]; Therefore, 'Pearl pear' can be used as an intermediate genetic material to improve pear varieties. However, embryo development in hybrid offspring of 'Pearl pear' is incomplete, the embryo contains few substances, and seedling emergence rate is low. Therefore, obtaining sufficient seedlings using conventional methods is a challenge, which limits the utilization of 'Pearl pear' [7].

The dormancy of pear seeds enables them to cope with adverse environmental conditions, and to promote the survival and reproduction of pear populations; however, the phenomenon remains unclear [8]. The reasons for pear seed dormancy are complex, and primarily include incomplete embryo development, embryo dormancy, and mechanical restriction of seed coats, which lead to low seed germination rates and uneven seedling emergence [9-11]. Present studies on breaking dormancy and promotion of pear seed germination have focused on low temperature stratification, hormone, and seed coat breaking treatments, among other methods [2,11]. Few studies have investigated the effects of different temperature treatments on breaking the dormancy of pear seeds. Temperature is essential for breaking seed dormancy, as well as promoting seed germination and growth. Generally, high ambient temperatures can increase seed dormancy, whereas low temperatures are conducive to promoting the release of seed dormancy. Some plants can germinate at a specific temperature, while some plants require varying ambient temperatures to facilitate dormancy release [12,13]. Different temperature treatments have varied effects on breaking seed dormancy. Wang applied various low-temperature stratification treatments to Ilex pubescens seeds and observed a seedling emergence rate of 28.8% after two months of cold stratification at 0°C, whereas seed germination rate was 92.0% after cold stratification at 4°C [14]. Xianliang et al. observed relatively high seed germination rates after the seeds of 12 shrubs were treated at variable temperatures of 5-25°C [14].

In this study, the seeds of sand pear variety ‘Pearl pear’ were used to investigate the effects of different temperature treatments on breaking seed dormancy and on seedling growth after germination. First, the weights and water absorption characteristics of ‘Pearl pear’ seeds were evaluated. Different stable and variable temperature gradients were applied to break the dormancy of ‘Pearl pear’ seeds. The results of this study could provide a practical and theoretical framework for fruit and forest tree breeding, and seedling production.

Materials and Methods

Plant Material Collection and Disinfection

The new variety of high quality pear, ‘Pearl pear’ was used as the experimental material. The fruits were collected in July 2022 from the fruit tree germplasm nursery of the platform for the protection and utilization of agricultural germplasm resources in Jiangsu Province, Xuanwu District, Nanjing City, Jiangsu Province. The fruits were stored at 25°C for a week. After completion of the fruit ripening process, the fruits were cut into smaller pieces and the seeds were removed. The seeds were washed with water and dried under a shade at room temperature for subsequent use.

Determination of the Thousand Grain Weight

A total of 100 naturally dried and plump ‘Pearl pear’ seeds were randomly selected, and the thousand grain weight was measured using a precision electronic balance. The procedure was repeated three times.

Determination of Seed Imbibition

A total of 30 naturally air dried, whole, and plump seeds were randomly selected and soaked in water at 25°C after weighing. Thereafter, the seeds were removed from water after 2, 4, 6, 8, 10, 12, 14, and 16 h of soaking and their weights measured after removing moisture from their surfaces using filter papers. The procedure was repeated three times and seed imbibition was calculated as follows: seed water absorption = (weight of seeds after water absorption - dry seed weight)/dry seed weight × 100%.

Breaking Seed Dormancy using Different Temperature Treatments

Stable temperature (4, 8, and 12°C) and variable temperature (8-4-8 and 4-8-12°C) treatments were used as the test conditions for breaking ‘Pearl pear’ seed dormancy. The total duration of each temperature treatment was 45 days and temperature treatment at 25°C was used as the control. The ‘Pearl pear’ seeds were washed with clean water, disinfected with 75% ethanol for 1 min, washed 3-5 times with sterile water, and then soaked in sterile water for 14 h. After the seeds absorbed water, wet filter papers were used to evenly distribute the seeds in the Petri dishes. Each Petri dish contained 100 seeds and each temperature treatment was repeated three times. To avoid contamination, the seeds were cleaned and the filter papers were replaced every five days.

Evaluation of Growth Characteristics of Seedlings

The seeds stored in wet Petri dishes were removed and placed in a culture room at 25 °C to promote germination. The number of germinated seeds was counted after 2, 4, 6, 8, and 10 days. In addition, rooting rate, cotyledon expansion rate, root length, length of the aboveground parts, and weight of individual seedlings after germination were determined on the 10th day. The formulas used to calculate each parameter were as follows:

germination rate = (number of germinated seeds/number of tested seeds) × 100%

Germination potential = (number of germinated seeds on the 4^{th} day/number of tested seeds) × 100%

Germination index = $\Sigma(G_n/D_n)$

where G_n is the germination potential of a seed on the nth day and Dn is the number of days counted after planting.

Average germination time = $\Sigma(D_n)/\Sigma n$

where D is the number of days counted from the beginning of germination and n is the number of germinated seeds on the corresponding days.

Rooting rate = number of seedlings with root lengths > 0.5 cm/total number of tested seeds × 100%

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Cotyledon expansion rate = number of seedlings with expanded cotyledons/total number of tested seeds × 100%.

Statistical Analysis

Data were analyzed using MS Excel (Microsoft Corp., Redmond, WA, USA) and IBM SPSS Statistics (IBM Corp., Armonk, NY, USA).

Results

Thousand Grain Weight and Seed Imbibition Characteristics

The thousand grain weight of 'Pearl pear' seeds was approximately 19.51 g after natural shade drying and the seeds were relatively complete and rich in nutrients. According to the results of water absorption characteristics of 'Pearl pear' seeds (Figure 1), water absorption efficiency was the highest between 4 and 6 h and water absorption rate was 21.09%. The water absorption rate of 'Pearl pear' seeds decreased between 12 and 16 h. The degree of protoplast hydration tended to be saturated and seed weights became stable, approximately 4.21 g, which was 2.15-fold higher than that observed before water absorption.

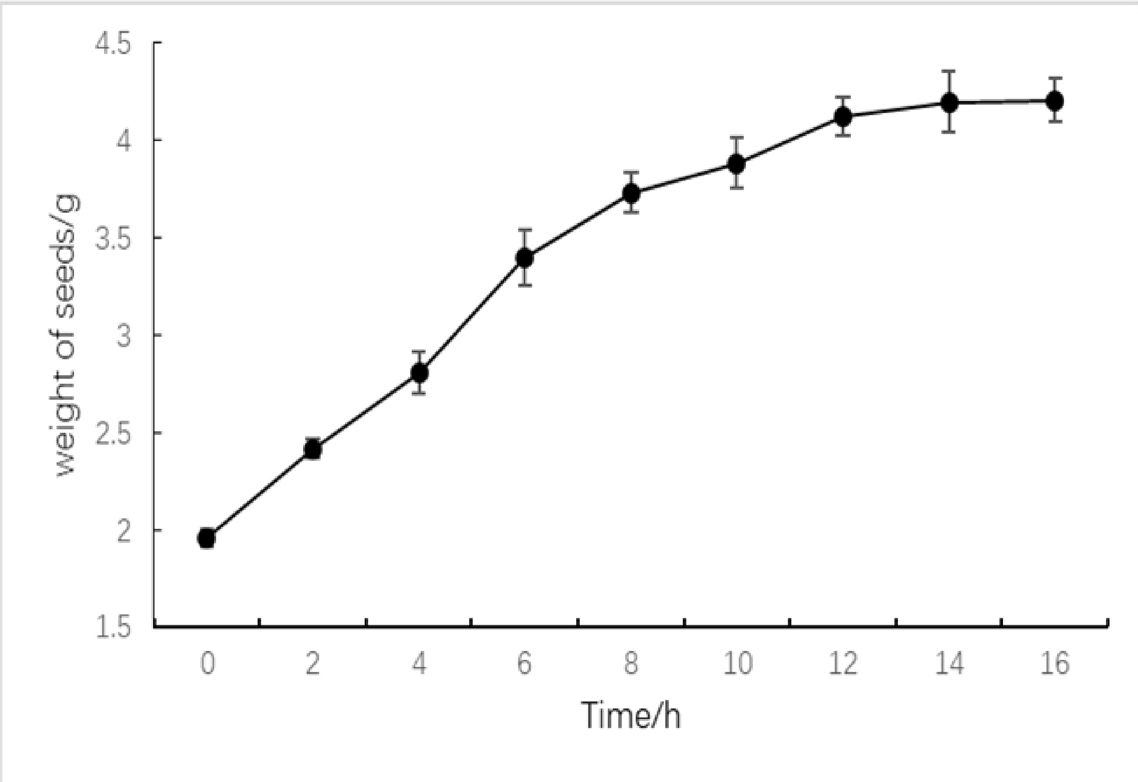

Figure 1: Water absorption characteristics of 'Pearl pear' seeds.

Effects of different temperature treatments on seed germination

The results of the effects of different temperature treatments on the germination of 'Pearl pear' seeds are shown in Table 1. The germination rates of treated seeds initially increased gradually with an increase in time and then remained stable. The germination rates of seeds in each treatment was the highest on the 10th day; seed germination rates in the variable temperature treatment of 4-8-12℃ were significantly higher (76.43%,$P<0.05$) than those in other temperature treatments, followed by the germination rates of seeds in the stable temperature treatment of 8℃ (56.10%) and variable temperature treatment of 8-4-8℃ (41.76%). The germination rates of seeds treated at stable temperatures of 12℃ and 4℃ were 26.42% and 19.34%, respectively. The germination rate of seeds treated at a constant temperature of 25℃ (control) was 0. The germination rates of 'Pearl pear' seeds in each treatment are shown in Figure 2.

In addition to germination rates, significant differences were observed in the germination potential, germination index, and average germination time of 'Pearl pear' seeds ($P<0.05$) after exposure to the different temperature treatments (Table 2). The germination potential (11.50%) and germination index (9.53%) of 'Pearl pear' seeds in the variable temperature treatment of

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4-8-12°C were the highest. The germination rate and potential of seeds treated at constant temperatures were lower than those of seeds treated at variable temperatures. In addition, the average germination time varied among the treatments. The average germination time of seeds treated at 4-8-12°C was relatively short (3.75 days), whereas that of seeds treated at 4°C was the longest (6.20 days). The average germination time of seeds treated at 8°C was the shortest (3.69 days). However, the germination rate and index of seeds treated at 8°C were significantly lower than those of seeds treated at 4-8-12°C.

Table 1: Germination rates of 'Pearl pear' seeds after exposure to different temperature treatments.

Temperature/°C	0 d	2 d	4 d	6 d	8 d	10 d
4	0.00±0.00d	2.44±0.48c	4.88±0.69e	11.57±1.53e	17.84±2.14e	19.34±2.86e
8	16.48±2.35a	29.27±6.18a	34.15±4.88b	45.59±9.51b	51.58±6.86b	56.10±9.37b
12	5.89±0.84c	12.50±2.79b	14.93±2.93d	19.42±3.77d	21.83±3.12d	26.42±3.77d
8-4-8	7.89±1.13b	11.11±1.59b	25.00±3.71c	27.94±3.64c	38.39±5.48c	41.76±6.64c
4-8-12	6.38±0.91c	29.64±4.23a	52.39±9.48a	61.90±8.84a	72.29±11.33a	76.43±8.92a

Note: Different lowercase letters within the same column indicate significant differences ($P<0.05$) among treatments.

Table 2: Germination rates, potential, indices, and average germination times of 'Pearl pear' seeds after exposure to different temperature treatments.

Temperature /°C	Germination rate/%	Germination potential/%	Germination index/%	Average germination time/d
4	19.34±2.86e	1.22±0.09c	1.71±0.14c	6.20±0.95a
8	56.10±9.37b	4.42±0.85b	4.80±0.84b	3.69±0.48b
12	26.42±3.77d	2.26±0.38bc	2.37±0.42bc	4.36±0.62b
8-4-8	41.76±6.64c	4.28±0.74b	3.28±0.51bc	4.72±0.72b
4-8-12	76.43±8.92a	11.50±1.85a	9.53±1.72a	3.75±0.81b

Note: Different lowercase letters within the same column indicate significant differences ($P<0.05$) among treatments.

Effects of Different Temperature Treatments on Seedling Growth and Development after Germination

The growth characteristics of seedlings on the 16th day after germination are presented in Table 3. According to the results, rooting and cotyledon expansion rates of 'Pearl pear' seeds treated at 4-8-12°C were the highest, with values of 76.67% and 45.83%, respectively, being observed, followed by those of seeds treated at 8°C (rooting and cotyledon expansion rates of 61.67% and 35.83%, respectively). The rooting rates of seeds treated at 12°C and 4°C were 28.94% and 22.37%, respectively, and cotyledon expansion rates at 12°C and 4°C were 26.29% and 20.65%, respectively.

Significant differences were observed in the growth of roots and aboveground parts of seedlings, and the weight of individual seedlings (Figure 3). The growth of seeds germinated at 12°C was significantly higher than that of seeds in other temperature treatments. The average root length was 4.27 cm, aboveground part length was 4.06 cm, and the average weight of an individual seedling was 193.06 mg; however, the seedlings were thin and weak. The growth of seeds treated at a variable temperature of 4-8-12°C was relatively low, the average root length was 3.03 cm, aboveground part length was 2.11 cm, the average weight of an individual seedling was 154.68 mg, and the seedlings were strong. The growth of seeds treated at a constant temperature of 4°C was the lowest, the average root length was 1.38 cm, aboveground part length was 0.71 cm, the weight of an individual seedling was 99.01 mg, and the seedlings were weak.

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Table 3: Growth characteristics of seeds treated at different temperatures after germination.

Temperature/℃	Rooting rate%	Cotyledon expansion rate%	Root length/cm	Aboveground part length/cm	Weight/mg
4	12.11±3.52e	7.51±1.54e	1.38±0.95d	0.71±0.12c	99.01±12.54d
8	54.26±11.26b	41.84±8.39b	2.87±0.46bc	1.94±0.25bc	115.39±16.35cd
12	25.91±3.51d	16.73±2.86d	4.27±0.68a	4.06±0.48a	193.06±34.12a
8-4-8	39.58±5.43c	21.08±3.94c	1.69±0.25cd	1.05±0.21c	134.61±19.84bc
4-8-12	75.27±9.85a	54.53±6.51a	3.03±0.51b	2.11±0.48b	154.68±21.57b

Note: Different lowercase letters within the same column indicate significant differences (P<0.05) among treatments.

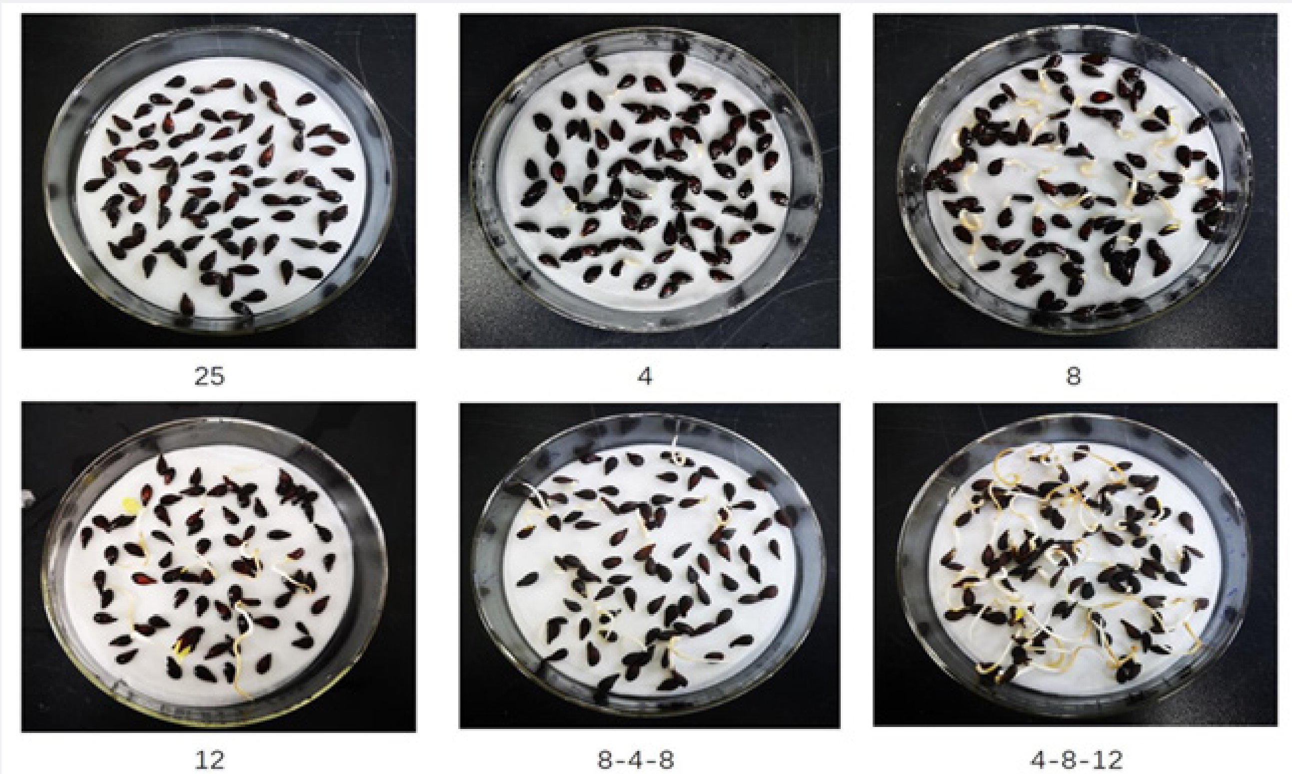

Figure 2: Germination of 'Pearl pear' seeds after exposure to different temperature treatments.

Discussion and Conclusion

Seed dormancy, which enables plants to avoid adverse climatic conditions and maintain plant populations through reproduction, is a common phenomenon in nature [15]. The release of seed dormancy is a complex process. Seeds can adjust their own material transformations by sensing subtle external environmental changes and then regulate the dormancy state to prepare them for germination under appropriate conditions [16,17]. Cell membrane permeability and metabolic enzyme activities in seed embryos increase after the seeds imbibe water, which is conducive to breaking dormancy and promoting germination. In this study, the weights of 'Pearl pear' seeds gradually became stable after

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12 h of water absorption. The water absorption rate of seeds reduced due to the seed coats that were less permeable to water, and seed weights remained unchanged thereafter [18]. Therefore, seeds should be soaked for 14 h in subsequent temperature and hormone tests to ensure maximum absorption of water by seeds, which is conducive to dormancy breaking and promotion of seedling germination at later stages.

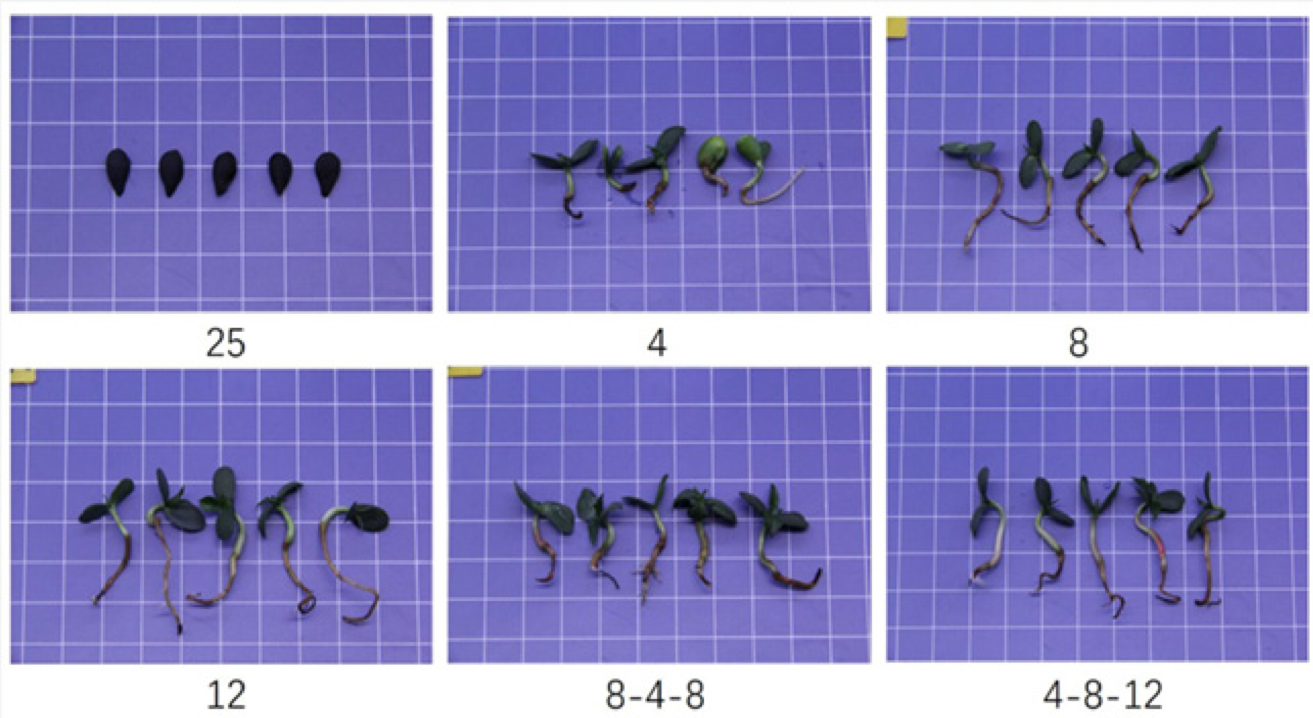

Figure 3: Growth characteristics of germinated 'Pearl pear' seeds after exposure to different temperatures.

Seed germination is an important process in plant ontogeny and the initial stage of the plant life cycle. Temperature, light, water, among other factors in the external environment, can affect seed dormancy and regulate seed germination [12,19]. Li et al. found that the germination rate of *Pyrus betulifolia* seeds was 80% after cold stratification at 4℃ for 30 days [20]. Wang et al. observed that subjecting early maturing sand pear cultivars, 'Cuiguan' and 'Cuiyu' seeds to a combination of low temperature and shelling treatment enhanced breaking of seed dormancy and improved the germination rate of the seeds [11]. In this study, we observed that the germination rate of seeds treated at 25℃ was 0%, suggesting that low temperature was a key factor influencing the breaking of 'Pearl pear' seed dormancy, which is consistent with the findings of a previous study on pears [21]. The germination rate of 'Pearl pear' seeds after exposure to different temperature treatments initially increased with an increase in germination time, then became stable, and reached a maximum rate on the 10th day.

The germination rates, potential, and indices of seeds treated at a variable temperature of 4-8-12℃ were the highest, whereas the average germination time was short. The effect of the 4-8-12℃ treatment on seed germination was greater than those of stable temperature treatments of 4, 8, and 12℃. The results suggested that a variable temperature treatment was more suitable for breaking seed dormancy and promoting seed germination than single temperature treatments. The variable temperature treatment had a substantial effect on dormancy release of late ripening and hard seeds, which is consistent with the findings of previous studies on temperature treatment effects on breaking the dormancy of *Carex spp, Schisandra chinensis*, and *Heracleum moellendorffii* [22-24]. The 4-8-12℃ treatment had a positive effect on seed germination and seedling growth after germination was robust. Seedlings emerged evenly, rooting and cotyledon expansion rates were high, and the survival rate of young seedlings after refinement and transplantation was 96% after 'Pearl pear' seeds were exposed to the 4-8-12℃ treatment. The results suggested that a variable temperature treatment was more suitable for promoting metabolism and conversion of storage substances in 'Pearl pear' seeds. The 4-8-12℃ treatment simulated the natural temperature changes occurring during seed germination, which enhanced enzyme activity in seed embryos, promoted various physiological and biochemical processes, facilitated the conversion of substances stored in seed embryos, and promoted embryo germination and growth [25-27]. In addition, variable

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temperature treatments accelerated the mechanical changes in seed embryos and increased seed membrane permeability, which promoted seed germination [28,29].

The 4°C stable temperature treatment had an insignificant effect on breaking the dormancy of 'Pearl pear' seeds. The germination rate of seeds treated at 4°C was low, the average germination time was long, and seedling germination was uneven. The 8°C stable temperature treatment had a moderate effect on the germination of 'Pearl pear' seeds, which was more suitable for breaking the dormancy of 'Pearl pear' seeds and promoting germination than that of the 4°C treatment. The observation may be because the climate of sand pear producing areas in southern China is mild and humid, and the temperature for breaking the dormancy of sand pear seeds under natural conditions is slightly higher than that in northern China. Therefore, the treatment time of 8°C can be appropriately extended in subsequent variable temperature treatments. The effect of the 8-4-8°C variable temperature treatment on seed germination and growth was lower than that of the 8°C stable temperature treatment, which could be because the low temperature treatment of 4°C prevented the conversion of storage substances in seed embryos and was not conducive to seed germination. The average germination time of 'Pearl pear' seeds treated at 12°C was the shortest and seedling growth was the highest; however, the germination rate of the seeds was low, seedling roots and stems were slender, and seedlings were weak. Furthermore, the survival rate of seedlings after seedling refinement and transplantation was lower than that of seedlings in other treatment groups. The effect of the 12°C treatment on breaking seed dormancy was insignificant, although the treatment promoted growth at later stages. Therefore, the duration of the 12°C treatment could be appropriately decreased during subsequent variable temperature treatments. The effects of the temperature combinations used in this study on promoting the breaking of 'Pearl pear' seed dormancy were in the order of 4-8-12°C > 8 °C > 8-4-8°C > 12°C > 4°C > 25°C.

Dormancy release and seed germination are closely associated with external environmental conditions and are the result of natural evolution over time [30]. Low temperature is a key factor that influences seed dormancy, affects changes associated with enzyme activities in seed embryos, affects water absorption by seeds, respiration, nutrient transformations and other metabolic activities, and ultimately affects seed germination and growth [31,32]. This study investigated the effects of different temperature treatments on breaking the dormancy of 'Pearl pear' seeds and subsequent seedling growth. The results revealed that a variable temperature treatment of 4-8-12°C was effective for breaking the dormancy of 'Pearl pear' seeds, thereby providing a reliable and practical method of breeding new pear varieties. However, further studies should be conducted to determine nutrient and hormonal changes in seed embryos during the breaking of seed dormancy by fluctuating temperatures, and to elucidate the physiological processes and molecular mechanisms associated with seed dormancy breaking [33,34].

Acknowledgments

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Conflict of interest

The authors declare that they have no conflict of interest.

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