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Selecting Appropriate Seedling Age for Restoration Using Comparative Analysis of Physiological Characteristics by Age in *Abies koreana* Wilson

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Abstract

The aim of this study was to investigate the sensitivity to environmental stress, and changes in the photosynthesis capacity in *Abies koreana* seedlings by age and to suggest the most effective age for restoration. To identify these physiological characteristics of *A. koreana*, the chlorophyll fluorescence and photosynthetic capacity of 1-, 2-, 3-, 5- and 6-year-old *A. koreana* seedlings were observed from June 2020 to June 2021. The maximum quantum efficiency of Photosystem II (Fv/Fm), a chlorophyll fluorescence measurement parameter, was strongly positively correlated with the monthly average temperature (1-year-old seedling: r=0.8779, 2-year-old seedling: r=0.8605, 3-year-old seedling: r=0.8697, 5-year-old seedlings: r=0.8085, and 6-year-old seedlings: r=0.8316). The Fv/Fm values were the lowest in winter (November 2020-March 2021). In addition, the Fv/Fm values of 1-, 2-, and 3-year-old seedlings in winter were lower than that of 5- and 6-year-old seedlings, while the Fv/Fm values in summer were relatively higher than those in winter. Further, the Fv/Fm values of seedlings of all ages decreased in August 2020, when the monthly average temperature was the highest. In particular, 1-year-old to 3-year-old seedlings showed Fv/Fm values less than 0.8. Further, the photosynthetic capacity measured in August 2020 increased with increasing seedling age. The analysis of variance results for summer Fv/Fm values showed significant differences in age-specific averages ($p<0.05$), and Duncan's multiple range test showed significant differences between 5- and 6-year-old seedlings and 1-, 2-, and 3-year-old seedlings ($p<0.05$). These results suggested that the 5- and 6-year-old seedlings were less sensitive to environmental stress and showed better photosynthetic capacity than the 1-, 2-, and 3-year-old seedlings. Therefore, 5-year-old or older *A. koreana* seedlings can be used as restoration materials because they can show increased adaptability and stable growth during transplantation due to their relatively high environmental resistance and photosynthetic capacity.

Key Words: chlorophyll fluorescence, Fv/Fm, light response curve, photosynthetic capacity, *Abies koreana* Wilson

Introduction

The genus *Abies* comprises approximately forty species that are mainly distributed in the Northern Hemisphere and subalpine areas (Liu 1971); correspondingly, the plants belonging to this genus grow in cold climatic conditions (Farjon 1990). This species is diminishing due to its inability to adapt to increasing temperatures caused by global

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warming (Crawford 1989; Koo et al. 2001). The *Abies* genus in Korea includes *A. koreana* Wilson, *A. nephrolepis* (Trautv.) Maxim., and *A. holophylla* Maxim., which are mainly distributed in subalpine areas having an elevation of more than 1,000 m (Yang et al. 2015). Among these species, *A. koreana* is native to Korea and is distributed in the middle and southern parts of the Korean Peninsula at an elevation of more than 1,200 m in the subalpine zone (Park et al. 2015). Further, it is extensively distributed in Mt. Halla and Mt. Jiri, and on a small scale in Mt. Deokyu, Mt. Gaya, Mt. Geumwon, and Mt. Yeongchuk (Yang et al. 2015; Koo and Kim 2020). However, the population of *A. koreana* is rapidly declining due to various causes, such as temperature increases due to climate change, water stress arising from spring droughts and typhoons, and soil environment changes (Koo and Kim 2020). In addition, *A. koreana* seedlings that are established naturally in an understory environment are decreasing (Kim et al. 1991; Song et al. 2014; Song et al. 2020). Thus, the International Union for Conservation of Nature and Natural Resource (IUCN) has designated *A. koreana* as an endangered species.

The IUCN Restoration Guidelines (2013) explain that many of the biological aspects of organisms used in species restoration are related to restoration strategies. In addition, these founder organisms should show good properties in morphology, physiology, and genetic conformity (IUCN/SSC, 2013). When selecting a founder, the natural sex ratio and age classes of the founders can be considered to enhance successful restoration. Juvenile trees differ from mature trees in their ability to photosynthesize and grow, and may be more sensitive to environmental stress (Kohyama 1983; Howe et al. 2003; Smith et al. 2003; Greenwood et al. 2008; Reinhardt et al. 2009). Thus, selecting the most appropriate age group of plant founders is important for a successful transplant (IUCN/SSC 2013).

During external environment stresses, reactive oxygen species (ROS), a highly reactive toxic oxygen type, are produced in plants. ROS causes physiological disorders, such as denaturation of nucleic acids, proteins, and lipids, inhibits photosynthesis, and causes plant necrosis (Alscher and Hess 1993; Asada 1999). Among the various mechanisms that protect plant cells from ROS, non-photochemical quenching releases excess light energy as heat and photons (fluorescence) (Muller et al. 2001; Tanaka 2007; Ruban 2016). Chlorophyll fluorescence estimation method can easily, quickly, and nondestructively measure the effect of various environmental stresses on the photometric activity of plant leaves (Oh and Koh 2004; Kumar et al. 2015). Various researchers have used chlorophyll fluorescence in their studies. For example, Cregg et al. (2004) evaluated the chlorophyll fluorescence in Fir (*Abies* sp.) according to the differences in soil pH, Lovelock et al. (1994) evaluated the relationship between plant growth and light, and Falqueto et al. (2017) studied plant reactions according to drying conditions.

Moreover, other studies have investigated the physiological differences between leaves of different ages (Katahata et al. 2007; Zhou et al. 2015; Wang et al. 2020). Teskey et al. (1984) reported that *A. amilis* shows increased photosynthetic capacity when its leaves are young. However, few studies have compared the photosynthetic capacity among plants with different ages. Therefore, this study aimed to suggest the most effective seedling age of *A. koreana* for its use as restoration material by measuring the age-specific chlorophyll fluorescence and photosynthetic capacity.

Materials and Methods

Plant materials and environmental factors

The study was conducted using 1-, 2-, 3-, 5-, and 6-year-old seedlings of *A. koreana* in the National Forest Science Institute, Korea (altitude: 40 m). Under the second basic plan for the management of biological resources (2014-2018), *A. koreana* seeds, which were collected from Mt. Jiri and conserved in the seed bank of the National Institute of Forest Science, Korea, were used for this study.

Table 1. Average height (cm) and average root collar diameter (mm) for each age class

Age	Height (cm)	Root collar diameter (mm)
1-year-old seedlings	4.68±0.33	1.12±0.17
2-year-old seedlings	5.08±1.81	1.21±0.29
3-year-old seedlings	6.27±0.81	1.74±0.35
5-year-old seedlings	13.93±3.16	6.26±1.26
6-year-old seedlings	34.38±6.01	9.78±1.18

Values are mean±SD (n=20).

The seeds were planted in March 2014, 2015, 2017, 2018, and 2019, and grown under the same environmental conditions as those for the experimental analysis. The respective average height (cm) and average root collar diameter (mm) of 20 seedlings belonging to each of the five age classes are listed in Table 1. Further, the daily air temperature and precipitation data were measured from June, 2020 to June, 2021 at the Suwon Meteorological Observatory, located near the study site (Fig. 1).

Chlorophyll fluorescence

Chlorophyll fluorescence was measured using Handy PEA-Advanced continuous excitation chlorophyll fluorimeter (Hansatech, UK) every month during the entire study period. According to Kumar et al. (2015), measurements should be conducted in dark light conditions. Therefore, we measured the chlorophyll fluorescence from 7 pm to 9 pm Moreover, before measuring the chlorophyll fluorescence, light was blocked for at least 20 min for the leaves to adapt to darkness. After adapting to darkness, the minimum fluorescence (Fo) was read and the light was saturated to induce maximum fluorescence (Fm). The maximum quantum efficiency of Photosystem II (Fv/Fm), a variable of chlorophyll fluorescence meters, is widely used by many researchers as an index to determine the health of plants under stress conditions (Hazrati et al. 2016). The maximum quantum efficiency of Photosystem Ⅱ was calculated as the ratio of Fv to Fm, i.e., Fv/Fm, where Fv=Fm-Fo represents fluorescence (Robakowski and Bielinis 2017). Bjorkman and Demmig (1987) explained that healthy plants show an Fv/Fm value of up to 0.83. In general, an Fv/Fm value below 0.8 can indicate that the reaction center of Photosystem II is under stress with deteriorated activity or irreversible inactivity (Bolhar-Nordenkampf et al. 1989). We measured the Fo, Fm, and Fv/Fm values of 20 seedlings for each of the five age classes using three leaves per tree to evaluate the response of *A. koreana* seedlings to seasonal stress. Because current-year leaves are not completely grown, a large variation in net photosynthesis can be observed (Teskey et al. 1984). Therefore, randomly selected leaves of 1-year-old seedlings and 2-year-old leaves of older seedlings (2-, 3-, 5- and 6-year-old) were used for measuring the chlorophyll fluorescence. Further, the sensor of chlorophyll fluorimeter with 4 mm diameter was used to measure the chlorophyll fluorescence at the center of the leaf front side.

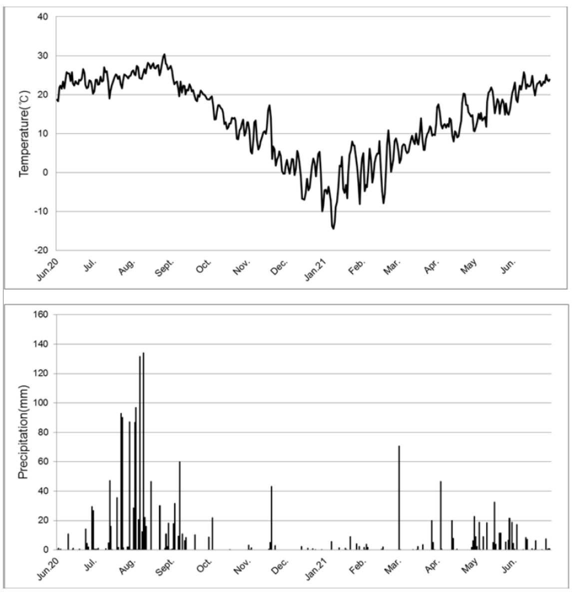

Fig. 1. Seasonal variations of the daily averages of temperature and precipitation determined from June 2020 to June 2021, in the Suwon Meteorological Observatory located near the study site.

Light response curve calculation

To compare the photosynthetic capacities of the five age classes of *A. koreana* seedlings, the net photosynthesis rate was measured for four seedlings of each of the five age classes in August 2020 using a portable photosynthesis meter (Li-6800, Li-cor Inc., USA). The photosynthetic capacities were measured in summer (August), during which the photosynthesis rate is the highest. In the *Picea* genus, which is taxonomically close to the *Abies* genus (Weng et al. 2005), both photosynthetic capacity and Fv/Fm values are generally high in August as the monthly average temperature is the highest. In addition, according to Teskey et al. (1984), the net photosynthesis rate of *A. amabilis* is the highest in July-August, when the temperature is high; additionally, Oh et al. (2001) reported that the Fv/FM value of *A. koreana* was the highest in summer. Moreover, Lim et al. (2006) reported that the photosynthetic capacities of healthy and unhealthy *A. koreana* seedlings can be evidently distinguished in August, and thus, it is appropriate to compare the photosynthetic capacities of *A. koreana* by age in this period. To minimize the difference between laboratory environmental conditions and internal conditions of the cuvette, the flow rate of air into the leaf chamber, chamber temperature, CO_2 concentration, and relative humidity were set to 600 μmol s^{-1}, 25°C, 500 μmol mol^{-1}, and 60%, respectively. Further, light response curves were produced by artificially irradiating the light at photosynthetic photon flux densities of 0, 100, 200, 300, 500, 700, 1000, and 1500 μmol m$^{-2}\cdot$s^{-1}. Subsequently, the resultant curve was used to calculate the light saturation point and net photosynthesis (Kim and Lee 2001). The size of this photosynthesis measuring chamber was 6.6×5.9×5.8 cm (L×W×H). To measure the photosynthetic capacity, all leaves of the 1-, 2- and 3-year-old seedlings were used, while branches containing 1- and 2-year-old leaves were randomly selected from 5- and 6-year-old seedlings, and placed in the chamber. The lengths and widths of all leaves were measured to calculate the leaf area, which in turn was used to calculate the net photosynthesis. According to Kim and Lee (2001), the light compensation point, dark respiration, and net apparent quantum yield are calculated through a linear relationship between light intensity and net photosynthesis at a light intensity of less than 100 μmol m$^{-2}\cdot$s^{-1}.

Statistical analyses

To compare the differences in the chlorophyll fluorescence and photosynthetic capacity among the five age classes of *A. koreana* seedlings, analysis of variance (ANOVA) and Duncan's multiple range test were conducted at $p<0.05$. In addition, correlation analysis was performed to analyze the relationship between chlorophyll fluorescence reactions and temperature. All analyses were conducted using the agricolae package in R statistical software (4.0.5 for Windows).

Results and Discussion

Seasonal changes in environmental factors

The air temperature and precipitation data of the study site from June 2020 to June 2021 are presented in Fig. 1. Various environmental factors, such as seasonal rainfall, temperature, and altitude, affect the natural regeneration of tree species, tree growth rate, photosynthetic, respiration, and phenological cycles (Asanok and Marod 2016; Krishnamoorthy et al. 2016; Chae and Yun 2018). Tanaka (2007) reported that among the various environmental conditions, temperature affected the photosynthetic capacity in the evergreen coniferous species of *Taxus cursidate* Siebold & Zucc.. The minimum and maximum temperatures at the study site in August 2020 were 24.1°C and 30.2°C, respectively, with an average monthly temperature of 26.7°C. The highest temperature was recorded in August 2020, which gradually decreased to an average of -2.4°C in January 2021 (minimum and maximum temperatures reached -7.8°C and 2.6°C, respectively). During the study period, the total annual precipitation was 1854.4 mm, of

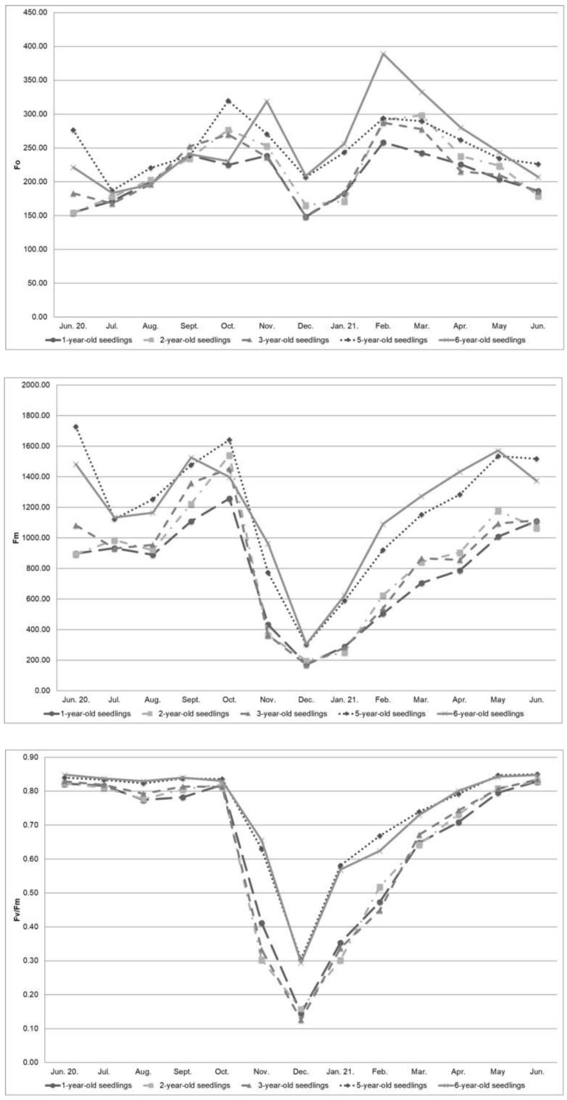

Fig. 2. Seasonal differences in the Fo, Fm, and maximum quantum efficiency of Photosystem II (Fv/Fm) by five age classes of *Abies koreana*.

which 76% was concentrated between June and September 2020 when the average monthly temperature was 20°C or higher, while 56% of the total precipitation was concentrated in July and August 2020. December received the lowest precipitation.

Age-wise differences in the chlorophyll fluorescence of A. koreana according to the season

The Fo, Fm, and Fv/Fm values were the lowest in winter (December 2020). Fo values decreased from November to December 2020, after which the Fo values rapidly increased until February 2021, and subsequently, gradually decreased again (Fig. 2). Fo is affected by environmental stress, and damage in PSII due to high temperatures increases the Fo values (Krause and Weis 1984). The results of this study also suggested that high temperatures in summer decreased the Fo value. Damage Fm values showed a pattern similar to that of Fv/Fm values, with values decreasing from July to August 2020, when the temperature was high (Fig. 2).

The Fv/Fm value was the lowest in winter (November 2020-March 2021), which gradually increased, with high values observed in summer (June 2020-September 2020) (Fig. 2). Further, the ANOVA results showed that the Fv/Fm values were significantly different among all seasons and ages ($p < 0.05$). This trend of Fv/Fm values was consistent with that of monthly average temperature, i.e., the Fv/Fm values were strongly correlated with the monthly average temperature (1-year-old seedling: $r = 0.8779$, 2-year-old seedling: $r = 0.8605$, 3-year-old seedling: $r = 0.8697$, 5-year-old seedlings: $r = 0.8085$, and 6-year-old seedlings: $r = 0.8316$ at $p < 0.01$).

The Fv/Fm values in winter were below 0.8 for all ages. This result was consistent with the results of Oh et al. (2001), who reported significantly lower Fv/Fm values of *A. koreana* in winter than in summer. Tanaka (2007) stated that the Fv/Fm values in *Taxus cuspidate* were reduced due to the effects of low temperatures and that adjusting the leaves, which show low Fv/Fm values in winter, to 20°C increases the Fv/Fm value. Moreover, Teskey et al. (1984) indicated that *A. amabilis* showed a low photosynthesis rate at low temperatures of 1°C, and most carbon gain occurred during summer when the temperatures are relatively high; additionally, they stated that the photosynthesis rate decreased in winter due to low soil and air temperature and low incident radiation. In this study, the decrease in the Fv/Fm values in winter was higher in the 1-, 2-, and 3-year-old *A. koreana* seedlings than in the 5- and 6-year-old seedlings, thus, indicating that the 1-, 2-, and 3-year-old seedlings were more sensitive to low-temperature stress than the 5- and 6-year-old seedlings.

The chlorophyll fluorescence results of summer (Fig. 3) showed a high average Fv/Fm value of 0.8324 in June (1-year-old: 0.8221, 2-year-old: 0.8231, 3-year-old: 0.8289, 5-year-old: 0.8399, and 6-year-old: 0.8482) possibly because of the increased solar radiation and plant growth in summer. In August 2020, when the monthly average temperature was the highest, the Fv/Fm values declined to an average of 0.7992 (1-year-old: 0.7742, 2-year-old: 0.7770, 3-year-old: 0.7923, 5-year-old: 0.8230, and 6-year-old: 0.8293) and recovered to an average of 0.8156 in September. Moreover, the Duncan's multiple range test results showed that the Fv/Fm values in August for all ages were statistically significant ($p < 0.05$). Lim et al. (2006) reported that the alpine *A. koreana* plant is adapted to low temperatures, thus, it shows reduced ability to photosynthesize at high temperatures. In August 2020, which showed high temperature (26.7°C), the Fv/Fm values decreased in all age groups and was particularly less than 0.8 in 1-, 2-, and 3-year-old seedlings; therefore, these seedlings were under more stress in high temperatures of summer than the 5- and 6-year-old seedlings.

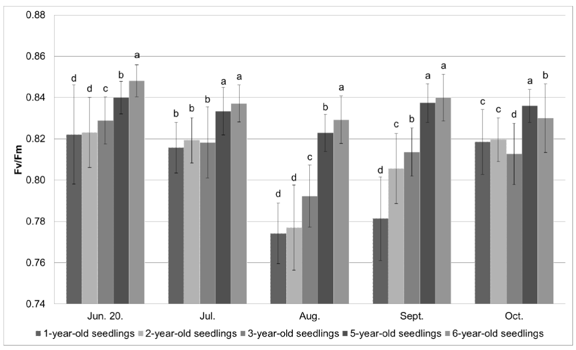

Fig. 3. Differences in the maximum quantum efficiency of Photosystem II (Fv/Fm) by the five age classes in *A. koreana* in summer. Significant differences were observed using the ANOVA and Duncan's multiple range test at $p < 0.05$.

Comparison of photosynthetic capacity of A. koreana seedlings by age through the light response curve

The photosynthesis rate measured with varying light intensity indicated that the light saturation point by age classes ranged from 700 to 1000 μmol $m^{-2} \cdot s^{-1}$, with no significant difference among the age classes. This finding was consistent with that of Teskey et al. (1984), who reported that light saturation points were equal to or greater than 1,000 μmol $m^{-2} \cdot s^{-1}$ in all age classes, with no age-dependent differences in photosynthetic capacity. Further, photosynthetic capacity increased with age (Fig. 4). The ANOVA results for summer photosynthetic capacity showed significant differences in age-specific averages ($p < 0.05$), and Duncan's multiple range test showed significant differences between 5- and 6-year-old seedlings and 1-, 2-, and 3-year-old seedlings ($p < 0.05$). Teskey et al. (1984) studied the photosynthetic capacity of foliage with age (current foliage and 1-, 3-, 5-, 7-, and 9-year-old foliage) and found that young foliage had better photosynthetic capacity than older foliage. Conversely, in this study, older seedlings showed higher photosynthetic capacity than young seedlings.

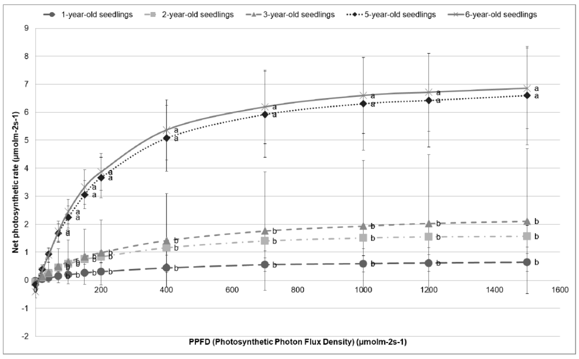

Fig. 4. Light response curve showing differences in the photosynthetic capacity of *A. koreana* with age differences in August. Significant differences were observed using the ANOVA and Duncan's multiple range test at $p < 0.05$.

The light compensation point, dark respiration rate, apparent quantum yield, and maximum photosynthesis rate were calculated using the linear regression equation of light intensity and photosynthesis at weak light conditions (0-100 μmol $m^{-2} \cdot s^{-1}$) (Table 2). The light compensation point was the lowest at 3.3835 μmol $m^{-2} \cdot s^{-1}$ for the 3-year-old seedlings, and photosynthesis was efficient at low light intensities. Conversely, the light compensation point for the 6-year-old seedlings was the highest at 10.6722 μmol $m^{-2} \cdot s^{-1}$. Apparent quantum yield is used as an indicator of photosynthetic capacity at low light intensities and represents the activity of the photochemical system that converts light energy into chemical energy (Evans 1987; Strasser et al. 2000; Kim and Lee 2001). In the absence of environmental stress, the apparent quantum yield ranges from 0.04-0.06 μmol CO_2 $m^{-2} \cdot s^{-1}$ (Bjokman and Demmig 1987), and if it is less than 0.04, the photochemical system is reported to have been damaged (Kim and Lee 2001). In this study, the *A. koreana* seedlings of all ages had a value of less than 0.04 μmol CO_2 $m^{-2} \cdot s^{-1}$, thus, suggesting that an unstable state even in the photochemical system. Particularly, the photochemical system was judged to be unstable in relatively young *A. koreana* seedlings (3-year-old or less), which exhibited an apparent quantum

Table 2. Photosynthetic parameters of *Abies koreana* seedlings

Age	Light compensation point (μmol $m^{-2} \cdot s^{-1}$)	Dark respiration rate** (μmol CO_2 $m^{-2} \cdot s^{-1}$)	Apparent quantum yield*** (μmol CO_2 $m^{-2} \cdot s^{-1}$)	Maximum photosynthesis rate*** (μmol CO_2 $m^{-2} \cdot s^{-1}$)
1-year-old seedlings	5.8253±3.7452[a]	-0.0118±0.0068[a]	0.0022±0.0005[b]	1.5458±0.3405[b]
2-year-old seedlings	5.2503±3.7543[a]	-0.0372±0.0337[a]	0.0066±0.0019[b]	4.6003±1.3320[b]
3-year-old seedlings	3.3835±1.7546[a]	-0.0155±0.0108[a]	0.0070±0.0083[b]	4.9020±5.7818[b]
5-year-old seedlings	3.7718±0.8997[a]	-0.0906±0.0235[a]	0.0242±0.0044[a]	16.8669±3.0574[a]
6-year-old seedlings	10.6722±7.5100[a]	-0.2930±0.1988[b]	0.0283±0.0034[a]	19.5345±2.4231[a]

Values are mean±SD (n=4).
Significant differences were observed using ANOVA at $p < 0$ '***', $0 < p < 0.001$ '**', $0.001 < p$ '*', and Duncan's multiple range test at $p < 0.05$.

yield value of 0.007 or less. The highest photosynthesis rate (19.5345 μmol CO_2 $m^{-2}\cdot s^{-1}$) was observed for 6-year-old seedlings, and it decreased with age.

Conclusions

A. koreana, a native species of Korea, is endangered due to climate change. Therefore, a restoration strategy to conserve *A. koreana* is required; additionally, biological information on the restoration material is essential to implement a successful restoration strategy. This study compared the chlorophyll fluorescence and photosynthetic capacities of *A. koreana* seedlings by age and suggested the seedling age that is most suitable for restoration. The results of this study showed that the response to environmental stress, and photosynthetic capacities of *A. koreana* seedling varied by age. On measuring the chlorophyll fluorescence by age of *A. koreana* seedlings, it was found that the lower the age, the more environmental stress it experienced. In particular, Fv/Fm values decreased significantly in low temperature environments (for example, winter). In addition, the results of the photosynthetic capacity analysis by age showed that photosynthetic capacities improved with age. The results suggested that the 5- and 6-year-old seedlings were less sensitive to environmental stress and have better photosynthetic capacities than the 1-, 2-, and 3-year-old seedlings. Therefore, the 5-year-old or older *A. koreana* seedlings can be effectively used as restoration material because they are expected to show increased adaptability and stable growth during transplantation due to their relatively high environmental resistance and photosynthetic capacity. The findings of this study can be used to implement effective strategies to preserve and restore the endangered *A. koreana* species.

References

Alscher RG, Hess JL. 1993. Antioxidants in Higher Plants. CRC Press, Boca Raton, FL, 174 pp.

Asada K. 1999. The Water-Water Cycle in Chloroplasts: Scavenging of Active Oxygens and Dissipation of Excess Photons. Annu Rev Plant Physiol Plant Mol Biol 50: 601-639.

Asanok L, Marod D. 2016. Environmental Factors Influencing Tree Species Regeneration in Different Forest Stands Growing on a Limestone Hill in Phrae Province, Northern Thailand. J For Environ Sci 32: 237-252.

Bjorkman O, Demmig B. 1987. Photon yield of O2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta 170: 489-504.

Bolhar-Nordenkampf HR, Long SP, Baker NR, Oquist G, Schreiber U, Lechner EG. 1989. Chlorophyll Fluorescence as a Probe of the Photosynthetic Competence of Leaves in the Field: A Review of Current Instrumentation. Funct Ecol 3: 497-514.

Chae HM, Yun YJ. 2018. Comparison of the Meteorological Factors on the Forestland and Weather Station in the Middle Area of Korea. J For Environ Sci 34: 249-252.

Crawford RMM. 1989. Studies in Plant Survival: Ecological Case Histories of Plant Adaptation to Adversity. Blackwell Scientific, Oxford, pp 253-254.

Cregg BM, Duck MW, Rios CM, Rowe DB, Koelling MR. 2004. Chlorophyll Fluorescence and Needle Chlorophyll Concentration of Fir (*Abies* sp.) Seedlings in Response to pH. HortScience 39: 1121-1125.

Evans JR. 1987. The Dependence of Quantum Yield on Wavelength and Growth Irradiance. Funct Plant Biol 14: 69-79.

Falqueto AR, da Silva Júnior RA, Gomes MTG, Martins JPR, Silva DM, Partelli FL. 2017. Effects of drought stress on chlorophyll a fluorescence in two rubber tree clones. Sci Hortic 224: 238-243.

Farjon A. 1990. Pinaceae, Drawings and Descriptions of the Genera: *Abies*, *Cedrus*, *Pseudolarix*, *Keteleeria*, *Nothotsuga*, *Tsuga*, *Cathaya*, *Pseudotsuga*, *Larix* and *Picea*. Koeltz Scientific Books, Konigstein.

Greenwood MS, O'Brien CL, Schatz JD, Diggins CA, Day ME, Jacobson GL, White AS, Wagner RG. 2008. Is early life cycle success a determinant of the abundance of red spruce and balsam fir? Can J For Res 38: 2295-2305.

Hazrati S, Tahmasebi-Sarvestani Z, Modarres-Sanavy SA, Mokhtassi-Bidgoli A, Nicola S. 2016. Effects of water stress and light intensity on chlorophyll fluorescence parameters and pigments of *Aloe vera* L. Plant Physiol Biochem 106: 141-148.

Howe GT, Aitken SN, Neale DB, Jermstad KD, Wheeler NC, Chen THH. 2003. From genotype to phenotype: unraveling the complexities of cold adaptation in forest trees. Can J Bot 81: 1247-1266.

International Union for Conservation of Nature and Natural Resources. Species Survival Commission. 2013. Guidelines for Reintroductions and Other Conservation Translocations. Version 1.0. Gland, Switzerland IUCN Species Survival Commission.

Katahata SI, Naramoto M, Kakubari Y, Mukai Y. 2007. Seasonal changes in photosynthesis and nitrogen allocation in leaves of different ages in evergreen understory shrub *Daphniphyllum humile*. Trees 21: 619-629.

Kim GT, Kim JS, Choo GC. 1991. Studies on the Structure of Forest Community at Banyabong Area-*Abies koreana* Forest. Korean J Environ Ecol 5: 25-31. (in Korean with English abstract)

Kim PG, Lee EJ. 2001. Ecophysiology of Photosynthesis 1: Effects of Light Intensity and Intercellular CO_2 Pressure on Photosynthesis. Korean J Agric For Meteorol 3: 126-133. (in Korean)

Kohyama T. 1983. Seedling stage of two *subalpine Abies* species in distinction from sapling stage: a matter-economic analysis. Bot Mag Tokyo 96: 49-65.

Koo KA, Kim DB. 2020. Review Forty-year Studies of Korean fir (*Abies koreana* Wilson). Korean J Environ Ecol 34: 358-371. (in Korean with English abstract)

Koo KA, Park WK, Kong WS. 2001. Dendrochronological Analysis of *Abies koreana* W. at Mt. Halla, Korea: Effects of Climate Change on the Growths. Korean J Ecol 24: 281-288.

Krause GH, Weis E. 1984. Chlorophyll fluorescence as a tool in plant physiology: II. Interpretation of fluorescence signals. Photosynth Res 5: 139-157.

Krishnamoorthy M, Palanisamy K, Francis AP, Gireesan K. 2016. Impact of Environmental Factors and Altitude on Growth and Reproductive Characteristics of Teak (*Tectona grandis* Linn. f.) in Southern India. J For Environ Sci 32: 353-366.

Kumar J, Pratap A, Kumar S. 2015. Phenomics in Crop Plants: Trends, Options and Limitations. Springer India, New Delhi, pp 181-194.

Lim JH, Woo SY, Kwon MJ, Chun JH, Shin JH. 2006. Photosynthetic Capacity and Water Use Efficiency under Different Temperature Regimes on Healthy and Declining Korean Fir in Mt. Halla. J Korean For Soc 95: 705-710. (in Korean with English abstract)

Liu T. 1971. A Monograph of the Genus *Abies*. National Taiwan University, Taipei, 608 pp.

Lovelock CE, Osmond CB, Jebb M. 1994. Photoinhibition and recovery in tropical plant species: response to disturbance. Oecologia 97: 297-307.

Muller P, Li XP, Niyogi KK. 2001. Non-photochemical quenching. A response to excess light energy. Plant Physiol 125: 1558-1566.

Oh SJ, Koh JG, Kim ES, Oh MY, Koh SC. 2001. Diurnal and Seasonal Variation of Chlorophyll Fluorescence from Korean Fir Plants on Mt. Halla. Korean J Environ Biol 19: 43-48. (in Korean with English abstract)

Oh SJ, Koh SC. 2004. Chlorophyll Fluorescence and Antioxidative Enzyme Activity of *Crinum* Leaves Exposed to Natural Environmental Stress in Winter. Korean J Environ Biol 22: 233-241. (in Korean with English abstract)

Park HC, Lee JH, Lee GG, Um GJ. 2015. Environmental features of the distribution areas and climate sensitivity assesment of Korean Fir and Khinghan Fir. J Environ Impact Assess 24: 260-277. (in Korean with English abstract)

Reinhardt K, Johnson DM, Smith WK. 2009. Age-class differences in shoot photosynthesis and water relations of Fraser fir (*Abies fraseri*), southern Appalachian Mountains, USA. Can J For Res 39: 193-197.

Robakowski P, Bielinis E. 2017. Needle age dependence of photosynthesis along a light gradient within an *Abies alba* crown. Acta Physiol Plant 39: 83.

Ruban AV. 2016. Nonphotochemical Chlorophyll Fluorescence Quenching: Mechanism and Effectiveness in Protecting Plants from Photodamage. Plant Physiol 170: 1903-1916.

Smith WK, Germino MJ, Hancock TE, Johnson DM. 2003. Another perspective on altitudinal limits of alpine timberlines. Tree Physiol 23: 1101-1112.

Song KM, Kang YJ, Hyeon HJ. 2014. Vegetation Structure at the Slope Direction and Characteristic of Seedlings of *Abies koreana* in Hallasan Mountain. J Environ Sci Int 23: 39-46. (in Korean with English abstract)

Song KM, Kim JH, Choi HS. 2020. Growth Changes in *Abies koreana* Seedlings of the Hallasan Mountain Over a 10-year Period. J Environ Sci Int 29: 209-218. (in Korean with English abstract)

Strasser RJ, Srivastava A, Tsimilli-Michael M. 2000. The Fluorescence Transient as a Tool to Characterize and Screen Photosynthetic Samples. In: Probing Photosynthesis: Mechanism, Regulation and Adaptation (Yunus M, Pathre U, Mohanty P, eds). CRC Press, Boca Raton, pp 445-483.

Tanaka A. 2007. Photosynthetic activity in winter needles of the evergreen tree Taxus cuspidata at low temperatures. Tree Physiol 27: 641-648.

Teskey RO, Grier CC, Hinckley TM. 1984. Change in photosynthesis and water relations with age and season in *Abies amabilis*. Can J For Res 14: 77-84.

Wang S, Li Y, Ju W, Chen B, Chen J, Croft H, Mickler RA, Yang F. 2020. Estimation of Leaf Photosynthetic Capacity from Leaf Chlorophyll Content and Leaf Age in a Subtropical Evergreen Coniferous Plantation. J Geophys Res Biogeosci 125: e2019JG005020.

Weng JH, Liao TS, Sun KH, Chung JC, Lin CP, Chu CH. 2005. Seasonal variations in photosynthesis of Picea morrisonicola growing in the subalpine region of subtropical Taiwan. Tree Physiol 25: 973-979.

Yang JC, Yi DK, Joo MJ, Choi K. 2015. Phylogeographic study of *Abies koreana* and *Abies nephrolepis* in Korea based on mitochondrial DNA. Korean J Pl Taxon 45: 254-261. (in Korean with English abstract)

Zhou H, Xu M, Pan H, Yu X. 2015. Leaf-age effects on temperature responses of photosynthesis and respiration of an alpine oak, Quercus aquifolioides, in southwestern China. Tree Physiol 35: 1236-1248.