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Viability Determination of *Pinus rigida* Seeds Using Artificially Accelerated Aging

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노화처리를 이용한 리기다소나무 종자의 활력 평가

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ABSTRACT

We tested the seed viability of *Pinus rigida* using accelerated aging to discover optimum times and temperatures for artificially accelerated aging. Seeds were artificially aged at different temperatures and during different times. The seed viability was affected by the accelerated aging and by temperature with a decline in germination and seed vigor. The aging index of *P. rigida* seed was 0.31 at 35°C and seed viability was nearly lost after aging treatment at 40°C for 15 days. The optimum temperature of *P. rigida* for the aging test was decided to be approximately 37°C on the basis of the aging index. Inorganic materials and conductivity of leaching solution from aging seeds increased with the increase of aging period. The accelerated aging test was considered to be a suitable method to evaluate the seed viability of tree species. Because seed characters are much different among tree species, however, more studies need to be done to discover the optimum conditions for aging by tree species.

Key words **: Seed germination, Aging index, Inorganic material, Conductivity**

I. INTRODUCTION

Seed germination tests are usually conducted as part of seed-quality testing. The standard germination test is based on estimating the maximal potential for seed viability, or the ability of a seed to produce a normal plant under favorable conditions, so it is not adequate for assessing field emergence (McDonald, 1980). Therefore a vigor test based on stress conditions is more appropriate for testing seed emergence since it implies the ability of the seed to germinate under both favorable and unfavorable conditions (Kneebone, 1976).

Those tests that have been evaluated for tree seeds can be grouped into four types: seedling growth tests, stress tests, biochemical tests, and germination rate (Bonner, 1998). Among these tests, vigor evaluation by stress tests requires seed samples to be germinated either under stress conditions or under the standard germination test following a separate stress treatment (AOSA, 1983). The accelerated aging (AA) test is the one stress test that has received extensive testing among tree seeds. Initially this test was developed to evaluate storage potential of seed lots (Delouche and Baskin, 1973), and it has evolved into an indicator of seed vigor in many agricultural crops. Recently, the AA technique which has utility as a seed vigour test of agronomic crops has attracted the attention of tree seed researches as a means for evaluating the efficacy of *ex-situ* genetic resources

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conservation method (Chaisurisri *et al.*, 1993; El-Kasaby and Edwards, 1998).

In AA test, tree seeds were exposed on high temperature and high relative humidity, factors that can cause rapid seed deterioration, and seed vigor is measured by subsequent germination testing. The differences in germination before and after aging provide a relative measure of seed vigor, but simple percentage differences lack the precision required for a real quantitative test (Hampton and TeKrony, 1995). For this reason Wang *et al.* (1992) proposed the use an "Index of Aging" (AI) with lodgepole pine (*P. contorta* var. *latifolia*). The need for precise control of the test environment and procedures makes the accelerated aging test more difficult than it may seem, but the basic concept of the test has been attractive to tree seed researchers.

Most AA studies with tree seeds have focused on the finding the optimum times and temperatures for aging, which have been reported a number of species (Downie and Wang, 1992; Thapliyal and Connor, 1997). However many tree species that have been tested had inconclusive results regarding AA conditions (Chaisurisri *et al.*, 1993; Bonner, 1998), In special, there was few information about AA conditions of tree species in Korea.

Therefore we investigated the decrease of seed vigor with the increase of storage period using the accelerated aging test in order to decide optimum conditions and length during the storage of tree seeds.

II. MATERIALS AND METHODS

2.1. Seed materials

Pinus rigida was the representative silviculture tree species in Korea. Recently, most forest stand of *P. rigida* are required regeneration. Their seeds, like other pines, are easy to store and suitable for the research of seed physiology during germination due to short-term germination period. In general, *P. rigida* seed has about 136 seeds per gram and the possible storage period is 11 years. Germination standards of *P. rigida* seed is 28 days at 20-30°C. Seeds require light condition more than 8 hrs/day, prechilling for 21 days at 3-5°C, and prefers good moisture (Ellis *et al.*, 1985; ASOA, 1985).

Dry seeds of *P. rigida* harvested from the seed orchard of Forest Seed Research Institute, Republic of Korea in October, 2003 represented a moisture content of 5.1% (on dry weight basis). Before experiment, all seeds were soaked in 1% sodium hypochlorite solution for 10 min.

2.2. Accelerated Aging

Preliminary investigation of seeds on accelerated aging effects was conducted at different temperatures (4, 10, 20, 30, 40°C) and 80% relative humidity (RH) for 35 days. The experiments on accelerated aging were performed by incubating the 100 seeds in a closed plastic box. Rapidly-aged seeds were sampled at 3-day intervals, then air-dried for 24 hours at 25°C in order to restore the initial weight. Seed incubated at 20°C and 40% RH were used as control (non aged seed).

Secondary accelerated aging test based on the result of preliminary investigation was applied at 35°C for 4 aging periods from 0 to 12 days at 3-day intervals. Each test consisted of 10 samples of 100 seeds. Each sample was placed separately on a wire-mesh screen in a tightly-closed incubation box containing 50 mL of water. The distance between the seeds and the water surface was 1.4 cm. Samples were obtained in succession so that the samples for the longest accelerated-aging treatment (12 days) were placed in the incubator first and the shortest-aged samples (3 days) were the last. At the end of the test, all samples were removed and 3 samples from each accelerated aging treatment were used for moisture content determination. The remaining 7 samples were kept at room temperature overnight to reduce the effect of rapid temperature change on seed germination, as recommended by Bourland and Ibrahim (1982).

2.3. Germination test

All germination tests were conduced under ISTA rules (International Seed Testing Association, 1985). Three replications of 100 seeds were placed in presoaked germination paper and then were placed in a seed germinator at 25°C for 10 days. All experiments were based on completely random designs with three replicates. Seeds were recorded as germinated when the radicles were visible on the surface of germination paper.

Percent germination (PG%) was calculated as the proportion of germinants of the total number of viable seeds at 10 days. Seed vigor was represented as aging index (AI) which is defined as initial germination % minus germination % after aging, divided by the initial germination (Wang *et al.*, 1992).

2.4. Conductivity and inorganics

To analyze inorganics and to measure conductivity, the 0.1g of the non-aged and aged seeds were soaked into 50 mL of triple-distilled water for 24 hours, and were

put into the incubator at 25°C. In this analysis, 50 mL of extracted sample solution was filtered. Conductivity tests were measured with seeds of three replications (CONSORT Model C533, Belgium). Results were recorded in microSiemens (μS). K, Na, and Fe in the sample collected (leaked material) were analyzed using atomic absorption spectrophotometer (SHIMADZU AA-6701F, Japan).

III. RESULTS AND DISCUSSION

3.1. Germination characteristics

Time and temperature of accelerated aging treatment affected the seed viability (Fig. 1). The reduction of seed germination was started at temperature above 20°C, decreased up to 20% at 30°C after 25 days of aging treatment, and germination was almost stopped up to 2% at 40°C after 15 days of aging treatment. The aging index (AI) after 15-day accelerated aging treatment was calculated. AI was 0.14 at 30°C aging temperature but sharply increased up to 0.98 at 40°C aging temperature.

The research of tree seed aging was focused on finding the optimum temperatures and times (Bonner, 1998). It was difficult to find out the detailed physiological characteristics with fast aging rate at 40°C accelerated aging treatment. For slow aging rate, it took too long time for experiment (El-Kassaby and Edwards, 1998).

The second experiment was conducted to find out optimum aging conditions. After 12 days aging treatment, germination rate was 66.0% at 35°C and AI was 0.31 (Fig. 2). Based on 50% AI, optimum aging temperature of *P. rigida* seed was approximately 37°C. Other research groups reported the similar condition of 37.5°C as optimum aging temperature (Chaisurisri *et al.*, 1993;

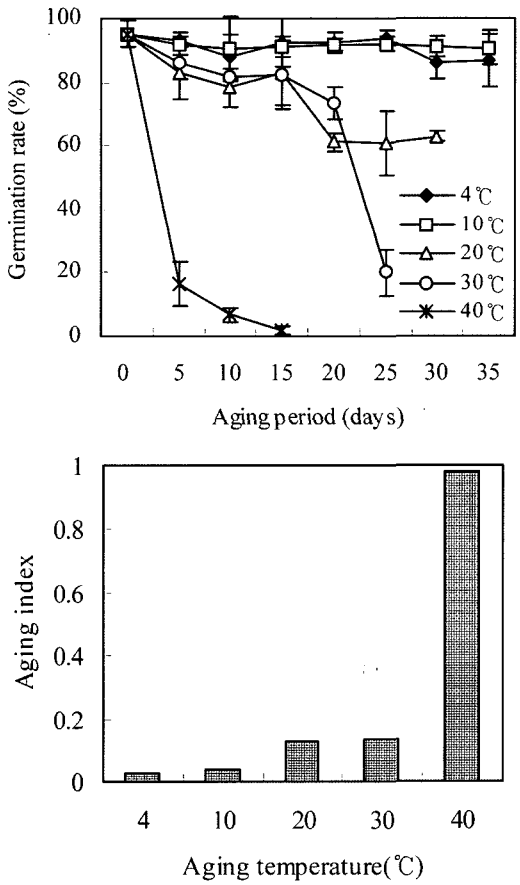

Fig. 1. Changes in germination rate (left) and aging index (right) of *P. rigida* seeds after first accelerated aging treatments at different temperatures. Each point represents means ± s.d. of three replications of 100 seeds.

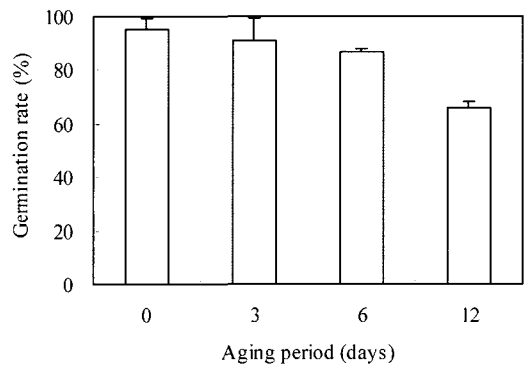

Fig. 2. Changes in germination rate of *P. rigida* seeds after accelerated aging treatments at 35°C. Each point represents means ± s.d. of three replications of 100 seeds.

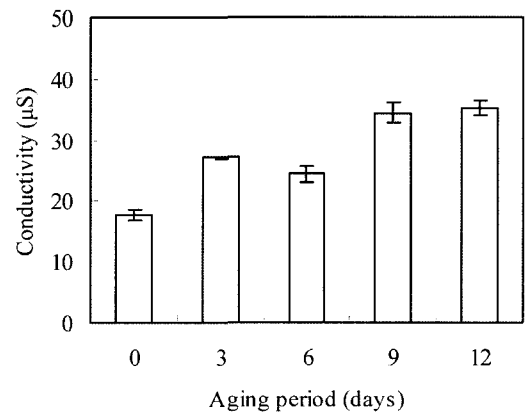

Fig. 3. Changes in conductivity of electrolyte leakage from *P. rigida* seeds after accelerated aging treatments at 35°C. Each point represents means ± s.d. of three replications.

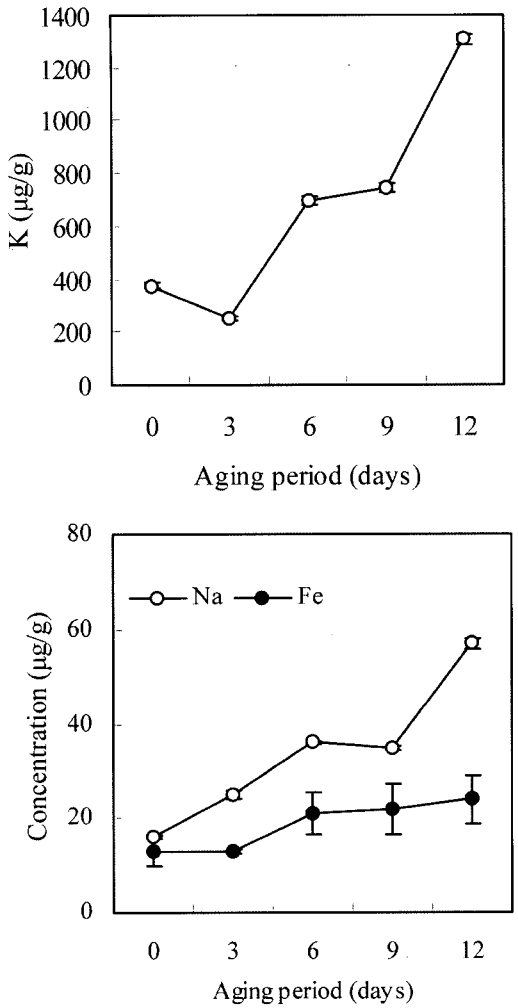

Fig. 4. Changes in the contents of inorganic components in the leachates from *P. rigida* seeds after accelerated aging treatments at 35°C. Each point represents means±s.d. of three replications.

El-Kassaby and Edwards, 1998)

3.2. Conductivity and inorganics

The conductivity changes coming from electrolyte leakage of *P. rigida* seed depending on the aging length was shown in Fig. 3. Conductivity in control was 17.8 μS, but it increased up to double (35.2 μS) in conductivity of 12-day treated seed with increasing conductivity by extended aging length. Because more inorganics were leached out from seed, the conductivity of leaching solution was increased with the extended aging treatment time.

Fig. 4 showed the content of aging treated seed's leachate. With increasing the aging time, inorganic contents of K^+, Na^+ and Fe^{2+} were increased. Potassium ion was the highest amount of leached out ion as 376 μg/g for the non-aged and 3 times more in 12-day aged seed than that of control. With 12-day aging treatment, sodium ion content was tripled and double for Fe compared with those of control.

When the seed absorbs water, they are exposed to the high pressure, which cause the cell membrane damage. In case of high activity seeds, they are recovered from the damage quickly. However in the low activity seeds, they cannot recovered from damage and the water-soluble components in cell was leached out. Leachate components includes phenolics, sugars, organic acids, ions, and protein (Simon and Mills, 1983). Those components are leached out from both living and dead seeds, but leached out more dead ones (Powell and Matthews, 1981). This result may come from the inappropriate arrangement of cell membrane in initial stage of water absorption, not function in cell membrane, or damaged cell wall or seed skin (Powell and Matthews, 1979).

Tree seed aging treatment can use to evaluate the stored seed activity because aging treatment can provide the information about the seed's biochemicals and germination properties. However, the extended researches are needed for the each species' optimum conditions because tree species has very different seed property.

적 요

노화 처리를 이용하여 리기다소나무 종자의 활력을 평가하고, 적정 노화 처리 기간 및 온도를 결정하고자 하였다. 리기다소나무 종자의 활력은 노화 처리 기간과 온도에 큰 영향을 받았으며, 노화 처리가 진행되는 동안 종자 발아율과 종자 활력은 크게 감소하였다. 리기다소나무 종자의 노화 지수는 35°C에서 0.31이었으며, 40°C에서 15일 동안 노화 처리한 종자는 활력을 완전히 상실하였다. 노화 지수를 기준으로 한, 리기다소나무 종자의 노화 처리 최적 온도는 대략 37°C 부근으로 판단되었다. 노화 처리된 종자로부터 빠져나온 용출액을 분석한 결과, 용출액내 무기물과 전기전도도는 노화 기간이 증가함에 따라 증가하였다. 노화 처리 시험은 수목 종자의 활력을 평가하는데 매우 적절한 방법으로 판단되었으나, 수종에 따라 종자 특성이 매우 다르므로 다양한 수종에 대해 노화 처리의 최적 조건을 찾기 위해서 더 많은 연구가 필요하다.

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