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Seed Conservation Methods According to the Prediction of Suitable Distribution of Endangered Conifer *Abies nephrolepis* Maxim.

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Abstract: This study predicted habitat distribution changes according to the current distribu seedtion 14 and future climate using the MaxEnt model for endangered *Abies nephrolepis* Maxim., which is vulnerable to 15 climate change and is a least-concerned species. This study aimed to predict the current distribution and future habitat distribution changes of the endangered *A. nephrolepis* under climate change using the MaxEnt model. The purpose was to predict the future habitat of the declining *A. nephrolepis*, to identify the necessity of in situ conservation, and to devise appropriate ex situ seed storage methods. The study utilized climate data from 513 GPS coordinates of *A. nephrolepis* habitats in South Korea to predict the changes in habitat distribution using the MaxEnt model. The seeds used in the seed experiment were collected from Pyeongchang-gun, Gangwon-do, Republic of Korea in 2019. After confirming an initial seed filling, germination tests were performed under constant temperatures of 15, 20, and 25 °C and alternating day/night temperatures of 25/5, 25/10, 25/10, 20/15, 25/15, 30/15, and 35/15 °C. The seed germination conditions were investigated under 10 different temperature settings. For the determination of storage behavior, seeds were dried at a consistent temperature of 15 °C and relative humidity (eRH) levels of 15, 20, 30, 40, and 50%. Subsequently, the seeds were stored for three months at temperatures of −20 °C and 5 °C, and the vitality tests of the seeds were conducted. Based on these experiments, the storage characteristics of seeds were identified. The results indicated that in all SSP scenarios, it is predicted that *A. nephrolepis* will become extinct in its habitat by the 2090s. Therefore, it has been shown that on-site and ex situ conservation is necessary. As a result of the seed germination characteristics, the highest germination rate (52.5 ± 16.01%) was achieved at a constant temperature of 20 °C, followed by 25 °C (50.0 ± 10.81%) and 25/10 °C (47.5 ± 4.79%). The highest viability was obtained under 20% eRH (64.0 ± 0%) but was not statistically different from that determined immediately after seed collection. The moisture content was approximately 4.33% fresh weight under 15% eRH at 15 °C. *A. nephrolepis* seeds are classified as orthodox-type seeds, which do not lose viability at 3%–7% moisture content and after drying under 15% eRH conditions at 15 °C. In conclusion, it can be observed that the seeds can be stored long-term at −20 °C. This research was conducted as a basic study to predict the habitat distribution of the endangered species *A. nephrolepis* and to establish seed conservation methods. According to the results, it is deemed necessary to conduct both domestic and international analyses of the habitat of *A. nephrolepis*. In addition, the germination and storage characteristics of *A. nephrolepis* seeds were confirmed, and based on this, effective seed conservation methods were suggested.

Keywords: *Abies nephrolepis*; germination characteristic; MaxEnt; orthodox; storage behavior

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1. Introduction

The changes in temperature and precipitation are not occurring uniformly over the earth. Temperatures are increasing faster/more at high than at low elevations, in winter than in summer, and at night than during the day [1,2]. The extent of winter snow cover at high latitudes is decreasing, and with increased temperatures, snowmelt is occurring earlier in the spring than in the past, resulting in an increase in the length of the growing season.

While conifer defoliation is occurring worldwide due to temperature rise and drought [3], sub-alpine tree species are also affected by abnormal weather conditions, such as extreme drought and abnormally high temperatures and climate change, including global warming [4].

Approximately 50 species of *Abies* are distributed in the north temperate zone [5,6]. Among them, *A. nephrolepis* is listed as a species of least concern in the International Union for Conservation of Nature Red List [7] and has been designated as a climate change biomarker because it is expected to be vulnerable to climate change [8].

On the Korean Peninsula, if the temperature rises due to global warming by climate change, the distribution of evergreen broadleaf forests in the southern coast of Korea expands, and ecological side effects, such as the decline or extinction of vegetation in alpine and subalpine regions, may occur [9,10]. *A. nephrolepis* is an endangered alpine coniferous species that must be conserved both in situ and ex situ worldwide.

Accordingly, it is necessary to develop measures for the conservation of *A. nephrolepis*. Currently, the Korea Forest Service has been implementing conservation policies by selecting seven species of alpine conifers (*Abies nephrolepis*; *Abies koreana*; *Pinus pumila*; *Juniperus chinensis*; *Thuja koraiensis*; and *Taxus cuspidata*) as intensive conservation targets since 2016. It identifies and monitors distribution and damage through a nationwide survey and implements conservation and restoration measures to lay the foundation for restoration, such as creating an ex situ conservation center and evaluating genetic diversity.

In the four scenarios, due to rising temperature as a result of the approaching climate change, it was predicted that the habitat decrease will start from the 2030s, that the habitat will decrease sharply from the 2030s depending on the scenario, and that the species will be completely extinct in the 2090s in all scenarios. *A. nephrolepis* is an endangered species (LC: Least Concern) and has been identified as a climate change biomarker due to the influence of various climatic factors, such as annual average temperature.

A number of studies using distribution models have been conducted to predict the distribution of habitats according to climate change. Park et al. [11] evaluated the environmental characteristics and sensitivity of the distribution area of *Abies koreana* and *A. nephlolepis* to climate change, and Yoo et al. [12] reported the specificity of the Korean forest and predicted changes in the habitat distribution according to the current climate change of *A. nephrolepis*.

The MaxEnt model, which is based on the theory of maximum entropy, is an effective model of species distribution. The model simulates the potential geographical distribution of a species using information on its current (present-day) distribution as well as various environmental data [13,14].

Common practices implemented in the management of species with conservation importance include seed collection, banking, and propagation [15–17]. Species-specific knowledge of seed germination characteristics and storage behavior is essential for successful ex situ species conservation and in situ reintroduction and restoration projects.

Seeds age during storage, resulting in a decline in quality and ultimately loss of viability under improper storage conditions [18]. The practical categorization proposed by Roberts [19] and then supplemented by Ellis [20] considers three categories of seeds based on desiccation sensitivity: orthodox (seeds tolerate desiccation below 5% moisture content on a fresh weight basis), recalcitrant (seeds do not tolerate desiccation), and intermediate (seeds exhibit characteristics of the first two categories; i.e., they tolerate moderate desiccation but lose viability during subsequent storage at subzero temperatures).

The stability of *A. nephrolepis*'s current habitats on mountaintops is threatened by the changing climate conditions by global warming, and the species faces an uncertain future in its natural habitats in Korea. Given the significant commercial role and potential threat to the natural habitats of *A. nephrolepis*, the maintenance of genetic variation (i.e., gene conservation of the species) is an urgent task.

The specific objectives of this study were as follows: (1) to predict the potential suitable distribution of *A. nephrolepis* under different climate change scenarios in different time periods and (2) to determine seed germination characteristics and storage behavior for confirming conservation methods.

Based on this study, it is planned to establish management and conservation methods for *A. nephrolepis*. This will serve as basic data for the restoration and conservation of endangered conifers on the Korean Peninsula by predicting habitat changes due to the upcoming climate change.

2. Materials and Methods

2.1. Species Occurrence Data

The location data of the appearance of *A. nephrolepis* was used through the data collected from the direct field survey. The field survey was conducted randomly across the country, and the GPS coordinates of a total of 513 points were recorded and analyzed.

2.2. Climate Variable

The climate variable data used in this study were downloaded from the WorldClim-Global Climate Data website (Table 1) [21]. The data include 19 climate variables, which were derived from the monthly temperature and precipitation values to generate more biologically meaningful data that reflect a range of temperature and precipitation summaries (e.g., trends, seasonality, and extremes) [22].

Table 1. List of bioclimatic variables used in the model development.

Index	Description	Unit
bio01	Annual Mean Temperature	°C
bio02	Annual Mean Diurnal Range	°C
bio03	Isothermality	%
bio04	Temperature Seasonality	°C
bio04a	Temperature Seasonality	%
bio05	Max Temperature of Warmest Month	°C
bio06	Min Temperature of Coldest Month	°C
bio07	Annual Temperature Range	°C
bio08	Mean Temperature of Wettest Quarter	°C
bio09	Mean Temperature of Driest Quarter	°C
bio10	Mean Temperature of Warmest Quarter	°C
bio11	Mean Temperature of Coldest Quarter	°C
bio12	Annual Precipitation	mm
bio13	Precipitation of Wettest Month	mm
bio14	Precipitation of Driest Month	mm
bio15	Precipitation Seasonality (CV)	%
bio16	Precipitation of Wettest Quarter	mm
bio17	Precipitation of Driest Quarter	mm
bio18	Precipitation of Warmest Quarter	mm
bio19	Precipitation of Coldest Quarter	mm

In this study, global Shared Socioeconomic Pathway (SSP) scenario data provided through Coupled Model Intercomparison Project Phase 6 of the World Climate Research Program were used (https://esgf-node.llnl.gov/search/cmip6/, accessed on 1 March 2021).

In the IPCC 6th Assessment Report, SSP1-2.6, SSP2-4.5, SSP3-7.0, and SSP5-8.5 were used as standard routes. SSP1-2.6 is a scenario that minimizes the use of fossil fuels and assumes eco-friendly and sustainable economic growth, SSP2-4.5 assumes the mitigation of climate change and socio-economic development as an intermediate stage, SSP3-7.0 is a climate change mitigation policy, and SSP5-8.5 assumes a social structure susceptible to climate change due to a delayed technological development scenario [23].

2.3. MaxEnt Model

The occurrence data of *A. nephrolepis* was selected based on location information collected through field surveys, considering spatial representation and ecological characteristics. Subsequently, 19 climate factors affecting the growth of the species were selected as environmental variables, and redundant variables with a correlation coefficient of 0.7 or higher were removed through correlation analysis. Important variables significantly contributing to model performance were chosen through variable importance analysis.

The MaxEnt model was trained using occurrence data and environmental variables, and after evaluation of the model performance through cross-validation and independent validation data, the final model was selected. The selected MaxEnt model predicted the distribution within the habitat and visualized the results in the form of a distribution map. The range of AUC (Area Under the ROC Curve (ROC: Receiver Operating Characteristic)) values obtained during the modeling process was between 0.5 and 1, with higher values indicating better predictive power of the model [24]. According to the AUC values, model prediction accuracy is classified into five grades: 0.5–0.6 is considered a failure, 0.6–0.7 low, 0.7–0.8 medium, 0.8–0.9 good, and 0.9–1.0 very good.

The ROC (Receiver Operating Characteristic) curve is a graphical representation used to evaluate the performance of a binary classification model. It plots the True Positive Rate (Sensitivity) against the False Positive Rate (1-Specificity) at various decision thresholds. The area under the ROC curve (AUC-ROC) is a widely used metric to assess the model's ability to distinguish between the positive and negative classes. A higher AUC-ROC value, closer to 1 indicates a better-performing model [25].

The MaxEnt model predicted habitat suitability from the lowest (0) to the highest (1) based on the current climate, and this was projected onto future habitat suitability maps through four scenarios. Habitat suitability was subdivided into six grades using an equal interval approach: 0–0.17 as unsuitable areas, 0.17–0.33 as low suitability areas, 0.33–0.50 as moderate suitability areas, 0.50–0.67 as medium suitability areas, 0.67–0.83 as high suitability areas, and 0.83–1.0 as very high suitability areas.

MaxEnt modeling was implemented using MaxEnt software version 3.4.4 provided by the American Museum of Natural History (American Museum of Natural History, New York, NY, USA) [26,27]. Pearson's correlation coefficient was calculated for the 19 generated bioclimatic factor grid maps to remove highly correlated variables, thus addressing multicollinearity. The final selection of environmental variables was used to train the model, and model performance was evaluated using cross-validation and independent validation data.

An important aspect of this process was eliminating redundancy among variables and selecting those that contribute to optimizing model performance. This allowed the MaxEnt model to more accurately predict the habitat suitability of *A. nephrolepis*.

2.4. Seed Collection

On 26 September 2019, *A. nephrolepis* seeds were collected from Balwang Mountain in Pyeongchang-gun, Gangwon-do, South Korea during the time when the seeds had matured. The collected seeds were brought in cone form, dried at room temperature, and then immediately used for experiments after the seeds were released from the cones.

2.5. Determination of Morphometrics of Seeds

Seeds of *A. nephrolepis* were cut in half using a double-sided knife to observe internal and external morphology under a light microscope (Leica DVM6 Digital Microscope; Leica, Wetzlar, Germany). The areas of the embryo and seed of *A. nephrolepis* were measured and their shapes were observed. The ratio of embryo length to seed length (E:S) was calculated.

Seed weight was measured and expressed as 1000-seed weight. For the 1000-seed weight, 100-seed samples were measured in 4 replications to calculate the 1000-seed weight.

The collected seeds were tested for seed filling by X-ray radiography (EMT-F70; Softex, Japan), and the percentage of filled seeds was calculated using the formula below. The seed viability was assessed by taking X-rays of 25 seeds in four replicates and calculating the mean value ± standard error.

$$\text{Percentage of filled seeds (\%)} = \text{number of filled seeds}/\text{number of seeds} \times 100$$

2.6. Seed Germination Tests

Ten seeds were placed in a 60 × 20 mm Petri dish on 1% agar in four replicates and incubated at 10 different temperatures between 5 and 35 °C, along a thermal gradient plate (TGP, ONSOL. Co., Suwon, Republic of Korea). The experiment was conducted under constant temperatures of 15, 20, and 25 °C and alternating day/night temperatures of 25/5, 25/10, 25/10, 20/15, 25/15, 30/15, and 35/15 °C.

A seed was considered germinated when the root protruded more than 2 mm through the seed coat. The number of germinated seeds was registered every day. Germination (G), mean germination time (MGT), T50 (time to reach 50% of germination), and germination index (GI) were calculated using the following formulas [28]:

$$G = (N/S) \times 100$$

The germination percentage represents the total number of seeds that have germinated (N) divided by the total number of seeds (S), multiplied by 100 to express it as a percentage.

$$MGT = \Sigma(Tx \cdot Nx)/N$$

The mean germination time is calculated by summing the product of the number of days after sowing (Tx) and the number of seeds germinated on that day (Nx), divided by the total number of germinated seeds (N).

$$T50 = Ti + (Tj - Ti) \times (N/2 - Ni)/(Nj - Ni)$$

Ti, which is the time of the first observation where the cumulative number of germinated seeds is less than 50% of the total; Tj, the time of the first observation where the cumulative number of germinated seeds exceeds 50% of the total; N, the final total number of germinated seeds; Ni, the number of germinated seeds at the time Ti; and Nj, the number of germinated seeds at the time Tj.

$$GI = \Sigma(Tx \cdot Nx)/Ti,$$

The germination index is calculated by summing the product of the number of days after sowing (Tx) and the number of seeds germinated on that day (Nx), divided by the time of the first germination (Ti).

2.7. Determination of Seed Equilibrium Moisture Content

To determine storage behavior, the relative humidity (RH) and moisture content of the seeds were measured immediately after collection. Moisture equilibrium was previously set in sealed containers at 15, 20, 30, 40, and 50% eRH at 15 °C. Different RH values were obtained using LiCl solutions of various concentrations in airtight containers. The seeds of *A. nephrolepis* were placed in an airtight box and dried under RH conditions to equilibrate moisture. As a temperate tree species, the temperature was set to 15 °C. The moisture equilibrium was confirmed using a hygrometer (HP23-AW; Rotronic, Crawley, UK) [29,30]. The moisture content (MC) of the seeds was investigated using the oven moisture test at 103 °C for 17 h following the guidelines of the International Rules for Seed Testing [31]. Germination testing was performed on seeds with moisture equilibrium in relation to the

MC. MC was calculated on a fresh weight basis and was measured for approximately 50 seeds per each moisture content four times.

2.8. Determination of Seed Storage Behavior

The germination test was performed following the same protocol described in Section 2.6. Seeds that germinated were considered viable, while those that did not germinate were subjected to a tetrazolium test to verify their viability. For the tetrazolium test, the seeds were submerged in the solution for 24 h, after which it was checked whether they had been stained [31].

After the tetrazolium test, total viability was calculated by calculating the ratio of germinated seeds. In addition, through the seed bank storage method, the seeds were sealed in an aluminum bag with silica gel and stored at −20 °C and 5 °C for 3 months, followed by a final verification experiment through a germination test. The protocol for the determination of storage behavior was adapted from Hong and Ellis (1996) [32] and Chau et al. [33] (Figure 1).

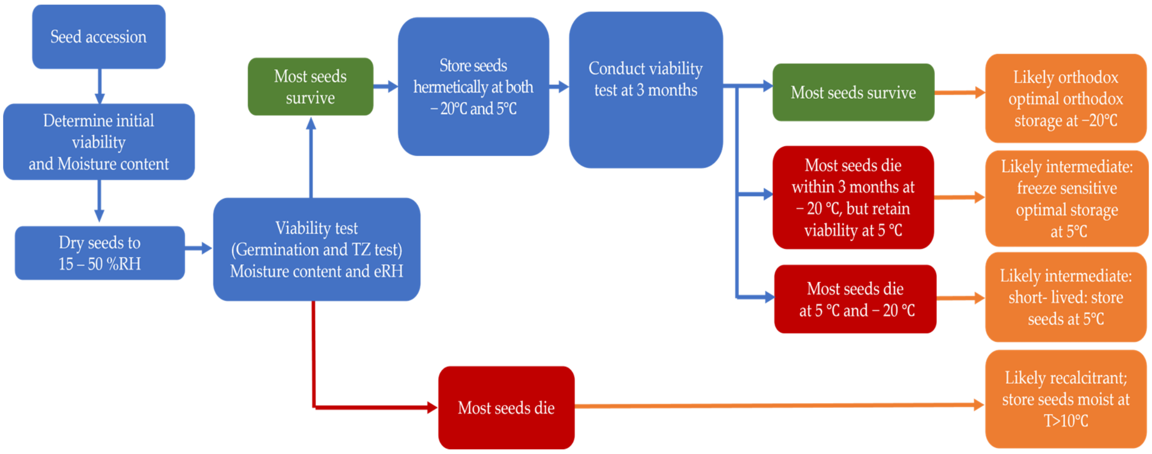

Figure 1. Protocol to determine seed storage behavior of unknown species (adapted from Hong and Ellis (1996) [32] and Chau et al. (2019) [33]).

2.9. Statistical Analysis

The results were analyzed by one-way analysis of variance (ANOVA) using SPSS Statistics v21 (SPSS Inc., Chicago, IL, USA). The differences in means between treatments were verified at a 5% significance level using Duncan's multiple range test.

3. Results

3.1. Model Evaluation

L0.5 regularization is a technique used in machine learning to address the problem of overfitting. It is a hybrid approach that combines the properties of L1 (Lasso) and L2 (Ridge) regularization [34]. As a result of 10-fold cross-validation for the finally selected L0.5 model, the average test AUC value was calculated to be 0.985, which is 0.9 or higher (excellent), so the model was judged to have reliable and suitable performance (Figure 2) [14]. In this study, the distribution of potential habitats of *A. nephrolepis* was predicted using the MaxEnt model. The habitat of *A. nephrolepis* was distributed along the Baekdudaegan Mountains with an altitude of about 1000 m. Analysis was performed to select climate factors to be used in the MaxEnt model of *A. nephrolepis*. As a result, bio01 (annual average temperature), bio03 (isothermal), bio07 (annual temperature difference), bio12 (annual cumulative precipitation), bio13 (precipitation in the wettest month), and bio14 (precipitation in the driest month) were finally selected as final environmental variables.

The percentage contribution and permutation importance of the six environmental factors in the distribution were calculated (Table 2). We found that the climatic factor showing the highest contribution to the distribution of *A. nephrolepis* was bio01 at 97.2%, followed by bio14 at 1.5%, bio12 at 0.6%, bio03 at 0.5%, and bio07 and 13 at 0.1%. The climatic factor shows the highest importance low of bio01 at 97.8%, followed by bio12 at 1.2%, bio07 at 0.7%, and bio03 at 0.3%. Therefore, temperature appears to be more sensitive than precipitation, and the contribution and importance of the annual average temperature were as high as 97%, which seems to have the greatest influence on the prediction of the habitat of *A. nephrolepis*. Based on the results of the main response curve of bio01 (Figure 3), the habitat suitability of *A. nephrolepis* rapidly decreases when the average annual temperature is 6 °C or higher.

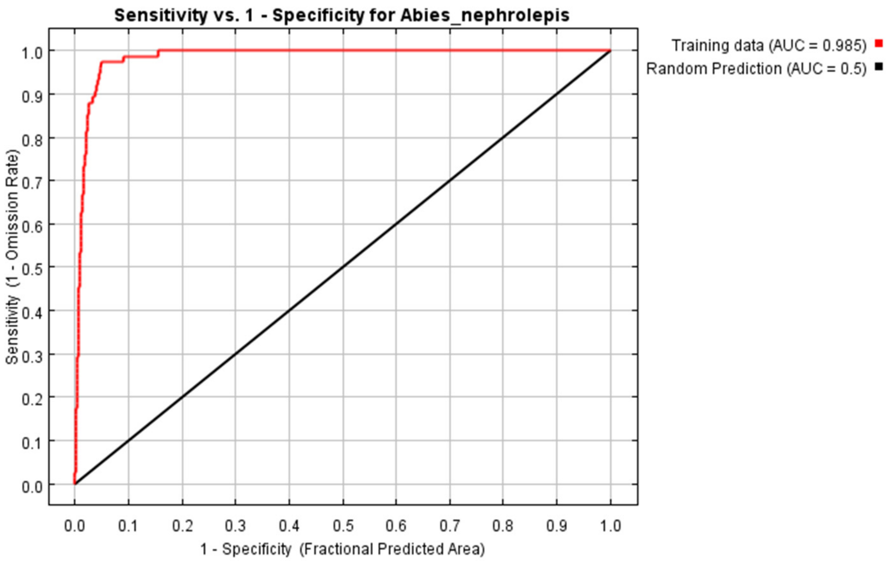

Figure 2. The AUC curves of the climate suitability model for *Abies nephrolepis* Maxim.

Table 2. Relative importance of environmental variables in MaxEnt model.

Environmental Variables	Percent Contribution (%)	Percent Importance (%)
bio01	97.2	97.8
bio14	1.5	0
bio12	0.6	1.2
bio03	0.5	0.3
bio07	0.1	0.7
bio13	0.1	0

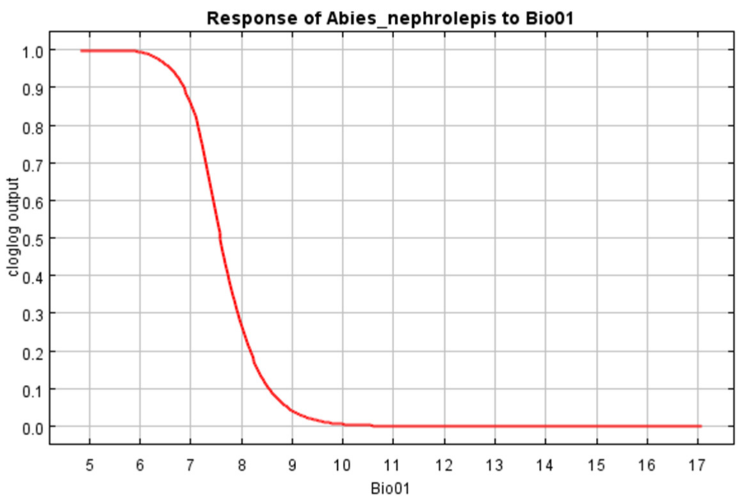

Figure 3. Response curves of Bio01(Annual Mean Temperature) variable affecting MaxEnt prediction.

3.2. Habitat Distribution Prediction

Using 0–0.17 (non-suitable area), 0.17–0.33 (low-suitability area), and 0.33–0.50 (general-suitability area), each MaxEnt value was calculated by categorizing it into 0.50–0.67 (medium-suitability area), 0.67–0.83 (high-suitability area), and 0.83–1.00 (very high-suitability area) (Figures 4 and 5). According to the SSP1-2.6 scenario, it was confirmed that compared to the present, medium-suitable areas and high-suitable areas will disappear in the 2030s and that the percentage will decrease by 2.54% from 9.18% in the case of *A. nephrolepis*. On the other hand, the proportion of unsuitable land will increase by 91.21%, and the habitat of *A. nephrolepis* will rapidly decrease, and in the 2090s, 100% of unsuitable land will be confirmed. According to the SSP2-4.5 scenario, the proportion of unsuitable land and low-suitable land is decreasing compared to the present, and habitats of medium-suitable land and high-suitable land were confirmed to be disappearing. Even in the 2090s, a 100% unsuitable area was confirmed for *A. nephrolepis*. In the case of the SSP3-7.0 scenario, 100% of non-conforming land was confirmed for the 2030s. In the SSP5-8.5 scenario, it was confirmed that low-suitable land, general-suitable land, and medium-suitable land disappeared in the 2030s, and low-suitable land increased to 8.40%. In the case of the 2090s, 100% non-conforming land was confirmed. We detected through habitat prediction that *A. nephrolepis* is on the verge of extinction from the Korean Peninsula in the 2030s, the near future. Habitat is expected to decrease due to a sharp decrease in habitat fitness over time, and climate change is predicted to be the biggest factor.

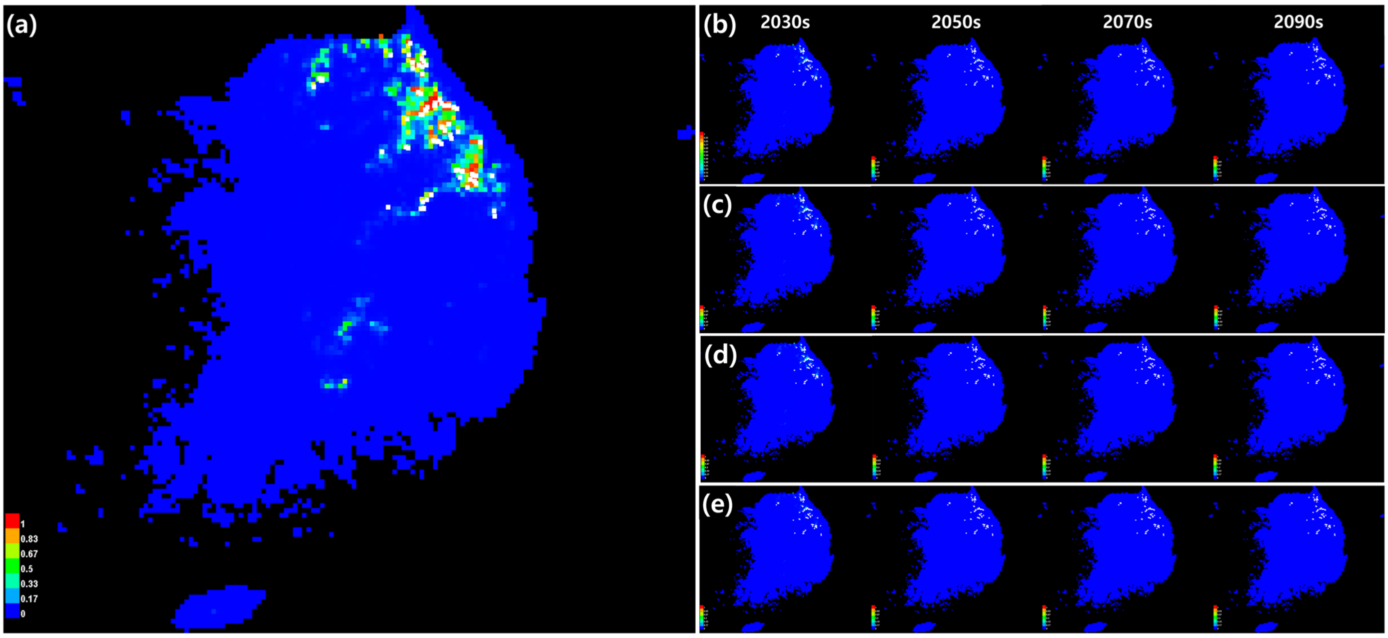

Figure 4. Change in potential distribution of *Abies nephrolepis* Maxim.: (**a**) present, (**b**) SSP1-2.6, (**c**) SSP2-4.5, (**d**) SSP3-7.0, (**e**) SSP5-8.5.

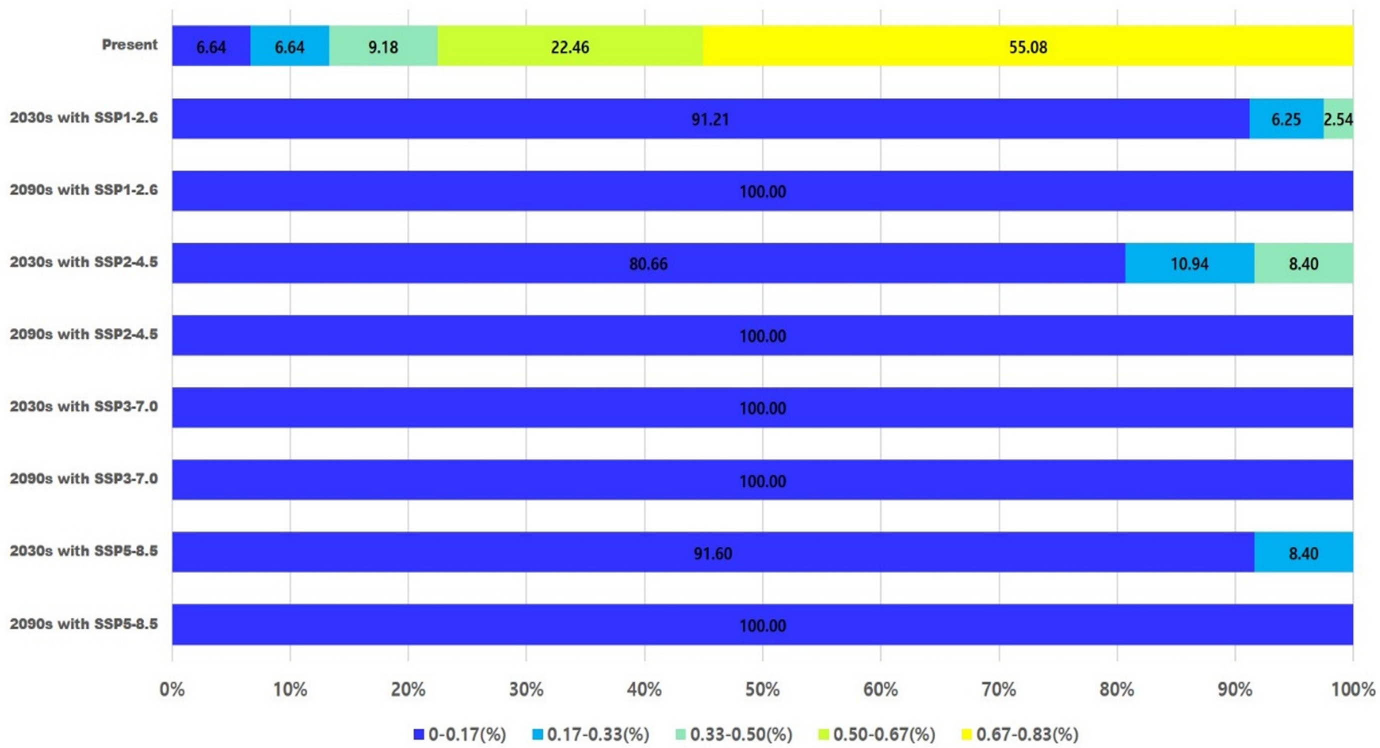

Figure 5. Change in potential distribution area of *Abies nephrolepis* Maxim. under SSP scenarios.

3.3. Seed Morphometrics

The cone of *A. nephrolepis* is cylindrical, and the seed coat is brown, shiny, and winged (Figure 6). The seeds were ovate, with an average length of 6.86 ± 0.12 mm, width of 2.89 ± 0.05 mm, and 1000-seed weight of 10.960 ± 0.165 g. The E:S ratio measured immediately after seed collection and used as an indicator of embryo size relative to seed length was 0.32 ± 0.018; the embryos were approximately 32% of the seed size (Table 3) [35].

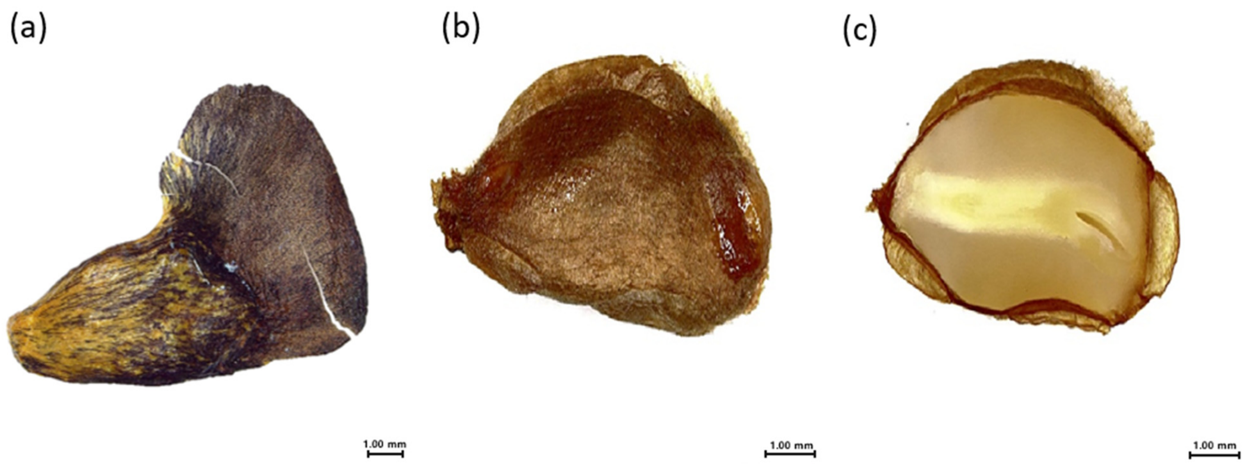

Figure 6. Seed morphology. (**a**) External view with wing; (**b**) external view without wing; (**c**) internal view (scale bar = 1 mm).

Table 3. Seed morphometrics of *Abies nephrolepis* Maxim.

Length (mm)	Width (mm)	1000-Seed Weight (g)	E:S Ratio [1]
6.86 ± 0.12 [2]	2.89 ± 0.05	10.960 ± 0.165	0.320 ± 0.018

[1] Embryo: seed ratio. [2] Values are expressed as standard deviation from mean (n = 10).

3.4. Seed Germination

The initial percentage of filled seeds determined by X-ray radiography was 74.0 ± 2.00%. The first seeds germinated after 13 days. The highest germination of the *A. nephrolepis* was obtained at a constant temperature of 20 °C (52.5 ± 16.01%), followed by that at 25 °C (50.0 ± 10.81%) and 25/10 °C (47.5 ± 4.79%) (Figure 7). There was no significant difference in the final germination rates among these temperature treatments. The germination rate was high when the daily average temperature was high. The time required to reach 50% germination (T50) was the shortest at 25/20 °C. The mean germination time was reduced at an average daily constant temperature of 20 °C or higher, with the shortest times of 14.47 days at a constant temperature of 25 °C and 14.29 days at 25/20 °C. The germination index (GI) was 3.91 ± 0.59 at 25/10 °C, 3.54 ± 1.16 at a constant 20 °C, and 3.34 ± 1.10 at 25/15 °C. The GI showed the highest value of 3.91 ± 0.59 at 25/10 °C and the lowest value at 25/5 °C. No statistical difference was confirmed among the other treatment groups.

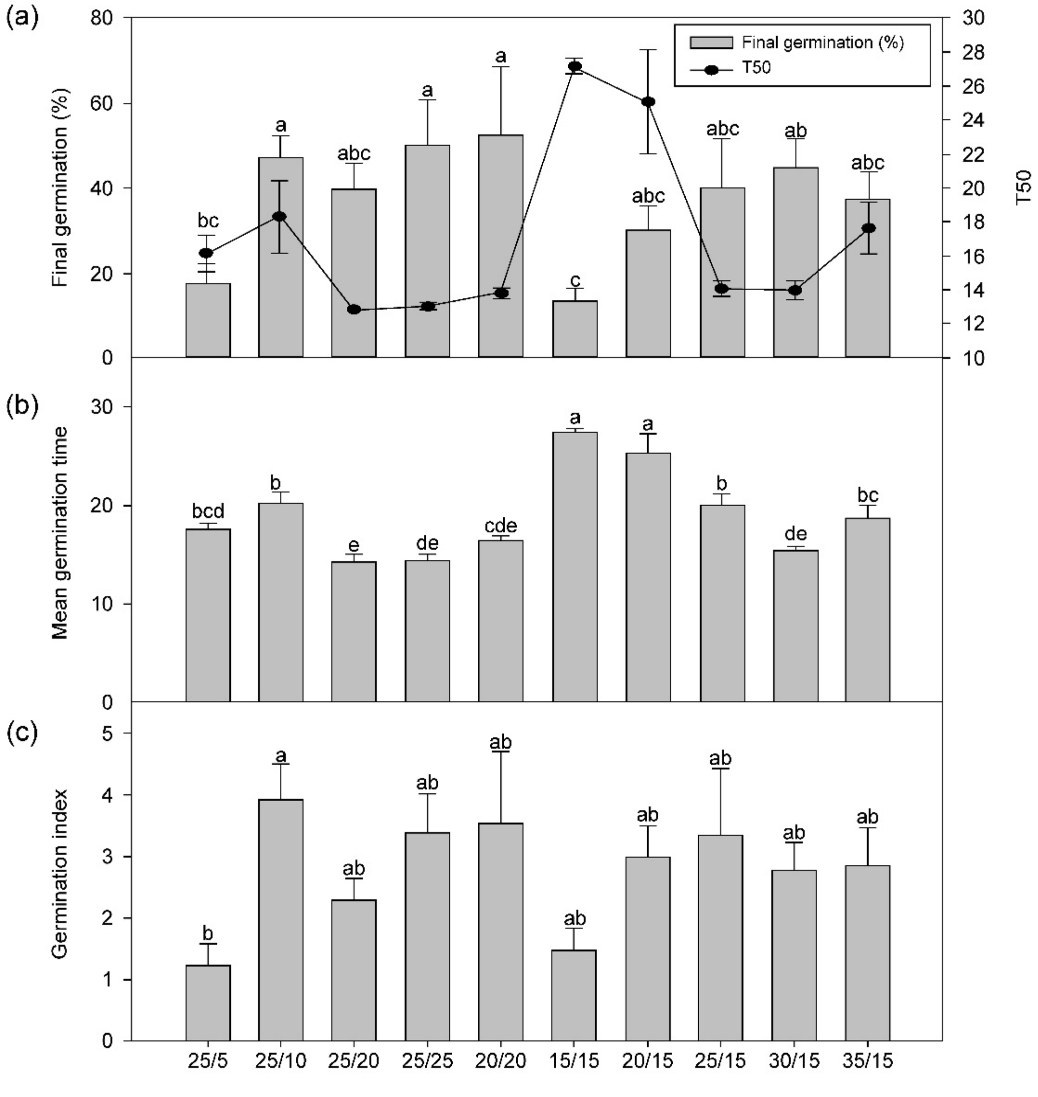

Figure 7. Final germination, T50 (**a**), mean germination time (**b**), and germination index (**c**) of *Abies nephrolepis* Maxim. seeds in temperature treatments. Seeds were pre-treated with temperatures from 5 °C to 35 °C. Different letters on the same column indicate significant differences based on Duncan's multiple range test ($p \leq 0.05$). Vertical bars represent the standard deviation from the mean.

3.5. Seed Equilibrium Moisture Content

Immediately after collection, the moisture content of *A. nephrolepis* seeds was 5.39%. As the equilibrium RH (eRH) was increased by 15%–50% in 15 °C treatments, the moisture content increased from 4.33% to 7.03%. At eRH of 15%–20%, the moisture content was 4.33%; at eRH of 30%–40%, it was under 5% (Figure 8).

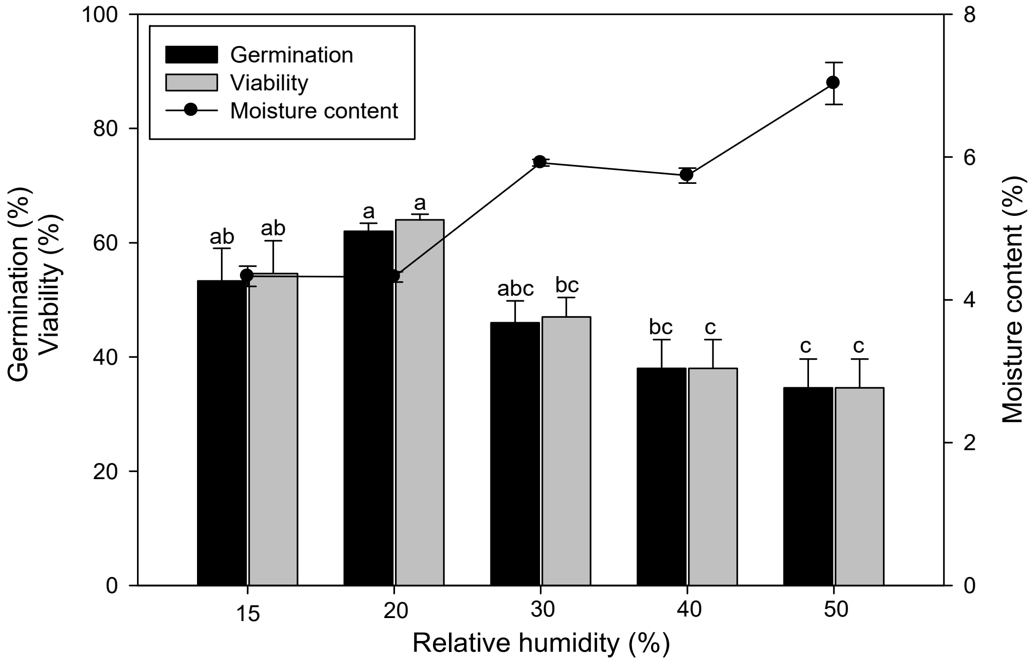

Figure 8. The total viability of *Abies nephrolepis* Maxim. seeds in desiccation treatments. Different letters in the same column indicate significant differences based on Duncan's multiple range test ($p \leq 0.05$). Vertical bars represent the standard deviation from the mean.

3.6. Seed Storage Behavior

Germination and the total viability of seeds were determined as the moisture content of the seeds decreased. The MC was within 3%–7% at 15%–50% eRH. When dried at 20% eRH and 15 °C, the germination and viability were found to be the highest. As the eRH increased, the germination decreased (Figure 8). Tetrazolium tests were performed on non-germinated seeds to select viable seeds and determine total viability. The data revealed that the viability decreased with increasing eRH, suggesting that an increased moisture content lowers the viability of the seeds. The MC was divided into 15%, 20%, 30%, 40%, and 50% according to the eRH, but there was no difference in the germination and viability between different eRHs. Orthodox seeds can be stored at a moisture content of 2%–6% and sub-zero temperatures [32]. All the conditions were satisfied at 15%–50% eRH *for A. nephrolepis* seeds. As the moisture content decreased, the seed germination and total viability increased. In general, for each 1% decrease in seed moisture (when seed MC ranges between 5 and 14%) and for each 5 °C decrease in storage temperature (between 0 °C and 5 °C), the life of the seed is doubled [18]. After drying at 15 °C and 15% RH, the seeds were stored under different conditions (−20 °C, 5 °C) for 3 months. The viability test of the seeds stored at different temperatures revealed a viability rate of 52.0 ± 5.6% after 3 months of storage at −20 °C and 43.0 ± 1.0% at 5 °C, but the difference between the two treatments was not statistically significant. The seeds of *A. nephrolepis* exhibited the characteristics of orthodox seeds (Table 4).

Table 4. Change in viability response of *Abies nephrolepis* Maxim. seeds under different storage temperature treatments.

	Germination (%)	Viability (%)
Stored seeds at −20 °C	39.0 ± 2.5	43.0 ± 1.0
Stored seeds at 5 °C	46.0 ± 7.4	52.0 ± 5.6
p-value	0.4046	0.1682

4. Discussion

In this study, future habitat distribution prediction using the MaxEnt model was performed for *A. nephrolepis*, a species vulnerable to climate change in Korea. It was confirmed that when the average annual temperature increased to more than 6 °C, the habitat suitability of *A. nephrolepis* rapidly decreased.

Seed banks for plants are a recognized strategy to conserve genetic diversity, providing insights into Earth's biodiversity, human need for biodiversity, and the consequences for our planet if we do not protect biodiversity [36]. If we want to perform ex situ conservation through seeds, we need to know exactly how to store seeds first, so that we can store seeds in a healthy way. Therefore, this study examined conservation measures for endangered coniferous trees in the field of seed conservation.

For the conservation and restoration of *A. nephrolepis*, an experiment was conducted on the germination of *A. nephrolepis*. The first seeds germinated after 13 days. Dormancy was not confirmed before or after drying. It was confirmed that seed germination was the highest at temperature conditions of 25/10, 25/25, and 20/20 °C. The germination of seeds of *Abies koreana*, which is similar to *A. nephrolepis*, was found to be higher at 20 °C and 17 °C than at 23 °C under the same light conditions in alpine areas [37]. Furthermore, it was confirmed that the highest germination occurred at 20 °C. In contrast to *A. nephrolepis*, it was confirmed that the germination rate increased at high temperatures (25 and 25/10 °C) and decreased at a low temperature (15 °C).

A. nephrolepis seeds were identified herein to be non-dormant. The germination of non-dormant seeds occurs in nature when soil moisture and temperatures are favorable. If temperatures are favorable but the onset of the wet season is delayed, the timing of germination will be delayed [38]. Even if soil moisture is favorable, germination will be delayed until temperatures (in temperate regions) increase or decrease enough to overlap with those required for germination [39].

Thus, changes in the time when soil moisture and temperature are favorable for germination in the habitat can shift the time of germination. Although seeds may germinate, water stress [40–42], late frosts, strong light [43], low depth of winter snowpack [41], a combination of high soil surface temperatures in the growing season, unfavorable soil microsites, and low winter snowpack [44] may limit seedling establishment. There is a particular concern not only about the effects of elevated temperatures on regeneration in high mountains but also about the effects of drought on seedling survival in these habitats [45].

In the case of *A. nephrolepis*, the occurrence of young trees with a diameter of 6–10 cm at breast height is decreasing [4]. Timing germination such that conditions are favorable for growth after seedling emergence is an important aspect of the adaptation of a plant species to its habitat [46]. Accordingly, it is judged that the germination environment of seeds and the environment in which the germinated seeds grow are important.

The main factors determining the storage behavior of seeds are the type of fruit, seed number, and seed traits. *A. nephrolepis* has cones, and its dried seeds are potentially classified as orthodox seeds. The 1000-seed weight (<25 g) and moisture content of mature seeds (<20%) support this classification and suggest that the seeds will survive at 2%–6% moisture content. After sufficient drying at 15% RH and 15 °C, the seeds should be stored at −20 °C. Based on this study, *A. nephrolepis* seed was identified as an orthodox seed and was confirmed to be healthy at 15% RH and −20 °C in the seed bank.

Storing seeds in gene banks is the most effective way of conserving and sharing most of our existing agrobiodiversity (orthodox species). It is also a relatively simple activity; seeds are dried and stored at low temperatures. However, within that simple statement lies a whole series of operations, which, if not carefully followed and controlled, will pose a risk of losing the agrobiodiversity that gene banks seek to conserve [47].

In the future, it is necessary to first expand genetic diversity as a conservation method for alpine conifers, and through this, continuous renewal will be necessary to maintain genetic diversity. Therefore, we must preserve the seed or cloned trees (ex situ conservation) in a safe place, and not in their native land, to maintain the genetic diversity of present-day alpine conifers. Genetic diversity refers to the genetic variation between a population or individuals within a species. To increase adaptability to impossible prediction of environmental changes, forests (in situ restoration) of trees with various genetic variations (high genetic diversity) should be reforested [31].

It is also necessary to conduct a local adaptation experiment for adaptation to an area using the germinated seed produced based on basic information on seed germination and storage. This study was conducted as a basic study for the prediction of the habitat distribution of *A. nephrolepis* and for the determination of the seed conservation method.

5. Conclusions

This study aimed to predict the current distribution and future habitat distribution changes in *A. nephrolepis* under climate change, confirm the necessity of in situ conservation, and devise appropriate ex situ storage methods. It was predicted that *A. nephrolepis* would become extinct in its habitat by the 2090s under all SSP scenarios. Therefore, the need for both in situ and ex situ conservation was revealed. According to the seed germination characteristics, the highest germination rate (52.5 ± 16.01%) was observed at a constant temperature of 20 °C, followed by 25 °C (50.0 ± 10.81%) and 25/10 °C (47.5 ± 4.79%). The highest viability was obtained under 20% eRH (64.0 ± 0%). *A. nephrolepis* seeds were classified as orthodox-type seeds, which do not lose viability after drying. They can be stored long-term at −20 °C. This study provides valuable insights for predicting the habitat distribution of the endangered species *A. nephrolepis* and establishing seed conservation methods, contributing to future research and conservation efforts.

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References

1. Wuebbles, D.J. Climate Science Special Report: 4th US National Climate Assessment, Volume I. In *World Scientific Encyclopedia of Climate Change: Case Studies of Climate Risk, Action, and Opportunity*; World Scientific Publishing: Singapore, 2021; Chapter 2; pp. 213–220.
2. Intergovernmental Panel on Climate Change (IPCC). *Summary for Policymakers. Global Warming of 1.5 °C. Special Report on the Impacts of Global Warming of 1.5 °C Above Pre-industrial Levels and Related Global Greenhouse Gas Emission Pathways, in the Context of Strengthening the Global Response to the Threat of Climate Change, Sustainable Development, and Efforts to Eradicate Poverty*; Masson-Delmotte, V., Zhai, P., Portner, H.-O., Skea, J., Shukla, P.R., Pirani, A., Eds.; United Nations Environment Programme: Nairobi, Kenya, 2018.
3. Intergovernmental Panel on Climate Change (IPCC). *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II And III to The Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; Core Writing Team, Pachauri, R.K., Meyer, L.A., Eds.; IPCC: Geneva, Switzerland, 2014; 151p.
4. National Institute of Forest Science (NIFS). *Endangered Alpine Coniferous Species in Korea*; NIFS: Seoul, Republic of Korea, 2019.
5. Farjon, A. *Pinaceae. Drawings and Descriptions of the Genera Abies, Cedrus, Pseudolarix, Keteleeria, Nothotsuga, Tsuga, Cathaya, Pseudotsuga, Larix and Picea*; Koeltz Scientific Books: Königstein, Germany, 1990.
6. Hunt, R.S. *Abies*. In *Flora of North America North of Mexico*; Editorial Committee, Ed.; Oxford University Press: New York, NY, USA; Oxford, UK, 1993; pp. 353–362.
7. Zhang, D.; Katsuki, T.; Rushforth, K. *Abies nephrolepis*. The IQHRWLUCN Red List of Threatened Species, Version 2014.3. 2013. Available online: https://www.iucnredlist.org/species/42292/76095986 (accessed on 13 June 2024).
8. Lee, B.Y.; Nam, G.H.; Yun, J.H.; Cho, G.Y.; Lee, J.S.; Kim, J.H.; Park, T.S.; Kim, K.; Oh, K. Biological indicators to monitor responses against climate change in Korea. *Korean J. Plant Taxon.* **2010**, *40*, 202–207. [CrossRef]
9. Lee, D.K.; Kim, J.U. Vulnerability assessment of sub-alpine vegetations by climate change in Korea. *J. Korean Soc. Environ. Restor. Technol.* **2007**, *10*, 110–119.
10. Chun, J.H.; Lee, C.B.; Yoo, S.M. Shifts of geographic distribution of *Pinus koraiensis* based on climate change scenarios and GARP model. *Korean J. Agric. For. Meteorol.* **2015**, *17*, 348–357. [CrossRef]
11. Park, H.C.; Lee, J.H.; Lee, G.G.; Um, G.J. Environmental features of the distribution areas and climate sensitivity assesment of Korean Fir and Khinghan Fir. *J. Environ. Impact Assess.* **2015**, *24*, 260–277. [CrossRef]
12. Yoo, S.; Lim, C.-H.; Kim, M.; Song, C.; Kim, S.J.; Lee, W.-K. Potential distribution of endangered coniferous tree species under climate change. *J. Clim. Chang. Res.* **2020**, *11*, 215–226. [CrossRef]
13. Phillips, S.J.; Anderson, R.P.; Schapire, R.E. Maximum entropy modeling of species geographic distributions. *Ecol. Model.* **2006**, *190*, 231–259. [CrossRef]
14. Phillips, S.J.; Dudík, M. Modeling of species distributions with Maxent: New extensions and a comprehensive evaluation. *Ecography* **2008**, *31*, 161–175. [CrossRef]
15. Guerrant, E.O.; Fiedler, P.L. Accounting for sample decline during ex situ storage and reintroduction. In *Ex Situ Plant Conservation: Supporting Species Survival in the Wild*; Guerrant, E.O., Havens, K., Maunder, M., Eds.; Island Press: Washington, DC, USA, 2004; pp. 365–385.
16. Convention on Biological Diversity. Global Strategy for Plant Conservation. Available online: https://www.cbd.int/gspc/ (accessed on 1 March 2022).
17. Center for Plant Conservation. *CPC Best Plant Conservation Practices to Support Species Survival in the Wild*; Center for Plant Conservation: Escondido, CA, USA, 2019.
18. Harrington, J.F. Seed storage and longevity. In *Seed Biology: Insects, and Seed Collection, Storage, Testing, and Certification. Physiological Ecology*; Kozlowski, T.T., Ed.; Academic Press: New York, NY, USA; London, UK, 1972; pp. 145–245.
19. Roberts, E.H. Predicting the storage life of seeds. *Seed Sci. Technol.* **1973**, *1*, 499–514.
20. Ellis, R.H.; Hong, T.D.; Roberts, E.H. An intermediate category of seed storage behaviour? I. Coffee. *J. Exp. Bot.* **1990**, *41*, 1167–1174. [CrossRef]
21. WorldClim. Global Climate Data, Free Climate Data for Ecological Modeling and GIS. Available online: http://www.worldclim.org/ (accessed on 1 April 2020).
22. Fick, S.E.; Hijmans, R.J. WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. *Int. J. Climatol.* **2017**, *37*, 4302–4315. [CrossRef]
23. IPCC. Climate Change 2021. In *The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*; Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S.L., Péan, C., Berger, S., Caud, N., Chen, Y., Goldfarb, L., Gomis, M.I., et al., Eds.; Cambridge University Press: Cambridge, UK, 2021.
24. Swets, J. Measuring the accuracy of diagnostic systems. *Science* **1988**, *240*, 1285–1293. [CrossRef] [PubMed]
25. Hajian-Tilaki, K. Receiver operating characteristic (ROC) curve analysis for medical diagnostic test evaluation. *Casp. J. Intern. Med.* **2013**, *4*, 627.
26. Merow, C.; Smith, M.J.; Silander, J.A., Jr. A Practical Guide to MaxEnt for Modeling Species' Distributions: What It Does, and Why Inputs and Settings Matter. *Ecography* **2013**, *36*, 1058–1069. [CrossRef]
27. Phillips, S.J. A Brief Tutorial on Maxent. *AT&T Res.* **2005**, *190*, 231–259.

28. Ranal, M.A.; Santana, D.G. How and Why to Measure the Germination Process? *Rev. Bras. Botânica Braz. J. Bot.* **2006**, *29*, 1–11. [CrossRef]
29. Gold, K.; Manger, K. *Measuring Seed Moisture Status Using a Hygrometer*; Technical Information Sheet_04; Royal Botanic Gardens Kew: Richmond, UK, 2008.
30. Gold, K.; Hay, F. *Equilibrating Seeds to Specific Moisture Levels*; Technical Information Sheet_09; Royal Botanic Gardens Kew: Richmond, UK, 2014.
31. International Seed Testing Association (ISTA). *Chapter 5: The Germination Test, International Rules for Seed Testing*, 1st ed.; The International Seed Testing Association (ISTA): Bassersdorf, Switzerland, 2006.
32. Hong, T.D.; Ellis, R.H. *A Protocol to Determine Seed Storage Behaviour*; IPGRI Technical Bulletin No. 1; International Plant Genetic Resources Institute: Rome, Italy, 1996.
33. Chau, M.M.; Chambers, T.; Weisenberger, L.; Keir, M.; Kroessig, T.I.; Wolkis, D.; Yoshinaga, A.Y. Seed freeze sensitivity and ex situ longevity of 295 species in the native Hawaiian flora. *Am. J. Bot.* **2019**, *106*, 1248–1270. [CrossRef]
34. Louizos, C.; Welling, M.; Kingma, D.P. Learning sparse neural networks through L_0 regularization. *arXiv* **2017**, arXiv:1712.01312.
35. Vandelook, F.; Bolle, N.; Van Assche, J.A. Seed dormancy and germination of the European *Chaerophyllum temulum* (Apiaceae), a member of a trans-Atlantic genus. *Ann. Bot.* **2007**, *100*, 233–239. [CrossRef] [PubMed]
36. Van Dooren, T. Banking seed: Use and value in the conservation of agricultural diversity. *Sci. Cult.* **2009**, *19*, 373–395. [CrossRef]
37. Lee, H.J. *Effects of Pretreatments on Seed Germination of Abies koreana*; Korea University Graduate School: Seoul, Republic of Korea, 2007.
38. Kimball, S.; Angert, A.L.; Huxman, T.E.; Venable, D.L. Contemporary climate change in the Sonoran Desert favors cold-adapted species. *Glob. Chang. Biol.* **2010**, *16*, 1555–1565. [CrossRef]
39. Baskin, J.M.; Baskin, C.C. The annual dormancy cycle in buried weed seeds: A continuum. *BioScience* **1985**, *35*, 492–498. [CrossRef]
40. Kueppers, L.M.; Faist, A.; Ferrenberg, S.; Castanha, C.; Conlisk, E.; Wolf, J. Lab and field warming similarly advance germination date and limited germination rate for high and low elevation provenances of two widespread subalpine conifers. *Forests* **2017**, *8*, 433. [CrossRef]
41. Lubetkin, K.C.; Westerling, A.L.-R.; Kueppers, L.M. Climate and landscape drive the pace and pattern of conifer encroachment into subalpine meadows. *Ecol. Appl.* **2017**, *27*, 1876–1887. [CrossRef] [PubMed]
42. Lett, S.; Dorrepaal, E. Global drivers of tree seedling establishment at alpine treelines in a changing climate. *Funct. Ecol.* **2018**, *32*, 1666–1680. [CrossRef]
43. Shen, W.; Zhang, L.; Guo, Y.; Luo, T. Causes for treeline stability under climate warming: Evidence from seeds and seedling transplant experiments in southeast Tibet. *For. Ecol. Manag.* **2018**, *408*, 45–53. [CrossRef]
44. Davis, E.L.; Gedalof, Z. Limited prospects for future alpine treeline advance in the Canadian Rocky Mountains. *Glob. Chang. Biol.* **2018**, *24*, 4489–4504. [CrossRef] [PubMed]
45. Giménez-Benavides, L.; Escudero, A.; García-Camacho, R.; García-Fernández, A.; Iriondo, J.M.; Lara-Romero, C.; Morente-López, J. How does climate change affect regeneration of Mediterranean high-mountain plants? An integration and synthesis of current knowledge. *Plant Biol.* **2018**, *20*, 50–62. [CrossRef] [PubMed]
46. Baskin, C.C.; Baskin, J.M. *Plant Regeneration from Seeds: A Global Warning Perspective*; Academic Press: Cambridge, MA, USA, 2022.
47. Whitehouse, K.J.; Hay, F.R.; Lusty, C. Why seed physiology is important for gene banking. *Plants* **2020**, *9*, 584. [CrossRef]