

NEW METHOD FOR BREAKING KOREAN PINE SEED DORMANCY

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Abstract. *Pinus koraiensis* (Korean pine) seeds possess deep dormancy caused by mechanical barrier and embryo immaturity. It usually takes 9-12 months of cold stratification to break the deep dormancy which is a main problem in nursery practice. This study was initiated to find an effective method to shorten the duration of stratification and to hasten seed germination. Thirty-one treatments were examined, focusing on the stratification period. Increased temperature in the pre-soaking process reduced the duration of required stratification at the low temperature and increased the rate of germination. The best method for breaking seed dormancy was pre-soaking seeds at a starting water temperature of 70°C, then at room temperature for 6 days, followed by 3 months of stratification at a constant temperature of 3°C.

Additional Index Words: *Pinus koraiensis*, stratification, germination

Korean pine (*Pinus koraiensis*) is an important land regeneration and timber species, ranging through Korea and Northeast China into South-eastern Siberia, with outliers on the Japanese islands of Honshu and Shikoku (8). Numerous studies (4,5,6,7,10,14,15,16,17,18) indicated that Korean pine seed possess very deep dormancy caused by both a thick seed coat and an immature embryo, which slow down a series of physiological and biochemical activities (2,3,6,9,12,18). In nursery practice, cold stratification has been recognized as an essential method to break the seed dormancy. However, the duration of stratification is usually 9-12 months, which is a major problem in promoting seed germination (10,12,14,17). Following this 9-12 months stratification, the typical germination rate in nursery is 70-85% for freshly harvested seeds and usually lower for stored seeds because of the decrease in viability. Attempts to reduce the lengthy stratification requirement have not been so successful (2, 3, 5, 9,15). More effort is needed to find an effective method of shortening the stratification duration and hastening seed germination of Korean pine.

The first step of seed germination is imbibition of water and the amount of water imbibed by seed is greatly affected by soaking temperature and duration. In the expression of amount of water taken up in seed, calculation on basis of dry matter is the only safe method when describing imbibing processes in comparative experiments (1). This research compared 1) effects of temperature and duration on uptake of water in the seeds during pre-soaking processes, and 2) germinative responses to seed treatments based on the stratification of various levels of pre-soaking temperature and duration. The aim was to find the best method of breaking dormancy and hastening seed germination.

Literature on Korean pine shows that the most extensive work on hastening seed germination was done by Asakawa (2,3,4,5,6,7). He soaked seeds in tap water at room temperature for 48 hours and then focused on different stratification temperatures and durations. His recommendation for hastening germination is: the compound stratification of 25°C for 2 months and then 2°C for 3 months (5). However, the 2-month stratification at 25°C increases the chance of seed deterioration. By this method, his best germination in laboratory was 65.6% based on 200 seeds. No previous work was found which used the high temperature pre-soaking treatment plus low temperature stratification to treat Korean pine seeds. Indeed, in most publications, little attention was paid to pre-soaking temperature, although the pre-soaking process has always been used.

Materials and Methods

Stored Korean pine seeds were supplied by Heilongjiang Provincial Seed Bank of Northeast China, and shipped to the United States via air mail in Aug. 1988. The experiments were carried

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out from fall of 1988 to spring of 1991. The seed had an average moisture content of 12% on an oven-dry weight basis. To maintain uniformity, seeds were first selected based on size and the smaller ones were discarded. Then they were floated in water and the floaters were discarded.

Seed Moisture Analysis. Three 500 ml beakers were each filled with 300 ml of distilled water: one was kept at room temperature (25°C), one was heated to 45°C, and one was heated to 70°C. One batch of 100 dormant seeds was dropped in each beaker. Contents of the hot beakers were stirred by a glass rod until the water cooled to room temperature. The seeds in all three beakers were then soaked at room temperature for nine consecutive days. The water was changed daily. Ten seeds were randomly picked daily from each beaker for moisture test. Each seed was blotted with a filter paper and de-coated carefully by a nutcracker. The fresh weight of the embryo with endosperm and seed coat was measured separately. The oven-dry weight of each part was determined following drying for 48 hours at 80°C. Moisture content was expressed as a percentage of oven-dry weight. Moisture contents of the seed coat and the embryo with endosperm were calculated for each selected seed. A complete factorial design analysis of variance with the 3 soaking temperatures and 9 durations (1, 2, ..., 9 days) was performed for moisture content of each part of the seeds. Mean separation was accomplished with Duncan's multiple-range test at the 5% level. The moisture contents of 50 air dry seeds were analyzed as control.

Seed Treatments. A total of 31 treatments, based on various pre-soaking and stratifying conditions, were designed for breaking seed dormancy. They were included into the following three categories:

1. Control (air dry seeds, no pre-soak/no stratification).
2. Alternating temperature stratification (18 treatments). Prior to stratification, nine pre-soaking treatments were applied to seeds based on:
 - a. Pre-soaking durations: 3, 6, 9 days.
 - b. Pre-soaking temperature regimes: 25°C room temperature throughout; 45°C beginning temperature and then cooled to the room temperature;

70°C beginning temperature and then cooled to the room temperature.

Each treatment contained 600 seeds. Following treatment, one half of the treated group was immediately tested for germinative response. The other half was stratified at alternating 20°C-10°C day-night temperatures and 12-hour photoperiod for 50 days before germination test. T-tests were performed for germination percentage between stratified and unstratified groups. Treatment of no pre-soak/alternating temperature stratification was omitted as a result of overall low germination rates observed from the experiments in categories 1 and 2.

3. Constant low temperature stratification (16 treatments). Prior to stratification, the seeds were treated with four pre-soaking treatments (no pre-soak, pre-soak at 25°C, pre-soak at 45°C beginning temperature, and pre-soak at 70°C beginning temperature, where the pre-soaking period was 6 days for all treatments). Each treatment included 1200 seeds, which were divided into four lots with 300 seeds per lot. Each lot was assigned randomly to one of four low temperature stratification durations (0, 3, 5, and 7 months at 3°C). Germination percentage was analyzed by a complete factorial design analysis of variance with 4 pre-soaking treatments and 4 stratification durations, and mean separation was accomplished with Duncan's multiple-range test at the 5% level. It should be noted that 4 treatments from categories 1 and 2 (no pre-soak/no stratification, pre-soak at 25°C for 6 days/no stratification, pre-soak at 45°C for 6 days/no stratification, and pre-soak at 70°C for 6 days/no stratification) were repeatedly used here in order to have a complete experimental design.

Germination Test. Germination tests of all treatments were conducted in an incubator at 30°C-20°C day-night temperatures and 12 hour photoperiod for 60 days. Each treatment was replicated three times with 100 seeds per replication. Seeds were considered germinated when the radicle emerged to 1 cm. Daily and total germination were recorded.

Results

Moisture Content of Embryo with Endosperm. The moisture uptake by the embryo with en-

dosperm was significantly affected by pre-soaking temperature and duration (Figure 1). In unsoaked seeds, the embryo with endosperm had an average moisture content of 4.6%. After pre-soaking for 24 hours, the moisture content increased to 18% for 25°C, 33% for 45°C, and 37% for 70°C. The higher the pre-soaking temperature, the greater the moisture uptake, and the earlier the embryo with endosperm was saturated (Figure 1).

Moisture Content of Seed Coats. In unsoaked seeds, the moisture content of seed coats averaged about 17%. After pre-soaking for 24 hours it increased to 44% for 25°C, 43% for 45°C, and 46% for 70°C (Figure 2). The continued soaking periods (2, 3, ... 9 days) did not significantly increase the moisture content; the range of moisture contents of seed coats was from 43% to 50% regardless of the individual treatments (Figure 2).

Germinative Responses to the Various Treatments. Germination of untreated seeds was extremely low, less than 4% during the 60-day germination test. The earliest germination occurred on the 43rd day (Figure 3, Control treatment). Pre-soaking treatments increased germination up to 21%, and pre-soak plus alternating temperature stratification was better than pre-soaking treatments alone (Table 1). However, the highest germination rate was only 32%. Because of the low germinative responses to control treatment

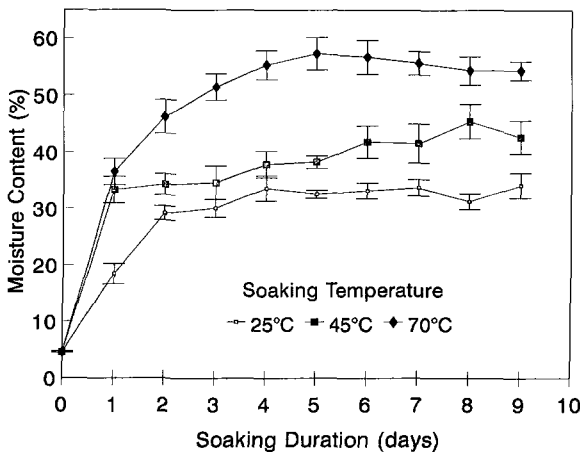


Figure 1. Moisture content (%) of embryo with endosperms in Korean pine seeds, by pre-soaking duration at three temperature regimes (vertical bars are 1 standard errors).

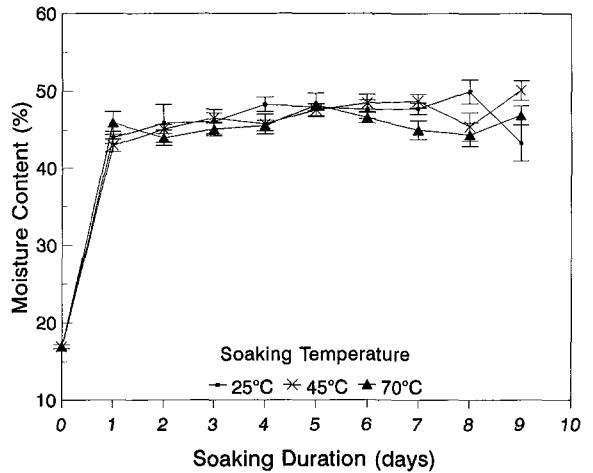


Figure 2. Moisture content (%) of seed coats in Korean pine seeds, by pre-soaking duration at three temperature regimes.

(category 1) and to all treatments in Table 1 (category 2), the test of no pre-soak/alternating temperature stratification treatment was omitted technically.

Seed germination was significantly affected by pre-soaking treatments, cold stratification durations, and their interactions (Table 2). Germination of unsoaked seeds, as well as of the seeds pre-soaked at 25°C, increased significantly with increasing duration of cold stratification. Germination of the seeds pre-soaked at 45°C and 70°C

Table 1. T-test results for total germination (%) between alternating temperature stratified and unstratified Korean pine seeds under 9 pre-soaking treatments.

Pre-soaking T°C & Days	Germination(%) Stratified	Germination(%) Unstratified	t-value	Two-tailed prob.
25 ^o 3	14.3	8.0	-4.36	0.012*
25 ^o 6	13.3	7.3	-5.45	0.006**
25 ^o 9	9.0	5.3	-1.68	0.169
45 ^o 3	11.3	8.6	-1.21	0.294
45 ^o 6	12.3	8.7	-1.08	0.341
45 ^o 9	16.0	9.3	-2.77	0.050*
70 ^o 3	31.7	21.3	-2.01	0.115
70 ^o 6	26.0	16.7	-1.88	0.134
70 ^o 9	23.3	5.3	-3.27	0.031*
Combined all	17.5	10.1	-3.72	0.000**

** Indicated significant difference at alpha=0.01 level;
* Indicated significant difference at alpha=0.05 level;

Table 2. Germination (%) of Korean pine seed by 4 pre-soaking treatments and 4 durations of the constant low temperature stratification.

Pre-soaking treatment	Mean total germination(%)			
	Low temperature stratification duration (months)			
	0	3	5	7
No pre-soak	3.3a ¹	15.7b	26.0bc	27.7c
Pre-soak 25°C	7.3a	19.0bc	28.7c	48.7d
Pre-soak 45°C	8.7a	60.0de	62.0e	62.7e
Pre-soak 70°C	16.7b	60.0de	52.7de	48.7d

1 Within the table, means sharing a common letter are not significantly different at the 5 percent level according to Duncan's multiple range test.

regimes did not increase significantly with the stratification duration from 3 to 7 months (Table 2). Maximum germination rates (49%-63%) were obtained by pre-soaking seeds at 45°C or 70°C and stratifying for at least 3 months, or by pre-soaking at 25°C and stratifying for at least 7 months (Table 2). Increased temperature in the pre-soaking process reduced the duration of required cold stratification and speeded up seed germination in *Pinus koraiensis*. Based on the speed and total percentage of germination (Figure 3), the best germinative response was obtained by the treatment of pre-soaking seeds at 70°C plus 3-month cold stratification.

Discussion

Proteinaceous and fatty seeds demonstrate a rapid imbibitional water uptake, which is temperature dependent (1). Some pine species contain as much as 50% lipid, mainly in the endosperm (megagametophyte) (11). Korean pine seeds are rich in fat: on the oven dry weight basis, the endosperms of unstratified Korean pine seeds contain 66.7% of oily substances (soluble in petroleum ether) and the embryos have 39.2% of oily substances (7). During the pre-soaking process, the temperatures differed only on the first day of soaking (then maintained at room temperature), but their impacts on water uptake in the seeds were significantly different (Figure 1).

Seeds could hardly germinate without any treatment (Figure 3, control treatment). Pre-soaking too long at the high temperature had

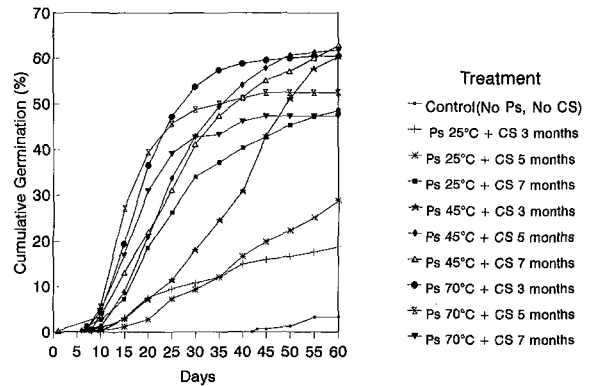


Figure 3. Germination time-course plots of Korean pine seeds of selected treatments, under the constant low temperature stratification (Ps, pre-soak; CS, cold stratification).

negative effects, causing a decline in germination (Table 1) and increasing seed deterioration. The average seed decay rate was about 15% in the two large experiments (categories 2 and 3), and the highest decay rates (23-26%) were found under the treatments of pre-soak for 9 days at the higher temperature. Germination was 60% when seeds were pre-soaked at 45°C or 70°C for 6 days and stratified for 3 months at the low temperature. Extended cold stratification (5 and 7 months) did not improve significantly the germination (Table 2 and Figure 3). After the 3-month cold stratification, germination speed in 70°C pre-soaking treatment was much greater than that of 45°C pre-soaking treatment (Figure 3).

The high temperature pre-soaking process increased imbibition by seeds and softened the hard seed coats. Then, seeds underwent an “after-ripening” process through the low temperature stratification, during which physiological and biochemical changes occurred (3,4,7,9,12,13,14,17,18), and the embryo continued its development and seed dormancy was broken. Much evidence shows that the embryos of Korean pine seeds enlarged during after-ripening (2,3,6,12). This study suggests using the following method to break Korean pine seed dormancy: pre-soak seeds beginning with 70°C water and remaining at room temperature after cooling for a period of 6 days, and then apply low temperature stratification at 3°C for 3 months. The seeds will germinate readily

by this method.

Acknowledgments. We thank Dr. C. A. Blanche, Southern University and A & M College, for reviewing the manuscript and his valuable comments. We appreciate partial financial support from College of Forestry, Stephen F. Austin State University.

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Résumé. Les semences du pin de Corée (*Pinus koraiensis*) possèdent une dormance triple causée par une barrière mécanique et physiologique et par une immaturité biochimique. Celle-ci requiert de 9 à 14 mois pour être brisée par une stratification à froid. Cette recherche était initiée afin de découvrir une méthode effective qui raccourcirait la durée de la stratification et hâterait la germination de la semence. L'accroissement de température lors du processus de prétrempage réduisait la durée de stratification requise à basse température. La meilleure méthode pour briser la dormance a été de tremper les semences dans de l'eau à 70° C et, par après, dans de l'eau refroidit à température de la pièce pour une durée de six jours avant une période de stratification de trois mois à une température constante de 3° C.

Zusammenfassung. Die Samen der Koreanischen Fichte (*Pinus koraiensis*) verfügen über eine dreifach bestimmte Ruhephase, die durch eine mechanische Schranke und physiologische und biochemische Unreife verursacht wird. Normalerweise wird eine Kälteperiode von 9-14 Monate benötigt, um die tiefe Ruhephase zu beenden. Diese Studie wurde eingeleitet, um eine wirkungsvolle Methode zu finden, die die Dauer der Kälteperiode verkürzt und um die Samenkeimung zu beschleunigen. Erhöhte Temperatur während des Vortränkungsverfahrens verminderte die Dauer der benötigten Kälteperiode. Es stellte sich heraus, daß die beste Methode zur Unterbrechung der Ruhephasen die Tränkung der Samen mit einer anfänglichen Wassertemperatur von 70°C ist. Anschließend erfolgt bei Zimmertemperatur eine 6tägige Abkühlung und darauffolgend eine dreimonatige Lagerung bei einer konstanten Temperatur von 3°C.